Dry deposition and canopy exchange for temperate tree species under high nitrogen deposition

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DRY DEPOSITION AND CANOPY EXCHANGE FOR TEMPERATE TREE SPECIES UNDER HIGH NITROGEN DEPOSITION

Thesis submitted in fulfillment of the requirements
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Dutch translation of the title:
Droge depositie en kroonuitwisseling bij gematigde boomsoorten blootgesteld aan hoge stikstofdepositie

Illustrations on the cover:
Front: Young beech (*Fagus sylvatica* L.) leaves in Wellin, Walloon region (April 2009)
Back: Autumn canopy of the beech tree studied in Chapter 5, 6 and 7 (October 2008)

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Woord vooraf

“Winter is gone and the leaves turn green”
(Nick Drake, 1969)

Het doorbreken van de lente in het bos heb ik de afgelopen vier jaar van zeer dichtbij meegemaakt. Die eerste groene blaadjes waren steevast het signaal om volop in actie te schieten en de data te verzamelen die de basis van dit doctoraat vormen. Alleen heb ik het altijd een beetje gek gevonden dat er maar één naam op de voorkant van zo’n doctoraat prijkt, want, naar mijn gevoel, is dit werk toch zeker niet het resultaat van enkel mezelf. Samenwerking was de basis en ook de rode draad van dit werkstuk. De voorbije vier jaar ben ik in het bos, in het labo, aan mijn bureau en op het thuisfront bijgestaan door een hele rits aan enthousiaste collega’s, vrienden en familie. Gelukkig bestaat er nog zoiets als een dankwoord om al deze mensen toch nog even naar voor te schuiven.

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Bij mijn start in Gontrode kwam ik in de 0.17 terecht, later geklasseerd als de koudste bureau van het gebouw. Maar dat “koude” gold allerminst voor de mensen daar aanwezig. Karen, je hebt mij zeer warm onthaald, met de nodige porties cake, raad en daad bij al mijn doctoraatsperikelen, maar ook met een zelfde humor die tot vele hilarische 0.17-momenten leidde. Tatiana, ik denk dat dit laatste na jouw komst er niet op is verslechterd. Bedankt voor al jullie hulp en steun! Darline, bedankt om de 0.17 ook gedurende meer dan een jaar te komen opvrolijken met, tussen het werk door, allerlei gesprekken over plantjes, tuinieren en muzikale ideeën. Van de lege 0.17, hierdoor echt wel een beetje koud, ging ik de laatste vier maanden naar de 0.02, de “warmste” bureau van het gebouw. Lander, super bedankt voor al het statistisch advies, om de R-microbe over te brengen, de vegetatiekunde-excursies mee te organiseren en af en toe een Miles Davis plaat op te leggen en samen een pintje te drinken. Margot, ongelooflijk bedankt voor het kritisch en belangeloos nalezen van een aantal hoofdstukken en het checken van de referenties, maar vooral voor de niet aflatende stroom aan supporter-kaartjes en –mails om de laatste maanden door te komen!!

Dit doctoraat bestaat uit vele tabellen en figuren, die op zich dan weer bestaan uit vele getallen. Deze getallen zouden niet bestaan zonder het werk van een aantal mensen. Luc, samen hebben we 400 boompjes in de Kangoo gekregen, specie gemengd, geplant, proefjes uitgevoerd, “wagonnetjes” gemaakt, waterstalen opgehaald en de volgens jou (en ook door jou) leukste proefopzet gebouwd. Bedankt voor al die creatieve oplossingen en om altijd klaar te staan om te helpen. Greet, bedankt om die tweewekelijkse stroom aan waterstalen zo goed bij te houden en te analyseren! Kris, zonder jou stond ik waarschijnlijk nu nog plantstalen te vermalen, bedankt! Katja, bedankt om die 2320 plantstalen steeds even opgewekt aan te nemen, te analyseren wanneer de getalletjes er moesten zijn en die 160 waterstalen ‘even’ om te zetten. Jan, bedankt om die waterstalen even fluks te analyseren. Het project waarop dit doctoraat gebeurde zou financieel en administratief waarschijnlijk in het honderd zijn gelopen zonder de strenge opvolging van Christel, bedankt! Ook een dikke merci aan de stage- en jobstudenten Ward en Noam.

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Contents

List of abbreviations and symbols

Abbreviations

AF	Artifical foliage
BC	Base cations (= $Na^+ + K^+ + Ca^{2+} + Mg^{2+}$)
BD	Bulk precipitation deposition (mmol$_c$ m^{-2} period^{-1})
CA	Contact angle (°)
CBM	Canopy budget model
CE	Canopy exchange (mmol$_c$ m^{-2} period^{-1} or kg ha^{-1} yr^{-1})
CL	Canopy leaching (CE > 0) (mmol$_c$ m^{-2} period^{-1})
CU	Canopy uptake (CE > 0) (mmol$_c$ m^{-2} period^{-1} or kg ha^{-1} yr^{-1})
dbh	Diameter at breast height
DD	Dry deposition (mmol$_c$ m^{-2} period^{-1} or kg ha^{-1} yr^{-1})
DD$_{CBM}$	Dry deposition calculated by the canopy budget model
DD$_{AF}$	Dry deposition calculated from artificial foliage
DD$_{vd}$	Dry deposition calculated from air concentration measurements multiplied with a deposition velocity
DDF	Dry deposition factor
DIN	Dissolved inorganic nitrogen
DM	Dry matter
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
L	Stomatal length (µm)
LAI	Leaf area index (m^2 m^{-2})
LiDAR	Light detection and ranging
MMD	Mean mass diameter (µm)
NTF	Net throughfall deposition (mmol$_c$ m^{-2} period^{-1} or µmol$_c$ g^{-1} DM)
PAR	Photosynthetic active radiation
PD	Precipitation deposition (bulk or wet-only) (mmol$_c$ m^{-2} period^{-1})
RH	Relative humidity (%)
SD	Stomatal density (number of stomata m^{-2} leaf area)

SEM	Scanning electron microscopy
SF	Stemflow deposition (mmol$_c$ m^{-2} period^{-1})
SPS	Stomatal pore surface (µm^2)
TD$_{ac}$	Total acidifying deposition (molc ha^{-1} yr^{-1})
TD$_N$	Total nitrogen deposition (kg ha^{-1} yr^{-1})
TF	Throughfall deposition (mmol$_c$ m^{-2} period^{-1} or µmol$_c$ g^{-1} DM)
TLS	Terrestrial laser scanning
W	Stomatal width (µm)
WA	Weak acids
WD	Wet-only precipitation deposition (mmol$_c$ m^{-2} period^{-1})
WSC	Water storage capacity (g m^{-2})

Symbols

δ_j	Time-averaged relative difference from the mean
g_{smax}	Theoretical maximal stomatal conductance (mol H_2O m^{-2} s^{-1})
p	Significance of a statistical test
r	Pearson correlation coefficient
r_s	Spearman rank correlation coefficient
R_s	Stomatal resistance (s m^{-1})
v_d	deposition velocity (cm s^{-1})

Chemical compounds

C	Carbon
Ca^{2+}	Calcium
Cl^-	Chloride
CO_2	Carbon dioxide
H^+	Proton
HCl	Hydrogen chloride
HCO_3^-	Bicarbonate
HNO_3	Nitric acid
K^+	Potassium
KCl	Potassium chloride
Mg^{2+}	Magnesium
N	Nitrogen

Na^+	Sodium
NH_4^+	Ammonium
NH_3	Ammonia
pNH_4^+	Particulate NH_4^+
NH_x	Reduced nitrogen ($NH_3 + NH_4^+$)
N_2O	Nitrous oxide
NO_3^-	Nitrate
NO_2	Nitrogen dioxide
pNO_3^-	Particulate NO_3^-
NO_y	Oxidised nitrogen (NO_3^-, HNO_3, NO_2,…)
PO_4^{3-}	Phosphate
S	Sulphur
SO_2	Sulphur dioxide
SO_4^{2-}	Sulphate
pSO_4^{2-}	Particulate SO_4^{2-}
^{15}N	N with atom mass of 15 g mol^{-1}
^{13}C	C with atom mass of 13 g mol^{-1}

1 Introduction

1.1 Atmospheric deposition on forest ecosystems

1.1.1 Definition

Atmospheric deposition is an important nutrient source in forest ecosystems (Lindberg et al. 1986). It represents the total input of ions, gases and organic compounds to a forest canopy and can be divided into several fractions according to Ulrich (1983) (Fig. 1.1). From the viewpoint of the depositing compound, a distinction can be made between i) wet deposition, i.e. the input of rainfall and snow, ii) dry deposition, i.e. the input of particulate and gaseous compounds and iii) occult deposition, i.e. the impaction of mist and fog (Ulrich 1983). An important contribution of occult deposition is generally limited to mountainous and coastal areas, while the importance of dry and wet deposition varies from region to region (Lovett 1994). From the viewpoint of the depositing surface (the forest canopy), a distinction is made based on whether this surface influences the deposition process. Precipitation deposition, i.e. rainfall, snow and large particles, is only influenced by gravitation, while interception deposition consists of small particles, gases, mist and fog and is determined by the state of the receiving surface (Ulrich 1983).

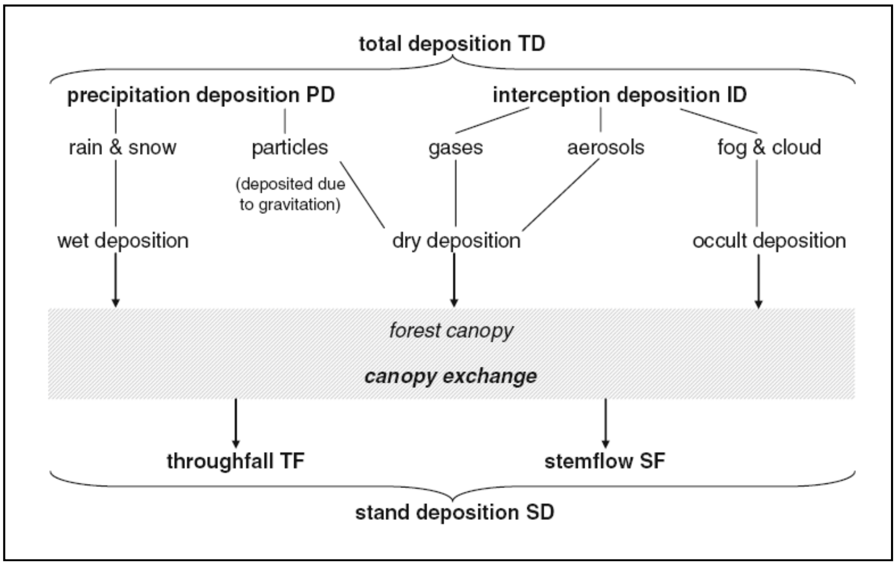

Fig. 1.1 Overview of the input and output pathways of a forest canopy (Talkner et al. 2010)

Although in the rest of this work the viewpoint of the depositing compound is considered, it is on interception deposition that the filtering efficiency of the forest canopy (size, structure and chemical state) exerts an influence (see 1.2). In general, the structure of forest canopies creates favourable micrometeorological conditions for the deposition of atmospheric particles and gases (Rodrigo and Avila 2002). Their higher roughness and greater exposed surface compared to other vegetation types promotes turbulence at the forest boundary layer and increases their ability to filter gases and particles (De Schrijver et al. 2007). Atmospheric deposition can then interfere with the forest canopy, which can act as a net source or sink for a specific chemical compound. Stand deposition is then the output of ions and organic compounds from the canopy and consists of throughfall and stemflow, which represent the dissolved nutrient input to the forest floor (Fig. 1.1; Talkner et al. 2010).

1.1.2 Human induced changes in atmospheric deposition

The study of atmospheric deposition on forest ecosystems has gained increasing interest when first anthropogenic emissions of sulphur (S) dioxide (SO_2) and then nitrogen (N) compounds such as nitric oxide (NO_y) and reduced N (NH_x) increased. High SO_2 emissions accounted for a strong acidity in rainfall dissolved as SO_4^{2-}, the so-called ‘acid rain’ (Martin 1984), which in turn could lead to acidification of forest soils (Johnson and Lindberg 1992; De Schrijver et al. 2006). Furthermore, it led to acute and chronic effects on stomatal functioning of tree foliage close to emission sources (Neighbour et al. 1988; McAinsh et al. 2002) and significantly affected leaf characteristics such as leaf wettability (Haines et al. 1985). In the 1970s and ‘80s, North America and Europe negotiated emission reductions internationally, which has led to a significant decrease in SO_2 emissions (Cape et al. 2003; Vestreng et al. 2007) and depositions (Staelens et al. in press). Currently, most SO_2 deposition occurs to forest ecosystems outside North America and Europe (Fowler et al. 2009).

From 1960 onwards, the global rate of increase in anthropogenic NO_x and NH_3 emissions has accelerated sharply in industrialized regions due to fossil fuel combustion and agricultural activities (Galloway et al. 2003). Before this emission increase, N was only available for the growth of plants and microbes when converted from N_2 in the atmosphere to reactive forms such as NH_4^+ and NO_3^-, or through biological N fixation (Driscoll et al. 2003). The productivity of most natural ecosystems was limited by the supply of biologically available N, particularly in temperate and boreal regions, and many native plant species are adapted to and function optimally in soils with low N levels. The more than doubled atmospheric input of reactive N into these ecosystems has significantly altered the N cycle (Vitousek et al. 1997). As forest ecosystems tend to have a high accumulation potential for this additional N (Galloway et al. 2003), several studies have observed increased tree growth with N deposition (Solberg et al. 2009), although not for all tree species (Thomas et al. 2010). Within the framework of climate change, much attention has also been given to the impact of this increased N availability to carbon (C) storage in forests; however, this effect is still under debate (Nadelhoffer et al. 1999; Magnani et al. 2007; Sievering et al. 2007; Högberg 2011).

Research conducted during the last 20 years has caused a major shift in the perspective of forest ecologists on N. The large reservoir size and the relatively low turnover rate of forest ecosystems implicates that these human induced changes in N availability can have long-term effects (Aber et al. 2003). Chronic N input from air pollution exceeds the normal growth requirement of forests in large areas in Europe and some parts of North America, and has led to a conceptual model of "N saturation" that describes the temporal sequence of changes in ecosystem functioning in response to chronic increased N input (Aber et al. 1989; Gundersen et al. 2006). After the first phase of increasing primary production and accelerated N cycling, the composition of the forest floor vegetation may gradually change towards more nitrophilic species (Bobbink et al. 1998) and other essential resources (light, water and other nutrients) may at least periodically limit tree growth. In the accelerated N cycle, net nitrification becomes important, nitrate (NO_3^-) starts to appear in soil water and the ecosystem approaches N saturation. When elevated NO_3^- leaching becomes a chronic condition, soil acidification becomes significant due to the depletion of so-called base cations such as calcium and magnesium and the mobilization of aluminium (Gundersen et al. 2006; De Schrijver et al. 2007). In this regard, NO_3^- acts in concert with sulphate (SO_4^{2-}) leaching, thus N and S pollution can have additive effects. Nitrate leaching will continue to increase with deposition (Dise et al. 2009) and losses of NO and the greenhouse gas N_2O from the ecosystem was shown to increase significantly (Pilegaard et al. 2006). This N saturation concept is supported by many studies, although not all studies have supported the temporal sequence in which the ecosystem responses occur (Emmett 2007; Lovett and Goodale 2011). Recently, a new conceptual model of the N saturation process was presented and supported with data by

Lovett and Goodall (2011), focusing on the mass balance of N and distinguishing kinetic N saturation from capacity N saturation (Fig. 1.2). This illustrates the complexity of the N cycle in forest ecosystems, as a result of which ecosystem N stocks and processes are not as well studied as those of C (Butterbach-Bahl and Gundersen 2011).

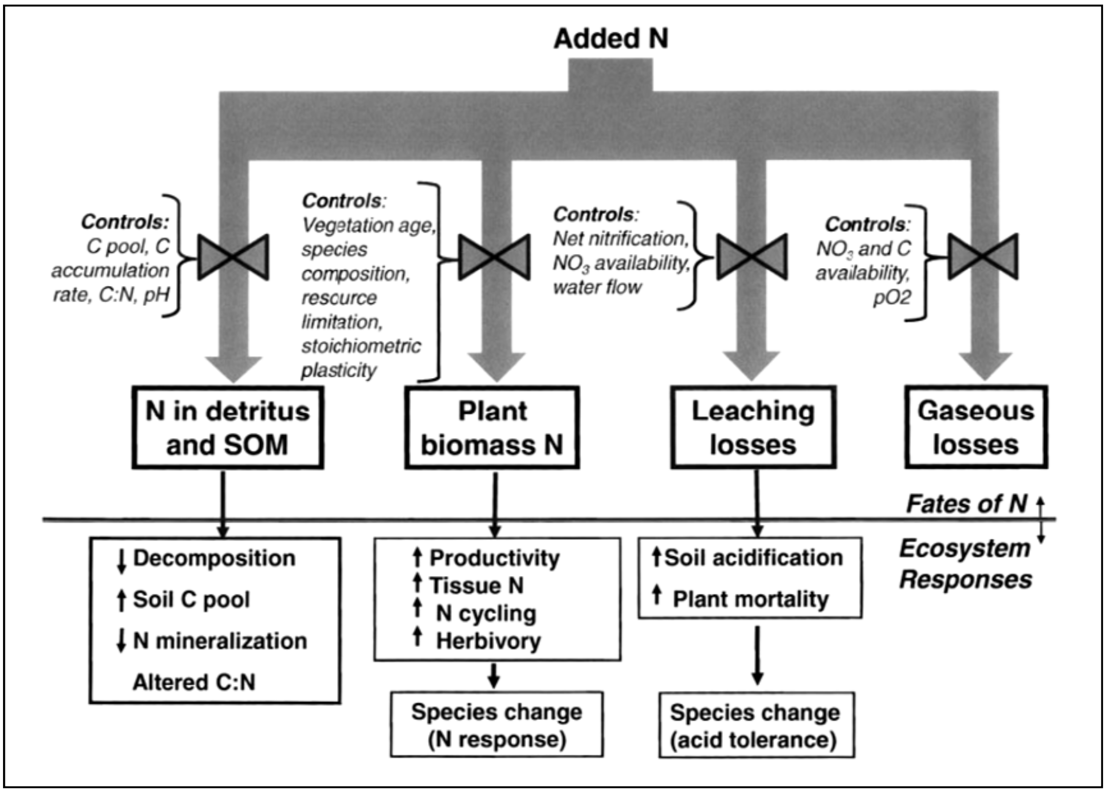

Fig. 1.2 Conceptual model of N saturation in an ecosystem, illustrating N flow to various fates (internal sinks and losses from the ecosystem), examples of controlling factors, and ecosystem responses associated with N flow to those fates. Detritus includes the forest floor and woody debris, and SOM represents soil organic matter (Lovett and Goodale 2011)

The implementation of international agreements and conventions, e.g. the European Commissions' National Emission Ceilings Directive (NECD) or the UNECE Convention on Long-range Transboundary Air Pollution (CLRTAP) and the establishment of critical loads, must lead to emission reductions and increased ecosystem protection against adverse effects of air pollution. Critical loads are defined as "a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge" (Nilsson and Grennfelt 1988). At the European level, emissions of NH_x, NO_y and SO_x

have steadily decreased from 1990 to 2005 (Hertel 2011), but this has not always resulted in decreased atmospheric depositions on a local level (Erisman et al. 2005; Fowler et al. 2005). Long-term monitoring of N and S fluxes and their effects on total nutrient cycling is imperative to assess ecosystem control on N and S losses and allow detection of forest ecosystem recovery from historical pollution effects (Neirynck et al. 2008). However, deposition processes to forest ecosystems are generally still poorly understood and accurate quantification of N and S deposition remains a key issue (Wesely and Hicks 2000; Erisman et al. 2005). As the acidifying effect of N and S can be neutralised by the base cations K^+, Ca^{2+} and Mg^{2+}, quantification of the atmospheric deposition of these ions is also of importance to evaluate forest nutrient cycling.

1.2 The forest canopy as an interceptor of atmospheric nitrogen and sulphur

As mentioned in 1.1.1, forest canopies act as very efficient sinks for atmospheric gases and particles. For example, atmospheric N deposition to forest ecosystems is enhanced up to a factor of 2 to 3 compared to open land (Fowler et al. 2004). Although forest canopies receive the same amount of wet deposition as other vegetation types, their large aboveground biomass in contact with incident rainfall can alter the chemical composition of precipitation significantly. This section reviews the effect forest canopies can exert on these processes and addresses existing knowledge gaps.

1.2.1 Wet and dry deposition processes

Wet deposition is controlled by the concentration and size distribution of atmospheric particles, the concentration, solubility and reactivity of gases and by the meteorological factors that control precipitation (Lovett 1994; Beverland et al. 1998). Its composition will therefore vary across regions. Especially small aerosols enclosing SO_4^{2-}, NO_3^- and NH_4^+ are efficiently removed from the atmosphere through wet deposition (Fowler et al. 2001). The atmospheric chemistry of S and N compounds is complex and beyond the scope of this thesis. In general, the wet deposition process is the result of gravity acting on droplets that have grown large enough to fall, and the primary control on the rate of deposition is the number and size of droplets in the air and the chemistry of those droplets (Lovett 1994). Wet deposition can be reasonably well estimated by bulk or wet-only precipitation collectors. When using bulk precipitation collectors, wet deposition is overestimated due to dry deposition onto the collector surface, which is washed away into the sample during the next precipitation event (Staelens et al. 2005).

Deposition of dry particles and gases is a complex process governed by air concentrations and turbulent transport processes in the boundary layer, as well as by the chemical and physical nature of the depositing species and the capability of the surface to capture or absorb gases and particles (Erisman and Draaijers 2003). It is generally conceptualized as a resistance analogy (Hicks et al. 1987; Fig. 1.3). First, the transport of gases and particles from the atmosphere to the near surface is governed by the level of atmospheric turbulence (aerodynamic resistance; R_a), generated by both wind shear and buoyancy. Tree height, leaf area density profile and the leaf area index (LAI) have been shown to influence the dry deposition of particles through the level of atmospheric turbulence (Erisman and Draaijers 2003; Katul et al. 2011), but research questions on the impact of the canopy on dry deposition still remain to be answered and supportive data are lacking (Petroff et al. 2008).

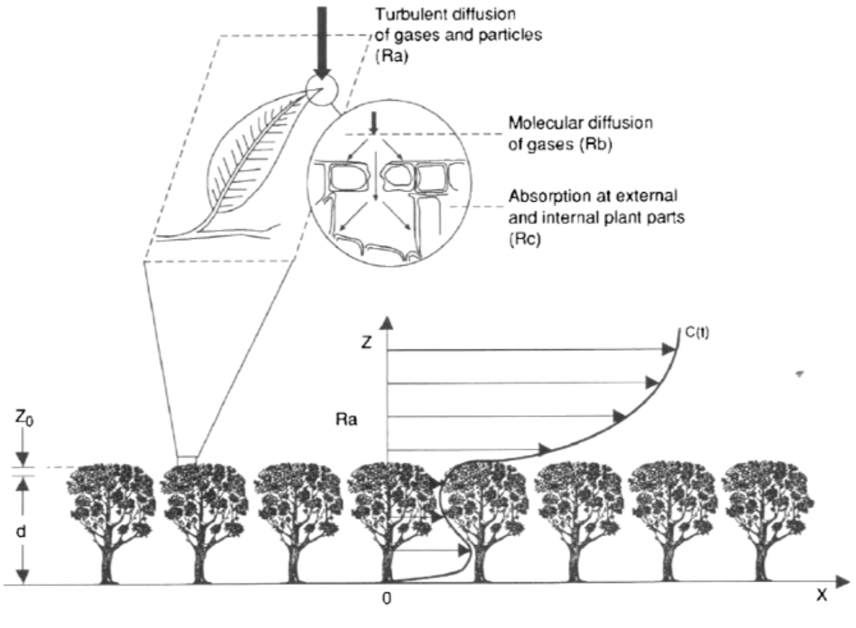

Fig. 1.3 The dry deposition process as the sum of the aerodynamic resistance R_a, the boundary layer resistance R_b and the surface resistance R_c. R_a is determined by the roughness length (z_0) and the displacement height (d) and influences the concentration gradient (C(t)) above the receptor surface (Erisman and Draaijers 2003)

Second, deposition on the forest canopy is determined by the boundary layer resistance (R_b) (Hicks et al. 1987). Particles at the large end of the spectrum (i.e. with an aerodynamic diameter > 5 μm) such as aerosols containing Na^+, Cl^-, Ca^{2+}, Mg^{2+} and NO_3^- (Lindberg et al. 1986; Ruijgrok et al. 1997) are transferred through the boundary layer mainly by gravitational sedimentation and inertial impaction, while smaller particles (< 0.2 μm) can be transferred through Brownian diffusion (Davidson and Wu 1991). Particles between this size range and particularly from 0.2 to 2 μm, such as NH_4^+, SO_4^{2-} but also NO_3^- are not readily dry deposited and tend to travel long distances in the atmosphere until their eventual deposition, most likely through incorporation into precipitation (Lovett 1994). However, they can still account for a large part of total mass deposition (Davidson et al. 1982). Since particles are not able to diffuse through stomatal openings, their deposition is mainly determined by microstructure and turbulence intensity (Erisman and Draaijers 2003). Tree shoots with a more complex structure, such as needles or more hairy leaves are more efficient in capturing dry deposition (Beckett et al. 2000; Freer-Smith et al. 2004). Gases such as NH_3, HNO_3, NO_2 and SO_2 can easily traverse the boundary layer by molecular diffusion, but then final deposition is determined by the chemical, physical and biological nature of the canopy, summarized as the canopy resistance R_c which consists of several resistances in parallel (Erisman and Draaijers 2003). Surface deposition (defined as the cuticular resistance, R_{cut}) is controlled by leaf characteristics, such as surface wetness chemistry and leaf surface wetness, (Burkhardt and Eiden 1994; Neirynck et al. 2005) and the nature of the gas (highly reactive gases such as HNO_3 vs less reactive such as SO_2 or NO_2). Stomatal transport (or stomatal resistance, R_s) is regulated by all the physiological factors and meteorological conditions that regulate stomatal opening (Hicks et al. 1987). The sum of R_a, R_b and R_c is the total canopy resistance R_t (s cm^{-1}), the inverse of the deposition velocity (v_d; cm s^{-1}). The deposition flux of a certain atmospheric gas or particle to a forest canopy is then calculated by the product of v_d with its atmospheric concentration.

Despite significant developments and research throughout the years, measurements of dry deposition (see Chapter 6 for an overview) are still subject to several problems and cannot be quantified with sufficient accuracy (Erisman et al. 1994). Process oriented studies are needed to derive insight into deposition processes for different atmospheric components and to obtain process descriptions and parameters to be used in models. Micrometeorological methods provide the highest accuracy for this purpose, but in heterogeneous forest stands throughfall measurements might be a more suitable alternative. These process oriented studies can also be used to test or verify other simple/cheap measuring methods (Erisman et al. 1994).

1.2.2 Canopy exchange processes of N

Uptake of atmospheric N deposition is potentially possible through foliage, twigs, branches and stems (Harrison et al. 2000). The ability of tree canopies to take up ammonium (NH_4^+) and NO_3^- from wet deposition is well known, as observed from changes in N composition of rainfall and throughfall (Parker 1983; Lindberg et al. 1986; Cappellato et al. 1993; Lovett and Lindberg 1993; Neary and Gizyn 1994; Houle et al. 1999), from ^{15}N labelling studies (Bowden et al. 1989; Brumme et al. 1992; Eilers et al. 1992; Garten et al. 1998; Dail et al. 2009) and from comparisons of N fluxes beneath real and plastic trees of similar morphology (Ignatova and Dambrine 2002; Stachurski and Zimka 2002). It is believed that the transport of NO_3^- and NH_4^+ ions at the leaf surface primarily occurs through cuticular diffusion (Peuke et al. 1998; Fig. 1.4), however, the exact mechanism is still unclear as it also is for the uptake through the bark of twigs, branches and stems. Based on precipitation and throughfall measurements it is concluded that NH_4^+ uptake is an ion-exchange process for which base cations are leached from the foliage (Draaijers et al. 1997; Stachurski and Zimka 2002; Umana and Wanek 2010), but passive diffusion through stomata and cuticular transport have also been suggested as mechanisms (Schaefer et al. 1988; Bowden et al. 1989; Wilson 1992; Boyce et al. 1996).

Generally, uptake or emission of gaseous N dioxide (NO_2) and ammonia (NH_3) occurs via the stomata and cuticle (Rennenberg and Gessler 1999) and has been observed from micrometeorological measurements (Neirynck et al. 2007; Wu et al. 2011), ^{15}N-labelling experiments (Vose and Swank 1990; Bruckner et al. 1993; Gessler et al. 2002) and flux chamber experiments (Van Hove et al. 1987a; Thoene et al. 1996; Gessler et al. 2000; Gessler et al. 2002; Raivonen et al. 2009). Nitrogen dioxide and NH_3 dissolve in the leaf apoplast to primarily form nitrous acid (HONO, HNO_2) and nitric acid (HNO_3) that then dissociate to nitrite (NO_2^-), NO_3^-, and protons (H^+) and NH_4^+, respectively (Wellburn 1990). After dissolution NO_3^- is rapidly reduced to NO_2^-, which is in turn reduced to NH_4^+ after transport to the chloroplast (Stulen et al. 1998). Ammonium is assimilated by the glutamine synthetase and glutamate synthase systems (Pearson et al. 2002; Krupa 2003). Atmospheric NH_3 and NO_2 may also be adsorbed at the leaf cuticle and dissolve in the thin water film. They can then either be taken up through the cuticle, washed off with incident rainfall or be re-emitted when the water films are dried (Rennenberg and Gessler 1999).

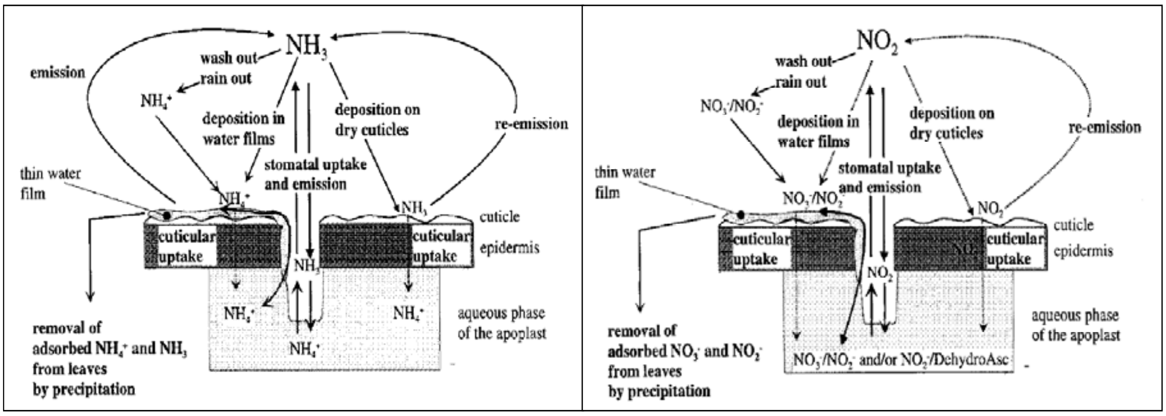

Fig. 1.4 Uptake mechanisms of NH_3/NH_4^+ (left) and NO_2/NO_3^- (right) through tree leaves (Rennenberg and Gessler 1999)

The uptake of N compounds is greatly controlled at the leaf level by chemical and physiological leaf characteristics (Sparks 2009). The dissolution of NH_3 in the apoplast is driven by the concentration difference between NH_3 in the substomatal cavity and NH_3/NH_4^+ in the aqueous phase of the apoplast, resulting in a stomatal compensation point which determines if emission or deposition occurs (Farquhar et al. 1980). Stomatal compensation points for NH_3 are shown to depend on leaf development stage (Wang et al. 2011), temperature and N nutritional stage (Farquhar et al. 1980; Sutton et al. 1995; Husted and Schjoerring 1996). As forest ecosystems become more N saturated, tree canopies may emit significant amounts of NH_3 (Rennenberg and Gessler 1999; Neirynck and Ceulemans 2008). Some studies have also reported on a leaf compensation point for NO_2 (Thoene et al. 1991; Gessler et al. 2000; Raivonen et al. 2009), but this has not always been confirmed (Chaparro-Suarez et al. 2011). The primary physiological leaf control over gaseous uptake of NH_3 and NO_2 is stomatal conductance (Hanson and Lindberg 1991; Hanstein et al. 1999; Gessler et al. 2000; Chaparro-Suarez et al. 2011), which in turn depends on the microclimate (Van Hove et al. 1990), internal CO_2 concentration (Farquhar and Sharkey 1982) and plant water status (Hutchinson et al. 1972). Dissolved uptake through leaf cuticles is suggested to be enhanced by the degree of wettability of the foliar surface (Harrison et al. 2000; Sase et al. 2008) and was shown to occur mainly during the growing season when the foliage is physiologically active (Harrison et al. 2000).

Canopy uptake of N may contribute substantially (16-42 %) to the total N demand of trees (Harrison et al. 2000), depending on the atmospheric N concentration (Schaefer et al. 1988; Eilers et al. 1992; Krupa 2003), N status of the plant (Muller et al. 1996; Stulen et al. 1998) and the tree species (Morikawa et al. 1998). Canopy uptake of atmospheric N may support additional growth, development and storage in trees (Fangmeier et al. 1994). The rest of the N demand is met by N uptake through the roots, mainly as NH_4^+, NO_3^- and to a lesser extent as organic N (Vallano and Sparks 2007). Plant available N in the soil is mainly the result of litter decomposition, biological N_2 fixation and atmospheric deposition (Butterbach-Bahl and Gundersen 2011). The uptake of N by roots is a well regulated process controlled by shoot-related signals (Stulen et al. 1998; Rennenberg and Gessler 1999). Enhanced canopy N uptake was shown to reduce N uptake via roots (Pérez-Soba and Van der Eerden 1993; Muller et al. 1996) through the translocation of amino acids generated from N taken up via foliage via the phloem to the roots, which then interact with inorganic N uptake in the roots (Rennenberg et al. 1998). However, a feedback mechanism also exist in the opposite direction, leading to decreased canopy uptake at increasing root N availability (Dyckmans and Flessa 2001; Vallano and Sparks 2008; Tateno and Takeda 2010).

Estimates of canopy N uptake compared to soil-derived N are highly variable, ranging from 0-80 % (Harrison et al. 2000; Gaige et al. 2007; Sievering et al. 2007), mainly due to the difficulty of estimating canopy uptake from throughfall measurements and an incomplete closure of the N budget at any given site (Sparks 2009). However, as pointed out by Sparks (2009), the quantification of canopy N uptake separately from soil N uptake is important since (i) canopy uptake leads to a direct addition of NO_3^- and NH_4^+ to the trees metabolism without competition with soil organisms, (ii) detrimental effects of atmospheric N mainly occur in the forest soil, while canopy uptake may influence tree growth and (iii) the partitioning of total deposition between canopy uptake and deposition to other surfaces could be very different across environments due to varying transport distances of N compounds.

1.2.3 Canopy exchange processes of S

In general, forest canopy interactions of deposited S are small due to the counteracting effects of foliar uptake and leaching (Lindberg and Lovett 1992). Uptake occurs most likely in the form of dry deposited SO_2, which is known to enter the substomatal cavity of the leaf or needle (Winner et al. 1985) and which is mainly controlled by stomatal conductance (Bache 1977). There is also some evidence for cuticular uptake (Winner et al. 1985), which is strongly enhanced by wind and abrasive damage on the cuticle (Hoad et al. 1992). Lindberg and Lovett (1992) estimated that annually 20-70 % of dry deposited SO_2 is fixed in tree canopies over North America and Europe. Foliar leaching of SO_4^{2-} results from root uptake and subsequent transport to the canopy. Garten (1990) and Cape et al. (1992) estimated this leaching to contribute little to throughfall by means of radioactive $^{35}SO_4$ measurements, although the results of these studies were not conclusive and they were conducted at regions with high S deposition. Lindberg and Lovett (1992) concluded that canopy leaching of SO_4^{2-} is unimportant at sites with high S deposition where deposition wash off dominates the below canopy flux and cannot be detected in the canopy balance because of uptake of SO_2. This low canopy interaction of SO_4^{2-} makes throughfall plus stemflow an excellent indicator of total atmospheric S loading to forests (Lindberg and Lovett 1992). However, as S deposition fluxes have decreased, canopy leaching might contribute more significantly to SO_4^{2-} fluxes in throughfall.

1.3 On the importance of studying processes at different spatio-temporal scales

To be able to quantify total atmospheric deposition of pollutants and to understand the pathways by which airborne pollutants enter and react within ecosystems, measurements at different spatio-temporal scales are highly valuable. Due to the complexity of forest structure, measurements on particular components of it, such as leaves, branches, or a horizontal canopy layer are often conducted (Nadkarni et al. 2008), leading to leaf area based flux rates, such as for NH_3 (Van Hove et al. 1989a; Bruckner et al. 1993; Gessler et al. 2000; Gessler et al. 2002; Wang et al. 2011), NO_2 (Nussbaum et al. 1993; Thoene et al. 1996; Gessler et al. 2000; Gessler et al. 2002; Raivonen et al. 2009) and SO_2 (Van Hove et al. 1989a) and HNO_3 (Vose and Swank 1990). For fluxes of dissolved NH_4^+ and NO_3^- such experiments are relatively scarce (Wilson and Tiley 1998; Sparks 2009; Umana and Wanek 2010), especially at realistic concentrations (Cape et al. 2003). Research on a within canopy spatial scale allowed assessing the effect of local canopy structure on horizontal atmospheric deposition patterns (Beier et al. 1993b; Staelens et al. 2006). A few studies also compared vertical throughfall patterns within tree canopies, but without quantifying canopy structure (Schaefer et al. 1988; Hansen 1996). Since it is currently possible to more accurately quantify local canopy structure (Lefsky et al. 2005; Van der Zande et al. 2006), such within canopy studies could be valuable to aid the understanding of dry deposition and canopy exchange processes (Nadkarni and Sumera 2004). These leaf-level and canopy-level measurements should then be integrated in stand-level estimates of atmospheric deposition fluxes, although this remains challenging (Sparks 2009). Stand level estimates of atmospheric deposition are necessary to be able to relate the total input of atmospheric pollutants to the ecosystem response (de Vries et al. 2007), to compare different forest types (De Schrijver et al. 2007) and to assess long term trends (Neirynck et al. 2002; Vanguelova et al. 2010). It is also essential to conduct studies at a fine temporal scale, since several studies have already demonstrated the impact of leaf phenology (Houle et al. 1999; Hill et al. 2002; Staelens et al. 2007; Sase et al. 2008; Wang et al. 2011) and varying canopy wetness due to temperature and relative humidity differences (Cape et al. 1998; Neirynck et al. 2005) on canopy exchange and dry deposition processes. Results of these studies should be integrated in annual deposition estimates to evaluate long term trends.

1.4 Aims and outline of the thesis

The first general aim of this thesis was to gain better insight in spatio-temporal dry deposition and canopy exchange processes in forest canopies, with the emphasis on atmospheric N as knowledge is still lacking here. The second general aim was to evaluate the often-used canopy budget model (Draaijers and Erisman 1995) to calculate total atmospheric deposition of N and S from throughfall measurements. To achieve both aims, experiments were conducted at several spatial and temporal scales with several tree species and the relationship of the observed fluxes to chemical, physiological and structural canopy parameters was assessed. The specific aims were:

- To quantify canopy N uptake for different tree species and phenological stages and relate this N uptake to differences in leaf characteristics;
- To assess differences in dry deposition and canopy exchange along a vertical gradient within the canopy of different tree species and relate these to differences in canopy structure and canopy phenology;

- To assess the effect of the considered canopy budget model on patterns of total N and acidifying deposition.

We focused on temperate tree species common in Flanders (Belgium) where critical loads (CL) are exceeded on large parts of the forested area. In 2010, CL for acidifying deposition were exceeded at 67 % of the forested area (Flemisch Environmental Agency; www.milieurapport.be), and in 2011, all forests in Flanders still received higher N deposition than the CL for protection of biodiversity (Overloop et al. 2011).

These aims are addressed in the next six chapters, which are grouped in three main parts based on the spatial scale of study (Fig. 1.5). **In the first main part, which comprises experiments conducted at the level of a foliated twig, the uptake of inorganic N from wet (NH_4^+, NO_3^-) and dry (NH_3) deposition by foliage and twigs of four different tree species, i.e. silver birch (*Betula pendula* Roth), European beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.) and Scots pine (*Pinus sylvestris* L.), was assessed by means of stable N isotope (^{15}N) tracing.** Both ambient and elevated N concentration levels were used. Chapter 2 quantifies the foliar uptake of NH_4^+ and NO_3^- at different leaf phenological stages (period of leaf development, fully leafed period, period of leaf senescence) and assesses whether this uptake is related with leaf wettability and leaf water storage capacity. In Chapter 3 the uptake of NH_3 by foliage and twigs is quantified at three different treatment dates within the growing season and the relationship with leaf characteristics was investigated. Chapter 4 assesses the NH_4^+ and NO_3^- retention by twigs, the retention process (adsorption vs absorption) and relates the NH_4^+ and NO_3^- retention of leaves and twigs to fluxes of NH_4^+, NO_3^- and other major ions in throughfall water.

In the second main part, comprising two observational studies at the canopy level, dry deposition and canopy exchange are studied along a vertical gradient within a tree canopy. Chapter 5 assesses the variability of throughfall deposition and calculated canopy exchange and dry deposition along a vertical gradient within a beech (*Fagus sylvatica* L.) and two Norway spruce (*Picea abies* Karst.) canopies. In Chapter 6 the calculated dry deposition by the canopy budget model is compared with dry deposition calculated from air concentration measurements and from dry deposition onto artificial foliage. Furthermore, the effect of canopy structure on dry deposition is assessed by means of terrestrial laser scanning.

In the third part of the thesis (Chapter 7), the effect of varying forms of the canopy budget model, partly based on results derived from the previous chapters, on patterns of total N and acidifying deposition are evaluated. A schematic overview of the structure of the thesis is presented in Fig. 1.5.

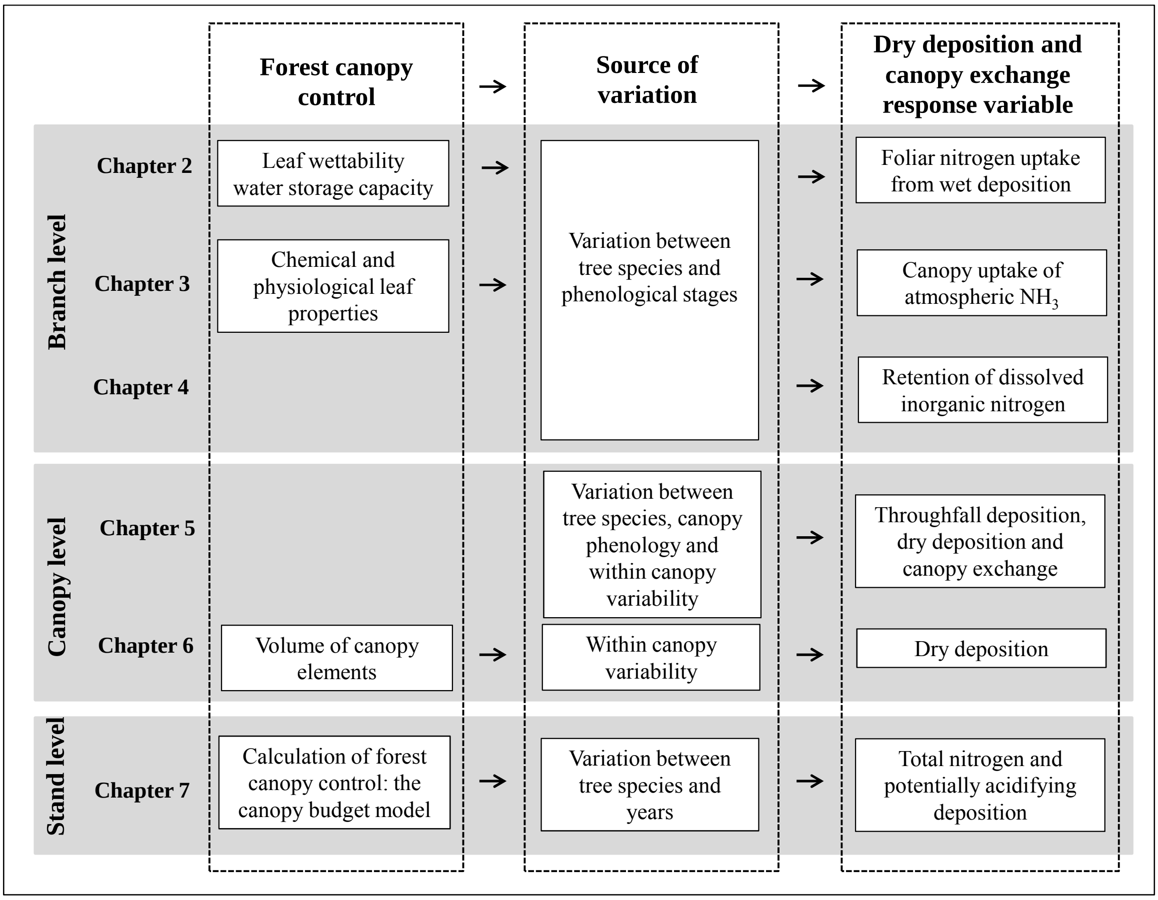

Fig. 1.5 Outline of the thesis

2 Foliar nitrogen uptake from wet deposition and the relationship with leaf wettability and water storage capacity

After: Adriaenssens S, Staelens J, Wuyts K, De Schrijver A, Van Wittenberghe S, Wuytack T, Kardel F, Verheyen K, Samson R, Boeckx P (2011) Foliar nitrogen uptake from wet deposition and the relation with leaf wettability and water storage capacity. Water, Air and Soil Pollution 219, 43-57

2.1 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^-), NO_3^- to NH_4^+ 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 NO_3^- to NH_4^+ ratio did only affect the amount of N uptake by senescent leaves. The negative correlation between measured leaf 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.

2.2 Introduction

Increased deposition of atmospheric reactive nitrogen (N) oxides (NO_y) and ammonia (NH_3) 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). This atmospheric N can be taken up either by aboveground 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 physicochemical processes rather than by N assimilation (Dail et al. 2009; Chapter 4). 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 retention 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 characterized 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 rainwater droplets and the cuticle (Sase et al. 2008). Similarly, a higher WSC would prolong the contact period between incident rainwater 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, if the foliage is rinsed prior to analysis, to distinguish between N assimilated by foliage or N adsorbed to the cuticle (see Chapter 4). 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; Chávez-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 hypothesize that foliar N uptake is influenced by tree species, N form, applied NO_3^- to NH_4^+ 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.

2.3 Materials and methods

2.3.1 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, 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 February 27th 2008 in a mixture of peat and slow releasing fertilizer (8.6 g N kg^{-1} dry soil $year^{-1}$) 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 characterized by relatively high atmospheric N and sulphur (S) deposition. In the period 2002-2006, average estimated annual total deposition fluxes to a mixed beech forest nearby amounted to 30 kg N ha^{-1} yr^{-1} and 20 kg S ha^{-1} yr^{-1} (Verstraeten et al. 2007).

2.3.2 Experimental setup and sample analysis

2.3.2.1 ^{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 mg L^{-1}) based on average precipitation data for this area in 2002-2006 (VMM 2009): 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 atom %) NH_4^+; the other was sprayed with ^{15}N-labelled (99 atom %) NO_3^-. Two types of solutions were applied as separate treatments, subsequently called the 'low NO_3^- to NH_4^+ ratio' and the 'high NO_3^- to NH_4^+ ratio' treatments. The concentration of NH_4^+-N was similar in both solutions (0.84 mg L^{-1}). However, in the 'high NO_3^- to NH_4^+ ratio ratio treatments, the concentration of NO_3^--N (1.26 mg L^{-1}) and Cl^- (5.00 mg L^{-1}) were 3 and 2.42 times higher than those in the 'low NO_3^- to NH_4^+ ratio treatments, (0.42 mg NO_3^--N L^{-1} and 2.07 mg Cl^- L^{-1}). As such, the NO_3^- to NH_4^+ ratio differed by a factor three between the treatments (1:2 versus 3:2), 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, 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, 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.

2.3.2.2 Measurement of leaf wettability and visualisation of leaf surface structure

Contact angles (CAs) of standardized 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 visualized by digital photographs at different magnifications. First, 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.

2.3.2.3 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; (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.

2.3.2.4 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 analyzed 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.

2.3.2.5 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 & 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.

2.3.3 Data processing

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

$$Y = X.\frac{at\%_{treated} - at\%_{untreated}}{at\%_{solution} - at\%_{untreated}}.10^3 \tag{2.1}$$

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

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. Calculated N uptake for each combination of tree species, NO_3^- to NH_4^+ ratio, N form and leaf phenological stage was tested for significant difference from zero by means of a one sample t-test. An ANOVA on the measured ^{15}N uptake was not possible due to heteroscedasticity across tree species, leaf phenology, N form and NO_3^- to NH_4^+ 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 NO_3^- to NH_4^+ 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, NO_3^- to NH_4^+ ratio and phenological stage. For pine, an additional univariate analysis was done including needle age as extra factor. Correlations between WSC, CA, foliar N content and $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake were assessed by calculating Spearman rank coefficients. All statistical analyses were performed using SPPS 15.0 with a minimum significance level of $p < 0.05$.

2.4 Results

2.4.1 Foliar N uptake

The foliar uptake of $^{15}NH_4^+$ ranged from 0.70 µg g^{-1} DM for pine to 3.76 µg g^{-1} DM for birch, while the uptake of $^{15}NO_3^-$ varied between 0.11 µg g^{-1} for pine and 0.86 µg g^{-1} DM for birch. This uptake was significantly higher than zero in most cases (Table 2.1).

Table 2.1 Mean $^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}NO_3^-$ uptake (µg g^{-1} DM) by the leaves of four tree species in three leaf phenological stages after applying artificial rainwater with low (1:2) or high (3:2) NO_3^- to NH_4^+ ratio. Bold values indicate that N uptake is significantly different from zero ($p < 0.05$)

Tree species	Leaf phenology	Low NO_3^- to NH_4^+ ratio		High NO_3^- to NH_4^+ ratio	
		$^{15}N\text{-}NH_4^+$	$^{15}N\text{-}NO_3^-$	$^{15}N\text{-}NH_4^+$	$^{15}N\text{-}NO_3^-$
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 NO_3^- to NH_4^+ ratio.
† Only last year's needles

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 NO_3^- to NH_4^+ ratio and senescent birch and developing beech leaves under low NO_3^- to NH_4^+ ratio. On average, the uptake of NH_4^+ was approximately seven and three times higher than the uptake of NO_3^- in the low (2:1) and high (2:3) NO_3^- to NH_4^+ 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 NO_3^- to NH_4^+ ratio and by the two-factor interactions of these three factors (Table 2.2). Under both the low and high NO_3^- to NH_4^+ ratio, the tree species effect on $^{15}NH_4^+$ uptake was pronounced by a higher uptake for deciduous species vs pine (Table 2.1). 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 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 to fully developed and senescent leaves. The effect of NO_3^- to NH_4^+ ratio is largely manifested on senescent leaves which showed a lower $^{15}NH_4^+$ uptake and a higher $^{15}NO_3^-$ uptake under the high NO_3^- to NH_4^+ ratio. However, the ratio of $^{15}NH_4^+$ to $^{15}NO_3^-$ uptake was 7.4 in the low NO_3^- to NH_4^+ ratio treatment and only 3.4 in the high NO_3^- to NH_4^+ ratio treatment.

Table 2.2 Degrees of freedom (df) and F and p values of a three-way ANOVA: effect of tree species, leaf phenology and NO_3^- to NH_4^+ ratio on the observed foliar ^{15}N uptake of NH_4^+ and NO_3^-. Bold values are significant at $p < 0.05$

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**
NO_3^- to NH_4^+ 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 * NO_3^- to NH_4^+ ratio	3	3.23	**0.027**	3	7.61	**<0.001**
Leaf phenology * NO_3^- to NH_4^+ ratio	2	16.21	**< 0.001**	2	17.91	**< 0.001**

Univariate ANOVA 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 to last year needles was only visible during summer (Fig. 2.1). 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 NO_3^- to NH_4^+ 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.

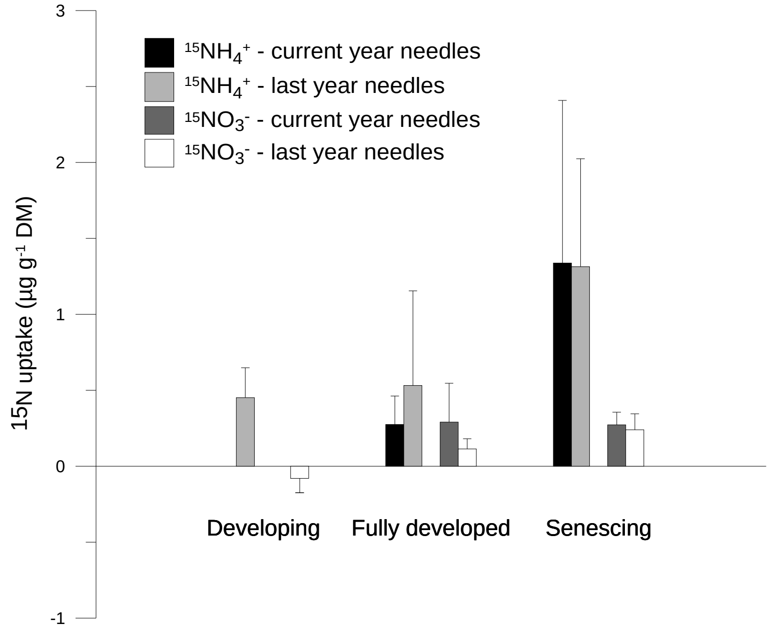

Fig. 2.1 ^{15}N uptake (µg g^{-1} DM) (± standard deviation) by current and last year needles of Scots pine per phenological stage

2.4.2 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. 2.2).

The contact angle (CA) of droplets (Table 2.3) 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.

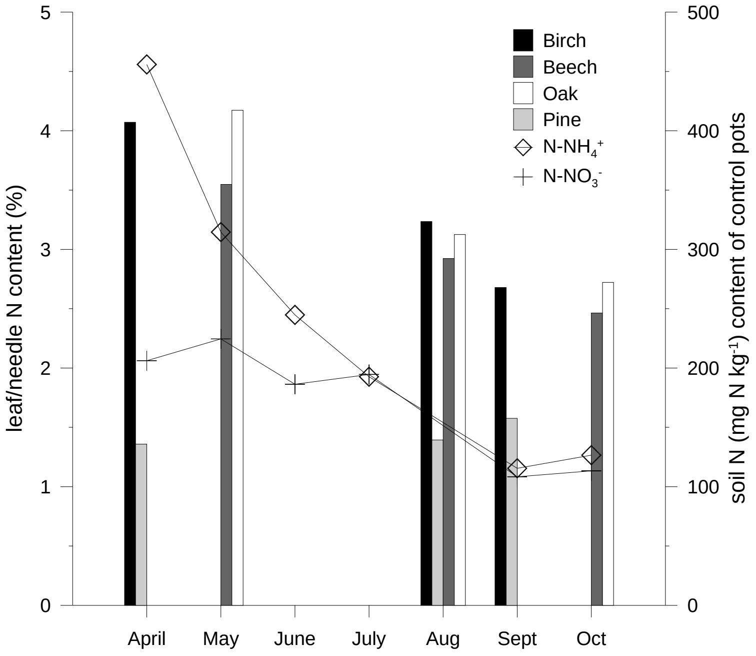

Fig. 2.2 Total N content (%) of birch, beech and oak leaves and pine needles in the different phenological stages and KCl-extractable soil NH_4^+ and NO_3^- content (mg N kg^{-1} dry soil) in the control pots through the growing season of 2008

Table 2.3 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, capitals indicate significant ($p < 0.05$) differences between phenological stages for a specific tree species.

† Only last year's needles

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. 2.3). 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 water storage capacity (WSC) of fully wetted leaves and WSC after dripping differed significantly between tree species, but the ranking depends on the phenological stage (Table 2.3). 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. 2.4). 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 ($p = 0.048$; $r = -0.407$) and no significant relationship ($p > 0. 05$; $r < 0.418$) 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. 2.2). 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).

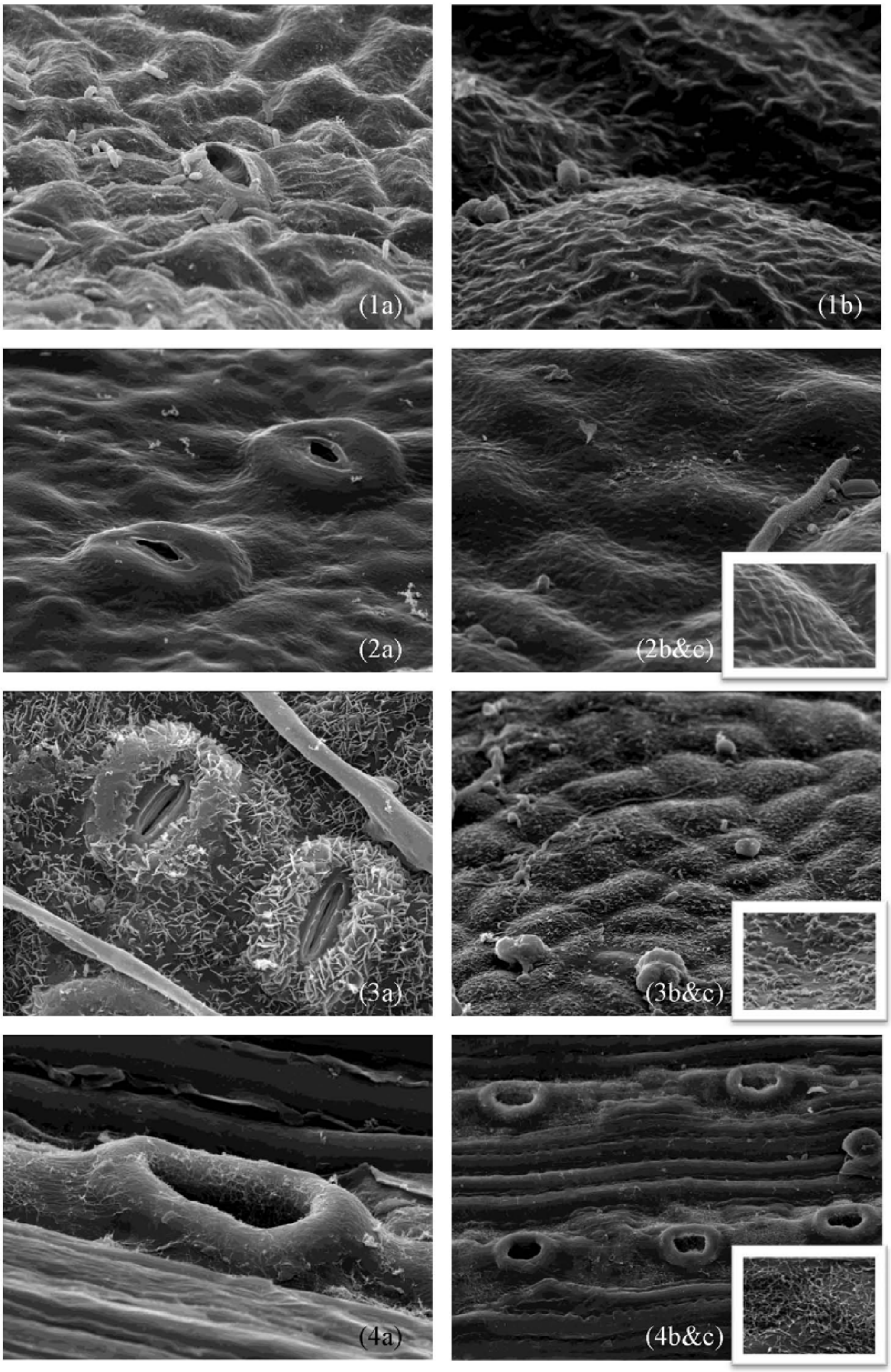

Fig. 2.3 Scanning electron microscope images taken from the (a) abaxial and (b-c) adaxial leaf side of respectively (1) birch (x 1200; x 4780), (2) beech (x 2620; x 2720; x 10900), (3) oak (x 2500; x 1010; x 9600) and (4) pine (x 2500; x 625; x 8400)

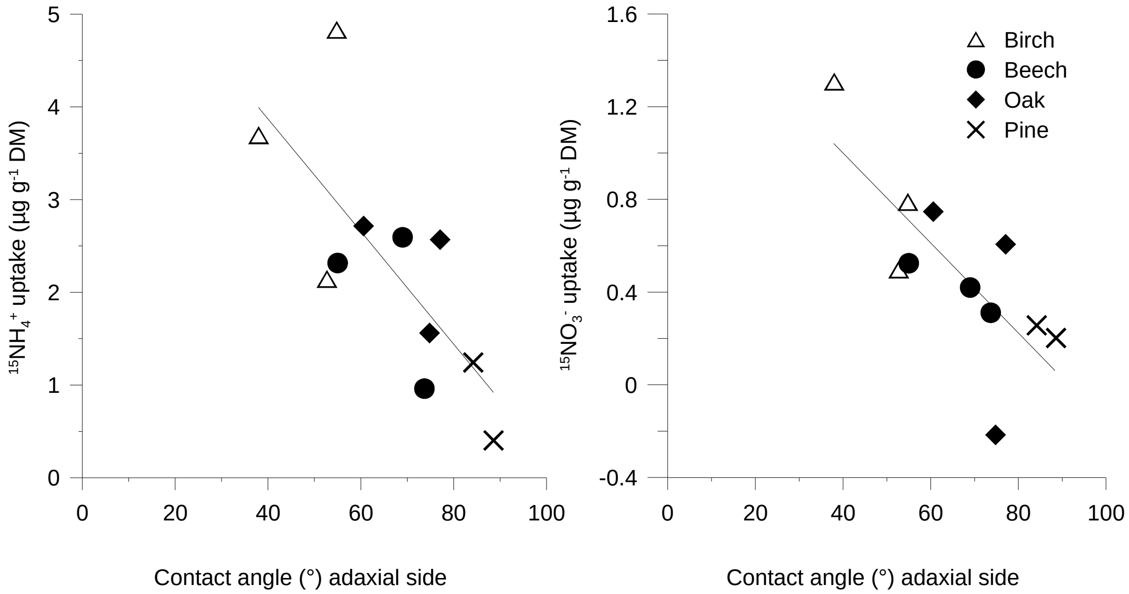

Fig. 2.4 Relationship between the leaf contact angles (°) measured at the adaxial leaf side and the $^{15}NH_4^+$ (p = 0.019; r = -0.70) and $^{15}NO_3^-$ uptake (p = 0.011; r = -0.73; n=11) (µg g^{-1} DM)

2.5 Discussion

2.5.1 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 to 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 low NO_3^- to NH_4^+ ratio treatment to 3.0 for oak under the high NO_3^- to NH_4^+ 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 NO_3^- to NH_4^+ 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.

2.5.2 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 2.5.5), 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 to 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. 2.2). Fourthly, the cuticular pore density (see 2.5.1) 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 to only six months for deciduous species. Extrapolating the average results of the low NO_3^- to NH_4^+ ratio treatment from one branch to mature stands with a LAI of 1.4 for silver birch (Wuyts et al. 2008a), 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 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 $cm^2\ g^{-1}$ DM for birch, beech, oak and pine. Considering a wet N deposition of 10 kg^{-1} N ha^{-1} yr^{-1} (N level as in the low NO_3^- to NH_4^+ 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 kg N ha^{-1} yr^{-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 2.5.1), since these have been shown to contribute significantly to the total water storage capacity (Herwitz 1985; André et al. 2008), and the effect of varying rain intensity (Hansen et al. 1994).

2.5.3 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 to 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.

2.5.4 Effect of NO_3^- to NH_4^+ ratio

In the present study, no distinct effect of the applied NO_3^- to NH_4^+ ratio was found, except for senescent leaves, in contrast to previous reports for European beech (Brumme et al. 1992), sacred fir (*Abies religiosa*) (Chávez-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 to the factor of three used in this experiment. In the other studies mentioned, NO_3^- and NH_4^+ 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 NO_3^- to NH_4^+ ratio, indicates that the affinity of leaves and needles for NH_4^+ is much higher than for NO_3^- (see 2.5.1), and that the ratios of NO_3^- to NH_4^+ used in this experiment are not sufficient to counter this balance. This implies that the potentially increasing future NO_x emissions in industrialized 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.

2.5.5 Leaf surface characteristics and N uptake

Leaf wettability is species-dependent, since the physicochemical 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. 2.3) 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 hypothesize that this lack of relationship 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 to 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 relationship between N uptake and wettability was only observed for saplings growing in the same climatic conditions, it is important to further assess this relationship within one species under varying conditions.

2.6 Conclusion

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 NO_3^- to NH_4^+ 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.

3 Canopy retention of $^{15}NH_3$ by four temperate tree species and the interaction with leaf properties

After: Adriaenssens S, Staelens J, Wuyts K, Van Wittenberghe S, Wuytack T, Verheyen K, Boeckx P, Samson R. Canopy uptake of $^{15}NH_3$ by four temperate tree species and the interaction with leaf properties. Submitted to Water, Air and Soil Pollution

3.1 Abstract

Tree canopies are believed to act as a sink of atmospheric ammonia (NH_3). However, few studies have compared the retention efficiency of different tree species. This study assessed the retention of ^{15}N-labelled NH_3 at 5, 20, 50 and 100 ppb_v by leaves and twigs of potted silver birch, European beech, pedunculate oak and Scots pine saplings in June, August and September 2008. Additionally, foliar retention of ^{13}C-labelled carbon dioxide ($^{13}CO_2$) and leaf stomatal characteristics were determined per species and treatment date and the relationship with $^{15}NH_3$ retention was assessed. Both $^{15}NH_3$ and $^{13}CO_2$ retention were affected by tree species and treatment date, but only $^{15}NH_3$ retention was influenced by the applied NH_3 concentration. Depending on the treatment date, optimal $^{15}NH_3$ retention by leaves and twigs was at 5 (September), 20 (June) or 50 (August) ppb. Birch, beech and oak leaves showed the highest retention in August, while for pine needles this was in June, and except at 5 ppb in June the $^{15}NH_3$ retention was always higher for the deciduous species than for pine. For all species except beech $^{13}CO_2$ retention was highest in August and on every treatment date the $^{13}CO_2$ retention by leaves of deciduous species was significantly higher than by pine needles. Leaf characteristics and $^{13}CO_2$ retention did not provide a strong explanation for the observed differences in $^{15}NH_3$ retention. This study shows that on the short term a high interspecific variability exists in NH_3 retention, which depends on the time in the growing season.

3.2 Introduction

At the global scale, ammonia (NH_3) is considered to be the most important nitrogen (N) component in the atmosphere deposited to terrestrial vegetation (Krupa 2003). It originates mainly from intensive agriculture and to a lesser extent from biomass burning (Fangmeier et al. 1994). Since NH_3 is either deposited to surfaces or converted into ammonium (NH_4^+), high concentrations and consequently high deposition rates of NH_3 are only found close to emission sources (Ferm 1998; Krupa 2003). Particularly forest ecosystems are exposed to high dry deposition levels of NH_3, related to their surface roughness, leaf area index and physiological leaf characteristics (Erisman and Draaijers 2003). In contrast to fertilized agricultural ecosystems which are known to emit NH_3 (Sutton et al. 1995), historically N-limited forests are considered to act as a net sink of NH_3 (Duyzer et al. 1992) although there is also evidence of net emission under high N deposition levels (Neirynck and Ceulemans 2008).

This anthropogenic NH_3 input can have several adverse acute effects on plant functioning when the rate of foliar uptake of NH_3 is greater than the rate and capacity for in vivo detoxification by the plants (see Krupa (2003) for an overview). Dry deposited NH_3 that is not taken up by foliage, contributes as NH_4^+ to throughfall deposition to the forest floor (Lindberg et al. 1986). This increased NH_x input, as N input in general, can significantly affect forest functioning, with both adverse and beneficial consequences (see Chapter 1).

The deposition and uptake of atmospheric NH_3 to vegetation is controlled by stomatal characteristics, but also by non-stomatal characteristics such as those of the leaf cuticle (Bruckner et al. 1993; Sutton et al. 1998; Wyers and Erisman 1998; Gessler et al. 2002). Stomatal NH_3 uptake is determined by stomatal conductance, which is considered to be an active process, and the concentration gradient between the atmosphere and the substomatal cavities where NH_3 is dissolved in the water film of the mesophyll cells, which is a passive diffusion process (Farquhar et al. 1980; Van Hove et al. 1987). No internal mesophyll resistance to NH_3 uptake has been detected (Van Hove et al. 1987). Stomatal opening depends on microclimatic parameters such as temperature and photosynthetic photon flux rate (Gessler et al. 2000), internal CO_2 concentration (Farquhar et al. 1980) and plant water status. The concentration gradient determining the direction of the NH_3 flux depends on the atmospheric NH_3 concentration and on the ratio between the apoplastic NH_4^+ concentration and pH (Asman et al. 1998). As the apoplastic

NH_4^+ concentration is very sensitive to leaf N status and external N supply, plants receiving high N inputs may emit NH_3 while those receiving low N inputs take up NH_3 at the same ambient concentrations (Husted and Schjoerring 1996). Deposition to the cuticle is a passive process, occurring either to thin cuticular water films formed by hygroscopic dry deposited particles at relative humidity (RH) values as low as 50 % (Burkhardt and Eiden 1994; Van Hove and Adema 1996), or directly onto dry plant surfaces, adsorbed to surface waxes (Jones et al. 2007). Dissolved NH_4^+ can then be assimilated via cuticular diffusion (Peuke et al. 1998) or can be re-emitted. Cuticular deposition velocity has been found to be affected by RH (Wyers and Erisman 1998; Gessler et al. 2000), leaf wetness (Neirynck et al. 2005), atmospheric NH_3 concentration (Jones et al. 2007), leaf surface chemistry (Burkhardt 2010), temperature (Neirynck et al. 2005) and atmospheric acid concentration (Neirynck et al. 2011). Variation in foliar retention capacity, i.e. cuticular adsorption and stomatal uptake, between species is observed (Morikawa et al. 1998; Jones et al. 2007) and can be explained to some extent by variation in stomatal dynamics and surface waxes (Vallano and Sparks 2007). Next to tree species, leaf development stage has a clear influence on NH_3 exchange between plants and the atmosphere. Peaks in NH_3 emission have been observed due to a higher apoplast pH (Hill et al. 2002) and NH_4^+ concentration (Wang et al. 2011), which are related to N mobilization during leaf establishment (Millard and Proe 1993) and N remobilization during leaf senescence (Schjoerring et al. 1998). It is likely that tree species with different leaf structure and longevity, e.g., deciduous vs coniferous trees, exhibit different NH_3 patterns throughout the year.

Therefore, the first aim of this study was to assess canopy retention, i.e. adsorption to the canopy surface and uptake, of NH_3 by four different tree species under increasing NH_3 air concentration levels and to examine how this varies over the growing season. For this purpose, retention of ^{15}N-labelled NH_3 was measured, which is an accurate and convincing method that correlates well to flux chamber experiments (Jones et al. 2008). The second aim was to relate on the one hand photosynthetic activity through $^{13}CO_2$ retention and on the other hand stomatal characteristics and theoretical maximal conductance to the observed $^{15}NH_3$ retention.

3.3 Materials and methods

3.3.1 Plant material and experimental site

A full description of the plant material and experimental site is given in Section 2.3.1.

3.3.2 Experimental setup and sample analysis

3.3.2.1 $^{15}NH_3$ and $^{13}CO_2$ application

Two twigs with a length of approximately 30 cm each were selected per tree and sealed with an air-tight polyethylene bag of 15 L, which was then filled completely with ambient air. Next, a mixture of ^{15}N-labelled NH_3 (99 atom %), ^{13}C-labelled CO_2 (99 atom %) and unlabelled CO_2 were injected in the plastic bag with a syringe. In total 12 ml of CO_2 (13.3 atom %) was added to keep photosynthetic activity possible during the experiment. The ^{13}C-labelled CO_2 allowed determining the photosynthetic level. The volume of added CO_2 was determined after a preliminary test on other twigs where the decrease in CO_2 concentrations in the 15-L bag during one hour was measured. Four different concentrations of NH_3 were used, representing 1, 4, 10 and 20 times the average (2002-2006) ambient NH_3 concentration in Ghent, which is 5.1 ppb_v and ranges from 1.9 to 9.8 ppb_v NH_3-N (VMM 2009). The first and second level (5 and 20 ppb_v NH_3-N) were applied on the same tree, and the third and fourth level (50 and 100 ppb_v NH_3-N) on another tree. Four replicated trees were used per NH_3 level. The plastic bags were removed 1 h after the gas mixture addition, and branches were cut off 2 h after the start of the experiment. Just before the removal of the plastic bags, a gas sample was taken and air temperature and RH were recorded with a portable digital temperature/RH meter (DM509, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). The experiment took place on sunny days between 11 am and 3 pm to ensure optimal conditions for photosynthesis. However, during the 2-h experiment the trees were put in the shadow to prevent condensation due to excessive transpiration. The air inside the bags was mixed regularly by means of a syringe. The experiment was conducted three times, i.e., in June, August and September 2008, to assess the effect of leaf development stage on N retention. During the experiment, air temperature inside the bags increased on average with 0.1°C (± 0.6 °C standard deviation) and RH with 17.3 % (±6 % standard deviation) in comparison with the temperature outside the bags, with no differences between species and NH_3 concentration levels. Before each experiment, a representative twig of each tree was sampled to measure natural abundance of ^{15}N and ^{13}C (Harrison et al. 2000) and the total surface area exposed to $^{15}NH_3$. 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. Twig surface area was estimated assuming a cylindrical shape and based on the mean twig diameter and length. Mean leaf/needle and twig surface area per tree species and treatment date are presented in Table 3.1, as for the air temperature and RH.

Table 3.1 Mean air temperature (°C), relative humidity (RH, %) and leaf and twig area (cm^2) exposed to $^{15}NH_3$ per tree species at the different treatment dates in 2008. Standard deviation is given in brackets.

	June	August	September
Air temperature	24.4 (1.8)	21.7 (1.0)	18.2 (1.0)
RH air	50.3 (5.1)	58.4 (4.2)	56.7 (3.7)
Leaf area exposed to $^{15}NH_3$			
Birch	261 (77)	252 (97)	213 (108)
Beech	608 (184)	423 (133)	420 (115)
Oak	973 (244)	401 (152)	441 (200)
Pine	1574 (309)	1723 (608)	2113 (694)
Twig area exposed to $^{15}NH_3$			
Birch	63 (7)	64 (7)	67 (9)
Beech	99 (8)	103 (7)	106 57)
Oak	53 (7)	55 (9)	68 (25)
Pine	49 (15)	50 (29)	68 (32)

3.3.2.2 Analysis of plant material and gas samples

After harvesting, leaves and needles were separated from branches, and needles were separated into current-year and last-year age classes. All plant material was 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). The samples were analysed in duplicate for total N, total C, ^{15}N and ^{13}C using an Elemental Analyzer (EA) connected to an Isotope Ratio Mass Spectrometer (IRMS) (20-20, SerCon, UK). The standard deviation (n = 10) of internal standards measured by this EA-IRMS system is 0.2‰ for $\delta^{15}N$ and 0.3‰ for $\delta^{13}C$. Carry-over effects were avoided by measuring one or two blanks in between natural abundance samples or enriched samples, respectively. The laboratory annually participates successfully in joint proficiency ring tests of WEPAL-IAEA on total N, C,

^{15}N and ^{13}C analysis of plant material. The CO_2 concentration of the gas samples was analysed by means of a gas chromatograph (Shimadzu GC-14B, Germany) equipped with an Electron Capture Detector.

3.3.2.3 Measurement of stomatal characteristics

Stomatal imprints were collected at the abaxial leaf side to determine stomatal density (SD), i.e. the number of stomata per mm^2 leaf area, and stomatal pore surface (SPS, μm^2), i.e. the surface area of a widely opened stomatal pore. Colourless nail varnish was applied over an area of 6 cm^2 next to the main vein of the leaves and over a length of 3 cm in the middle of the needles. Next, the nail varnish was peeled off the surface with a transparent adhesive tape, which was then fixed on a microscope slide. The imprints were analysed with a light microscope (Olympus BX51, UK) connected with a camera (Soft Imaging System’s Colorview III) at a magnification of 40 x 10 for SD and 100 x 10 for SPS (Balasooriya et al. 2009). The SPS (μm^2) was calculated by measuring length (L, µm) and width (W, µm) of widely opened stomata, and by assuming an elliptical shape.

3.3.3 Data analysis

Retention of ^{15}N and ^{13}C per unit dry plant material (µg N or C g^{-1} DM) was calculated, similar to Chapter 2, by accounting for the natural abundance of ^{15}N and ^{13}C in untreated branches (Harrison et al. 2000):

$$Y = X.\frac{at\%_{treated} - at\%_{untreated}}{at\%_{gas} - at\%_{untreated}}.10^3 \tag{3.1}$$

with Y being the ^{15}N or ^{13}C retention (µg g^{-1} DM) and X the total N or C concentration (mg g^{-1} DM) in the treated samples.

The theoretical maximal stomatal resistance R_s (s m^{-1}) was calculated as (Balasooriya et al. 2009):

$$R_s = \frac{4l}{SD\pi LWD} + \frac{L+W}{4SDLWD} \tag{3.2}$$

with l the depth of the stomatal pore (m), SD the stomatal density (m^{-2}), and D the diffusion coefficient of water vapour in the air (0.242×10^{-4} m^2 s^{-1} at 20°C). The depth of the stomatal pore was assumed to be 10 µm (Balasooriya et al. 2009). R_s was then inverted to a maximal stomatal conductance (g_{smax}, m s^{-1}), and converted to mol H_2O m^{-2} s^{-1} (Pearcy et al. 1989).

The effect of tree species, treatment date and applied NH_3 concentration on $^{15}NH_3$ and $^{13}CO_2$ retention by leaves and twigs was tested by means of a three-way factor analysis. A Generalized Least Squares Model was used to account for heteroscedasticy in $^{15}NH_3$ retention across the different treatment dates and concentrations levels. Due to a three-way interaction effect between species, treatment dates and applied NH_3 concentration for $^{15}NH_3$ retention, the analysis was conducted for each species separately. For Scots pine the effect of needle age was included. To test for differences in SPS, SD, g_{smax}, N and C content across species and treatment dates, a two-way factor analysis was performed. Means of SPS, SD, g_{smax} and $^{13}CO_2$ retention were separated by Tukey's Honestly Significant Difference test and p values were calculated for each factor. Correlations between SPS, SD, g_{smax}, N content, $^{13}CO_2$ retention and $^{15}NH_3$ by leaves and twigs were assessed by Pearson or Spearman rank correlation coefficients, depending on whether the variables were normally distributed. All statistical tests were performed in R 2.10.1 (R Development Core Team 2011).

3.4 Results

3.4.1 $^{15}NH_3$ retention

The $^{15}NH_3$ retention (µg N g^{-1} DM) by leaves and twigs of each tree species was significantly affected by treatment date, the applied initial NH_3 concentration level and their interaction, except for the effect of applied NH_3 concentration on the retention by twigs (Table 3.2). In June, the highest NH_3 retention by leaves was found at 20 ppb_v NH_3, while in August this was at 50 ppb_v (Fig. 3.1a). In September, retention was highest at 5 ppb_v for pine and oak and at 20 ppb_v for birch. No significant differences between applied NH_3 concentration levels were found for beech leaves in September. Furthermore, for birch, beech and oak leaves, the retention at 20 and 50 ppb_v was highest in August, while at 5 ppb_v the highest retention was observed in September. For pine needles, retention was generally highest in June. Current year needles generally assimilated more $^{15}NH_3$ than last year needles of the same branch, however, the difference was not always found to be significant (Fig. 3.2). Similar to the leaves, $^{15}NH_3$ retention by twigs was significantly higher in June at 20 ppb_v and in August at 50 ppb_v (Fig. 3.1b). However, in contrast to the leaves, the highest retention was observed in June, while in September retention approached zero.

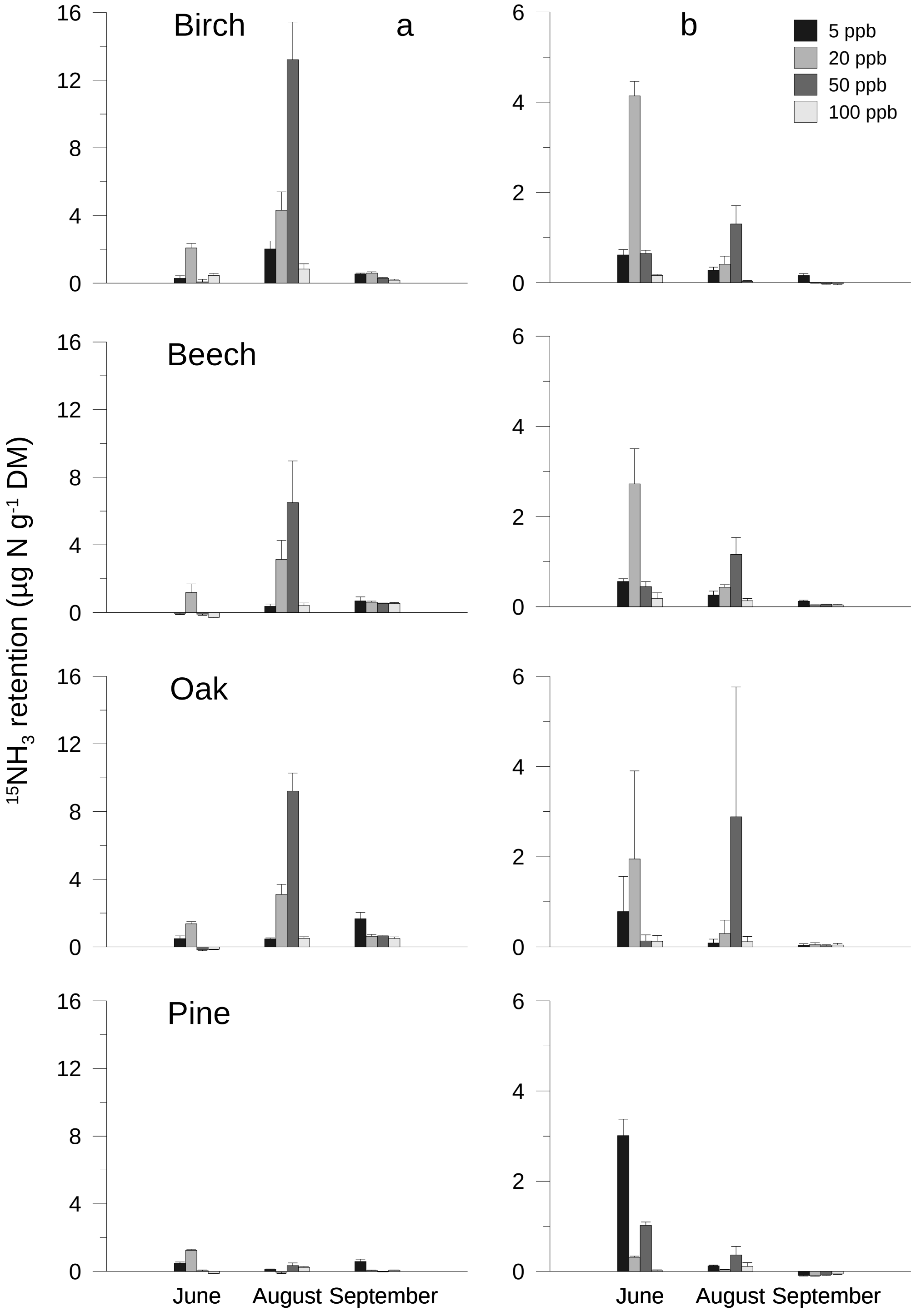

Fig. 3.1 Average $^{15}NH_3$ retention (µg N g^{-1} DM) by leaves/needles (a) and twigs (b) of birch (1), beech (2), oak (3) and pine (4) per treatment date and applied NH_3 concentration level. Error bars represent standard error. Negative values indicate a lower ^{15}N atom % in the treated than in the non-treated branches

Table 3.2 Degrees of freedom (df), F and P values for the significance of the effect of applied initial NH_3 concentration level, treatment date and their interaction on $^{15}NH_3$ retention by foliage and twigs of four tree species Bold values are significant at $p < 0.05$

Tree	Concentration			Treatment date			Interaction		
species	df	F	P	df	F	P	df	F	P
Leaves									
Birch	3	13.73	< **0.001**	2	11.62	< **0.001**	6	11.87	< **0.001**
Beech	3	5.57	**0.003**	2	12.78	< **0.001**	6	3.33	**0.010**
Oak	3	82.75	< **0.001**	2	50.52	< **0.001**	6	13.34	< **0.001**
Pine	3	31.90	< **0.001**	2	35.31	< **0.001**	6	54.47	< **0.001**
Twigs									
Birch	3	14.32	< **0.001**	2	47.83	< **0.001**	6	34.48	< **0.001**
Beech	3	12.96	**0.001**	2	47.95	< **0.001**	6	5.76	**0.002**
Oak	3	1.63	0.315	2	18.68	< **0.001**	6	12.99	< **0.001**
Pine	3	33.81	< **0.001**	2	120.43	< **0.001**	6	28.69	< **0.001**

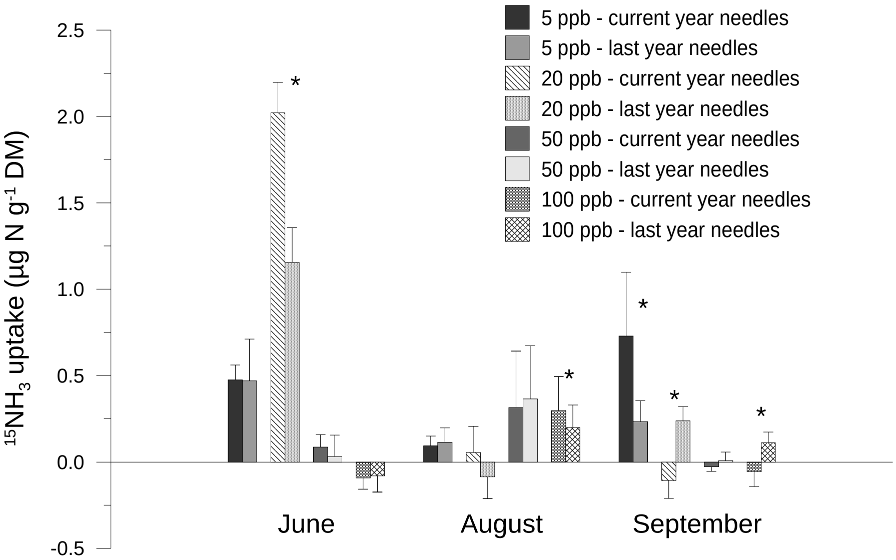

Fig. 3.2 Average $^{15}NH_3$ retention (µg N g^{-1} DM) by current and last year needles of pine per treatment date and applied NH_3 concentration level. Error bars represent standard error. Significant differences between current and last year needles are indicates by '*'. Negative values indicate a lower ^{15}N atom % in the treated than in the non-treated branches.

3.4.2 $^{13}CO_2$ retention and leaf characteristics

The $^{13}CO_2$ retention (µg C g^{-1} DS) by leaves was significantly affected by species, treatment date and the interaction between both ($p < 0.001$), but not by the applied NH_3 concentration ($p = 0.121$). For all species, $^{13}CO_2$ retention was significantly lower in June compared to August and September, and except for beech highest values were found in August (Fig. 3.3). With respect to tree species, the $^{13}CO_2$ retention by leaves of deciduous species was higher than by the pine needles and from June to September $^{13}CO_2$ retention by beech leaves became significantly higher than oak and birch. Current year pine needles took up significantly more $^{13}CO_2$ than last year needles in all cases (data not shown). No retention by branches was detected. The observed $^{13}CO_2$ retention by leaves was significantly correlated ($p < 0.001$; Spearman rho = 0.733; n=174) to the decrease in CO_2 concentration measured in the plastic bags at the end of the experiment.

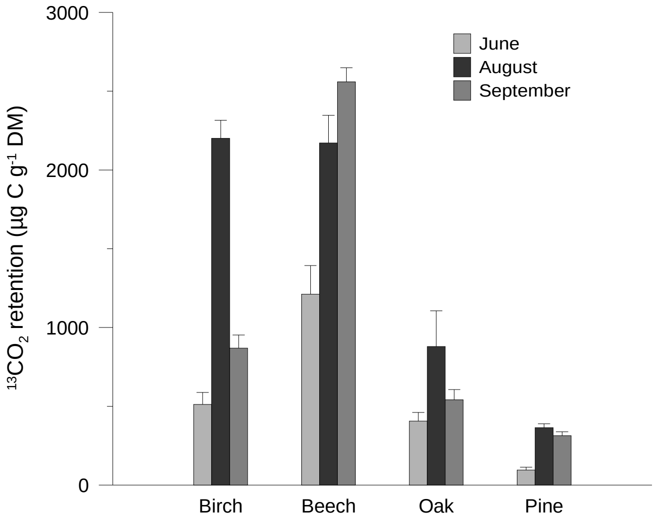

Fig. 3.3 Average $^{13}CO_2$ retention (µg C g^{-1} DM) by leaves/needles of birch, beech, oak and pine per treatment date. Error bars represent standard error

Leaf N content was significantly affected by tree species, treatment date and their interaction. For beech and oak N content decreased significantly from June to September, while for pine needles it was lowest in August and increased slightly in September (Fig. 3.4). Leaf C content remained stable between June and August, but decreased thereafter for beech, birch and pine. For oak leaves, the C content increased in September.

Stomatal density (SD), pore surface (SPS) and conductance (g_{smax}) were significantly influenced by tree species, treatment date and the interaction between both factors ($p < 0.001$), with a higher variation between species than within one species. Generally, SPS increased from beech and oak over birch to pine, with a maximum in August for birch and oak and in September for pine (Table 3.3). In contrast, stomatal density was highest for beech and oak and lowest for pine and birch.

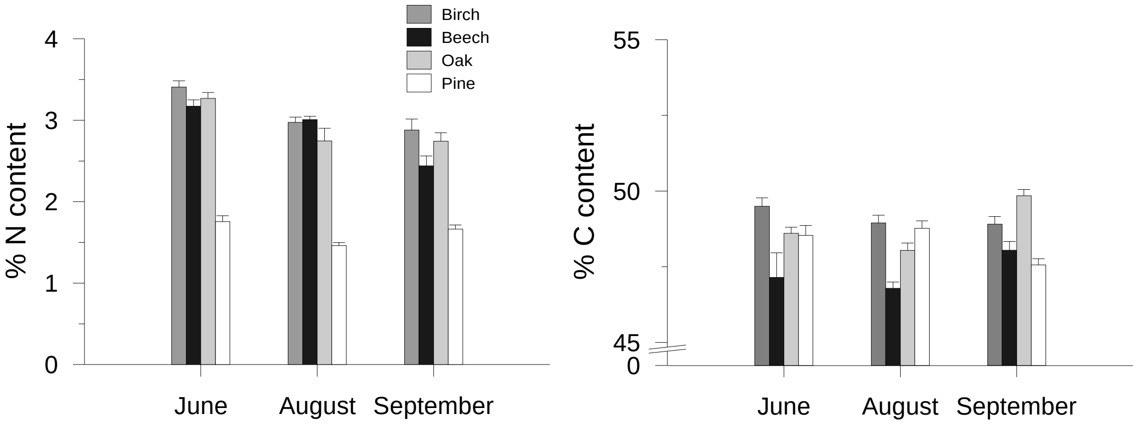

Fig. 3.4 Average N and C content (%) of birch, beech and oak leaves and pine needles at different treatment dates

The correlation coefficient between SPS and stomatal density amounted to -0.72 ($p < 0.0001$; $n = 39$), indicating a trade-off between both parameters. The highest g_{smax} was observed for pine and the lowest for beech and oak. Maximum g_{smax} was found in August for birch, oak and pine, while no differences between treatment dates were found for beech.

Table 3.3 Average stomatal length, width (µm, n = 32), pore surface (SPS, μm^2, n = 32) and density (SD, number of stomata m^{-2}, n = 36-88 depending on the tree species) and theoretical maximal stomatal conductance (g_{smax}, mol H_2O m^{-2} s^{-1}, n = 32) per species and treatment date. Standard deviation is given in parentheses

Tree species	Date	Length	Width	SPS	SD	g_{smax}
Birch	June	18.35 (2.10)	6.63 (0.93)	96.27 (21.42)	198 (50)	1.28 (0.24)
	August	22.39 (3.57)	8.41 (1.15)	148.65 (33.91)	160 (29)	1.48 (0.27)
	September	21.87 (4.64)	7.26 (1.49)	128.13 (49.76)	190 (56)	1.53 (0.48)
Beech	June	9.52 (1.27)	5.06 (0.55)	38.01 (7.69)	383 (55)	1.13 (0.20)
	August	9.59 (1.01)	5.17 (0.62)	39.19 (7.87)	383 (50)	1.17 (0.21)
	September	10.27 (1.06)	5.15 (0.5)	41.72 (6.95)	341 (33)	1.09 (0.16)
Oak	June	9.76 (1.69)	3.39 (0.54)	26.31 (7.97)	451 (116)	0.94 (0.25)
	August	14.79 (1.63)	4.75 (0.53)	55.49 (10.51)	343 (47)	1.38 (0.23)
	September	13.41 (1.35)	4.06 (0.64)	43.02 (9.13)	370 (45)	1.19 (0.23)
Pine	June	n.a.	n.a.	n.a.	n.a.	n.a.
	August	22.06 (2.97)	13.62 (2.41)	235.67 (51.09)	155 (24)	2.15 (0.37)
	September	25.50 (3.01)	15.31 (2.91)	308.74 (75.98)	110 (22)	1.89 (0.38)

n.a.: not available

3.4.3 Relationship between $^{15}NH_3$ retention and leaf characteristics

At 20 ppb$_v$, total $^{15}NH_3$ retention and retention by leaves was significantly correlated to the N content of both leaves and twigs (Table 3.4). A significant correlation was also found between $^{15}NH_3$ retention of leaves plus twigs and the N content of twigs at 5 ppb$_v$ (Fig. 3.5).

Table 3.4 Spearman correlation coefficient (n = 48) between the $^{15}NH_3$ retention (µg g^{-1} DM) by leaves, twigs and leaves plus twigs (total retention) at different NH_3 concentration levels on the one hand, and the N content (%) of leaves and twigs, the $^{13}CO_2$ retention (µg g^{-1} DM) and the C content (%) of leaves on the other hand. Bold values are significant at $p < 0.01$, underlined values at $p < 0.05$

		5 ppb$_v$	20 ppb$_v$	50 ppb$_v$	100 ppb$_v$
$^{15}NH_3$ retention leaves	N content leaves	-0.095	<u>0.335</u>	-0.002	0.026
	N content twigs	0.270	**0.390**	0.043	0.131
	^{13}C retention leaves	0.001	0.258	**0.411**	**0.410**
$^{15}NH_3$ retention twigs	N content leaves	<u>0.349</u>	0.271	-0.032	**0.382**
	N content twigs	0.164	<u>0.324</u>	0.071	0.070
	^{13}C retention leaves	-0.032	-0.020	0.000	0.000
Total $^{15}NH_3$ retention	N content leaves	0.037	**0.414**	0.050	0.101
	N content twigs	<u>0.297</u>	<u>0.346</u>	0.014	0.046
	^{13}C retention leaves	-0.17	0.192	<u>0.328</u>	**0.389**

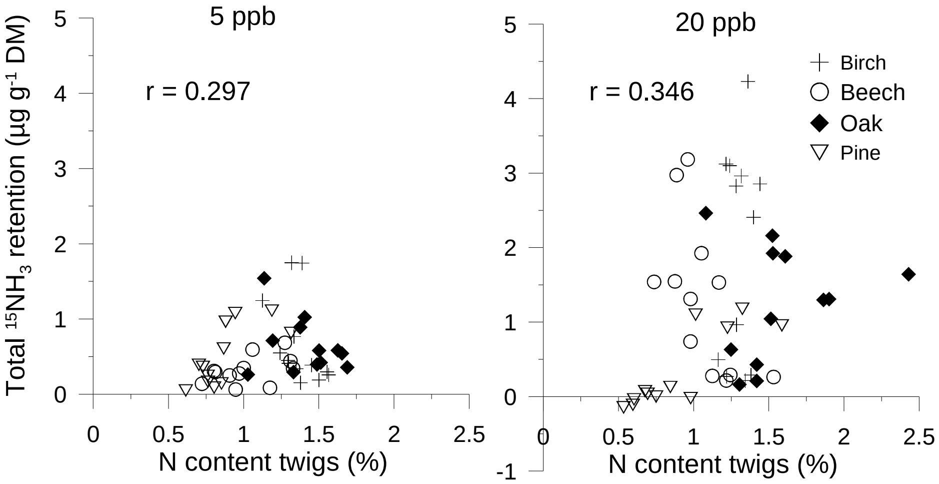

Fig. 3.5 Relationship between total $^{15}NH_3$ retention by leaves/needles and twigs and the N content of twigs at 5 (a) and 20 ppb (b) NH_3

At 50 and 100 ppb$_v$, branch N status did not influence total $^{15}NH_3$ retention and $^{15}NH_3$ retention by leaves. However, at these NH_3 concentrations a weak but significant relationship was found between the foliar ^{15}N retention and $^{13}CO_2$ retention (Fig. 3.6). Stomatal pore surface, density and conductance showed no significant relationship with $^{15}NH_3$ retention by leaves (Table 3.5). We also found a significant correlation between $^{13}CO_2$ retention and the N content of the leaves ($p < 0.001$; $r = 0.352$).

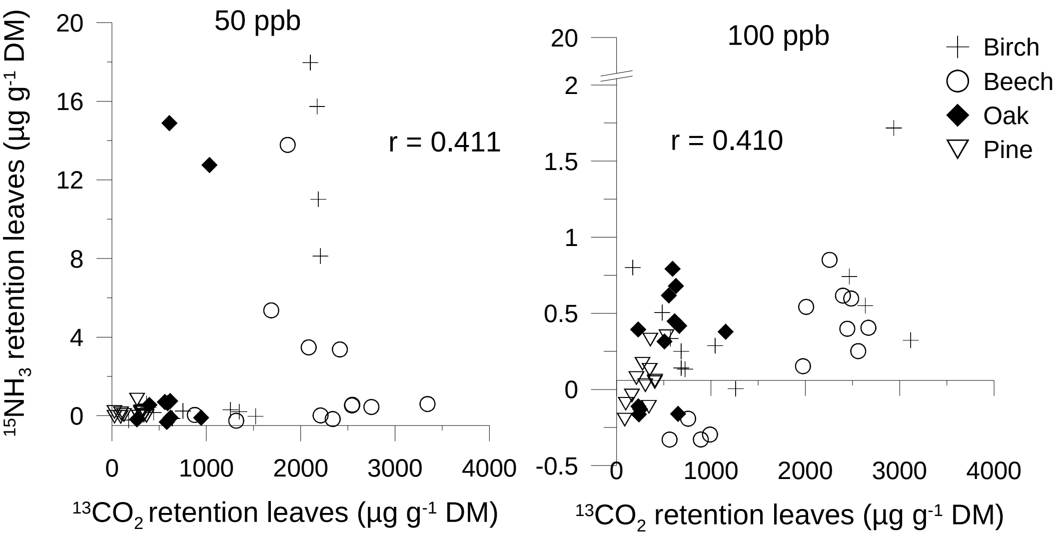

Fig. 3.6 Relationship between $^{15}NH_3$ retention by leaves and the $^{13}CO_2$ retention at 50 and 100 ppb$_v$ NH_3

Table 3.5 Pearson correlation coefficients (n = 12) between the $^{15}NH_3$ retention (µg g^{-1} DM) by leaves at different NH_3 concentration levels and the stomatal pore surface (SPS, µm^2), density (number of stomata m^{-2}) and conductance (g_{smax}, mol H_2O m^{-2} s^{-1}). Bold values are significant at $p < 0.01$, underlined values at $p < 0.05$

	$^{15}NH_3$ retention			
	5 ppb$_v$	20 ppb$_v$	50 ppb$_v$	101 ppb$_v$
SPS	0.044	-0.348	-0.112	-0.081
density	-0.152	0.136	-0.110	-0.204
gs_{max}	-0.057	-0.312	-0.006	0.037

3.5 Discussion

3.5.1 Tree species effect

To our knowledge, this is the first study that compares NH_3 retention between deciduous and coniferous canopies. Except for the retention at 5 ppb_v in June, $^{15}NH_3$ and $^{13}CO_2$ retention by Scots pine were always lower than those by the deciduous species. This also held true for the $^{15}NH_4^+$ retention from rainwater by saplings of the same four species (Chapter 2). Just as in Chapter 2, this can be explained by the low N demand of coniferous tree species, 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). Next to this, during the in-leaf season, coniferous species have lower assimilation rates than deciduous species. However, on a yearly basis, coniferous species are expected to grow faster because they start to assimilate earlier in the year and continue longer (Schulze et al. 2005).

Furthermore, the $^{15}NH_3$ retention by birch in June and September was higher than by beech and oak. This could be explained by a different susceptibility to NH_3 toxicity of birch compared to beech and oak. Birch is a fast growing species which generally assimilates nitrate by the roots. Assimilation of nitrate releases hydroxyl ions and hereby increases the physiological buffering potential of the leaves (Pearson and Stewart 1993), making them less prone to the acidifying effect of ammonia assimilation (Raven 1988). In contrast, beech and oak are slow growing species, which rely mostly on root ammonium assimilation for their N source (Smirnoff et al. 1984) and have therefore less buffering potential to assimilate NH_3. Additionally, birch was found to retrieve only 48-54 % of the N for new growth from storage, while for oak this was 80-100 % (Millard and Grelet 2010). This indicates that birch trees need to retrieve more N from external sources.

3.5.2 Effect of treatment date

We observed significant differences in the $^{15}NH_3$ retention between the treatment dates, which can be attributed to several internal and external parameters influencing photosynthesis rate and the apoplastic NH_4^+ concentration. External parameters include temperature, water supply and photosynthetic active radiation (PAR), while internal factors determine metabolic activity such as C skeletons, energy, and reduction equivalents (NAD(P)H, ferredoxin), which vary significantly between tissue developmental stages (Krupa 2003). The difference in air temperature of 5°C between

June and August or September could have doubled the stomatal compensation point (Farquhar et al. 1980; Schjoerring et al. 1998), which is the NH_3 concentration in the substomatal cavity when net uptake is zero. Increased stomatal compensation points decrease the concentration gradient necessary for passive diffusion and could potentially lead to higher net re-emission after the removal of the plastic bags. This could explain why $^{15}NH_3$ retention already decreased at concentrations higher than 20 ppb_v in June.

The net photosynthesis rate, measured by $^{13}CO_2$ retention, was higher by current year needles of Scots pine than by last year needles, and so was $^{15}NH_3$ retention. Ammonia retention at 20 and 50 ppb_v by the deciduous species was also highest in August when $^{13}CO_2$ retention was highest, although in this case the apoplastic NH_4^+ concentration influenced by internal factors might be as important: Wang et al. (2011) observed increased apoplastic and bulk tissue NH_4^+ levels in beech leaves during leaf development and senescence and low levels of glutamine synthetase during leaf development compared to other growth stages. This seasonal pattern was related to N mobilization during early leaf establishment and remobilization during late leaf senescence. The treatment dates used in this study did not coincide completely with leaf development and senescence, but there is some evidence that these processes were important. In June, the N content of leaves and twigs was highest for all species, indicating higher apoplastic or bulk tissue NH_4^+ concentrations (Schjoerring et al. 1998), while in September the N content of deciduous leaves had substantially decreased due to N remobilization (Fig. 3.4). Resorption of N has been found to coincide with the decomposition of the photosynthetic apparatus, including chlorophyll, thereby resulting in a decreased net CO_2 assimilation (Wang et al. 2011). Lower $^{13}CO_2$ assimilation in September compared to August was observed for birch and oak, but not for beech. This is possibly due to the fact that the senescence stage must be rather advanced before net CO_2 assimilation decreases and NH_4^+ accumulation starts (Mattsson and Schjoerring 2003). Leaves of beech were visually less discoloured than those of birch and oak.

For both current and last year needles of Scots pine no decrease in N content was observed with treatment date. Current year needles of Scots pine were fully developed at the end of the growing season, and, as indicated by other studies on Scots pine and by their increasing N content (Fig. 3.4), will serve as main N storage site during winter (Millard and Proe 1993). This higher N content might give rise to higher apoplastic NH_4^+ concentrations at this stage (Schjoerring et al. 1998).

3.5.3 Effect of increasing NH_3 concentration

Unlike many other studies (Farquhar et al. 1980; Van Hove et al. 1987; Bruckner et al. 1993; Gessler et al. 2000; Gessler et al. 2002), no increased $^{15}NH_3$ retention with increasing NH_3 concentration was observed in this study through both leaves and twigs. We assume that this cannot be attributed to a toxic effect at 100 ppb_v, since no decrease in $^{13}CO_2$ retention was observed at this level. Instead, an explanation for this unexpected effect might be found in the two-step retention process of atmospheric NH_3. Generally, the driving force for the first step in the retention of gaseous compounds through stomata or the cuticle is the concentration gradient between the gas phases inside and outside the leaf. The gas phase in the leaf is in equilibrium with the concentration of NH_3 and NH_4^+ in the aqueous phase of the apoplast (Rennenberg and Gessler 1999). There, NH_4^+ strongly dominates NH_3 (Krupa 2003) and is assimilated via the enzymes of the glutamine synthetase/glutamate synthase system (Pearson et al. 2002), which is the second step in the retention process. Although it is possible that some of the measured ^{15}N retention by twigs could be due to physicochemical adsorption rather than by actual assimilation (Dail et al. 2009; see Chapter 4), it is likely that the unexpected effect of the applied NH_3 concentration can be attributed to the second step in the retention process of atmospheric NH_3 due to the following arguments.

In the first step, NH_3 retention through stomata is mainly controlled by stomatal aperture, and at high ambient NH_3 concentrations more NH_3 will enter through the stomata compared to low ambient concentration levels at a given stomatal conductance (Gessler et al. 2000; Gessler et al. 2002). Furthermore, it is expected that at increasing NH_3 concentrations and RH, more NH_3 is deposited to the plant cuticle (Gessler et al. 2002), although at increasing NH_3 concentrations the total leaf conductance, i.e. the sum of stomatal and cuticular conductance, will decrease due to a saturation of the cuticle (Hanstein et al. 1999; Jones et al. 2007). The cuticle has been found to be nearly impermeable for NH_3 (Van Hove et al. 1989a,b), but the gas can be dissolved in thin water films present on the leaf cuticle at RH > 50 % (Van Hove and Adema 1996), from where it can be transported as NH_4^+ via cuticular diffusion (Peuke et al. 1998). Based on these observations found in previous studies and the assumption that stomatal aperture was sufficient for gaseous exchange (see 3.5.5), we hypothesize that also in this experiment more $^{15}NH_3$ has diffused into the substomatal cavities or deposited to the cuticle at increasing atmospheric NH_3 concentrations. However, it is likely that the NH_3

captured inside the leaves or deposited as NH_4^+ to the plant cuticle was not all assimilated during the experiment (i.e., an interruption of the second step in the retention process) and possible re-emitted when the plastic bags were removed, for which we propose two explanations. First, Grundmann et al. (1993) reported a two-phase NH_3-N after exposure to $^{15}NH_3$ of maize plants, one which was immediately assimilated during exposure and a second corresponding to a reversible storage compartment in the apoplast or in cellular vacuoles. In their experiment, the second phase was not retrieved immediately after the experiment but after a few days. Since the study by Grundmann et al. (1993) was conducted at very high concentrations and since no other studies reported such a storage at realistic concentrations, this phenomenon may be linked to higher NH_3 concentrations and we would have found higher $^{15}NH_3$ in the plant material at the 100 ppb_v level after a longer time period between the removal of the bags and the harvest of plant material. Second - this applies to both leaves and twigs - all leaves and twigs were after the removal of the polyethylene bags during the following hour again exposed to the ambient NH_3 concentration and RH before they were cut off. Ammonium dissolved in the thin water films on the plant cuticle could then be re-emitted as NH_3 from drying water films (Sutton et al. 1995; Husted and Schjoerring 1996) and NH_4^+/NH_3 in the substomatal cavities could be re-emitted if atmospheric NH_3 concentrations were lower than the concentration inside the leaf. Especially plants that were exposed to a period of high NH_3 deposition may emit significant amounts of NH_3 when atmospheric NH_3 concentrations are low (Rennenberg and Gessler 1999; Gessler et al. 2002), which could explain why very little $^{15}NH_3$ was retained in the leaves and twigs exposed to the highest NH_3 concentration.

Nevertheless, from this study we observed that all studied tree species were not able to instantaneously assimilate the higher NH_3 availability. Depending on the treatment date and the tree species, as discussed in Sections 3.5.1 and 3.5.2, the maximum NH_3 assimilation occurred at 5, 20 or 50 ppb_v, while at 100 ppb_v, leaves and twigs almost assimilated no NH_3. Further research combining a $^{15}NH_3$ tracing study with NH_3 flux measurements could help to explain these results.

3.5.4 Leaf characteristics

In contrast to $^{15}NH_3$ and $^{13}CO_2$ retention, g_{smax} was found to be higher for pine compared to the deciduous species. This can largely be attributed to its high SPS, since SD was lowest for pine and highest for beech and birch. No relationship between stomatal characteristics or g_{smax} and $^{15}NH_3$ retention was observed, in contrast to other studies where stomatal conductance was found to influence NH_3 fluxes (Van Hove et al. 1987; Gessler et al. 2000; Gessler et al. 2002). One explanation might be that stomatal conductance of pine needles was overestimated due to stomatal wax plugs or imbricacy (Brodribb and Hill 1997). Removing pine from the correlation analysis increased correlation coefficients, but they were still not found to be significant (data not shown). Additionally, towards the end of the growing season, it is possible that more stomata will stop functioning due to air pollutants (Paoletti and Grulke 2005), a process that cannot be determined by measuring g_{smax}. From this we can conclude that stomatal characteristics and g_{smax} are not suitable parameters to explain differences in NH_3 retention between species and throughout the growing season.

We only found a weak relationship of total $^{15}NH_3$ retention with N content of twigs (Fig. 3.5) at 5 and 20 ppb_v and with N content of leaves at 20 ppb_v (Table 3.4). The N content of both leaves and twigs can be considered as an indicator of the N status of the plant (Ma et al. 2005). Husted and Schjoerring (1996) showed that the ratio between leaf conductance for NH_3 and H_2O depends on the plant N status, with a relatively higher H_2O conductance in low-N plants, a similar conductance in medium-N plants and a much higher relative NH_3 conductance in high-N plants.

At 50 and 100 ppb_v NH_3, foliar $^{15}NH_3$ retention was related to $^{13}CO_2$ retention. One explanation for this observation might be that at higher NH_3 concentrations, $^{13}CO_2$ assimilation was restricting to the N assimilation process, although plants are shown to detoxify NH_3 by storing N in compounds with low C:N ratios (Krupa 2003). No relationship was found between measured stomatal characteristics and $^{15}NH_3$ retention, which is probably due to the fact that g_{smax} was not a good proxy for actual stomatal conductance to explain differences between tree species and tissue developmental stages.

3.5.5 Methodological considerations

This experiment was performed in closed chambers where temperature, RH and PAR could not be controlled. However, per treatment date the experiment was performed at the same moment for all species, subjecting them to similar PAR intensities. Moreover, the increase in temperature inside the bags was overall very low and the increase in RH (with 17 %) did not differ between tree species and NH_3 concentration levels (see 3.3.2.1).

Furthermore, all saplings were watered sufficiently, so differences in plant water status should be limited. We can assume that stomata were not closed, since significant $^{13}CO_2$ assimilation and transpiration (indicated by an increase in RH) occurred.

However, it is possible that RH increase affected the NH_3 retention by different species differently through a different response of stomatal and cuticular conductance. For example, stomatal aperture of fast growing species like birch has been shown to be less sensitive to changes in RH than for slow growing species (Aasamaa and Sõber 2011). RH had a significant effect on NH_3 fluxes to a beech canopy which was attributed to increased surface deposition (Gessler et al. 2000), but only a marginal effect on NH_3 fluxes to a Norway spruce canopy (Gessler et al. 2002). Especially at high RH, the capacity of leaf cuticles to adsorb NH_3 is linked to leaf surface chemistry and different species likely show a different interaction (Asman et al. 1998). Additionally, the leaf surface area exposed to $^{15}NH_3$ also differed between the tree species and the different treatment dates (Table 3.1), which may have influenced cuticular conductance. In general, when NH_3 availability is constant, NH_3 deposition increases with the leaf area exposed (Massad et al. 2010), but not in all cases (Loubet and Cellier 2001). In this study, a fixed amount of NH_3 was applied, which may not have been sufficient for trees with a large biomass such as the pines. However, in case of insufficient NH_3 availability, increased ^{15}N retention would have been measured at increasing NH_3 concentrations, which was not the case. The retention of $^{15}NH_3$ did not increase with increasing surface area (results not shown), so it is likely that the effect of leaf area was negligible.

Although no differences in $^{13}CO_2$ retention were found between the treatments with different NH_3 concentrations, it is possible that stomatal conductance varied between the concentration levels since stomata only marginally limit C gain (Farquhar and Sharkey 1982). However, no reduction in stomatal conductance caused by an increased NH_3 concentration has been reported yet. Contrary, Van Hove et al. (1989b) reported an increased stomatal conductance and CO_2 assimilation rate in poplar leaves after NH_3 exposure at 144 ppb_v for 6 to 8 weeks, but it is not likely that such an effect occurred during our experiments within one hour. Therefore, stomatal conductance may have varied randomly among individual trees and tree species, but likely without distinct differences between the applied different NH_3 concentrations levels.

3.6 Conclusion

To the best of our knowledge, this is the first study that compares the short-term $^{15}NH_3$ retention by leaves and twigs of four temperate tree species at different NH_3 concentration levels. We clearly observed a higher $^{15}NH_3$ retention by birch, beech and oak compared to pine, related to their net $^{13}CO_2$ assimilation. Further research based on long-term monitoring needs to clarify if this also holds true on a yearly basis, since needles may also assimilate during the dormant season. Furthermore, the capacity of all species to assimilate $^{15}NH_3$ reached a maximum at 5, 20 or 50 ppb_v NH_3 depending on the treatment date, which we attributed to increasing temporal storage of NH_3 or strong re-emission at increasing initial NH_3 concentration levels. The mechanism of temporal storage has been suggested in the past at high external NH_3 concentrations. This study suggests that this may also occur at lower NH_3 concentrations. However, this needs to be confirmed by further research. The relationships between $^{15}NH_3$ retention on the one hand and $^{13}CO_2$ retention and N content of leaves and twigs on the other hand were significant but weak. Probably a more extensive experiment focussing on one tree species could help to reveal the exact interaction between N and C processes.

4 Retention of dissolved inorganic nitrogen by foliage and twigs of four temperate tree species

After: Adriaenssens S, Staelens J, Wuyts K, Samson R, Verheyen K, Boeckx P. Retention of dissolved inorganic nitrogen by foliage and twigs of four temperate tree species. Submitted to Ecosystems

4.1 Abstract

Nitrogen (N) retention by tree canopies is believed to be an important process for tree nutrient uptake, and its quantification is a key issue in determining the impact of total atmospheric N deposition on forest ecosystems. Due to dry deposition and retention by canopy elements, the actual uptake and assimilation by the tree canopy is often obscured in throughfall studies. In this study, ^{15}N-labelled solutions ($^{15}NH_4^+$ and $^{15}NO_3^-$) were used to assess dissolved inorganic N retention by leaves/needles and twigs of European beech, pedunculate oak, silver birch and Scots pine saplings. The effects of N form, tree species, leaf phenology and applied NO_3^- to NH_4^+ ratio on the N retention were assessed. Retention patterns were mainly determined by foliar uptake, except for Scots pine. In twigs, a small but significant ^{15}N enrichment was detected for NH_4^+, which was found to be mainly due to physicochemical adsorption to the woody plant surface. The mean $^{15}NH_4^+/^{15}NO_3^-$ uptake ratio varied considerably among species and phenological stadia, which indicates that the use of a fixed ratio in the canopy budget model could lead to an over- or underestimation of the total N retention. In addition, throughfall water under each branch was collected and analysed for $^{15}NH_4^+$, $^{15}NO_3^-$ and all major ions. Net throughfall of $^{15}NH_4^+$ was, on average, 20 times higher than the actual retention of $^{15}NH_4^+$ by the plant material, indicating that a large part of the applied $^{15}NH_4^+$ could not be attributed to measured pools and fluxes. The retention of $^{15}NH_4^+$ was correlated with the net throughfall of K^+, Mg^{2+}, Ca^{2+} and weak acids during leaf development and the fully-leafed period, while no significant relationships were found for $^{15}NO_3^-$ retention. This suggests that the main driving factors for NH_4^+ retention might be ion-exchange processes during the start and middle of the growing season and passive diffusion at leaf senescence.

4.2 Introduction

The ability of forest canopies to retain and assimilate inorganic nitrogen (N) from wet and dry deposition is well known (Rennenberg and Gessler 1999; Harrison et al. 2000; Sparks 2009). In contrast to N uptake from the soil, canopy N retention is a direct addition to a tree's metabolism; there is no competition from soil microbial biomass involved (Gaige et al. 2007; Sievering et al. 2007). The contribution of canopy retention to the N cycling within a forest canopy, particularly in photosynthetically active foliage, may be substantial (Sievering et al. 2007). Estimates based on dry deposition measurements and throughfall analyses indicate a net canopy retention of 10 to 90 % of the atmospheric input (Lovett and Lindberg 1993; Sievering et al. 2007). The wide range of canopy retention values can be caused by differences in tree species and leaf phenology (Tukey 1970; Fritsche 1992; Van Ek and Draaijers 1994; Chapter 2), but also by differences in atmospheric N deposition. As N deposition increases, canopy N consumption also increases (Schaefer et al. 1988; Lovett and Lindberg 1993; Gaige et al. 2007). Therefore, canopy retention under high N deposition levels might meet a larger portion of the whole tree demand of N (Gaige et al. 2007) and cause growth responses in historically N-limited forests (Sparks 2009). This higher N retention might thus enhance carbon (C) sequestration, although the magnitude and the direction of this effect is still under discussion (Magnani et al., 2007; Sievering et al., 2007; Dail et al., 2009; Högberg, 2011).

Canopy N retention, i.e. the atmospheric N input to the canopy not reaching the forest soil, is usually attributed to assimilation by tree foliage (Eilers et al., 1992; Harrison et al., 2000; Gessler et al., 2002) and epiphytic lichens (Reiners and Olson, 1984; Bowden et al., 1989; Friedland et al., 1991), microbial activity (Papen et al., 2002), to physical uptake by twigs and stems (Schoenherr, 1982; Katz et al., 1989) and to physicochemical adsorption processes to twigs and branches (Dail et al., 2009). The variety of potential N sinks in tree canopies complicates the accurate quantification of canopy N uptake and its relative magnitude compared to other N sources for trees (Sparks 2009). The quantification uncertainty may then propagate into larger uncertainties when calculating the impact of enhanced N deposition on forest ecosystem processes, such as eutrophication by N, soil acidification and on shifts in biodiversity (Aber et al. 1998; Gilliam 2006; Gundersen et al. 2006; Bowman et al. 2008) and C sequestration (Nadelhoffer et al. 1999; Magnani et al. 2007; de Vries et al. 2009).

A widely used approach to measure N retention by tree canopies is to examine the differences in N composition of rainfall and throughfall water (Parker 1983; Gaige et al. 2007). Throughfall measurements are well suited for large-scale and long-term monitoring purposes. Canopy N retention is then calculated with the canopy budget model, in which N fluxes are assumed to be related to fluxes of protons (H^+) and base cations (Draaijers and Erisman 1995; Staelens et al. 2008). Although the results of this model generally correspond with the results of other methods, there is a relatively large uncertainty in the estimated canopy N retention (Draaijers et al. 1996) and no information on the actual sinks and processes involved. Moreover, direct gaseous uptake is not taken into account (Draaijers et al. 1997; Thimonier et al. 2005). A more mechanistic and sensitive method to measure canopy N retention is the application of ^{15}N-labelled sources in solution or as gaseous form. Yet, this approach is usually limited to short-term exposure (Bruckner et al. 1993; Gessler et al. 2002; Jones et al. 2008) and application to saplings (Bowden et al. 1989; Brumme et al. 1992; Eilers et al. 1992; Lumme and Smolander 1996; Garten et al. 1998; but see Gaige et al. 2007) or specific plant parts (Garten and Hanson 1990; Boyce et al. 1996). A combination of the two approaches, i.e., a comparison between the chemical composition of throughfall water and the canopy retention of ^{15}N-labelled sources, might yield new insights into the relationship between canopy N retention and throughfall N composition.

Furthermore, none of the ^{15}N studies so far has attempted to quantify N retention during winter, when deciduous species are leafless and coniferous trees are physiologically less active. Increasing evidence suggests that twig, branch and stem surfaces are an important sink of dissolved N in the canopy (Bowden et al. 1989; Boyce et al. 1996; Wilson and Tiley 1998; Dail et al. 2009), although it is not clear whether this retention results from an actual assimilation process or abiotic uptake process (absorption) or a physicochemical process (adsorption) (Dail et al. 2009).

In the present study, we applied artificial rainwater containing ^{15}N-labelled inorganic N to determine the N retention from wet deposition by the foliage (leaves or needles) and twigs of four temperate tree species, i.e., one coniferous and three deciduous species. We also compared the canopy N retention with the chemical composition of the throughfall water. The aims were (i) to determine $^{15}NH_4^+$ and $^{15}NO_3^-$ retention by twigs from wet deposition with a low vs high NO_3^- to NH_4^+ ratio at four dates throughout the year, (ii) to assess the relative importance of ^{15}N retention by twigs compared to foliage, (iii) to determine whether the observed ^{15}N retention results from assimilation or from adsorption to the plant surface and (iv) to relate the observed ^{15}N retention to the net throughfall of ^{15}N and other ions.

4.3 Materials and methods

4.3.1 Plant material and experimental site

A full description of the plant material and experimental site is given in Section 2.3.1.

4.3.2 Experimental set-up and sample analysis

4.3.2.1 ^{15}N application

A detailed description of the ^{15}N application is given in Section 2.3.2.1. Additionally, throughfall water under each branch was collected and weighed. To quantify the exact amount of solution applied to each branch, the initial and final weights of the sprayer were registered. The experiment was conducted in the same three phenological periods and on the same dates as Chapter 2 and additionally in the leafless period (6 Feb 2009 for all species) to assess the effect of plant phenological state on N retention.

In the leafless period, a dead twig of each species was included in each treatment to assess whether the observed ^{15}N retention resulted from actual assimilation or physicochemical adsorption. For this purpose, living twigs were cut from the study sapling, dried in the oven (24 h at 70°C) to stop all physiological activity, and placed back outside to moisten them again.

4.3.2.2 *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 foliage or branches. To test if all surface ^{15}N was removed from the twig surface and if the measured ^{15}N retention represents actual assimilation, half of each twig, i.e. dead as well as living twigs, from the ^{15}N applications in the leafless period was rinsed with 0.05 M KCl. Potassiumchloride is a more thorough rinsing solution than deionised water since $^{15}NH_4^+$ or $^{15}NO_3^-$ adsorbed to the twig surface can be exchanged for K^+ or Cl^- ions. Next, leaves, needles and twigs were dried, weighed, ground to a fine powder and analysed in duplicate for total N and ^{15}N as described in Section 2.3.2.4.

4.3.2.3 Analysis of water samples

Within 24 h after sampling, pH (ion-specific electrode) and electric conductivity were measured. After filtering through a 0.45-µm nylon membrane filter, NO_3^-, SO_4^{2-}, PO_4^{3-} and Cl^- were determined with ion chromatography (Dionex, Sunnyvale, USA) within one week after sampling. Ammonium concentrations were assessed by the photometric determination of a reaction product of NH_4^+ at 660 nm (Dutch standard method NEN 6567), while K^+, Ca^{2+}, Mg^{2+} and Na^+ were determined by flame atomic absorption spectrophotometry (AA240FS, Varian, USA). Proton concentrations were derived from the pH measurements. The quality of the chemical analyses was checked by including method blanks and repeated measurements of internal standards and certified reference samples. Repeated measurements of certified reference water samples (CRM 409) performed during the study period yielded coefficients of variation less than 5 % and recovery rates higher than 90 % for all ions.

The ^{15}N contents of NH_4^+ and NO_3^- in the water samples were analysed after conversion to N_2O (Saghir et al. 1993; Stevens and Laughlin 1994). Ammonium was converted by adding MgO to the samples and absorbing NH_3 into H_2SO_4, after which N_2O was produced by reaction with NaOBr (Saghir et al. 1993). Nitrate was reduced by Cd-Cu at pH 4.7 to produce nitrite and hydroxylamine as intermediates of N_2O (Stevens and Laughlin 1994). Nitrous oxide was determined using a trace gas preparation unit (ANCA-TGII, SerCon, UK) coupled to an IRMS (20-20, SerCon, UK). Instrument error ($n = 10$) of this TG-IRMS system is 0.3‰ for $\delta^{15}N$-N_2O. For the fully leafed periods (2008), all water samples were individually analysed for ^{15}N content. For the other periods, a mixed sample per treatment was analysed.

4.3.3 Data processing

Retention of ^{15}N per unit dry plant material (µg N g^{-1} DM) was calculated according to equation 2.1. The total ^{15}N retention by foliage and twigs together was calculated as the mass-weighted mean of the retention by foliage and twigs separately. Throughfall (TF) and wet deposition (WD) fluxes (µmol$_c$ g^{-1} DM) for each sample were calculated as the product of molar ion concentration (µmol l^{-1}) and the collected or sprayed volume, respectively, divided by the weight of the sprayed dry plant material (g DM). Throughfall deposition of weak acids (WA, µmol$_c$ g^{-1} DM) was calculated as the difference between cations and anions. Net throughfall fluxes (NTF) for all ions, including $^{15}NH_4^+$ and $^{15}NO_3^-$, were calculated as the difference between TF and WD fluxes.

For each combination of tree species, NO_3^- to NH_4^+ ratio, N form and leaf phenological stage, a one sample *t* test was used to test whether the calculated N retention by foliage and twigs and the NTF of all ions were significantly different from zero. The effect of N form on the N retention by leaves and twigs was tested by means of *t* tests per tree species, NO_3^- to NH_4^+ ratio and phenological stage. A three-way ANOVA was performed to assess the effect of tree species, leaf phenology and NO_3^- to NH_4^+ ratio on the measured $^{15}NH_4^+$ and $^{15}NO_3^-$ retention. The three-factor interaction was excluded from the model because of insufficient degrees of freedom. Means were separated by Tukey's honestly significant difference post-hoc tests. Data of ^{15}N retention were log-transformed to meet normality conditions. Significant differences in ^{15}N retention between twigs rinsed with distilled H_2O or with KCl were tested by means of a general linear model including rinsing method as fixed effect and twig ID as random effect. The relationship between ^{15}N retention and NTF of different ions was assessed by Spearman rank correlation coefficients (r_s). All analyses were performed with SPSS 15.0.

4.4 Results

4.4.1 ^{15}N retention by plant material

Retention of $^{15}NH_4^+$ and $^{15}NO_3^-$ by leaves and needles during the growing season was already reported in Section 2.4.1. In the present study, the effects of leaf phenology and the NO_3^- to NH_4^+ ratio on the canopy N retention were recalculated including the leafless period, during which needles of Scots pine were still present (Table 4.1). The retention of $^{15}NO_3^-$ by needles in the leafless period was significantly lower than in the fully leafed and leaf senescence period, while $^{15}NH_4^+$ retention was lowest in the leaf senescence period.

The mean $^{15}NH_4^+$ retention by twigs (Fig. 4.1) ranged from 0.54 for oak to 1.76 µg N g DM^{-1} for pine and was significantly different from zero in most cases. The retention of $^{15}NO_3^-$ ranged from -0.07 for birch to 0.32 µg N g DM^{-1} for oak but did not always differ from zero. Nitrogen uptake by twigs was higher for NH_4^+ than NO_3^-, except under the high NO_3^- to NH_4^+ ratio for beech, oak (leaf development) and birch (fully leafed period) and under the low NO_3^- to NH_4^+ ratio for oak (fully leafed period) and pine (leafless period).

In general, the calculated $^{15}NH_4^+$ and $^{15}NO_3^-$ retention by twigs (Fig.4.1) was significantly influenced by tree species, the applied NO_3^- to NH_4^+ ratio and the two-way interactions between these factors between tree species, leaf phenology and the applied NO_3^- to NH_4^+ ratio (Table 4.1). In contrast to the foliage (see Chapter 2), $^{15}NH_4^+$ retention by the twigs of pine was always higher than for the deciduous species. For beech, $^{15}NH_4^+$ retention was lowest in the leafless period compared with the growing season, while no significant differences between phenological periods were found for the other tree species. The applied NO_3^- to NH_4^+ ratio only influenced the $^{15}NH_4^+$ retention by pine twigs. Birch twigs retained less $^{15}NO_3^-$ than the other species during the growing season, while there was no difference in the leafless period. Both beech and oak twigs showed a higher $^{15}NO_3^-$ uptake in the 'high NO_3^- to NH_4^+ ratio' treatment, particularly during leaf development.

Table 4.1 P values of a three-way ANOVA: effects of tree species, leaf phenology and NH_4^+ to NO_3^- ratio on the observed $^{15}NH_4^+$ and $^{15}NO_3^-$ retention by foliage, twigs and the sum of foliage and twigs (total). Bold values are significant at $p < 0.05$

Source of variation	$^{15}NH_4^+$ retention			$^{15}NO_3^-$ retention		
	Foliage	Twigs	Total	Foliage	Twigs	Total
Tree species	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Leaf phenology	**< 0.001**	0.147	**< 0.001**	**<0.001**	0.140	**< 0.001**
NO_3^- to NH_4^+ ratio	0.537	0.174	0.061	**< 0.001**	**< 0.001**	**< 0.001**
Tree species * leaf phenology	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Tree species * NO_3^- to NH_4^+ ratio	**0.021**	**0.001**	0.293	**<0.001**	**0.005**	**0.018**
Leaf phenology * NO_3^- to NH_4^+ ratio	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

Since the N retention by foliage exceeded the retention by twigs, total (i.e. foliage + twigs) N retention patterns were mainly determined by the foliage, except for $^{15}NH_4^+$ retention by pine (Fig. 4.2). Total $^{15}NH_4^+$ retention by foliated twigs was three to ten times higher than total $^{15}NO_3^-$ retention and was two to three times higher for the deciduous species than for pine. All species showed the lowest total retention during the leafless season, which was driven by the difference between foliated and non-foliated twigs for the deciduous species and by the difference between growing and dormant season for pine. Within the growing season, all species showed the highest ^{15}N retention at leaf senescence and the lowest in the fully leafed period (birch and pine) or at leaf development (beech and oak). The high NO_3^- to NH_4^+ ratio treatment generally resulted in a 1.3 times higher $^{15}NO_3^-$ uptake and had no effect on $^{15}NH_4^+$ uptake. Total $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake by foliated twigs amounted to 2.27 ± 1.01 and 0.52 ± 0.44 % of the applied ^{15}N, respectively.

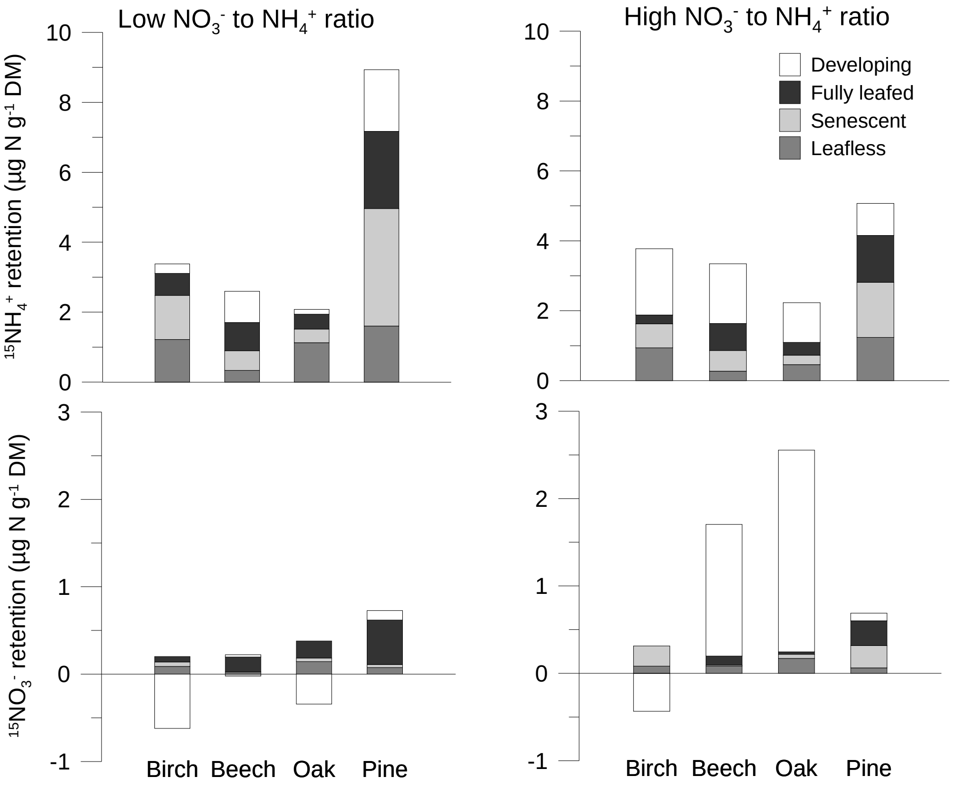

Fig. 4.1 Mean $^{15}NH_4^+$ and $^{15}NO_3^-$ retention (µg N g^{-1} DM) by twigs of birch, beech, oak and pine in the 'low NO_3^- to NH_4^+ ratio' treatment and in the 'high NO_3^- to NH_4^+ ratio' treatment in different phenological stages (developing leaves, fully developed leaves, senescent leaves and the leafless stage)

4.4.2 Rinsing experiment in the leafless period

The $\delta^{15}N$ values and ^{15}N retention of birch, oak and current year pine twigs sprayed with $^{15}NH_4^+$ were significantly lower when rinsed with KCl than with distilled water (Fig. 4.3). No significant differences were found for twigs sprayed with $^{15}NO_3^-$ and for current and last year pine needles sprayed with $^{15}NH_4^+$ or $^{15}NO_3^-$ (results not shown). The ^{15}N enrichment of dead pine twigs sprayed with $^{15}NH_4^+$ was also lower when rinsed with KCl than with distilled water. However, it was not possible to test the significance of this difference due to a lack of replicates. For both rinsing methods, $^{15}NH_4^+$ retention by the

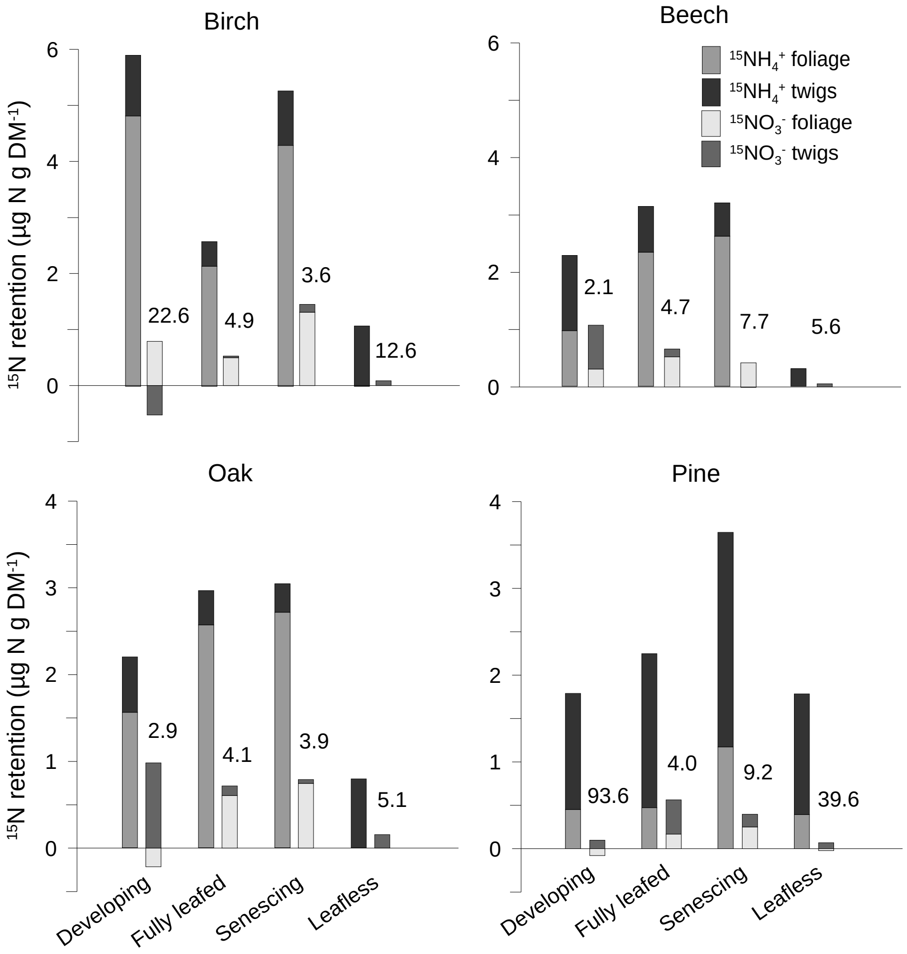

Fig. 4.2 Mean $^{15}NH_4^+$ and $^{15}NO_3^-$ retention (µg N g^{-1} DM) by foliage and twigs of birch, beech, oak and pine in different phenological stages. Numbers in the graph represent the ratio of $^{15}NH_4^+$ to $^{15}NO_3^-$ retention

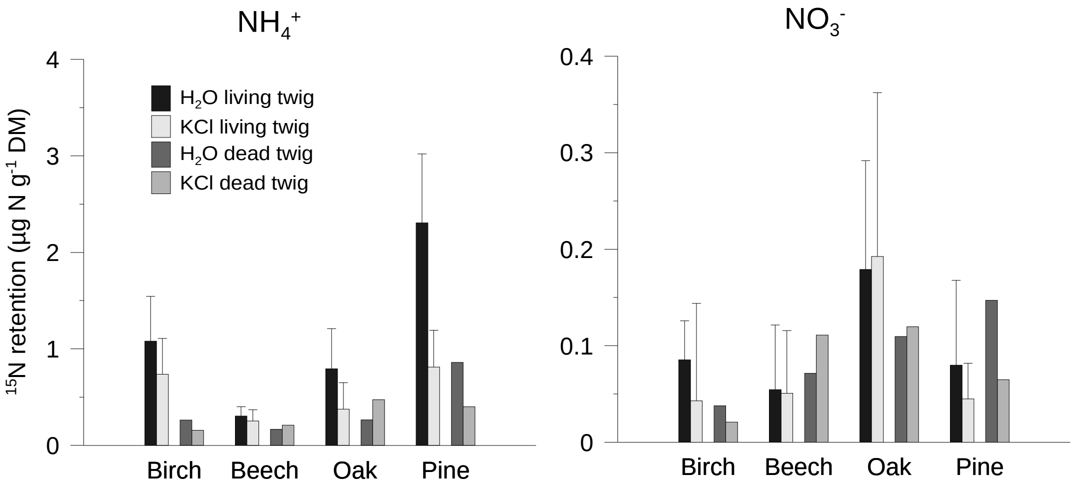

Fig. 4.3 Mean ^{15}N retention (µg N g^{-1} DM) of living and dead twigs of birch, beech, oak and pine sprayed with $^{15}NH_4^+$ and $^{15}NO_3^-$ and subsequently rinsed with distilled water or KCl. Vertical bars represent standard deviations

dead twigs ranged from 0.19 for beech to 0.63 µg g^{-1} DM for pine, and $^{15}NO_3^-$ retention from 0.03 for birch to 0.11 µg g^{-1} DM for pine

4.4.3 Water samples

The observed NTF of NH_4^+-N, NO_3^--N, Na^+, Cl^- and SO_4^{2-}-S differed significantly from zero in half of the cases (Fig. 4.4; $p < 0.05$), depending on the tree species, phenological period or NO_3^- to NH_4^+ ratio considered. Generally, NTF was significantly higher than zero, except for NH_4^+-N during leaf development of beech and oak and for NO_3^--N during leaf development of beech. The NTF of H^+ was always significantly lower than zero.

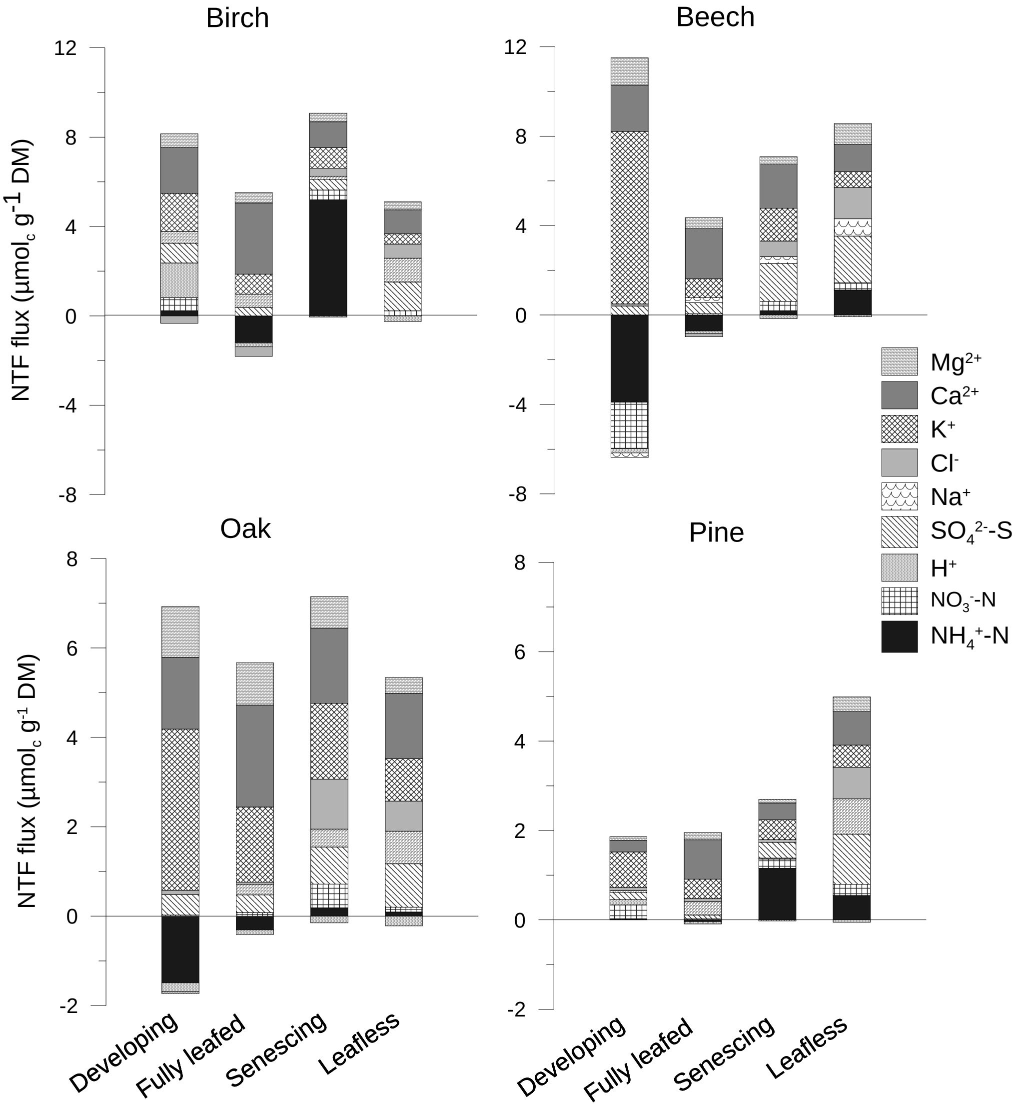

Fig. 4.4 Mean net throughfall fluxes (NTF, µmol$_c$ g^{-1} DM) for all major ions per tree species and phenological stage

The ^{15}N enrichment in TF ranged from 58.2 ± 19.6 atom % ^{15}N for NH_4^+ to 71.8 ± 11.4 atom % for NO_3^-. This corresponded to a mean recovery of 58.3 ± 17.5 % and 99.0 ± 12.8 %, respectively, of the applied ^{15}N (Fig. 4.5). So, based on the ^{15}N accounted for in the NTF, on average 41.7 % of the applied $^{15}NH_4^+$ and 1.0 % of $^{15}NO_3^-$ had been retained by the foliage and twigs.

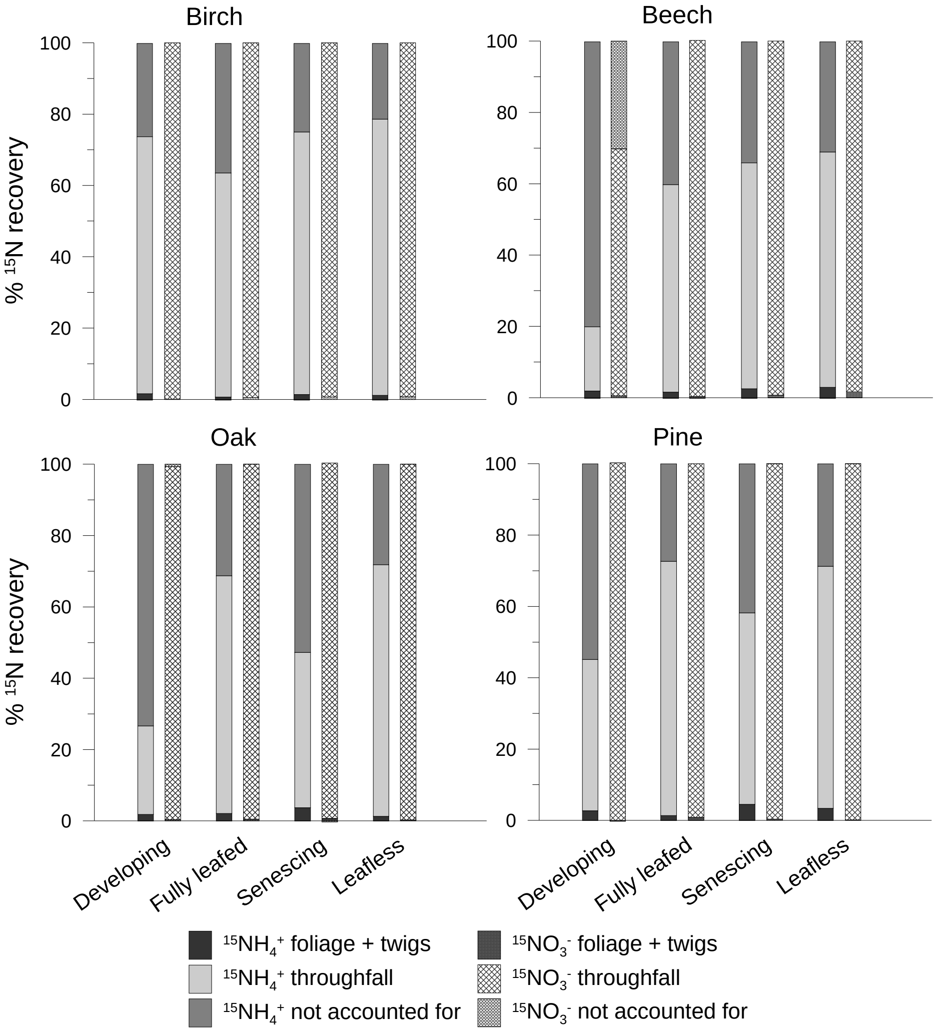

Fig. 4.5 Percentage recovery of ^{15}N applied as NH_4^+ or NO_3^- in foliage, twigs and throughfall water of birch, beech, oak and pine for the different phenological stages

The $^{15}NH_4^+$ retention by foliage and twigs was significantly correlated with the NTF of K^+, Ca^{2+}, Mg^{2+} and WA during leaf development and the fully leafed period (Fig. 4.6) and with the NTF of H^+ during the fully leafed period (Table 4.2). There was also a significant correlation with NTF of inorganic ^{15}N (Table 4.2). The $^{15}NO_3^-$ uptake was correlated with the NTF of K^+ during the leafless period only and with the NTF of Na^+ during leaf development and leaf senescence. Furthermore, the NTF of the sum of base cations ($K^+ + Ca^{2+} + Mg^{2+}$) was significantly correlated with the NTF of WA during leaf development ($p < 0.001$; $r_s = 0.81$), the fully leafed period ($p < 0.001$; $r_s = 0.88$), leaf senescence ($p < 0.001$; $r_s = 0.59$) and the leafless period ($p = 0.014$; $r_s = 0.31$).

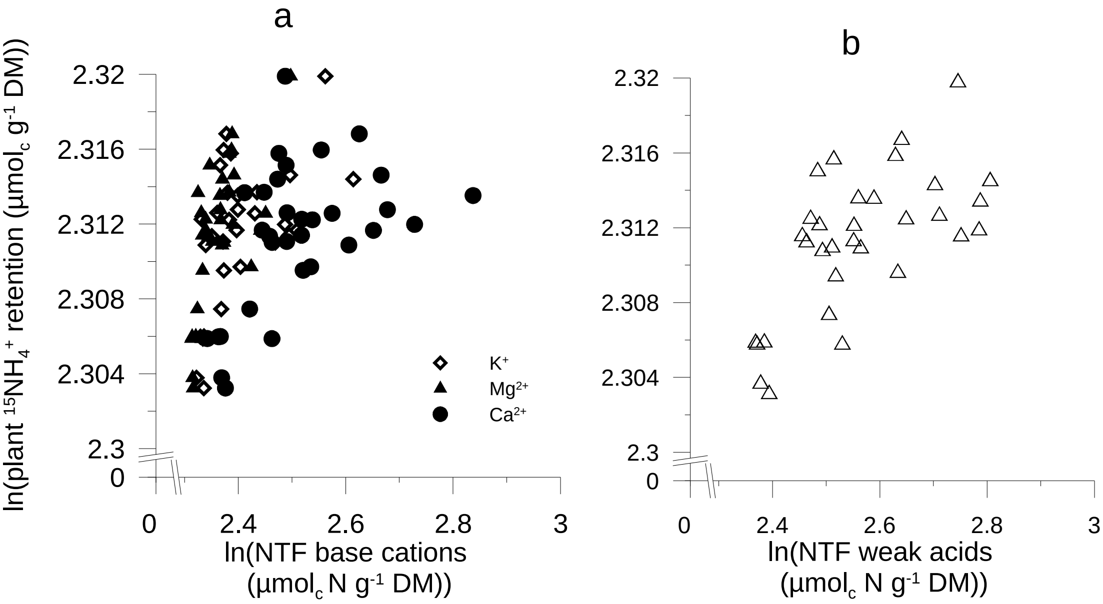

Fig. 4.6 Relationship between $^{15}NH_4^+$ uptake by foliage and twigs and (a) net throughfall (NTF) of K^+ ($r_s = 0.55$), Mg^{2+} ($r_s = 0.63$) and Ca^{2+} ($r_s = 0.48$) and (b) net throughfall of weak acids ($r_s = 0.59$) during the fully leafed period

4.5 Discussion

According to Gaige et al. (2007), N deposited on a forest canopy has the following possible fates: (1) retention and/or assimilation by foliar and bark surfaces within the canopy, (2) bypassing of the canopy at the time of the application, (3) wash off during subsequent rain events, followed by recovery as NO_3^-, NH_4^+or dissolved organic N (DON) in throughfall or stemflow and (4) gaseous loss by volatilization. Retention includes assimilation by tree foliage, twigs and stems, epiphytic lichens, microbial activity and physicochemical adsorption processes to twigs and branches. As no epiphytic lichens were observed on the young saplings, their contribution can be considered negligible. During leaf development, beech leaves were infested by woolly beech aphid (*Phyllaphis fagi* L.), which could have increased the presence of micro-

Table 4.2 Spearman correlation coefficients between the observed total (foliage and twigs) $^{15}NH_4^+$ and $^{15}NO_3^-$ retention (n = 126) and the net throughfall fluxes of different ions (n = 256). Bold values are significant at $p < 0.01$, underlined values at $p < 0.05$

^{15}N treatment and time period	^{15}N[a]	NH_4^+	NO_3^-	H^+	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	WA[b]
NH_4^+											
Leaf development	-0.21	-0.16	-0.26	0.3	0.12	-0.33	0.28	0.43	**0.47**	**0.44**	**0.51**
Fully developed	-0.22	-0.16	0.06	0.43	0.26	0.28	**0.55**	**0.48**	**0.63**	0.31	**0.59**
Leaf senescence	-0.64	0.26	-0.31	-0.09	-0.13	-0.09	0.05	0.11	-0.06	0.01	0.28
Leafless	-0.66	-0.23	-0.04	-0.40	0.21	0.06	0.08	0.26	0	0	0.06
Year	**-0.40**	-0.13	-0.13	0.05	-0.04	-0.17	**0.38**	**0.44**	**0.29**	0.02	**0.42**
NO_3^-											
Leaf development	-0.60	-0.16	-0.34	-0.15	**-0.65**	-0.23	0.03	0.19	0.02	-0.2	-0.04
Fully developed	0.24	-0.15	0	-0.13	0.02	-0.01	0.14	0.33	0.25	-0.01	0.29
Leaf senescence	-0.62	-0.14	**-0.51**	-0.06	**-0.46**	-0.18	0.27	0.1	0.28	-0.2	**0.47**
Leafless	-0.39	0.30	0.32	-0.13	0.03	0.16	**0.56**	0.29	0.21	0.26	0.26
Year	-0.25	-0.13	**-0.29**	-0.16	**-0.43**	-0.18	0.20	**0.28**	0.13	-0.18	0.28

[a] In case of NH_4^+, NTF of $^{15}NH_4^+$; in case of NO_3^-, NTF of $^{15}NO_3^-$

[b] Weak acids

organisms and their contribution to inorganic N assimilation later in the growing season (Stadler et al. 2001). In contrast to the studies of Gaige et al. (2007) and Dail et al. (2009), the possible fates (1) and (2) were measured almost instantaneously after one-time application in the present study while (3) was measured by analysing the rinsing water in a supplementary experiment (see 4.7 Appendix), which made it possible to recover N in all possible sinks, except for gaseous losses by volatilization. Nitrogen that bypassed the canopy is here referred to as throughfall, while wash off is used for N in rinsing water.

4.5.1 Nitrogen retention processes by canopy components

Several ^{15}N-labelling studies have indicated N retention by woody plant surfaces to be at least as important as foliar uptake (Bowden et al. 1989; Boyce et al. 1996; Wilson and Tiley 1998; Dail et al. 2009). Klemm (1989) demonstrated the existence of pathways for the absorption of water and solutes in the region of radial rays across the twig bark and suggested that the contribution of ion exchange via these water pathways may be significant for the whole plant ion balance. Boyce and McCune (1992) suggested that the bulk of intercepted precipitation remains on twig surfaces far longer than on foliage, which may provide more opportunity for ion uptake by twigs. In the field, the mass flow process through twigs should be most significant in the early phases of each precipitation event, because rainwater then generally has the highest concentration of elements (Hansen et al. 1994) and the xylem water potential of trees is lower than later in the precipitation event (Katz et al. 1989). Therefore, in this study we would expect a significant retention of NH_4^+ and NO_3^- after one spraying event. This was observed for NH_4^+ but not for NO_3^-. It is not clear whether tree bark has a negative charge like the leaf cuticle, but many studies have shown that bark has a high cation exchange capacity (Levia and Frost 2003), suggesting that cations are more easily retained by the bark surface. However, when relatively more NO_3^- was available, the beech and oak twigs retained more NO_3^-. So, by increasing the concentration gradient between the external mineral solution and the plant, anion diffusion may be enhanced for some species. On average, we found a 1.3 times higher NO_3^- uptake when the applied concentration was three times higher, in contrast to Brumme et al. (1992) and Eilers et al. (1992), who observed a proportional increase of NO_3^- with increasing concentration in the spray solution.

The higher N retention by twigs vs needles for Scots pine is concordant with the findings for Norway spruce (Wilson and Tiley 1998), red spruce (Bowden et al. 1989; Boyce et al. 1996) and hemlock (Dail et al. 2009). However, Eilers et al. (1992) found the opposite for 10-year-old Norway spruce trees. In the present study, the retention by the twigs of the three deciduous species, including beech, was lower than the foliar retention, which confirms the findings of Brumme et al. (1992) for beech twigs.

Furthermore, the ratio of NO_3^- to NH_4^+ retention by foliage and twigs was highly variable among the tree species and the phenological periods considered (Fig. 4.2), as was concluded in Chapter 2 for foliage only. For beech and oak, the mean ratio amounted to 4.7 and 4.0 respectively, while for birch and pine the ratio was significantly higher during leaf development and the leafless period compared to the other two periods. This indicates that the proposed efficiency factor of six suggested by de Vries et al. (2001) is not applicable for all species throughout the year. Using this fixed factor value could thus overestimate or underestimate total atmospheric N deposition to forest ecosystems.

Bulk tissue analysis does not allow distinguishing between physiological uptake into cells and adsorption to or diffusion into non-living bark. The higher ^{15}N retention values calculated for pine twigs compared to twigs of the deciduous species could be due to ^{15}N lodged in micro-crevices of the rough bark surface, as suggested for Norway spruce (Wilson and Tiley 1998). The bark of birch, beech and oak twigs is less rough, may have a smaller surface area and therefore has less potential adsorption sites. The hypothesis of physicochemical adsorption of N to the bark is confirmed by a decrease in $^{15}NH_4^+$ enrichment when twigs were rinsed with KCl compared to distilled water. This indicates that part of the $^{15}NH_4^+$ was only weakly adsorbed to the woody surface and was easily exchangeable for K^+. Moreover, dead twigs also showed an enrichment, which cannot be attributed to active assimilation. This leads to the conclusion that biological or physiological uptake by twigs was generally low, but that significant amounts of NH_4^+ were adsorbed to the bark surface. Since no difference in ^{15}N retention was found between needles of Scots pine rinsed with distilled water or with KCl in the leafless period or between the beech leaves rinsed with distilled water, KCl or artificial rainwater in 2010 (see supplementary experiment in 4.7. Appendix), it may be assumed that ^{15}N retention by leaves and needles results from actual uptake and assimilation processes. This was not verified for birch and oak in the present study, but can be confirmed by the findings of Garten and Hanson (1990) for red maple and white oak leaves.

Total ^{15}N retention by foliated twigs after this one-time application was generally low, e.g. 1-5 % of the applied ^{15}N for $^{15}NH_4^+$ and 0-2 % for $^{15}NO_3^-$. In general, higher retention was found in other previously performed ^{15}N studies with saplings (Brumme et al., 1992; Eilers et al., 1992; Garten et al., 1998; Wilson and Tiley, 1998), but these studies used higher N concentration levels comparable to throughfall deposition instead of wet deposition. Only the study of Bowden et al. (1989) applied similar N amounts to saplings of red spruce and found ^{15}N retention comparable to this study. In addition, it is possible that tree age might have an effect on the observed results. Until now, ^{15}N labelled rainwater has only been applied to a mature forest in an experiment by Dail et al. (2009), where 3-6 % of the applied ^{15}N was retrieved in living plant biomass but 20-25 % in twigs, branches and bark, of which the latter was also suggested to occur through physicochemical adsorption processes. So, mature forest trees may retain more N than saplings due to their higher bark surface area, but possibly also due to microbial activity or the presence of epiphytic lichens, which have been shown to occur mainly on older trees (Fritz et al., 2008; Ranius et al., 2008). Nitrogen assimilation by epiphytic lichens may contribute significantly to total canopy retention in coniferous forests (Lang et al. 1976; Johansson et al. 2010), although this fate was estimated to amount to only 5% of the applied ^{15}N for a spruce-fir forest (Friedland et al., 1991). Nitrogen uptake by lichens has yet not been assessed for deciduous stands.

4.5.2 Throughfall water

In several throughfall studies a reduced concentration of inorganic N was found in throughfall water compared to precipitation, indicating N retention by the canopy (Parker 1983; Neary and Gizyn 1994; Lovett et al. 1996). However, this canopy N retention is often obscured by the wash off of dry deposition, resulting in a positive NTF. This was also observed in the present experiment, except for beech and oak during the leaf development period (Fig. 4.5). The quantification of $^{15}NH_4^+$ and $^{15}NO_3^-$ retention by foliage and twigs and the concentrations of ^{15}N in the throughfall water allowed for discrimination between canopy retention from wet deposition and dry deposition. To our knowledge, only the analogous studies of Brumme et al. (1992) and Eilers et al. (1992) for beech and spruce and the study of Umana and Wanek (2010) in tropical rainforest have accomplished this previously.

Based on the calculated NTF of ^{15}N, we should have retrieved 42 % of the applied NH_4^+ and 1 % of the applied NO_3^- in foliage and twigs. This was confirmed by the ^{15}N analysis in plant tissue for NO_3^-. For NH_4^+, in contrast, only 1-3 % of the applied $^{15}NH_4^+$ was detected in leaves and twigs, leaving ca. 40 % NH_4^+ not accounted for. Similarly, Brumme et al. (1992) for beech and Umana and Wanek (2010) for tropical forest reported a difference of 15 % and 25.8 %, respectively, between ^{15}N retrieved in plant parts and the estimated value based on NTF. Several possible fates can be put forward to explain the difference between the calculated NTF and the actual retention values of ^{15}N. Firstly, N adsorbed to the plant surfaces, especially NH_4^+ could be washed off by rinsing solution (distilled water or KCl). Analysis of the rinsing water, however, explained only 2.8 % of the missing $^{15}NH_4^+$ (see 4.7. Appendix). Secondly, the N assimilated by the leaves may partly have been transported to stems and roots during the 2 h before they were harvested. After 72 h, Gessler et al. (2002) retrieved $^{15}NH_3$ taken up by adult spruce trees in plant parts at 17 cm distance from the fumigation chambers. After 24 h, however, this distance was only 5.5 cm. Therefore, we assume that transport of N assimilated into amino compounds is negligible after 2 h. Thirdly, gaseous losses of NH_3 from the canopy to the atmosphere can occur. Significant NH_3 emission from evaporating water layers to the atmosphere has been observed if atmospheric NH_3 concentrations are low (Rennenberg and Gessler 1999). Fourthly, dissolved inorganic N may convert rapidly into dissolved organic N within tree canopies, which is then released to the throughfall water (Gaige et al. 2007; Wu et al. 2010). Since the last two pathways were not measured in this study, the relative magnitude of these sinks remains to be investigated. In addition, the $^{15}NH_4^+$ retrieved in leaves and twigs was significantly correlated with the NTF of $^{15}NH_4^+$. Yet, the low correlation coefficient indicates that losses during the experiment vary among tree species and phenological periods.

We observed a significant correlation between $^{15}NH_4^+$ uptake and NTF of K^+, Mg^{2+} and Ca^{2+} during the fully leafed period. Uptake of NH_4^+ and H^+ in tree canopies is usually considered as an ion exchange process, accompanied by the release of base cations, which is necessary to maintain the ionic balance within the leaf (Staelens et al. 2008). Several studies have indicated a relationship between the loss of NH_4^+ (Stachurski and Zimka 2002; Umana and Wanek 2010) or H^+ (Schaefer et al. 1988; Lovett et al. 1996; Chiwa et al. 2004) and the leaching of base cations in throughfall water. This is supported by the relationship found in this study. Since NTF fluxes comprise the sum of wash off of dry deposition and canopy exchange processes, the relationship observed in this study may be obscured by K^+, Mg^{2+} and Ca^{2+} present on the plant surface prior to the experiment. For K^+, canopy leaching is the main mechanism of enrichment in throughfall water (Houle et al. 1999; Staelens et al. 2007), while for Ca^{2+} and Mg^{2+} dry deposition has been estimated to contribute up to 38 and 25 % during the growing season for an adult beech tree (Staelens et al. 2007). The NTF of the summed base cations exceeded by far the measured $^{15}NH_4^+$ uptake by leaves and twigs, indicating that base cation leaching was caused by factors in addition to NH_4^+ uptake. Previous research attributed base cation leaching partly to the leaching of weak organic acids (Chiwa et al. 2004; Zhang et al. 2006; Staelens et al. 2007). This was also observed in the present study. Furthermore, $^{15}NH_4^+$ uptake was also related to NTF of WA during leaf development and the fully leafed period, supporting the hypothesis that canopy uptake of NH_4^+ is related to the difference between canopy leaching of base cations and WA.

No relationship between NTF of base cations and $^{15}NH_4^+$ uptake was observed during leaf senescence and the leafless period. Several explanations can be put forward to explain this lack of correlation. During the leafless period, the positive NTF of K^+ and Ca^{2+} can be attributed to wash off of dry deposition and leaching from twigs and branches (Levia and Herwitz 2002); the NTF of Mg^{2+} is considered to be only due to wash off of dry deposition (Levia and Herwitz 2002; Staelens et al. 2007). Furthermore, it is likely that NH_4^+ is mainly adsorbed to the negatively charged plant surface during the leafless period, as discussed above, and is not exchanged for base cations. During leaf senescence, passive diffusion might be more important for NH_4^+ uptake than ion exchange. Firstly, the N content, which is a proxy for leaf apoplast NH_4^+ concentrations (Schjoerring et al. 1998), of senescing leaves decreases compared to fully developed leaves, thereby increasing the concentration gradient between the leaf and rainwater. Secondly, the contact surface between the leaf and the rainwater, defined as leaf wettability, increases significantly during leaf senescence (Chapter 2). However, it is important to further assess the relationship between NH_4^+ uptake and base cation leaching under varying NH_4^+ deposition levels, since increasing NH_4^+ deposition will likely increase NH_4^+ uptake (Schaefer et al. 1988), and for rain events with different intensity and duration.

4.6 Conclusion

This study demonstrated that dissolved inorganic N retention by foliage and twigs of tree saplings amounts to 1-3 % of the N in incident precipitation and is significantly influenced by N form, tree species, leaf phenology and the applied NO_3^- to NH_4^+ ratio. The observed canopy N retention patterns were mainly determined by the foliage for the deciduous species. For Scots pine, twigs were more important. Yet, the rinsing experiment indicated that the observed $^{15}NH_4^+$ enrichment of the twigs could mainly be attributed to adsorption to the woody plant surface rather than assimilation. Furthermore, the amount of $^{15}NH_4^+$ retrieved in plant material could not fully explain the loss of ^{15}N between the artificial rainwater and throughfall water, indicating that other possible sinks exist, which should be investigated in further research. Measured $^{15}NH_4^+$ retention by foliage and twigs was significantly correlated with base cation leaching during the leaf development and fully leafed period but not during the period of leaf senescence and the leafless period, indicating that different processes might prevail in different phenological periods. We conclude that, for the studied tree species, N is mainly assimilated by foliage, although a large amount of N can be temporally adsorbed to woody parts of the canopy. Further research should focus on assessing the relationship between throughfall measurements and N canopy retention for other tree species, tree ages and varying N deposition levels. Given the small amount of biological or physiological uptake measured in this study, the contribution of canopy N uptake from wet deposition to forest productivity is likely to be small. The contribution of canopy N uptake from gaseous deposition still remains to be assessed.

4.7 Appendix: Supplementary experiment

4.7.1 Aims and description

Since during the experiment conducted in 2008 a large amount of the applied $^{15}NH_4^+$ could not be retrieved in throughfall water and plant material (see 4.4.3), the ^{15}N spraying experiment was repeated on beech twigs only in June 2010. The aim of this experiment was to assess (i) if the “lost $^{15}NH_4^+$” could be attributed to the rinsing of leaves and twigs after the experiment and (ii) if this rinsing loss is affected by the type of rinsing solution, i.e. distilled water, KCl or a non-labelled rainwater solution.

For this experiment 3-year-old beech saplings were used of the same provenance as in 2008. They were planted on 16 March 2010 in a 1:1 mixture of river sand (1-5 mm) and sandy soil from a mature Corsican pine stand (20-100 cm depth). The saplings were also 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).

Similar to the experiment conducted in 2008, twelve foliated beech twigs were selected and sprayed with the low NO_3^- to NH_4^+ ratio treatment (only ^{15}N-labeled NH_4^+), after which the throughfall solution was collected. After harvesting, one-third of the treated beech twigs was rinsed with deionised water, one-third with 0.05 M KCl and one-third with an unlabeled artificial rainwater solution and subsequently the rinsing solution was collected. Plant material and water samples were analysed as described in 4.3.2.2. and 4.3.2.3.

4.7.2 Results

Foliar $^{15}NH_4^+$ retention did not differ between the three different rinsing methods (results not shown; $p = 0.22$). Average NH_4^+-N concentrations of the rinsing water collected in 2010 were 1.22 ± 3.15 µg N g^{-1} DM for deionised water, 10.40 ± 7.74 µg N g^{-1} DM for KCl and 32.24 ± 4.08 µg N g^{-1} DM for the artificial rainwater solution, with an average ^{15}N enrichment of 8.24, 4.29 and 1.45 atom %, respectively. This resulted in an average ^{15}N recovery of 0.5, 1.3 and 1.8 % for deionised water, KCl and artificial rainwater, respectively (Fig. 4.7). No significant differences in $^{15}NH_4^+$ concentration were found between the three different rinsing methods ($p = 0.966$).

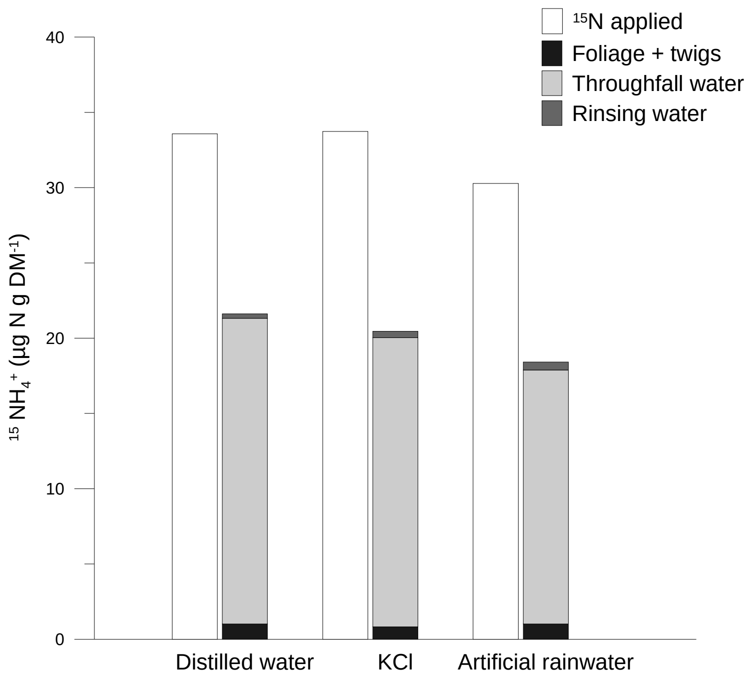

Fig. 4.7 Average $^{15}NH_4^+$ (µg N g DM^{-1}) retrieved in leaves + twigs, throughfall water and rinsing water compared with the $^{15}NH_4^+$ applied to foliated beech twigs

5 Throughfall deposition and canopy exchange processes along a vertical gradient within the canopy of beech and Norway spruce

*After: Adriaenssens S, Hansen K, Staelens J, Wuyts K, De Schrijver A, Baeten L, Boeckx P, Samson R, Verheyen K. Throughfall deposition and canopy exchange processes along a vertical gradient within the canopy of beech (*Fagus sylvatica *L.) and Norway spruce (*Picea abies *(L.) Karst.). Science of the Total Environment 420, 168-182*

5.1 Abstract

To assess the impact of air pollution on forest ecosystems, the canopy is usually considered as a constant single layer in interaction with the atmosphere and incident rainwater, which could influence the measurement accuracy. In this study the variation of througfall deposition and derived dry deposition and canopy exchange were studied along a vertical gradient in the canopy of one European beech (*Fagus sylvatica* L.) tree and two Norway spruce (*Picea abies* (L.) Karst) trees. Throughfall and net throughfall deposition of all ions other than H^+ increased significantly with canopy depth in the middle and lower canopy of the beech tree and in the whole canopy of the spruce trees. Moreover, throughfall and net throughfall of all ions in the spruce canopy decreased with increasing distance to the trunk. Dry deposition occurred mainly in the upper canopy and was highest during the growing season for H^+, NH_4^+, NO_3^- and highest during the dormant season for Na^+, Cl^-, SO_4^{2-} (beech and spruce) and K^+, Ca^{2+} and Mg^{2+} (spruce only). Canopy leaching of K^+, Ca^{2+} and Mg^{2+} was observed at all canopy levels and was higher for the beech tree compared to the spruce trees. Canopy uptake of inorganic nitrogen and H^+ occurred mainly in the upper canopy, although significant canopy uptake was found in the middle canopy as well. Canopy exchange was always higher during the growing season compared to the dormant season. This spatial and temporal variation indicates that biogeochemical deposition models would benefit from a multilayer approach for shade-tolerant tree species such as beech and spruce.

5.2 Introduction

During the last decades, atmospheric deposition of pollutants such as nitrogen dioxide (NO_2), ammonia (NH_3) and sulphur dioxide (SO_2) on forest ecosystems has dramatically increased due to anthropogenic activities. Reduced emissions have again decreased the deposition of SO_2 during the last decade. By means of deposition monitoring it is possible to (a) estimate deposition effects, (b) determine ecosystems at risk, (c) evaluate temporal and spatial trends, and (d) develop numerical models (Erisman et al. 1994). Wet deposition can be measured with wet-only or bulk precipitation collectors (Staelens et al. 2005). Methods to measure dry deposition can be divided in two categories, i.e. micrometeorological techniques such as the eddy correlation and the gradient method and surface wash techniques such as the throughfall method (Draaijers et al. 1997; see Chapter 6 for an overview), which each have their advantages and drawbacks. For long term monitoring programmes preferably throughfall measurements are used (de Vries et al. 2003), although here a distinction needs to be made between dry deposition and canopy exchange (Parker 1983).

One major drawback of the currently used deposition methods is the assumption that forest canopies consist of a homogenous array of components or one “big leaf”, which is in contact with homogenous throughfall water (Schaefer et al. 1988) or with homogenously distributed gaseous compounds. However, several arguments suggest that dry deposition and canopy exchange processes are likely to vary along a vertical gradient in a tree canopy. Firstly, foliar nitrogen (N) content per leaf area (N_A, g N m^{-2}), stomatal conductance and photosynthetic capacity strongly increase with height due to increasing light availability (Niinemets 2007; Eensalu et al. 2008). In addition, leaf wettability, i.e. water repellence by the leaf surface, decreased from the bottom to the top of a red spruce (Boyce et al. 1991) and European beech (Van Wittenberghe 2009) canopy. Dry deposition processes were found to be affected by surface wetness chemistry, wettability and stomatal opening (Erisman and Draaijers 2003). Differences in leaf wettability and physiological activity (determined by processes such as photosynthesis rate, transpiration and respiration) between tree species and phenological stages have been related to accelerated canopy leaching of K^+ and Mg^{2+} (Tukey 1970; Fritsche 1992) and enhanced canopy uptake of NH_4^+ and NO_3^- (Eilers et al. 1992; Sase et al. 2008; Chapter 2). Secondly, throughfall water is likely to be enriched for most elements on its way through the canopy (Hansen 1996), thereby also changing the concentration gradient between the surface water on the leaves and the interior and consequently enhancing canopy uptake or leaching processes (Schaefer et al. 1988). Thirdly, the decrease in intensity and volume of precipitation from the top to the bottom of the canopy and the increase in contact time between the rain water and leaves (Tukey 1970; Hansen 1995) could influence the canopy leaching of base cations (Fritsche 1992). Lastly, pollutant concentrations and turbulence patterns also vary from the top to the bottom of the canopy (Merony 1968; Lovett and Lindberg 1992). Comparisons between single-layer and multi-layer biochemical models for water, carbon (Samson 2001; Wu et al. 2003), ozone, SO_2 and NH_3 (Wu et al. 2003) showed that single layer models tend to overestimate or underestimate canopy processes, especially in trees where the structure of the canopy is rather complex and a high variation in microclimate exists (Samson 2001). Although multi-layer models are generally less applicable at a large scale due to the large amount of parameters needed (Nemitz et al. 2000), they provide important information on the errors made by single layer models and give more insight into the deposition and canopy exchange processes of various atmospheric gases (Lovett and Lindberg 1992). Furthermore, if different canopy layers are exposed to different concentrations of atmospheric pollutants, it is relevant to consider them separately when assessing the impact of air pollutants (Ould-Dada 2002). Throughfall measurements can add to these gaseous exchange models since they provide information on dry deposition processes and the canopy exchange of dissolved components.

To our knowledge, only few studies have assessed the variation in chemical composition of throughfall water within tree canopies. Throughfall water was sampled within coniferous canopies (Schaefer et al. 1988; Fritsche et al. 1989; Hansen 1995, 1996) and between the upper canopy and sub-canopy of a subtropical evergreen forest (Zhang et al. 2006). However, none of these throughfall studies in coniferous canopies quantified dry deposition and canopy exchange, and deciduous tree species remain altogether unstudied.

The within-canopy variation in chemical composition of throughfall water is likely to differ between deciduous and coniferous species, but also between the growing and the dormant season. Dry deposition processes are influenced by roughness length, a factor that is determined by the leaf area index (LAI), canopy height and closure (Erisman and Draaijers 2003). Coniferous species generally have an equal or higher LAI than deciduous species (Gower and Norman 1991; Malek and Astel 2008) and have needles present throughout the whole year, compared to only the leafed period for deciduous species. Moreover, leaf wetness and stomatal opening are absent for deciduous species during winter, but also vary significantly for coniferous trees (Neirynck and Ceulemans 2008). Along a vertical gradient, the variation in physical, physiological and chemical leaf characteristics will vary between deciduous and coniferous species due to their different leaf longevity (one leafed period vs multiple years) (Niinemets 2007).

In the present study, we determined the amount and chemical composition of throughfall water throughout the year within the canopy of a European beech (*Fagus sylvatica* L.) tree in Belgium and included the data of an earlier study in the canopy of two Norway spruce (*Picea abies* (L.) Karst) trees in Denmark (Hansen 1996). We applied a canopy budget model (Draaijers and Erisman 1995) to the throughfall data of both studies in order to distinguish the processes of dry deposition and canopy exchange. Based on the literature reviewed above, we hypothesized that within one species (i) dry deposition is highest in the upper canopy but canopy exchange is highest in the lower canopy and (ii) dry deposition and canopy exchange fluxes will differ between the growing season and winter. Furthermore, without testing this statistically, we expect that (iii) the lower canopy contributes more to dry deposition and canopy exchange in the cone-shaped spruce trees than in the beech tree where the highest leaf area density is found in the upper canopy (Fleck 2001; Samson 2001) and (iv) differences between the growing season and winter will be larger for beech than for spruce due to the absence of leaves during winter.

5.3 Materials and methods

5.3.1 Site description

Part of the study was conducted in the Aelmoeseneie forest (50°58.5’N, 3°48’E, 16 m a.s.l.), which is a mixed deciduous forest located near Ghent in northern Belgium, approximately 60 km from the North Sea. Climate variables are the same as in Chapter 2-4 (see 2.3.1). The region of Ghent is characterized by high atmospheric N and sulphur (S) deposition. In 2002-2006, average annual throughfall deposition fluxes to the forest stand amounted to 24 kg N ha^{-1} yr^{-1} and 20 kg S ha^{-1} yr^{-1} (Verstraeten et al. 2008).

The studied tree was located in a stand dominated by pedunculate oak (*Quercus robur* L.) and European beech. Mean stand basal area was 30 m^2 ha^{-1} and mean tree height was 25 m in 2007 (Verstraeten et al. 2008) and LAI was 5.52 in 1996 (Mussche et al. 2001). The stand is located on a poorly drained sandy loam soil containing a partly degraded B horizon and a clay substrate at 60 cm depth (Haplic Glossudalf) (Roskams et al. 1997). The studied beech tree was 29 m high with a diameter at breast height (dbh) of 64 cm and standing next to a measuring tower. Crown length and width were 21 and 13.42 m, respectively. The tree was foliated from 15 April 2009 until 19 November 2009 and from 9 May 2010 until 10 November 2010. The leafed and leafless period will subsequently be referred to as the growing and the dormant season to use the same terminology as for spruce. Within the growing season, three phenological periods were visually determined, i.e. leaf development, fully leafed and leaf senescence period.

The second part of the study was performed in an even-aged Norway spruce stand (74 years old at the study time) at Klosterhede Plantation (56°29’N, 8°24’E, 27 m a.s.l.) near Lemvig in the western part of Jutland, Denmark. The annual precipitation and temperature at the time of the study were 860 mm and 8.3°C, respectively. The mean total throughfall deposition (1988-1992) was approximately 23 kg N ha^{-1} yr^{-1} and 33 kg S ha^{-1} yr^{-1}. The forest is located 15 km from the North Sea coast and dominated by relatively strong, westerly winds, which resulted in high deposition of sea salt. The soil type is a Typic Haplorthod (podzol) developed on the sandy, nutrient poor deposits of an outwash plain (Hansen et al. 1995). The mean leaf area index, tree height and dbh were 4.8 m^2 m^{-2}, 20 m and 21.9 cm, respectively (Beier et al. 1993a).

The study was conducted in two spruce trees of 18 m height with a dbh of 23 cm. Crown length and width were 9 and 3.6 m for tree A and 8 and 3.0 m for tree B, respectively. Since Na^+ in throughfall water mainly originates from dry deposition (Ulrich 1983) and K^+ from the leaching of physiologically active canopy components (Tukey 1970), the K^+ to Na^+ ratio in the throughfall water can indicate the start and end of the growing period in coniferous stands. For the spruce stand the growing period derived in this way was from 8 May to 18 November 1993.

5.3.2 Data collection

5.3.2.1 Water fluxes and plant material

In Belgium, throughfall in the beech canopy could be measured using a 35-m high open tower with platforms at every 7 m. At 1.5, 15, and 22 m each, six funnels with a collecting area of 0.0460 m^2 were installed on two rails attached to the measuring tower

(Fig. 5.1). Two additional funnels were installed in the top of the canopy at 28 m. Above the canopy, at 36 m height, two similar funnels collected bulk precipitation. Placement of the rails was mainly constrained by the branch structure. We did not organize the funnels according to a spatial pattern around the stem, since the spatial variability of throughfall deposition under beech is mainly determined by canopy structure (Staelens et al. 2006), rather than by the distance from the stem. Care was taken not to place funnels below each other to avoid the influence of an upper funnel on the water volume of a lower one. In each funnel, a nylon 1-mm wire mesh was placed to minimize sample contamination by organic material. The throughfall water was led through black polyethylene tubes to the nearest platform where it was stored in polyethylene 5-L bottles in a wooden box to protect the samples from heat and sunlight. Stemflow was collected through a spiral gauge around the stem at 2 m above ground level and was led into three connected 200-L barrels at the ground level. All samples were collected every fortnight from 22 April 2009 to 21 May 2010. Funnels, wire meshes and bottles were replaced by equipment rinsed by demineralised water. Additionally, on several heights along the beech canopy (3.5, 8, 10.5, 15, 17.5, 22, 24.5 and 28 m), four twigs per height were collected on 9 June 2011 for the analysis of leaf N content.

In Denmark, data collection took place during 1992 and 1993 and has been thoroughly described by Hansen (1995, 1996). Horizontal girders were mounted at four levels (9.5, 11, 13 and 15 m above the ground where the lowest level was beneath the canopies) of an 18-m high tower located in between the two study trees (Fig. 5.1). The girders pointed towards the trunks of two trees (A and B). The girders were displaced in relationship to each other in order to avoid influence of a layer on its underlying layer. On the horizontal girders, 25 polyethylene funnels were installed at four distances from the trunks (0.1, 0.5, 0.9 and 1.3 m). Here, the funnels were installed in a specific spatial pattern (Fig. 5.1) since earlier research pointed to typical spatial variation in spruce forests (Beier et al. 1993b). The funnels had collecting areas of 0.0200 m^2. Two filters (mesh sizes of 1 and 0.3 mm) were placed in the funnels. One additional funnel was mounted in the top of the tower above the forest to collect bulk precipitation. The throughfall water was led through black polyethylene tubing to the ground where it was sampled in polyethylene bottles, stored in thermo-boxes which were placed in soil pits to protect the samples from heat and sunlight. From January 1992 to January 1994 sampling was performed approximately every fortnight, or when enough water was available for chemical analyses. The data analysis in Hansen (1996) was restricted to 270 days in 1993, so the annual fluxes are presented here for the first time. The throughfall data of 1993 were used, since these had the least missing data.

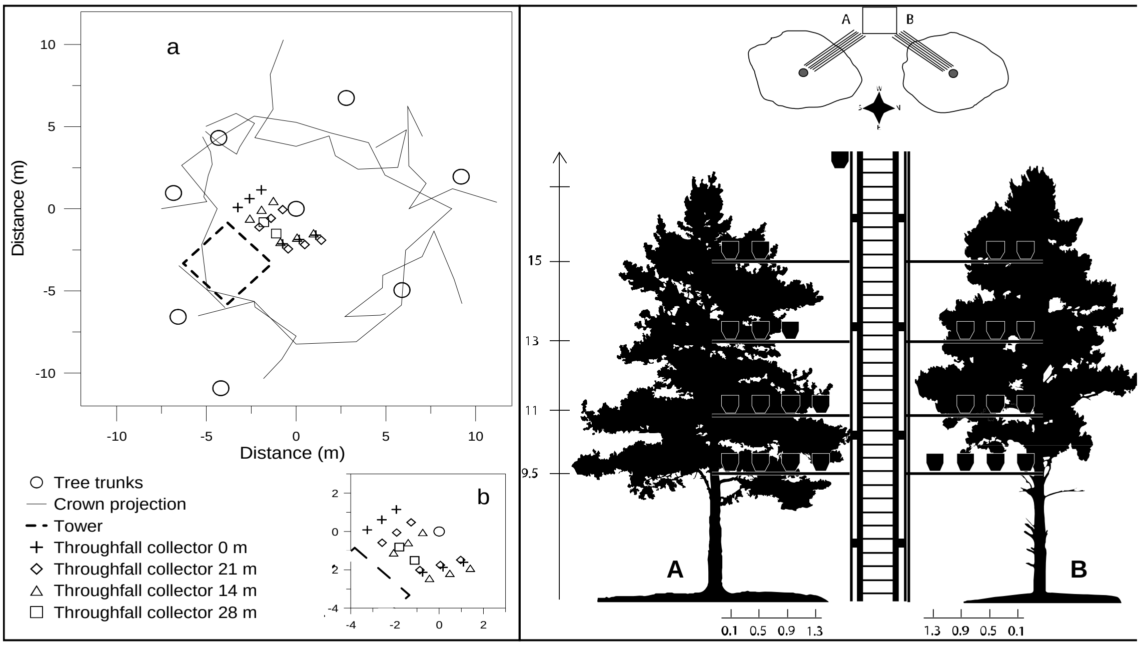

Fig. 5.1 Orthogonal overview (a) and detail (b) of the experimental set-up in the beech canopy (left side) and orthogonal view (top figure) and side-view of the set-up in the spruce canopy (right side; copyright iStockphoto & DeGarmo Creative)

5.3.2.2 *Chemical analysis*

Immediately after collection, sample volumes were determined and subsamples were stored at 4°C for chemical analysis. Water samples for the Belgian site and H^+, NO_3^-, SO_4^{2-}, PO_4^{3-}, and Cl^- for the Danish site were analysed as described in Section 4.3.2.3. NH_4^+ concentrations for the Danish site were assessed by continuous flow colorimetry on an autoanalyser, while K^+, Ca^{2+}, Mg^{2+}, and Na^+ were determined by flame emission spectrophotometry. Leaf material from the beech tree was dried at 70°C for 24 h, grinded, and analysed for total N content after total destruction (modified Kjeldahl method).

The quality of the analysed water samples was validated by checking the ion balance, the Na^+ to Cl^- ratio and by comparing the measured electric conductivity with a calculated electric conductivity. Next to this, samples of which the PO_4^{3-} concentration was 50 % higher than those of the surrounding collectors were excluded from further analysis since contamination by bird droppings was expected then. Missing data (e.g. wind-blown funnels) or rejected samples (3.8 %) for the Belgian site were estimated by the average of four products between the value of a nearby collector and the ratio of the missing collector to this nearby collector in a previous or subsequent collection time. For the Danish site, missing data (13.7 %) were estimated by regression analysis of throughfall water amount or ion concentration between the missing collector and the collector with which it had the highest Pearson correlation.

5.3.3 Data analysis

5.3.3.1 Ion fluxes

Biweekly bulk deposition (BD; $\text{mmol}_c\ \text{m}^{-2}$) above the canopy and throughfall deposition (TF; $\text{mmol}_c\ \text{m}^{-2}$) and stemflow deposition (SF, $\text{mmol}_c\ \text{m}^{-2}$) for each collector within the canopy were calculated by multiplying the ion concentration with the collected volume divided by the collecting area. Stemflow volume of the whole beech tree was calculated by multiplying the collected volume with the surface area of the orthogonal canopy projection, determined to be 149 m^2. Weak acids (WA; $\text{mmol}_c\ \text{m}^{-2}$) in throughfall and bulk deposition were estimated from the difference between the sum of cations and the sum of anions. Net throughfall fluxes (NTF) were calculated as the difference between throughfall and bulk deposition. The contribution of SF to TF+SF for the beech canopy was low (see 5.4.1), and therefore not included in the NTF calculations.

5.3.3.2 Canopy budget modelling

The NTF of an ion is defined as the difference between TF and BD, and equals the sum of dry deposition (DD) and canopy exchange (CE; $\text{mmol}_c\ \text{m}^{-2}$).

$$NTF = TF - BD = DD + CE \tag{5.1}$$

A distinction between the importance of these processes for the growing and the dormant season is made by means of a modified canopy budget model developed by Draaijers and Erisman (1995). Various approaches of this model have been reported, of which an overview is given in Chapter 7. Here, two particular approaches were chosen that are justified in the following Section and in Section 5.5.5. Positive CE represents canopy leaching (CL) and negative canopy uptake (CU). However, when we refer to CL or CU specifically, positive values are used. Furthermore, the calculated CU and CL gives no information on the actual sinks and processes involved (Chapter 4) and therefore the term "canopy retention" would be more appropriate. However, to be in line with literature, the terms CU and CL are kept in this Chapter 5-7.

In the canopy budget model, a filtering approach is used to estimate the DD of base cations (K^+, Ca^{2+} and Mg^{2+}) (Ulrich 1983). In this approach, aerosols containing base cations are assumed to be deposited with equal efficiency to the forest canopy as particles containing an inert tracer ion. This tracer ion is assumed not to be subject to canopy leaching. Na^+ is considered most suitable as tracer ion but also Cl^- and SO_4^{2-} have been used in the past, although they may overestimate DD of base cations if significant gaseous deposition of HCl or SO_2 occurs (Staelens et al. 2008). Because of the assumed negligible canopy exchange, the DD of Na^+, Cl^- and SO_4^{2-} equals their NTF. The DD of a base cation X (DD_X) is then calculated as:

$$DD_X = BD_X \cdot \frac{NTF_{tracer\ ion}}{BD_{tracer\ ion}} \qquad (5.2)$$

Sodium was used as a tracer ion for the beech canopy; however, SO_4^{2-} was chosen for the spruce canopy for the following reasons. Firstly, NTF of Na^+ under the spruce canopy was found to be rather high (see 5.4.2) due to the close proximity of the sea (15 km). It was shown by Ten Harkel (1997) that the diameter of Na^+ particles close to the sea is significantly larger (20 µm) than for base cations in general (1.6-7.7 µm; Ruijgrok et al. 1997) and as such are deposited more easily (Gallagher et al. 1999). Therefore, Na^+ was not a reliable tracer to estimate DD to the spruce canopies. Secondly, chloride depletion of NaCl particles with the formation of gaseous HCl is a significant process in coastal areas (Ten Harkel 1997), excluding also Cl^- as a suitable tracer ion. Thirdly, due the close proximity to the sea SO_4^{2-} aerosols contribute more to total S deposition (Lindberg and Lovett 1992), hereby reducing the bias caused by SO_2 deposition when SO_4^2 is used as a tracer. Canopy leaching of K^+, Ca^{2+} and Mg^{2+} (CL_X) was obtained from the difference between NTF_X and DD_X. Canopy uptake of NH_4^+ (CU_{NH4}) in the beech canopy was estimated from the CL of base cations (CL_{BC}) corrected for the simultaneous CL of weak acids (CL_{WA}).

$$CU_{NH_4^+} = CL_{BC} - CL_{WA} \qquad (5.3)$$

The CL_{WA} is calculated by subtracting the DD of WA from NTF of WA. The DD of WA is assumed to be equal to their BD, multiplied with the estimated percentage LAI above each canopy level. In the spruce canopy, the calculated CL_{WA} was found to be negative and CU_{NH4} was set equal to the CL_{BC}. Several field studies (Stachurski and Zimka 2002; Dail et al. 2009) and laboratory studies (Bowden et al. 1989; Chapter 2) have demonstrated that forest canopies can incorporate NO_3^-, but that preferentially NH_4^+ is retained. De Vries et al. (2001) suggested to calculate the CU of NH_4^+ and NO_3^- based on the TF of NH_4^+ and NO_3^-, using an efficiency factor of NH_4^+ vs NO_3^- uptake (xNH_4) with a proposed value of six:

$$CU_{\left(NH_4^+ + NO_3^-\right)} = \frac{xNH_4^+ . TF_{NH_4^+} + TF_{NO_3^-}}{xNH_4^+ . TF_{NH_4^+}} \tag{5.4}$$

To maintain the charge balance, CU of H^+ was set equal to the uptake of NO_3^- (Stachurski and Zimka 2002; Staelens et al. 2008). Dry deposition of NH_4^+, NO_3^- and H^+ were calculated as the difference between NTF and CE.

5.3.3.3 Statistical analysis

For each tree species the canopy level, precipitation volume and sampling time were related to the biweekly TF and NTF by means of a generalized additive mixed model. An additive model applies a smoothing method to model a non-linear relationship between the response variable and one or more explanatory variables, in this case the sampling time and the precipitation volume. This approach was chosen over an ANOVA repeated measures analysis because of the non-linear nature of throughfall data over time. Furthermore, in a repeated measures ANOVA analysis we would need to pool the data points per month in order to have sufficient power (Von Ende 2001), thereby removing relevant information on the bi-weekly variation. The factor “canopy level” was added to the model as a parametric term. Since the distance to the trunk affects the TF in Norway spruce stands (Beier et al. 1993b), this variable was also added to the spruce model. The use of a mixed model allowed the inclusion of the random factor “collector” for beech and “tree” for spruce in the model (since by the interaction of canopy level and distance to the trunk all collectors within one spruce tree were already captured). Data were log transformed to obtain homogeneity of variance for the model residuals. We used the *mgcv* package (Wood 2006) in R version 2.11.1 statistical software (R Development Core Team 2010), in which splines are used to fit a smoothing curve through the data without defining the functional form and the amount of smoothing in advance. The explanatory variable is divided in several segments, where a polynomial function is fit to the data. Where the different segments connect, a regression spline ensures that the different polynomial functions connect smoothly by imposing criteria on the function derivatives. We used a thin plate regression spline, which involves high order derivates (Wood 2003). The optimal amount of smoothing, defined by the number of segments, is determined automatically by minimizing the generalised cross-validation criterion. A Bonferroni post-hoc test was performed to assess the differences between the canopy levels.

We assessed the influence of leaf phenology (i.e., leaf development, fully leafed, leaf senescence and leafless periods) on the average NTF per canopy level in the beech tree by applying a linear mixed model (*nlme* package; Pinheiro et al. 2011) with phenological stage and canopy level as fixed effects and collector as random effect to account for the repeated observations on the same collector. Next, a similar linear mixed model was used both for the beech and spruce data to assess the effect of season (growing vs dormant), canopy level and distance to the trunk (spruce only) on the calculated DD and CE (*multcomp* package; Hothorn et al. 2008). Prior to that, DD and CE at the highest canopy level were tested to be significantly different from zero with a t test. Differences in leaf N content between canopy levels were assessed by means of a one-way ANOVA. The significance of differences between the levels of each factor was tested by means of Tukey's honestly significant difference test.

5.4 Results

5.4.1 Bulk deposition, throughfall deposition and leaf N content

The annual precipitation to the beech and the spruce canopy was 647 and 693 mm, respectively, of which 40 and 31 %, respectively, was evaporated after interception by the canopy (Table 5.1). The stemflow volume for beech amounted to 2 % of the total precipitation volume. The highest interception loss occurred in the top canopy layer and amounted to 22 % for beech (between 28 and 22 m height) and 28 % for spruce (between bulk deposition and 15 m) (Table 5.1). Although this trend was the same for the growing and the dormant season, the total interception for beech was higher in the growing period (50 %) than in the dormant period (26 %). For spruce the total interception was the same in both seasons (data not shown).

Table 5.1 Annual bulk deposition (BD), throughfall (TF) and stemflow (SF) (in mm yr^{-1} for water volume and mmol$_c$ m^{-2} yr^{-1} for ion fluxes) and TF/BD ratio at different heights (m) in the canopy of one beech and two spruce trees

	BD	TF at canopy height (m)				SF	TF/BD at canopy height (m)				SF/ (TF+SF)
Beech		28	22	15	1.5		28	22	15	1.5	
Volume	647	590	446	351	401	14.4	0.91	0.69	0.54	0.62	0.03
H^+	1.20	0.31	0.34	0.19	0.24	0.20	0.26	0.28	0.16	0.20	0.45
NH_4^+-N	42.2	44.7	57.7	59.0	72.3	3.15	1.06	1.37	1.40	1.71	0.04
Cl^-	31.1	67.7	72.5	73.9	86.9	2.48	2.18	2.34	2.38	2.80	0.03
NO_3^--N	21.5	35.3	32.2	34.6	34.7	1.61	1.65	1.50	1.61	1.61	0.04
SO_4^{2-}-S	24.3	83.4	75.0	91.2	114	3.06	3.43	3.08	3.74	4.67	0.03
K^+	2.5	27.6	67.7	70.8	94.7	1.96	11.0	27.0	28.2	37.7	0.02
Na^+	25.1	53.3	53.3	52.3	58.1	1.87	2.13	2.13	2.08	2.32	0.03
Ca^{2+}	18.0	40.1	38.9	48.5	63.3	1.11	2.22	2.16	2.69	3.51	0.02
Mg^{2+}	7.1	18.5	20.2	25.2	32.1	0.39	2.60	2.84	3.54	4.51	0.01
WA[b]	17.8	3.76	51.8	50.7	77.4	1.48	0.21	2.90	2.84	4.34	0.02
Spruce[a]		15	13	11	9.5		15	13	11	9.5	
Volume	693	496	557	488	476	n.a.	0.72	0.80	0.70	0.69	n.a.
H^+	26.7	11.9	8.70	7.33	8.26	n.a.	0.44	0.33	0.27	0.31	n.a.
NH_4^+-N	21.3	24.0	29.0	32.2	35.9	n.a.	1.13	1.37	1.52	1.69	n.a.
Cl^-	137	379	486	522	530	n.a.	2.76	3.54	3.80	3.86	n.a.
NO_3^--N	36.8	42.4	42.1	47.3	61.4	n.a.	1.15	1.15	1.29	1.67	n.a.
SO_4^{2-}-S	98.5	116	158	163	184	n.a.	1.17	1.61	1.66	1.86	n.a.
K^+	4.03	46.9	55.8	68.4	71.6	n.a.	11.7	13.9	17.0	17.8	n.a.
Na^+	88.5	308	403	448	440	n.a.	3.48	4.55	5.06	4.97	n.a.
Ca^{2+}	7.02	24.3	31.3	37.5	41.7	n.a.	3.46	4.45	5.34	5.93	n.a.
Mg^{2+}	23.7	67.2	91.8	99.7	104	n.a.	2.84	3.88	4.21	4.40	n.a.
WA[b]	0	0	0	0	0	n.a.	0	0	0	0	n.a.

n.a.: not available

[a] Average of the two trees

[b] WA: Weak Acids, negative values are set to zero

The bulk deposition (BD) of H^+, inorganic N (NH_4^+ + NO_3^-) and SO_4^{2-}-S in Belgium (beech) amounted to 1.20, 63.7 and 24.3 mmol$_c$ m^{-2} yr^{-1}, respectively, and to 26.7, 57.9 and 98.5 mmol$_c$ m^{-2} yr^{-1} in Denmark (spruce), respectively. The throughfall (TF) of all ions except H^+ and NO_3^- (beech only) increased with canopy depth for both species. Consequently, the TF/BD ratio was larger than one for all ions except H^+ and generally increased with canopy depth, except for NO_3^- in the beech canopy where it remained constant (Table 5.1). For H^+, the TF/BD ratio in the top canopy layer was 0.26 and 0.44 for beech and spruce, respectively, and decreased slightly to 0.20 and 0.31 at the ground level. The fraction of stemflow deposition (SF) to TF of the dissolved ions was only small (< 4 %) beneath beech, except for H^+ (45 %) (Table 5.1). Leaf N content in the beech canopy increased significantly ($p < 0.001$) from 2.25 % at 28 m height to 2.67 % at 3.5 m (Fig. 5.2).

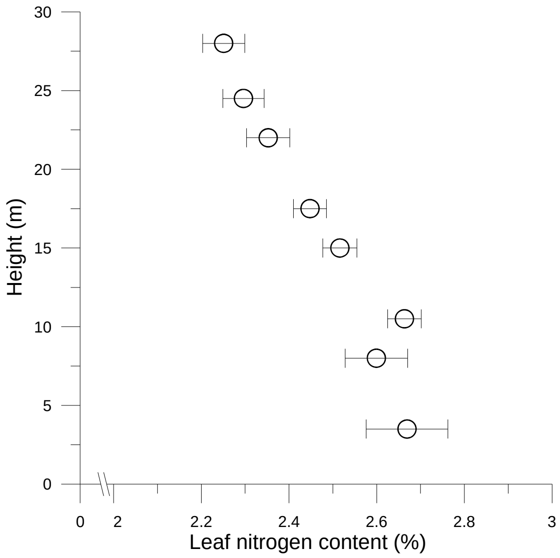

Fig. 5.2 Leaf nitrogen content (%) at various heights in the beech canopy. Error bars represent standard error (n = 4)

Throughfall fluxes were significantly ($p < 0.048$) influenced by sampling time and precipitation volume for all ions for both beech and spruce, and by canopy level for all ions except NO_3^- in the beech canopy. The interaction between time and canopy level was not significant ($p > 0.07$), indicating that TF at all canopy levels followed a similar pattern over time. Consequently, parallel smoothing curves were estimated for each canopy level, but with a different intercept. For beech (Fig. 5.3), this intercept was found to differ significantly between TF at 22 and 1.5 m for all ions, and additionally between 22 and 15 m for H^+ and Mg^{2+} and between 15 and 1.5 m for SO_4^{2-}, K^+ and Mg^{2+}. Only for the TF of K^+, the intercepts differed significantly between 28 and 22 m in the beech canopy. The temporal pattern indicates an increased TF during leaf senescence and the leafless period for NH_4^+, H^+, Cl^-, SO_4^{2-} and Na^+, while higher values were found during leaf development for K^+ and WA and during leaf senescence for K^+, WA, Ca^{2+} and Mg^{2+}. The TF of NO_3^- was lower during leaf development and approximately constant during the rest of the year.

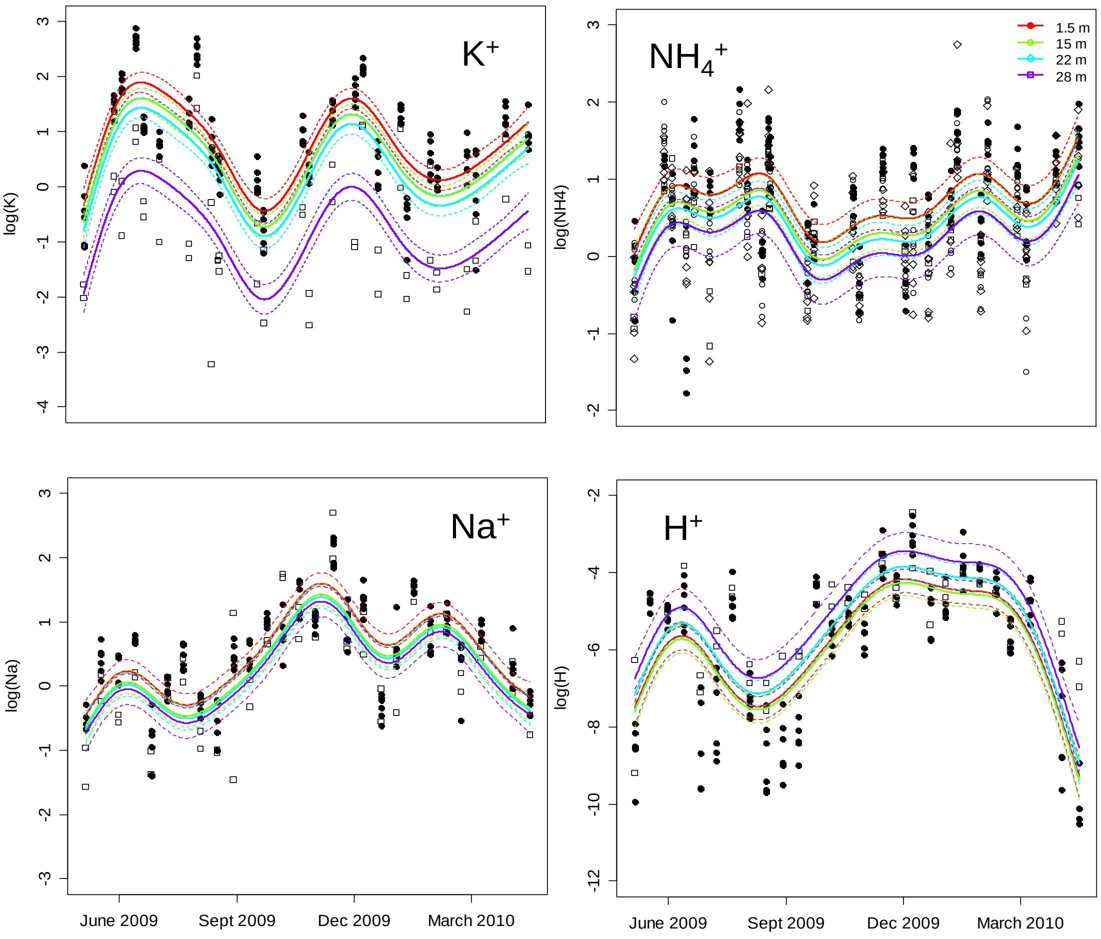

Fig. 5.3 Measured and modelled throughfall deposition (TF, log (mmol$_c$ m^{-2} 2 weeks^{-1})) of K^+, NH_4^+, Na^+ and H^+ within the studied beech canopy at the ground level (1.5 m) and at different canopy levels (15, 22 and 28 m). The non-linear relationship between the log transformed TF response variable and the sampling time was modelled using a generalized additive mixed model

Throughfall increased significantly between 15 and 13 m for SO_4^{2-}, K^+, Na^+, Ca^{2+} and Mg^{2+} and between 13 and 11 m for K^+, Ca^{2+} and Mg^{2+} in the spruce canopies (Fig. 5.4). An increase in TF between 11 and 9.5 m was found for K^+, Ca^{2+}, SO_4^{2-}, Na^+ and NO_3^-. Furthermore, TF within the spruce canopies was influenced by the distance to the trunk, but the interaction between distance to the trunk and canopy level was only significant ($p < 0.006$) for the throughfall water volume, H^+, NH_4^+ and Cl^- fluxes. The water volume decreased with canopy depth, but increased with increasing distance to the trunk. The slope of the relationship between the water volume and distance to the trunk increased with canopy level. In contrast, TF of NH_4^+ and Cl^- increased with canopy depth and decreased with increasing distance to the trunk, but here also the slope was higher for the upper canopy levels. Throughfall deposition of H^+ increased with canopy depth at 0.1 m from the spruce trunk, but decreased with canopy depth further away from the trunk. In general, higher TF was found during the dormant season in January 1994 for Na^+, H^+, Ca^{2+}, Mg^{2+}, SO_4^{2-} and Cl^-, while for NH_4^+ an increased TF was observed at the beginning of the growing season and for K^+ both at the beginning and at the end of the growing season.

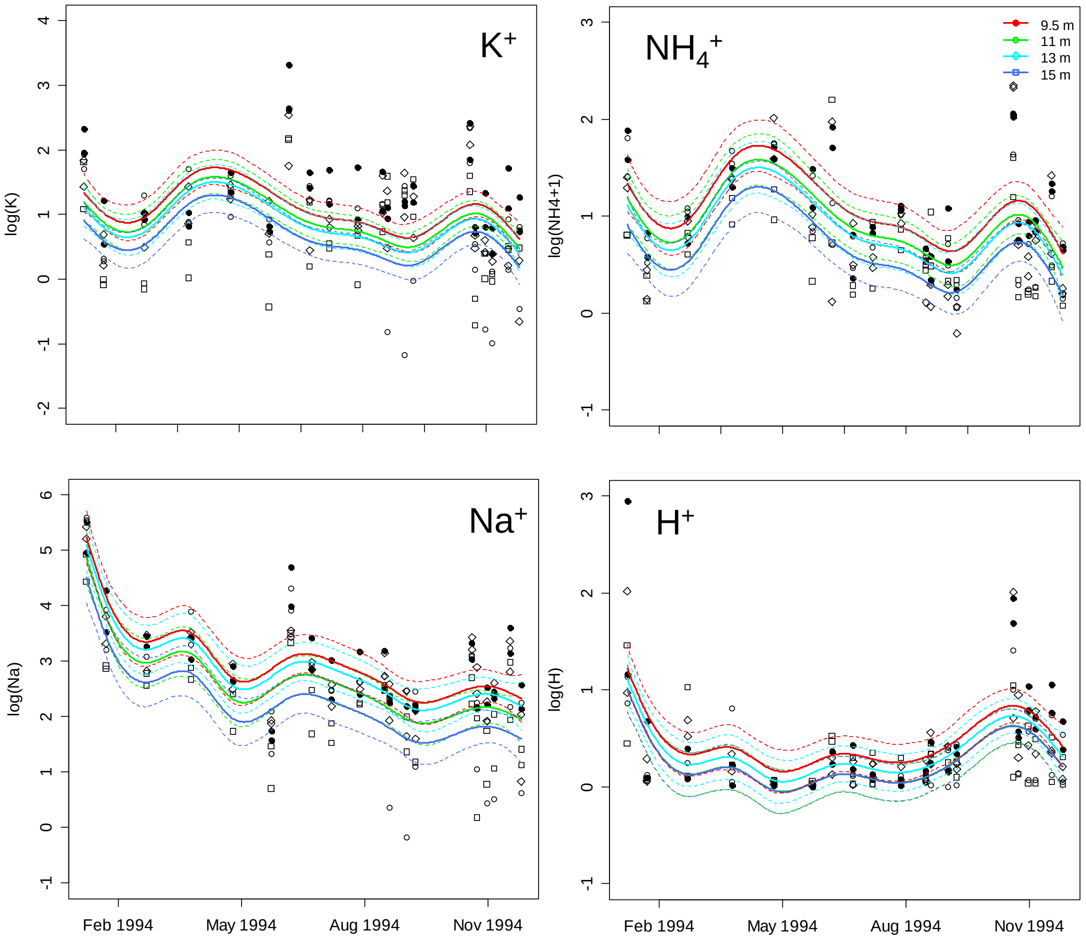

Fig. 5.4 Measured and modelled throughfall deposition (TF, log (mmol$_c$ m^{-2} 2 weeks^{-1})) of K^+, NH_4^+, Na^+ and H^+ within the studied spruce canopy at 0.1 m from the trunk and at different canopy levels (9.5, 11, 13, and 15 m). The non-linear relationship between the log transformed TF response variable and the sampling time was modelled using a generalized additive mixed model

5.4.2 Net throughfall

Net throughfall (NTF) in the beech canopy was significantly influenced by time and precipitation volume for all ions and influenced by canopy level for almost all ions.

Average NTF increased significantly towards the ground level (1.5 m) for all ions other than H^+, NO_3^- and Na^+, with a significant increase between 28 and 22 m for K^+ (Fig. 5.5), between 22 and 15 m for Mg^{2+} and between 15 and 1.5 m for SO_4^{2-}, K^+, Ca^{2+} and Mg^{2+}. The difference in NTF was significant for NH_4^+ between 28 and 1.5 m and for Cl^- between 22 and 1.5 m. Net throughfall of H^+, NO_3^- and Na^+ did not change significantly after passing the canopy at 28 m.

For spruce, NTF of all ions differed according to the sampling time, precipitation volume, canopy level and distance to the trunk (Table 5.2), but only for the volume, H^+ and Cl^- deposition the interaction between canopy level and distance to the trunk was significant ($p < 0.006$). The NTF patterns in the canopy were the same as for TF for all ions except NH_4^+. Instead, the NTF of NH_4^+ increased significantly between 15 and 13 m and between 13 and 9.5 m.

Average NTF per canopy level of the beech tree (Fig. 5.5) differed significantly between the phenological stages and the different canopy levels for all variables, except for the volume and H^+ where only the effect of phenological stage was significant. The interaction between both factors was also significant for all ions except SO_4^{2-}. The NTF was generally highest during the leaf senescence period, intermediate during the leafless period and lowest for the leaf development and fully leafed periods. This pattern was reversed for H^+ while NTF of NH_4^+ was highest during the leafless period (Fig. 5.5).

Net throughfall of NO_3^-in the upper canopy was positive and significantly different from the negative values in the lower canopy during leaf development. Since no differences in NTF volume were observed between the different canopy levels, this was caused by a decrease in NO_3^- concentration between 28 and 22 m (data not shown). Next to this, a negative NTF of NH_4^+ was detected in the upper canopy during the period of leaf development and the fully leafed period (Fig. 5.5).

Table 5.2 Average biweekly net throughfall volume (mm 2 weeks^{-1}) and net throughfall deposition (mmol$_c$ m^{-2} 2 weeks $^{-1}$) at different heights (H, m) and distances to the trunk in the canopy of one beech and two spruce trees

	D	H	Volume	H^+	NH_4^+-N	Cl^-	NO_3^--N	SO_4^{2-}-S	K^+	Na^+	Ca^{2+}	Mg^{2+}
Beech		28	-2.11^{b}	-0.03	0.1^{a}	1.36^{a}	0.52	2.19^{a}	0.83^{a}	1.04	0.81^{a}	0.42^{a}
		22	-7.46^{a}	-0.03	0.58^{ab}	1.54^{a}	0.4	1.88^{a}	2.42^{b}	1.04	0.77^{a}	0.49^{a}
		15	-10.97^{a}	-0.03	0.62^{ab}	1.59^{ab}	0.49	2.48^{a}	2.53^{b}	1.01	1.13^{a}	0.67^{b}
		1.5	-9.12^{a}	-0.03	1.12^{b}	2.07^{b}	0.49	3.31^{b}	3.42^{c}	1.22	1.67^{b}	0.93^{c}
Spruce$^{\$}$	0.1	15	-14.66	-1.04	0.02^{a}	11.68^{a}	-0.02^{a}	0.41^{a}	2.39^{a}	10.74^{a}	0.96^{a}	2.09^{a}
		13	-13.33	-0.94	0.76^{b}	22.85^{b}	0.63^{a}	3.90^{b}	3.05^{b}	22.19^{b}	1.65^{b}	4.86^{b}
		11	-17.04	-1.04	0.73^{b}	25.46^{bc}	0.55^{a}	3.19^{b}	3.20^{c}	24.98^{b}	1.77^{c}	4.70^{c}
		9.5	-14.04	-0.73	1.20^{c}	38.96^{c}	0.61^{b}	6.65^{c}	5.54^{d}	33.32^{c}	2.86^{d}	7.41^{d}
	0.5	15	-4.99^{b}	-0.44^{b}	0.25	12.48^{a}	0.59	1.31	1.9	11.23	0.77	2.27
		13	-5.99^{ab}	-0.92^{a}	0.42	18.29^{b}	0.2	2.95	2.9	15.41	1.21	3.3
		11	-9.52^{ab}	-0.89^{a}	1.14	24.08^{c}	1.52	5.31	4.46	22.66	2.2	5.19
		9.5	-16.90^{a}	-0.88^{a}	0.46	22.20^{bc}	-0.02	3.41	3.62	20.69	1.96	4.75
	0.9	13	-1.07^{b}	-0.84^{b}	-0.01	11.12^{a}	-0.03	2.09	1.82	9.58	0.77	2.06
		11	-7.40^{a}	-1.03^{a}	0.01	13.51^{a}	-0.09	2.12	2.63	11.51	1.05	2.59
		9.5	-8.71^{a}	-0.82^{ab}	1.48	20.24^{b}	0.76	5.84	3.94	21.65	2.42	5.08
	1.3	11	-3.79^{b}	-0.88	0.08	8.61	-0.25	1.41	1.93	7.54	0.63	1.66
		9.5	-7.25^{a}	-1.02	0.24	11.63	0.53	2.14	2.17	9.95	0.91	2.23

$^{\$}$Average of the two trees

Small letters within a column and, in case of spruce, within one distance to the trunk indicate significant differences ($p < 0.05$) between the different canopy levels for one species. If no interaction was found with distance to the trunk, significances are only given for 0.1 m to the trunk.

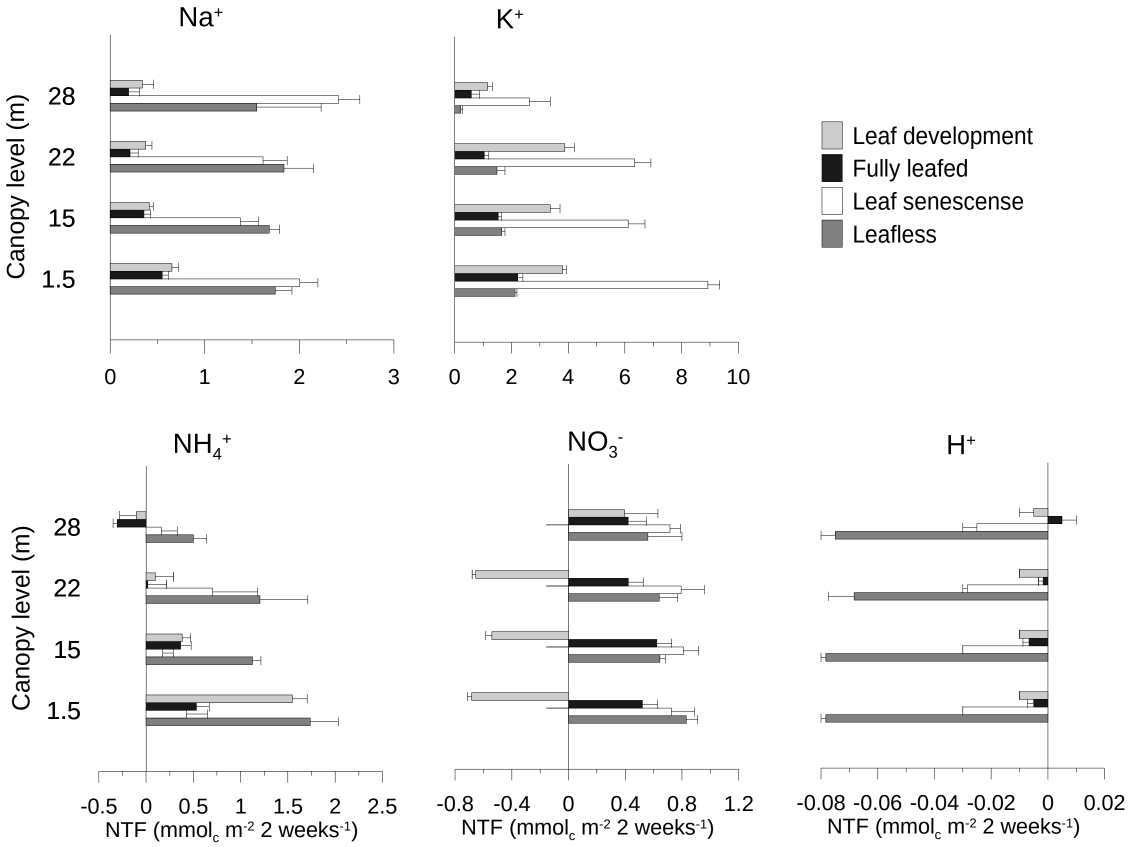

Fig. 5.5 Net throughfall deposition (NTF) with standard error of Na^+, K^+, NH_4^+, NO_3^- and H^+ in the beech tree as a function of canopy height for the four phenological stages considered. Error bars represent standard error

5.4.3 Dry deposition and canopy exchange

Annual dry deposition (DD) and canopy exchange (CE) at each canopy level are presented in Table 5.3. Net throughfall fluxes of K^+, Mg^{2+}, $Ca^{2+}$and H^+ were mainly determined by CE processes, while for the other ions DD was found to be more important. The DD to the beech canopy was higher during the growing season compared to the dormant season for K^+, Ca^{2+}, and H^+, while this was reversed for Na^+ (Table 5.4; Fig. 5.6). Dry deposition of K^+, Mg^{2+}, Ca^{2+}, Cl^- and NH_4^+ was significantly higher at 1.5 m than at 15 m and for NH_4^+ also higher at 15 m than at 22 m. In contrast, CE of all elements was significantly ($p < 0.05$) affected by canopy level and season (except for NH_4^+; $p = 0.06$ for season). Canopy leaching (CL) was significantly different between 28 and 22 m and between 15 and 1.5 m for K^+, Ca^{2+} and weak acids and between 22 and 15 m for Ca^{2+} and Mg^{2+}. Significant canopy uptake (CU) was detected for NH_4^+, NO_3^- and

H^+ between 22 and 15 m. At 22 m, only the calculated CE of Mg^{2+} in the dormant season and of Ca^{2+} in the growing season was not significantly different from zero.

Table 5.3 Annual bulk deposition (BD) and estimated dry deposition (DD) and canopy exchange (CE) ($mmol_c\ m^{-2}\ yr^{-1}$) at different heights (m) in the canopy of one beech and two spruce trees

	BD	DD at canopy height (m)				CE at canopy height (m)			
Beech		28	22	15	1.5	28	22	15	1.5
H^+	1.20	4.37	3.52	5.79	5.51	-5.26	-4.39	-6.79	-6.46
NH_4^+-N	42.2	40.1	58.1	85.3	108	-37.7	-42.6	-68.6	-77.6
Cl^-	31.1	36.7	41.5	42.9	55.9	0	0	0	0
NO_3^--N	21.5	19.1	15.1	19.9	19.7	-5.26	-4.39	-6.79	-6.46
SO_4^{2-}-S	24.3	59.1	50.6	66.8	89.2	0	0	0	0
K^+	2.50	2.79	2.48	2.48	3.34	19.6	62.7	65.9	88.8
Na^+	25.1	28.3	28.2	27.2	33.1	0	0	0	0
Ca^{2+}	18.0	20.1	18.0	18.0	24.0	1.98	2.87	12.5	20.9
Mg^{2+}	7.10	7.99	7.70	7.51	9.41	3.37	5.36	10.6	15.5
WA[b]	17.8	0.96	11.6	17.4	19.3	-12.7	28.3	20.3	47.7
Spruce[a]	WD	15	13	11	9.5	15	13	11	9.5
H^+	26.7	11.1	6.79	14.4	18.6	-26.0	-25.1	-33.8	-37.1
NH_4^+-N	21.3	92.8	122	146	151	-90.0	-113	-135	-137
Cl^-	137	242	360	385	392	0	0	0	0
NO_3^--N	36.8	31.6	29.4	44.4	61.7	-26.0	-25.1	-33.8	-37.1
SO_4^{2-}-S	98.5	24.2	59.0	65.3	85.1	0	0	0	0
K^+	4.03	1.39	3.64	3.68	4.72	41.5	50.5	60.7	62.9
Na^+	88.5	220	327	360	352	0	0	0	0
Ca^{2+}	7.02	2.12	5.41	5.64	7.28	15.2	19.8	24.8	27.4
Mg^{2+}	23.7	10.3	27.7	26.8	34.1	33.3	43.1	49.3	46.4
WA	0	0	0	0	0	0	0	0	0

[a] Average of the two trees

[b] WA: Weak Acids, negative values are set to zero

In the spruce canopy, canopy level and season significantly affected the DD fluxes of NH_4^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, Na^+ and SO_4^{2+} (Table 5.4; Fig. 5.7). The DD of NO_3^- was only affected by season. For NH_4^+ and NO_3^- the DD was highest during the growing season, while for the other elements DD was highest in the dormant season. Generally, the highest differences could be found between the upper two canopy levels, except for Na^+, Cl^- and Ca^{2+}, for which a significant increase was also observed between 13 and 9.5 m. However, calculated DD fluxes at 15 m did not differ significantly from zero, except for Na^+ and Cl^-. At 13 m, DD fluxes were significantly different from zero. Only for NH_4^+, Cl^- and Na^+, DD decreased with increasing distance to the trunk.

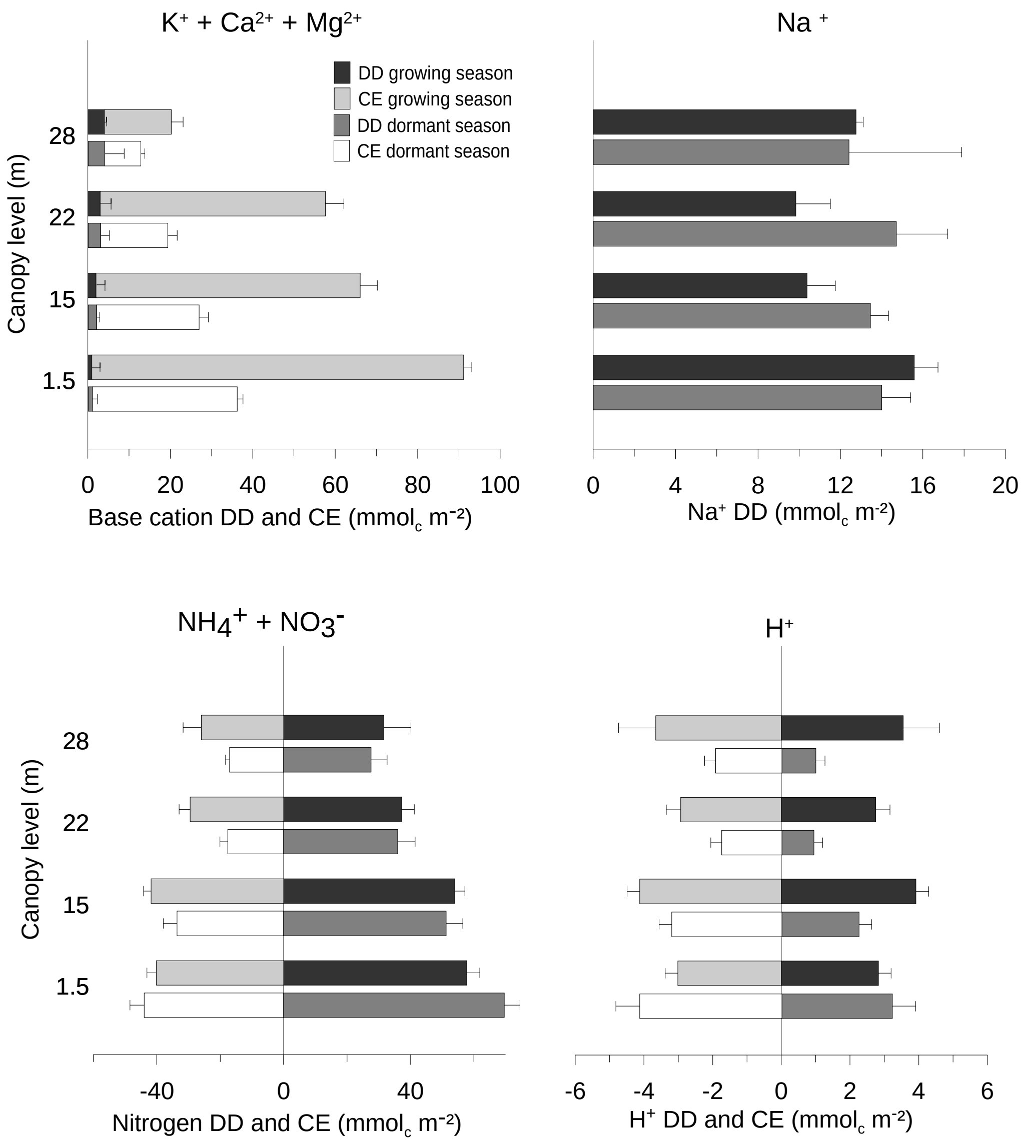

Fig. 5.6 Calculated dry deposition (DD) and canopy exchange (CE) of base cations (K^+ + Ca^{2+} + Mg^{2+}), Na^+, inorganic N (NH_4^+ + NO_3^-) and H^+ within a beech canopy at different canopy levels (1.5, 15, 22 and 28 m). Error bars represent standard error

For spruce, all ions that are assumed to be leached or taken up by the canopy were affected by canopy level and season. Leaching of K^+, Ca^{2+}, Mg^{2+} and uptake of NH_4^+ occurred mainly between 15 and 11 m in the upper and middle canopy and decreased with increasing distance to the trunk. Uptake of H^+ and NO_3^- was detected between 13 and 9.5 m in the middle and lower canopy. Calculated CE fluxes at 15 m differed significantly from zero, except for Mg^{2+} during the dormant season.

5.5 Discussion

5.5.1 Volume

An extensive discussion on the partitioning of throughfall volume within the tree canopies is beyond the scope of this study. However, for beech an increase in volume (50 mm yr^{-1}) could be observed between 15 m and the ground level (1.5 m), which had an impact on the calculated DD of K^+, Mg^{2+}, Ca^{2+}, Cl^- and NH_4^+ between these levels (Fig. 5.6). This increased volume at 1.5 m is possibly due to the diversion of stemflow to throughfall due to a low-hanging branch at 7 m. Stemflow of the beech tree amounted to 2 % of precipitation, which is markedly below the range of 7-20 % reported for beech (Chang and Matzner 2000). Assuming that the stemflow volume actually amounts to 10 % of the incident precipitation, as observed for a similar beech tree in the studied forest stand (Staelens et al. 2007), the annual stemflow volume would increase by 52 mm, which is exactly the observed increase in throughfall volume between 15 and 1.5 m. Since it is expected that the throughfall volume would decrease between 15 and 1.5 m, stemflow likely exceeds this 10 %.

5.5.2 Sodium, chloride and sulphate

Spatial and temporal throughfall variations of Na^+ and Cl^- are only driven by DD of particles and are therefore the easiest to interpret. As hypothesized, vertical patterns of both ions were similar during the growing season and reflect the impact of canopy structure on the DD processes (Erisman and Draaijers 2003). Significant DD was found in the top canopy of both species, and increased only little with canopy depth in the beech tree but significantly in the spruce tree (Fig. 5.6 and 5.7). The beech forest has a rather closed canopy with the majority of the leaves in the upper canopy layer (Samson 2001), while the spruce forest is more open at the top of the canopy due to the triangle-shaped tree contour of spruce causing a non-uniform distribution of the collecting surface, which enhances atmospheric turbulence within the canopy (Wuyts et al. 2008b). The same pattern was observed in the dormant season as in the growing season but with slightly higher fluxes for beech and doubled fluxes for

Table 5.4 P values of the effect of season, canopy level, distance to the trunk and their interactions on the estimated dry deposition (DD) and canopy exchange (CE) of beech and spruce according to linear mixed models

		H^+	NH_4^+-N	Cl^-	NO_3^--N	SO_4^{2-}-S	K^+	Na^+	Ca^{2+}	Mg^{2+}
DD	Season	**<0.001**	0.554	0.3	0.682	**0.04**	**<0.001**	**<0.001**	**<0.001**	0.576
beech	Canopy level	0.05	**<0.001**	**0.005**	0.056	**<0.001**	**0.019**	0.148	**0.02**	0.059
	Season: canopy level	0.064	0.519	0.659	0.057	0.885	0.059	0.247	0.064	0.142
CE	Season	**0.009**	0.059	/	**0.009**	/	**<0.001**	/	**0.025**	**<0.001**
beech	Canopy level	**0.043**	**<0.001**	/	**0.043**	/	**<0.001**	/	**<0.001**	**<0.001**
	Season: canopy level	0.073	0.183	/	0.073	/	**0.009**	/	**0.012**	**0.003**
DD	Season	0.084	**<0.001**	**<0.001**	**0.001**	**0.017**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
spruce	Distance	0.071	**<0.001**	**<0.001**	0.47	0.058	0.053	**<0.001**	**0.046**	0.087
	Canopy level	0.128	**<0.001**	**0.001**	0.097	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.0001**
	Season: canopy level	0.14	0.643	0.465	0.204	0.788	0.112	0.27	0.336	**0.028**
	Distance: canopy level	0.518	0.516	0.343	0.781	0.723	0.776	0.164	0.74	0.45
CE	Season	**<0.001**	**<0.001**	/	**<0.001**	/	**<0.001**	/	0.623	**0.008**
spruce	Distance	0.076	**<0.001**	/	0.076	/	**<0.001**	/	**<0.001**	**<0.001**
	Canopy level	**0.037**	**<0.001**	/	**0.037**	/	**<0.001**	/	**<0.001**	**0.001**
	Season: canopy level	0.116	0.464	/	0.116	/	0.452	/	0.436	0.373
	Distance: canopy level	0.951	0.631	/	0.951	/	0.834	/	0.821	0.132

Significant values are indicated in bold

/: P values not calculated because CE of Cl^-, SO_4^{2-} and Na^+ and DD of WA were assumed to be zero

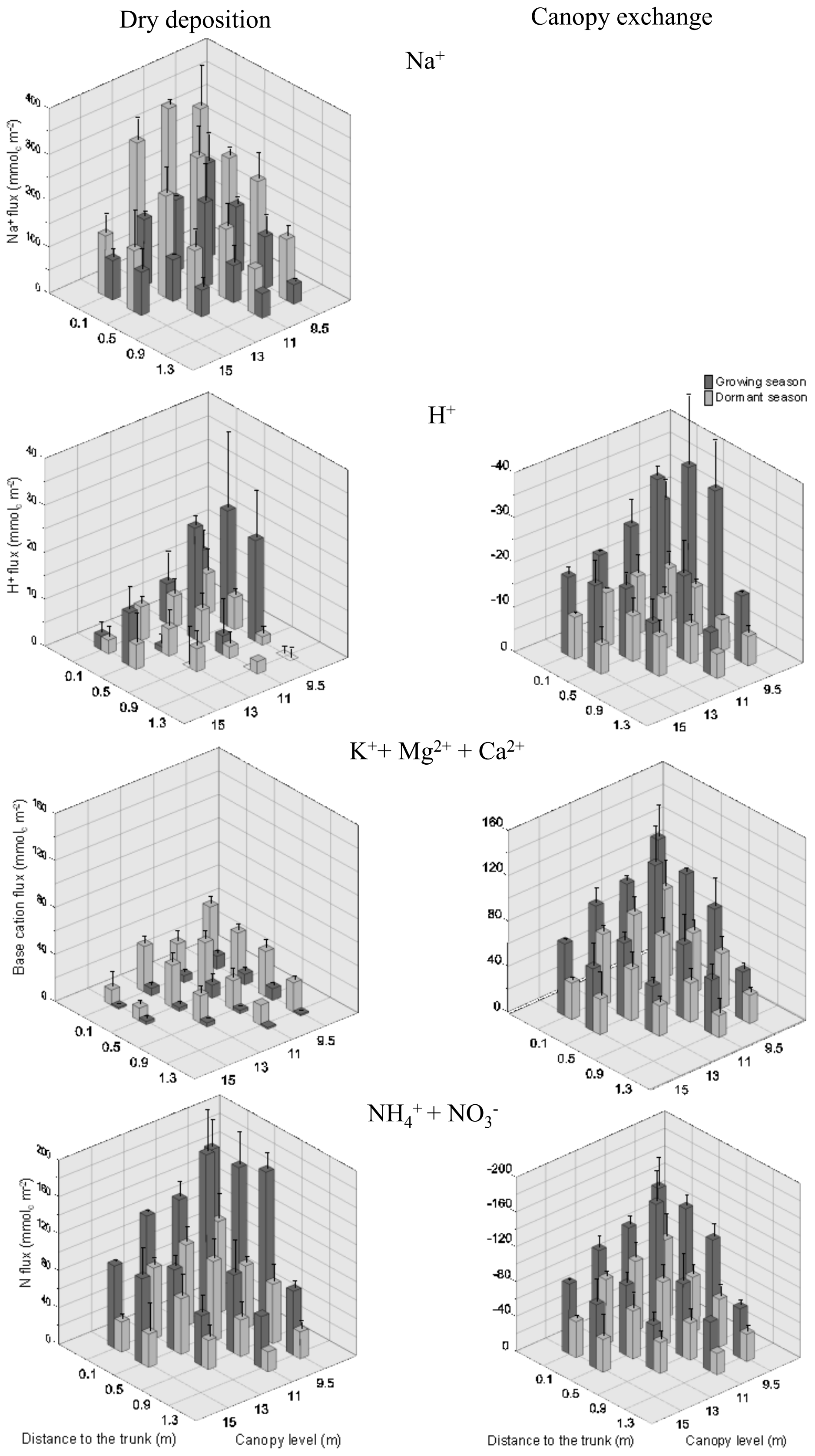

Fig. 5.7 Calculated dry deposition and canopy exchange of base cations ($K^+ + Ca^{2+} + Mg^{2+}$), Na^+, inorganic N ($NH_4^+ + NO_3^-$) and H^+ within a spruce canopy at different canopy levels (9.5, 11, 13 and 15 m) and as a function of the distance to the trunk. Error bars represent standard error

spruce in all canopy layers. Deposition fluxes of Na^+ and Cl^- are generally higher during winter (Neary and Gizyn 1994; Houle et al. 1999; Staelens et al. 2007) due to more frequent storms and generally higher wind speeds (Beckett et al. 2000) and enhanced atmospheric sea-salt aerosol concentrations (Gong et al. 1997). Since the canopy structure remains the same all year for the spruce trees, the higher fluxes in the dormant than the growing season can hereby be explained. For the beech tree, however, leaves are absent during the dormant season, which decreases the plant area index and thereby the collecting surface from a value of 5.2 to 1.1 (Holst et al. 2004). The observed high DD can then be explained by the greater capture efficiency of a large number of small twigs and branches at the top of the defoliated canopy, compared to large simply structured stems or leaves (Freer-Smith et al. 2004).

Since atmospheric concentrations of Na^+, Cl^- and SO_4^{2-} aerosols generally start to increase in autumn, NTF and thus DD is higher during the leaf senescence period compared to the leafless period in the beech canopy due to the higher collecting surface of leaves and stems compared to stems only.

Dry deposition of SO_4^{2-} occurs mainly as SO_2 in most forests, however, close to the sea, as for the studied spruce forest, an important sea-salt aerosol component can be present (Lindberg and Lovett 1992). Yet, in contrast to Na^+ and Cl^-, DD of SO_4^{2-} occurred mainly in the upper canopy for both species, indicating that plant physiology and surface wetness also influenced the deposition process. As stated in Section 5.5.4, these parameters were higher in the upper canopy, thereby favouring DD of gases.

5.5.3 Base cations

Net throughfall fluxes of base cations are determined by canopy leaching (CL) and DD of particles and are generally found to be positive. Throughfall fluxes of K^+ increased 17-37 times compared to bulk deposition in the beech and the spruce canopy, respectively, while for Ca^{2+} and Mg^{2+} the increase was 3-6 times (Table 5.1). This is concordant with previous findings (e.g. Houle et al. 1999; Staelens et al. 2007) and can be explained by the fact that K^+ forms only weak complexes in cells and is readily exchangeable, while Ca^{2+} and Mg^{2+} are bound tighter in cell walls, chloroplasts and enzymes (Marschner 1995). The difference in TF and CL of K^+ between spruce and beech was also found by Rothe et al. (2002) who compared 16 spruce-beech pairs in Europe, and is furthermore in line with results of a review of 24 case studies of coniferous/deciduous forest stands at comparable sites (De Schrijver et al. 2007). As the LAI of spruce trees is generally equal to or larger than for beech (Gower and Norman 1991; Mussche et al. 2001), higher K^+ leaching from beech canopies indicates that beech foliage is highly susceptible to leaching processes. One explanation for this can be the higher K^+ and Mg^{2+} content of beech leaves compared to spruce needles (unpublished results). Next to this, beech leaves show a higher wettability than spruce needles (Boyce et al. 1991; Van Wittenberghe 2009), which enhances foliar leaching (Tukey 1970; Parker 1983). This implicates a higher input of base cations to the forest floor in beech stands than in spruce stands. However, this applies only to the growing season, since during the dormant season base cation leaching in the beech canopy is strongly reduced (-60 % compared to the growing season), in contrast to spruce (-18 %) while DD is decreased in the beech canopy (-37 %) and strongly increased in the spruce canopy (+532 %) (Fig. 5.6 and 5.7). Thus, seasonal differences in CE are larger for beech compared to spruce for ions originating from the canopy.

Throughfall and NTF of base cations increased with canopy depth, as for CL in the beech canopy. In the spruce canopy, differences in CL were mainly found in the upper and middle canopy, although an increase was still visible in the lower canopy close to the tree trunk (Fig. 5.7). This contrasts with the findings by Schaefer et al. (1988) in a balsam fir canopy where NTF did not change substantially between the upper and the lower canopy, but agrees with Lovett et al. (1989). These patterns indicate that the canopy pools are not depleted when the throughfall water reaches the lower canopy, and that the concentration gradient between lower leaves and throughfall is still high enough to induce an additional release of base cations. It is also possible that the effect of a lower concentration gradient in the lower canopy is compensated by the higher wettability of leaves and needles (Boyce et al. 1991; Van Wittenberghe 2009).

Due to the assumptions of the canopy budget model, DD patterns of base cations in the beech and spruce canopy followed the patterns of Na^+ and SO_4^{2-}, respectively (see 5.5.2). Since base cations are only deposited as particles, these patterns are considered to be reliable, although it is difficult to verify this because NTF fluxes are mainly determined by leaching processes.

5.5.4 Nitrogen and hydrogen

Net throughfall fluxes of NH_4^+, NO_3^- and H^+ are the result of canopy uptake (CU) and dry gaseous and particulate deposition. Despite the uncertainty in the modelled CU and DD of N, this study provides valuable indications on the main driving factors for and the relative importance of these processes. In contrast to our first hypothesis, both NTF and the canopy model calculations indicate that a large part of total uptake of NH_4^+ and NO_3^- occurred in the upper canopy layer (Fig. 5.6 and 5.7). During the growing season, the upper canopy of beech and spruce accounted for 69 and 68 % of the NH_4^+ uptake and 100 and 87 % of the NO_3^- uptake, respectively. During the dormant season, 37 and 62 % of the NH_4^+ uptake and 48 and 64 % of the NO_3^- uptake occurred in the upper layers of beech and spruce, respectively. Higher inorganic N uptake by the upper canopy was confirmed by Schaefer et al. (1988) for balsam fir and by Zhang et al. (2006) for a tropical rain forest. Additionally, in the beech canopy, a strongly negative NTF was observed for NO_3^- during leaf development (Fig. 5.5), which is concordant with the findings of Koyama et al. (2008) who stated that the leaf expansion period in broadleaved species is the most important period for NO_3^--N assimilation. Also, DD of NO_3^- may have been lower during the leaf development period, but measured DD fluxes of NO_3^- aerosols to surrogate surfaces indicate similar deposition fluxes as during the rest of the growing season (Adriaenssens unpublished results).

Canopy N uptake is generally believed to be controlled by passive diffusion processes (Schaefer et al. 1988; Hansen 1996; Lovett et al. 1996) and by leaf physiological activity (Parker 1983; Marschner 1995; Lovett et al. 1996; Krupa 2003), of which the latter is characterized by e.g. stomatal opening and photosynthesis rate (Krupa 2003). From the present study it is not possible to determine which mechanism has the highest impact and determines the higher N uptake in the upper canopy. In the studied beech tree, both the N content per dry matter in the leaves and the throughfall inorganic N concentration increased from the top to the bottom of the canopy. However, the relative increase in inorganic N throughfall concentration (+147 %) from top to bottom was meaningfully higher than for the leaf N content (+19 %). Assuming that N per dry matter is a proxy for the foliar NH_4^+ and NO_3^- concentration (Schjoerring et al. 1998), this would indicate a higher concentration gradient between the throughfall water and the leaf interior at 28 m compared to lower canopy levels, which would favour passive diffusion. However, leaf thickness, chlorophyll content and chlorophyll fluorescence were highest at 22 m, indicating a high physiological activity, and lowest at 28 m, where leaves were exposed to high light conditions (Van Wittenberghe 2009).

In spruce trees, the upper canopy contains a higher proportion of young needles, which take up more N compared to older needles (Eilers et al. 1992) due to the higher physiological activity of young needles (Niinemets 2007). However, needles in the upper canopy also more easily form thin water films during a dry period than those in the lower canopy (Burkhardt and Eiden 1994). These water films are formed by dry deposited particles at relative humidity values as low as 50 % and decrease cuticular resistance. Consequently, gaseous deposition of NH_3, NO_y and SO_2 from the atmosphere to the plant surface increases and the concentration of these dissolved gases in the water film rises, driving passive diffusion into the needle. It is likely that this mechanism also exists for beech leaves. Van Hove and Adema (1996) found a water film on poplar leaves at a relative humidity exceeding 60 %, but no such water films have been reported for beech yet.

Based on the observed patterns in this study and the fact that the CU of N for spruce was higher in the growing season than in the dormant season, it seems likely that passive diffusion processes can drive CU of N, but only in case of sufficient metabolic activity.

Significant CU for both NH_4^+ and NO_3^- was calculated during the dormant season of the beech tree, amounting to 80 and 68 %, respectively, of the CU in the growing season. Although the main pathway of N uptake is generally believed to be via foliage, increasing evidence suggests that uptake via twig, branch and stem surfaces may be equally important (Bowden et al. 1989; Brumme et al. 1992; Dail et al. 2009). However, this may occur through physicochemical processes rather than by N assimilation (Dail et al. 2009; Chapter 4). Especially during the dormant season DD and CUof gases as NH_3, NO_2 and HNO_3 are determined by the ability of the plant surface to absorb the specific chemical species, including the presence of water films or the chemical nature of the surface (Erisman and Draaijers 2003). The distinction between actual assimilation or physicochemical adsorption by twigs during the dormant season remains to be investigated, since this may cause an overestimation of the contribution of canopy N uptake to tree growth or carbon sequestration by the current deposition models.

For both spruce and beech, the modelled DD of NH_4^+ increased with canopy depth, in contrast to the more constant DD of NO_3^-. The same pattern was observed for the measured NTF fluxes in the beech canopy. We hypothesize that this may be due to a difference in the relative importance of gases and aerosols contributing to the NTF of NH_4^+ and NO_3^-. Dry deposition of NH_x may be attributed to gaseous NH_3 and particulate NH_4^+, while NO_x is deposited as gaseous NO_2 and HNO_3 and particulate NO_3^- (Erisman et al. 1997). Dry deposition and CU of NH_3 and HNO_3 are controlled by both stomatal and cuticular conductance (Vose and Swank 1990; Sutton et al. 1998), while for NO_2 stomatal conductance is the main pathway (Chaparro-Suarez et al. 2001). Since stomatal uptake of gases cannot be retrieved in throughfall water, it is likely that the net NTF flux of NH_4^+ is due to the deposition of both gas (NH_3) and aerosols (NH_4^+), while for NO_3^- this is mainly due to aerosols (HNO_3 and NO_3^-). Since aerosol deposition occurs mainly in the upper canopy (see 5.5.2) and since HNO_3 deposition is relatively unimportant in the beech study region (Neirynck et al. 2007), this may explain the observed NO_3^- pattern for the beech canopy. Cuticular deposition of NH_3 that subsequently will be washed off to the throughfall water can occur over the whole canopy but is likely to be enhanced in the lower canopy due to higher leaf wettability and leaf wetness. In a spruce canopy HNO_3 deposition was more significant (Andersen and Hovmand 1995) and could increase NTF fluxes in the lower canopy. The fact that this was not observed in the calculated DD fluxes is possibly due to the weaker model reliability for NO_3^-.

A higher DD of NH_4^+ and NO_3^- was found for spruce during the growing season, while the opposite was observed for other elements. This can be due to higher atmospheric concentrations of NH_3 and HNO_3 (Andersen and Hovmand 1995; Andersen et al. 1999) and an increased physiological activity of the needles during the growing season, while DD of other elements is closely linked to sea-salt aerosol deposition which is higher during the dormant season (see 5.5.2). Calculated DD of NH_4^+ and NO_3^- was for both species more closely correlated to Na^+, Cl^- and SO_4^{2-} during the dormant season than during the growing season (data not shown), indicating that gaseous deposition was the prevailing process in the growing season while aerosol deposition was more important in the dormant season.

In contrast to N, TF of H^+ decreased from the top to the bottom of the canopy in both species, with a reduction of 60 % for spruce and 80 % for beech (Table 5.1). The highest CU of H^+ was also found in the upper canopy. Although we cannot compare beech and spruce in this study, these results confirm previous comparisons of beech and spruce (Rothe et al. 2002) that revealed a negative NTF of H^+ for both species with the same bulk deposition. However, as mentioned in Section 5.4.1, an increase in the TF of H^+ was observed for spruce at 0.1 m to the trunk. Closer to the stem, more old needles can be found, which have been shown to leach more H^+ than current year needles (Fritsche 1992). So, it is likely that leaf longevity can also influence the spatial variation of throughfall water within a tree canopy.

5.5.5 Limitations and implications of the study

This is the first study presenting estimates of DD and CE patterns within the canopy of two contrasting tree species based on throughfall measurements. The canopy budget model is based on several assumptions with regard to the DD and CE of base cations, N components and H^+. This can result in small errors that propagate further through the model into larger errors (Staelens et al. 2008). Previous studies have observed leaching of Na^+ (Reiners and Olson 1984; Staelens et al. 2007), Cl^- (Neary and Gizyn 1994; Houle et al. 1999; Staelens et al. 2007) and SO_4^{2-} (Houle et al. 1999; Staelens et al. 2007) from coniferous canopies or from deciduous canopies during leaf development or leaf senescence periods through increased throughfall to bulk deposition ratios (Talkner et al. 2010). We did not observe this, hereby justifying the assumption that these ions are inert in the canopy budget model. Furthermore, the filtering approach assumes that base cations are deposited with equal efficiency as the tracer ion used, which mainly depends on particle size. By using a different tracer ion between the beech and the spruce site, we tried to account for this assumption. The weakest results of the model are obtained for NH_4^+, NO_3^- and H^+. For this reason, the calculated fluxes were always compared with actual throughfall and net throughfall measurements and only used to compare relative differences between canopy levels and seasons.

With respect to the number of study trees, beech crowns have been found to have a deterministic growth pattern and an unidirectional development (Fleck 2001), suggesting that similar patterns can be found for other individuals, while for spruce trees this variability in growth pattern was accounted for by studying two trees. However, because both species were studied in different regions and different periods and because they were not replicated, the observed differences between beech and spruce can be related to a species or a regional effect. Furthermore, it is likely that light availability, tree age (Niinemets 2010) or N deposition will influence the observed patterns. Therefore, similar studies in other tree canopies in different forest stands could strengthen the conclusions drawn from this study. It is also difficult to extrapolate these results to other species. Beech and spruce are shade-tolerant species where light availability, relative humidity and air temperature decrease sharply in the canopy, and where leaves and needles are acclimated to this variation in microclimate (Herbinger et al. 2005; Niinemets 2010). In fast growing species such as silver birch (*Betula pendula* Roth) or Scots pine (*Pinus sylvestris* L.), this gradient will be less pronounced (Niinemets 2010), possibly resulting in less distinct DD and CE patterns.

The present study clearly showed that the upper canopy contributes most to the DD and CU of atmospheric components, although the patterns differed between beech and spruce due to their difference in canopy structure. In contrast, all canopy components contribute equally to the leaching of base cations. Despite the limitations of our study, important implications can be formulated. Firstly, atmospheric pollutants will have the highest impact on the upper canopy, where the physiological activity of leaves is highest and where often reproductive structures are situated (Lovett and Lindberg 1992). Many studies have assessed the physiological effects of air pollution (see Bytnerowicz and Grulke 1992 for an overview). From this study, it is likely that air pollution impact only accounts for the upper canopy layer, however, due to the importance of this layer, it will influence the whole tree. The sensitivity of reproductive structures to air pollution (Chauhan et al. 2004) has not yet been proven for tree species, but could have important consequences for forest regeneration. Secondly, inferential models using only one stomatal and one cuticular resistance to determine DD velocities are likely to overestimate or underestimate the DD to the forest ecosystem, depending if these values are based on sun or shade leaves. A multilayer approach as used by Pieterse et al. (2007) could help to improve their measure accuracy. Thirdly, the use of small artificial trees in combination with throughfall sampling in the field (Ignatova and Dambrine 2000; Stachurski and Zimka 2002) will probably overestimate DD, since these trees lack the structural heterogeneity that causes turbulence intensity and consequently DD to decrease from the top to the bottom of the canopy.

5.6 Conclusion

This study demonstrates that the chemical composition of throughfall and net throughfall changed significantly between the different canopy levels of beech and spruce trees, which can be linked to differences in dry deposition and canopy exchange. The observed patterns in throughfall, net throughfall, dry deposition and canopy exchange depended upon the ion considered, the season and the canopy structure of the studied tree species. Dry deposition of ions originating from gases (NH_4^+, NO_3^- and SO_4^{2-}) and canopy uptake of inorganic N was concentrated in the upper canopy of both species and could be linked to the higher physiological activity of this canopy layer, governed by stomatal conductance and photosynthesis. This implies that for these components multilayer models are highly recommended for tree species with a strong vertical light gradient in the canopy, such as beech and spruce. Whether this holds true for other tree species and under more increased N deposition levels remains to be investigated, as well as the actual processes determining this higher N uptake in the upper canopy. Surface wash experiments applying throughfall water with the same chemical composition to the different canopy layers could potentially reveal the exact mechanism occurring. Furthermore, ions originating from the canopy (K^+, Ca^{2+} and Mg^{2+}) were found to contribute to throughfall water at all levels, with a substantially reduced contribution in the dormant season for beech. Dry deposition of ions deposited as aerosols (K^+, Ca^{2+}, Mg^{2+}, Na^+ and Cl^-) occurred mainly in the upper canopy for beech, but increased with canopy depth and decreased with distance to the trunk for spruce, clearly reflecting the canopy structure of beech and spruce. However, further research is needed to verify if this can be related to a species effect and if the observed patterns also hold true for other tree species and under varying deposition levels.

6 Dry deposition along a vertical gradient within a beech canopy: comparison of methods and dependence on canopy structure

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6.1 Abstract

Dry deposition measurements using three different methods were conducted along a vertical gradient within a beech (*Fagus sylvatica* L.) canopy, and the effect of canopy structure measured by Terrestrial Laser Scanning (TLS) was assessed. Dry deposition calculated from throughfall measurements by the canopy budget model was compared with the deposition of aerosols on single and multi layer artificial foliage and with dry deposition calculated from air concentration measurements of NH_3, NO_2 and SO_2 multiplied with a literature-based deposition velocity. Air concentrations of NH_3 and SO_2 decreased with canopy depth, while this was not the case for NO_2. The multi-layered artificial foliage within the canopy yielded more realistic results than the single-layered foliage above the canopy and showed a good agreement with the canopy budget model for coarse aerosol deposition. Dry deposition of SO_x estimated by the canopy budget model was higher than estimated from air concentration measurements, especially during the leafless season. For NH_y, the canopy budget model and the air concentration measurements yielded similar results, but for NO_x dry deposition calculated by the canopy budget model was lower. A combination of throughfall measurements and multi-layered artificial foliage could aid to quantify dry deposition of NO_x. For all variables except throughfall volume, H^+ and NO_3^-, a significant correlation was found with the volume of canopy elements above the throughfall collectors, as measured by TLS, hereby contributing to current knowledge on spatial throughfall variability and deposition patterns.

6.2 Introduction

Dry deposition is an important pathway by which pollutants from anthropogenic origin such as nitrogen (N) dioxide (NO_2), ammonia (NH_3) and sulphur (S) dioxide (SO_2) enter ecosystems (Duyzer et al. 1992). Forest ecosystems in particular receive a high dry deposition compared with other vegetation types due to their higher surface roughness, leaf area index (LAI) and physiological leaf characteristics (Erisman and Draaijers 2003). The quantification of dry deposition is, therefore, essential to assess the complete impact of air pollution on these ecosystems.

Several methods have been developed yielding moderately to highly accurate estimates of dry deposition, with monitoring efforts and costs concordant to the measurement accuracy (Draaijers et al. 1997). The first group of methods are direct, micrometeorological techniques such as the eddy correlation and the gradient method, which are considered to be the most accurate but are often restricted to short term measuring periods and homogeneous surfaces. Therefore, gas and particle specific deposition velocities (v_d) have been derived from these micrometeorological techniques based on a resistance analogy. Those velocities can be multiplied by air concentration measurements in inferential models to obtain a dry deposition estimate on a regional scale for longer time periods and with lower monitoring efforts (Wesely and Hicks 2000; Zhang et al. 2002; Wu et al. 2003). However, these inferential models still suffer from large uncertainties, and depending on the model used, the dry deposition can differ with a factor of 2-3 (Flechard et al. 2011). Furthermore, the models typically require air concentration measurements at least 1 m above the canopy. Yet, at forest sites, air concentrations are often measured in clearings or in the trunk space for practical reasons (Flechard et al. 2011), which leads to a consistent underestimation of dry deposition fluxes, as was shown by Hicks (2006) for SO_2 and HNO_3. For NH_3 and HNO_3, very few vertical concentration profiles exist in literature (Flechard et al. 2011), while such data are necessary in the currently used multi-layer deposition models (Cox 2003; Pieterse et al. 2007).

Surface wash techniques are indirect, less intensive and less costly methods to quantify dry deposition. Of these techniques, the throughfall method, in which the amount and composition of water above and beneath the canopy are compared, is most often applied in long term monitoring networks such as the ICP Forests (de Vries et al. 2003). Total atmospheric deposition is then quantified by the so-called canopy budget model

(Draaijers and Erisman 1995), which estimates ion-exchange reactions between the canopy and throughfall water. However, different forms of this canopy budget model have been reported to yield different deposition estimates, e.g. for two deciduous plots (Staelens et al. 2008). Furthermore, direct gaseous uptake of NH_3, NO_2 and SO_2 is not taken into account, which may lead to an underestimation of the actual dry deposition when compared with an inferential method (Draaijers et al. 1997; Thimonier et al. 2005; Zimmermann et al. 2006). Nevertheless, advantages of the throughfall method are that it can be used in heterogeneous terrain and that it also provides useful information about processes in the canopy and the variation of fluxes within a stand (Erisman et al. 1994; Staelens et al. 2006).

Next to throughfall measurements, surrogate surfaces are used as an analogue to real trees to quantify dry deposition. Estimates of dry deposition are obtained by comparing throughfall fluxes under the surrogate surface (e.g., plastic trees) with rainfall (Ferm and Hultberg 1999; Ignatova and Dambrine 2000; Stachurski and Zimka 2002). These surrogate surfaces or artificial trees have proven to yield reasonable results for aerosols (Ferm and Hultberg 1999; Ignatova and Dambrine 2000). However, to our knowledge, the method has never been compared with the canopy budget model or with inferential models. Furthermore, it is likely that this method will overestimate dry deposition since the surrogate surfaces lack the structural heterogeneity of real canopies that causes dry deposition to decrease from the top to the bottom (Chapter 5).

On a within-stand spatial scale, surface wash experiments (Beier et al. 1993b; Draaijers et al. 1997; Whelan et al. 1998; Raat et al. 2002) and deposition models (Meyers et al. 1998) suggested that dry deposition on forests is related to their LAI. Although this influence of LAI on dry deposition seems obvious, it is more difficult to establish such relationships in heterogeneous forests because of the difficulty in practically relating LAI to, e.g., throughfall inputs observed in individual gauges (Levia and Frost 2006). Current methods of estimating canopy cover or LAI, such as hemispherical photographs, fail to operate on the same spatial resolution as throughfall orifice areas, so that the exact area of the canopy that affects the throughfall beneath it cannot be determined (Staelens et al. 2006). Only Nadkarni and Sumera (2004) managed to establish a relationship between throughfall volume and the canopy structure directly above the sampler by means of vertical canopy cylinder transects. Light Detection And Ranging (LiDAR) technology used by Terrestrial Laser Scanners (TLS) provides a novel tool for generating an accurate and comprehensive 3D mathematical description of tree and canopy structure (Lefsky et al. 2005; Van der Zande et al. 2006; Huang and Pretzsch 2010; Kane et al. 2010). This technique allows assessing the relationship between 3D canopy structure and spatial variability of throughfall chemistry on a detailed scale (i.e. cm-level).

In this study, we aimed to i) compare dry deposition estimates obtained by the canopy budget model with aerosol deposition on artificial foliage and gaseous deposition obtained by air concentration measurements along a vertical gradient in a European beech (*Fagus sylvatica* L.) tree, and ii) to relate the variability in throughfall fluxes and consequently dry deposition to 3D canopy structure by means of TLS data.

6.3 Materials and methods

6.3.1 Site description

A description of the Aelmoeseneie forest and the studied beech tree was given in Section 5.3.1.

6.3.2 Sample collection and analysis

6.3.2.1 Air concentrations of NH_3, NO_2 and SO_2

Air concentrations of NH_3, NO_2 and SO_2 were obtained four-weekly by means of passive samplers at 36, 22, 15 and 1.5 m height along the measuring tower (Fig. 6.1). Ammonia was measured with PK20 Radiello cartridges (Sigma Aldrich, St-Louis, USA), while NO_2 and SO_2 were measured with diffusive sampler types of IVL (Gothenburg, Sweden) (see Ferm and Rodhe 1997, for more detailed information). All measurements were made in duplicate.

Within one week after exposure, NH_3 samplers were desorbed with ultrapure water, which was then analysed using spectrophotometry (Eurofins, Oostkamp, Belgium). The air concentration of NH_3 was calculated from the measured ion concentration corrected for the average air temperature during the measuring period, as determined experimentally by Swaans et al. (2005). Air temperature at each canopy level was measured with Thermistor sensors (Omega, Manchester, UK). The IVL diffusive samplers for NO_2 and SO_2 were sent to Sweden for chemical analysis by spectrophotometry (NO_2) and ion chromatography (SO_2), from which air concentrations were determined accounting for temperature-dependent diffusivity (Ferm and Rodhe 1997; Ferm and Svanberg 1998).

6.3.2.2 Throughfall water

Throughfall fluxes inside the beech canopy were measured by means of two different types of throughfall collectors. The first type was a normal funnel of which the arrangement within the beech canopy is described in Section 5.3.2.1.

At 15, 22, 28 and 36 m each, two replicates of the second type, i.e., funnels with artificial tree foliage above it, were placed on the same rails as the throughfall collectors (Fig. 6.1). They were used to measure dry deposition between 36-28, 28-22, 21-15 and 15-0 m, according to an adapted version of the method described by Stachurski and Zimka (2002). The artificial foliage was shaped as Norway spruce (*Picea abies* (L.) Karst.) trees and consisted of one iron spiral (representing the 'stem') with attached shorter spirals ('branches') and polyethylene ribbon, densely woven into the spirals and cut into every 1 mm ('the needles') (Wuyts et al. 2008b). Stachurski and Zimka (2000) pointed out that polyethylene imitated the natural smoothness of beech leaves well and that a spruce surrogate yielded the same ionic load as a beech surrogate. The artificial foliage above each funnel had a LAI similar to the canopy layer below it, e.g., the LAI of the artificial tree foliage at 28 m equalled that of the canopy layer from 28 to 22 m. Consequently, the artificial foliage captured the same amount of dry deposition as this canopy layer, but no canopy exchange processes occurred. The stand LAI of 5.5 (Mussche et al. 2001) was divided into 0.3 (36-28 m), 3.0 (28-22 m), 1.7 (22-15 m) and 0.6 (15-0 m) based on Samson (2001) and visual observations. This method is subsequently referred to as the multi-layer approach. Additionally, two collectors with artificial foliage having a LAI equal to the whole stand (5.5) were placed at 36 m, subsequently referred to as the single-layer approach.

Throughfall water of the two different collector types and stemflow was collected as described in Section 5.3.2.1. All samples were collected every fortnight from 22 April 2009 to 21 May 2010. Samples from throughfall collectors without artificial foliage were analysed over the whole study period, while those from collectors with artificial foliage were only analysed from 7 May to 19 November 2009, because after that period they were no longer representative for the leafless tree. Analysis was conducted as in Section 4.3.2.3.

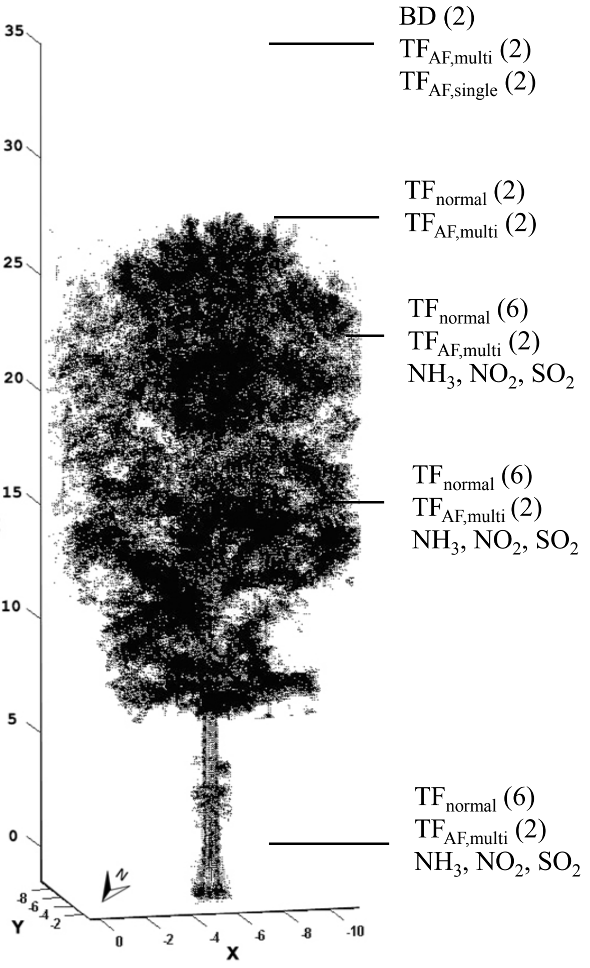

Fig. 6.1 3D view indicating the location of normal throughfall collectors (TF_{normal}), throughfall collectors with single- and multi-layered artificial foliage ($TF_{AFsingle}$; $TF_{AFmulti}$), bulk deposition collectors (BD) and air concentration measurements in the beech canopy. The 3D point cloud of the studied tree was obtained after merging 14 Terrestrial Laser Scans taken at different heights and separating the tree from the surrounding forest scene.

6.3.2.3 Terrestrial Laser Scanning (TLS)

If a detailed 3D structure description of a forest canopy is needed at an ecologically significant scale, TLS systems prove to be very valuable (Van der Zande et al. 2009). Its fine spatial resolution and small beam size allow measurements of the inner canopies of trees from the ground. In this study, the FARO LS880 was used to collect large quantities of range data, which is most commonly described as a cartesian LiDAR point cloud in which the 3D structure information is embedded. The non-contact optical active sensor uses Continuous Wave (CW) technology to determine the distance to a scanned object, e.g., leave or branch, by emitting light beams with a wavelength of 785 nm (nearinfrared). These beams are deflected in a panoramic field of view (FOV) of 320° x 360° using a mechanical direction system with an angular resolution of 0.018° both in the vertical and horizontal axis. This TLS system is therefore able to create 3D structure datasets with high point densities.

The FARO LS880 typically only registers the first object encountered by the beam. This means that spatial information related to the position of the vegetative elements located behind this first object therefore is not available. This is called shadowing and is a major obstacle when extracting structure information from the LiDAR datasets. Consequently, these background objects have to be measured from different angles to obtain comprehensive laser coverage (Van der Zande et al. 2006). To minimize this shadow effect, which can be high in dense beech stands (van Leeuwen and Nieuwenhuis 2010), scans were performed at the ground level (1.5m) and at the different platforms of the measuring tower at heights of 8.5, 15.5, 22.5 and 29.5 m in September 2009.

6.3.3 Data analysis

6.3.3.1 Air-concentration-based dry deposition (DD$_{vd}$)

The 'time stability' (Raat et al. 2002; Staelens et al. 2006) of four-weekly air concentrations at the different canopy levels was assessed for both the leafed and the leafless period. For *m* periods and *n* locations, the method calculates the temporal mean (δ_j) and standard deviation of the relative difference $\delta_{t,j}$ of a variable S at time *t* and location *j*:

$$\bar{\delta}_j = \frac{1}{m}\sum_{t=1}^{m}\delta_{t,j} \tag{6.1}$$

Where

$$\delta_{t,j} = \frac{S_{t,j} - \bar{S}_t}{\bar{S}_t} \tag{6.2}$$

and

$$\bar{S}_t = \frac{1}{n}\sum_{j=1}^{n}S_{t,j} \tag{6.3}$$

By plotting δ_j for each canopy level, the time-averaged ranking of the air concentration at a certain canopy level can be observed.

Dry deposition of inorganic N and S for the year, the leafed and the leafless period and for the artificial foliage period (see 6.3.3.3) was obtained by multiplying the air concentration derived from passive samplers with a fixed gas-specific deposition velocity (v_d). This v_d depends on the vegetation type and can vary considerably throughout the year due to varying meteorological and derived conditions such as wind speed, irradiance, relative humidity and leaf wetness. In case of a compensation point, as for NH_3, the ambient air concentration also determines whether emission or uptake occurs (Neirynck et al. 2005). Based on a literature survey of peer-reviewed gradient or inferential model studies, a range and mean of v_d values on deciduous forest in Flanders were determined for NH_3, SO_2, and NO_2 (Staelens et al. in press; Table 6.1). We multiplied these v_d values with the measured air concentrations at 36 m to calculate dry deposition onto the entire canopy (0-36 m). Dry deposition onto the lower canopy levels (0-22 m, 0-15 m, 0-1.5 m) was calculated by multiplying the air concentration at 22, 15 and 1.5 with an adjusted v_d, calculated as a percentage of v_d at 36 m based on the wind speed profile (Samson 2001). To evaluate the effect of v_d, dry deposition calculated as the measured air concentrations multiplied with a minimum, average and maximum v_d ($DD_{vd,min}$, $DD_{vd,avg}$, $DD_{vd,max}$)was compared with dry deposition derived by the canopy budget model (see 6.3.3.2).

6.3.3.2 Ion fluxes and dry deposition derived by the canopy budget model (DD_{CBM})

Biweekly wet deposition (WD; $mmol_c\ m^{-2}$), throughfall deposition (TF, $mmol_c\ m^{-2}$), weak acid deposition ($mmol_c\ m^{-2}$) and net throughfall fluxes (NTF) were calculated as in Section 5.3.3.1. Dry deposition (DD) and canopy exchange ($mmol_c\ m^{-2}$) for the whole year, the leafed and the leafless period and for the artificial foliage period (see 6.3.3.3) were calculated by means of the canopy budget model described in Section 5.3.3.2.

6.3.3.3 Dry deposition calculated from the artificial foliage (DD_{AF})

Ion dry deposition (DD_{AF}) for a canopy layer Y ($mmol_c\ m^{-2}$ 2 $weeks^{-1}$) was calculated as the average of the two differences (one for each rail) in TF between a collector with artificial foliage and the average of the three collectors without artificial foliage located on the same rail. The dry deposition estimate of Y was summed with all canopy layers above Y for comparison with the canopy budget model. In this way, the multi-layer approach resulted in a dry deposition ($DD_{AF,multi}$) between 28-36 m, 22-36 m, 15-36 and 1.5-36 m and the single-layer approach in a dry deposition ($DD_{AF,single}$) between 1.5-36 m.

Table 6.1 Percentage (%) of full wind speed as measured by Samson (2001) and dry deposition velocity (v_d, cm s^{-1}; minimum, maximum, and mean value) above and inside the canopy of the beech tree for NH_3, NO_2 and SO_2. Values of v_d at 36 m were derived from a literature study and used by Staelens et al. (in press), while values at 22, 15 and 1.5 m were calculated as a percentage from those at 36 m based on the wind speed profile

Height (m)		36	22	15	1.5
% Wind speed*		100	36	27	17
NH_3	Min.	0.80	0.28	0.22	0.14
	Max.	3.00	1.05	0.81	0.51
	Avg.	1.90	0.67	0.51	0.32
NO_2	Min.	0.10	0.04	0.03	0.02
	Max.	0.40	0.14	0.11	0.07
	Avg.	0.25	0.09	0.07	0.04
SO_2	Min.	0.30	0.11	0.08	0.05
	Max.	1.50	0.53	0.41	0.26
	Avg.	0.90	0.32	0.24	0.15

*Measured by cup anemometers above the canopy and at the respective canopy level

Since Na^+ in NTF is assumed to originate only from dry deposition, $DD_{AF,multi}$ and $DD_{AF,single}$ of Na^+ was compared with the NTF at each canopy level over the growing period. Both methods were significantly correlated ($p < 0.001$) at 0-36 (Spearman r_s = 0.809 for the multi-layer approach; r_s = 0.923 for the single-layer approach), 15-36 (r_s = 0.760), 22-36 (r_s = 0.870) and 28-36 m (r_s = 0.890). However, both $DD_{AF,multi}$ and $DD_{AF,single}$ of Na^+ above the stand were always higher than the NTF measurements (Fig. 6.2). This could be due to the fact that sea-salt particles are retained more efficiently on artificial foliage than on real foliage (Draaijers et al. 1997). Moreover, at the end of the growing period, when the top of the beech tree was already leafless, the difference between both methods became increasingly larger. Therefore, the period for comparison of the dry deposition methods was taken from 7 May to 9 October 2009, subsequently referred to as the artificial foliage period. The data of all ions other than Na^+ were corrected by multiplying the dry deposition with the mean ratio of Na^+ NTF to dry deposition at each canopy level, which was 0.52 (1.5-36 m), 0.40 (15-36 m), 0.27 (22-36 m) and 0.56 (28-36 m) for the multi-layer approach and 0.16 for the single-layer approach.

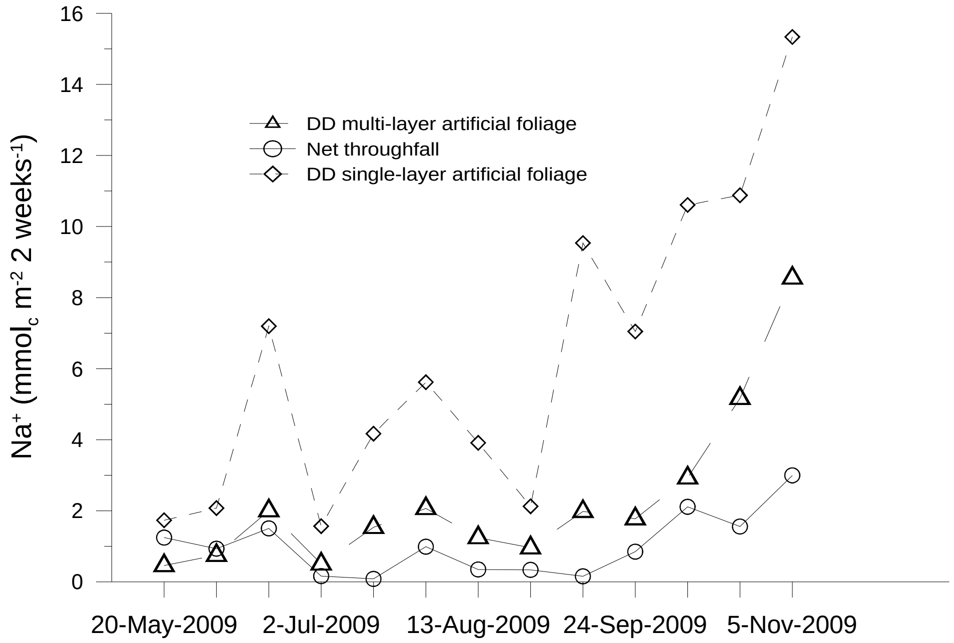

Fig. 6.2 Dry deposition of Na^+ ($mmol_c$ m^{-2} 2 $weeks^{-1}$) on the canopy of a beech tree measured by net throughfall, multilayer artificial foliage and single-layer artificial foliage between 20 May and 19 November 2009

6.3.3.4 Terrestrial Laser Scanning

Prior to structure extraction, raw TLS datasets were pre-processed using the following procedure. First, TLS data were transformed from polar to Cartesian coordinates. Then the datasets of the 11 scans were registered and merged into one central coordinate system using the Iterative Closest Point Algorithm (Besl and McKay 1992). Clearly recognizable features of the measuring tower were used as reference points for the registration procedure. Next, a transformation of vector to raster data was performed with cubic voxels of 0.05 m side. A voxel is defined as a volume element in a regular grid in a 3D space and can be interpreted as the 3D variant of a 2D pixel. Each voxel was characterized by its laser beam/voxel interaction: attribute F (filled) for voxels with at least one intercepted laser beam, attribute E (empty) for intersections by laser beams without interception. Afterwards, the voxels describing the tree were separated from the voxels describing the surrounding forest scene by segmentation using manual in situ measurements of the canopy size and visual interpretation of the TLS data, resulting in a 3D representation of the tree (Fig. 6.1). Finally, a filter procedure was performed enabling the extraction of a cuboid with a ground surface of 1, 2 and 3 m in side around each collector which was located manually in the 3D point cloud. The cuboid reached from the height of the collector to the top of the canopy. For each cuboid a vertical profile was generated describing the number of filled voxels, which can be considered as a proxy of the vegetation biomass present in that specific column.

6.3.3.5 Statistical analysis

Spearman rank correlation coefficients (r_s) were calculated between the biweekly NTF of Na^+ and $DD_{AF,multi}$ and $DD_{AF,single}$ of Na^+, and between NTF, dry deposition and canopy exchange of all ions and the number of filled voxels above each throughfall collector.

6.4 Results

6.4.1 Vertical profiles of NH_3, NO_2 and SO_2

During the leafed period, the air concentrations of SO_2 and NH_3 increased from 1.5 m (0.8 µg SO_2 m^{-3}; 1.8 µg NH_3 m^{-3}) to 36 m (2.8 µg SO_2 m^{-3}; 3.0 µg NH_3 m^{-3}). The largest differences were found between 1.5 and 15 and between 22 and 36 m (Fig. 6.3). The concentrations of SO_2 at 1.5 and 36 m had a larger deviation from the mean than those of NH_3. During the leafless period, the difference in concentration between 36 and 22 m persisted for NH_3 (2.5 and 2.3 µg m^{-3}, at 36 and 22 m respectively), but almost disappeared for SO_2 (3.3 and 3.2 µg m^{-3}, respectively). For SO_2 the concentration difference between 1.5 m (1.7 µg m^{-3}) and 15 m (3.3 µg m^{-3}) decreased compared with the leafed period, but for NH_3 the difference increased (1.8 and 2.5 µg m^{-3} at 1.5 and 15 m, respectively). Mean concentrations of NO_2 amounted to 15.9 and 23.6 µg m^{-3} in the leafed and the leafless period, respectively, and showed little differences along the vertical profile. Only at 1.5 m NO_2 concentrations were slightly lower than within and above the canopy, particularly during the leafless period.

6.4.2 Effect of deposition velocity on semi-annual (leafed and leafless period) and yearly dry deposition

Fig. 6.4 compares the semi-annual and yearly DD of N and S gases at the different canopy levels calculated from air concentration measurements multiplied with a minimum, average and maximum v_d ($DD_{vd,min}$, $DD_{vd,avg}$, $DD_{vd,max}$) with DD calculated by the CBM (DD_{CBM}). Since the actual DD could not be measured and the throughfall measurements were conducted with a higher temporal and spatial resolution than the air concentration measurements, DD_{vd} was tested against DD_{CBM}.

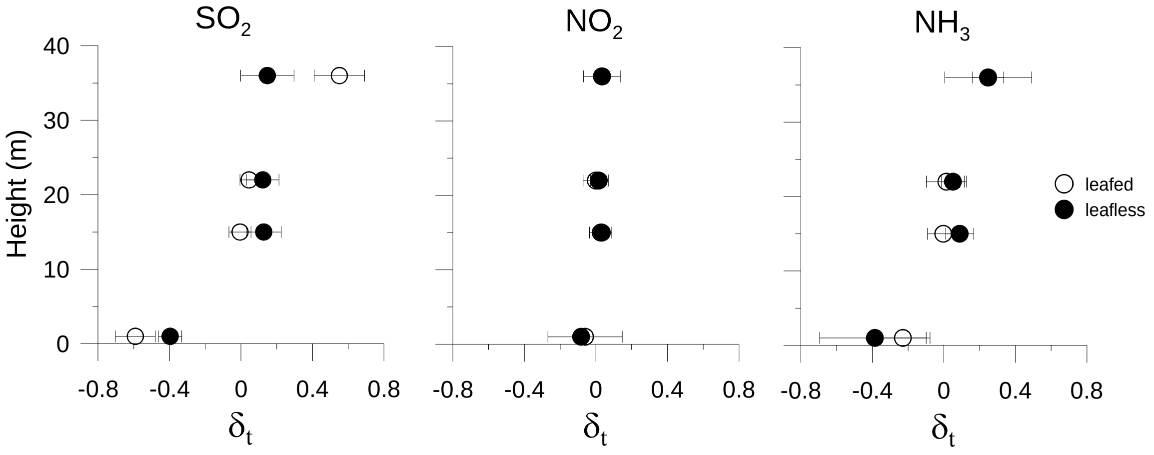

Fig. 6.3 Time stability plots for the air concentration of SO_2, NO_2 and NH_3 at 1.5, 15 and 22 m within the beech canopy and at 36 m above the canopy. δ_t represents the time-averaged relative difference from the mean for the leafed and the leafless period. Error bars represent standard deviations

In the leafed period, DD_{CBM} of SO_x onto the 22-36 m canopy layer equalled $DD_{vd,max}$, but for 15-36 and 0-36 m the CBM always yielded higher values. In the leafless period, DD_{CBM} of SO_x was, on average, 138 % higher than $DD_{vd,max}$, while this was 65 % for the yearly DD. Assuming that particulate SO_4^{2-} (pSO_4^{2-}) amounts to on average 30 % of total dry S deposition (Erisman et al. 1997), total SO_x deposition would be 16, 48 and 80 $mmol_c\ m^{-2}\ yr^{-1}$ for the beech canopy with a minimum, average and maximum v_d, respectively, of which only the result obtained with a maximum v_d approaches the CBM (87 $mmol_c\ m^{-2}\ yr^{-1}$).

Furthermore, the DD_{CBM} of NH_x during the leafed period was intermediate between $DD_{vd,min}$ and $DD_{vd,avg}$. A slightly higher DD_{CBM} was obtained in the leafless period compared with the leafed period, in contrast to DD_{vd} which was 50 % lower, resulting in approximately equal DD_{CBM} and $DD_{vd,max}$. Yearly DD_{CBM} of NH_x is generally equal to $DD_{vd,avg}$, except for 22-36 m. In both the leafed period and the leafless period, DD_{CBM} of NO_y was intermediate between $DD_{vd,min}$ and $DD_{vd,avg}$ (Fig. 6.4a and b). Consequently, the annual DD of NO_y showed the same pattern (Fig. 6.4c). For the entire canopy (0-36 m), the DD_{CBM} was similar to $DD_{vd,min}$ for NO_y.

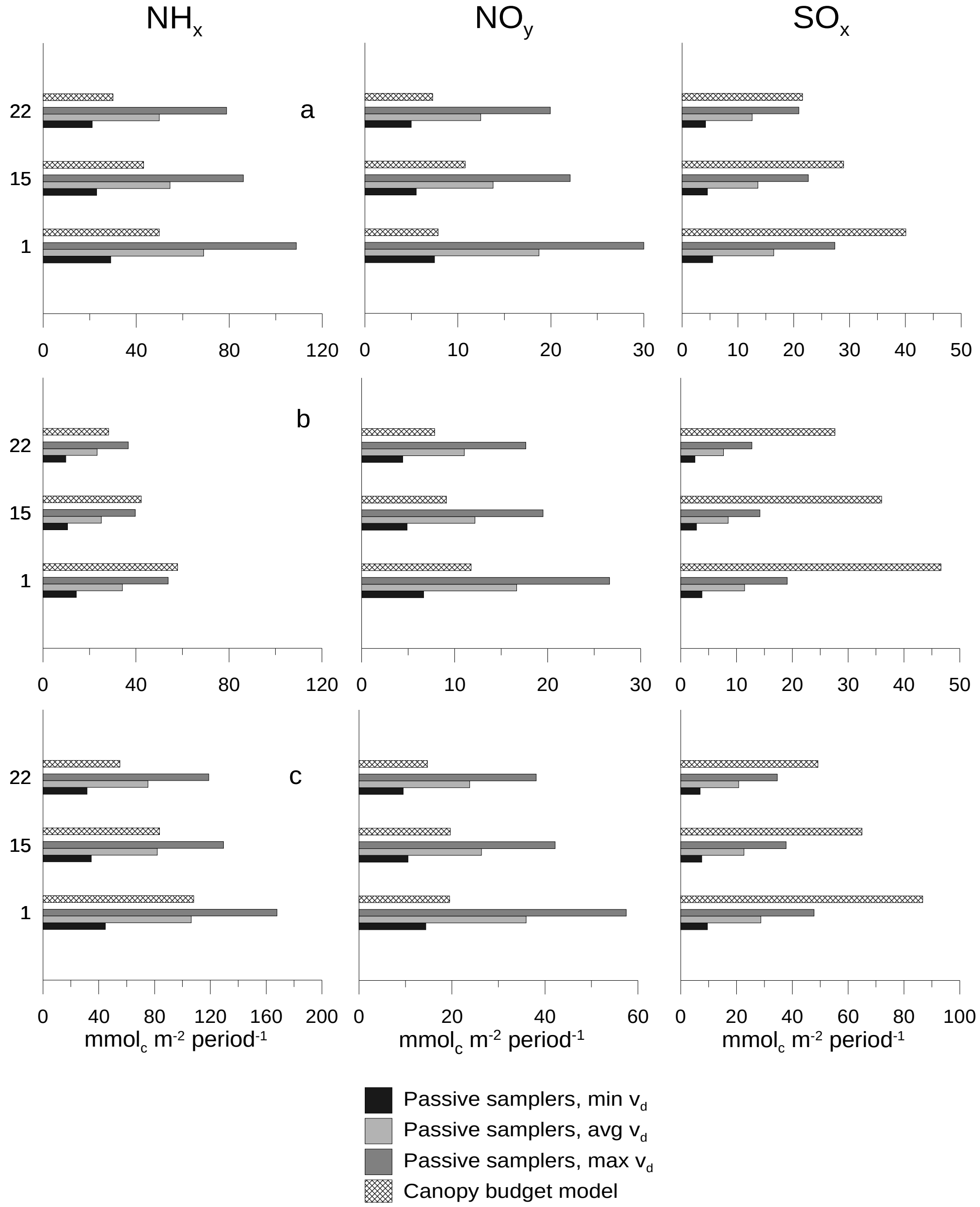

Fig. 6.4 Dry deposition of NH_x, NO_y and SO_x in the (a) leafed and (b) leafless period and (c) over the whole year, calculated by the canopy budget model, and air concentrations measured by passive samplers multiplied by a minimum, average and maximum deposition velocity (v_d)

6.4.3 Comparison of the three dry deposition methods

Table 6.2 compares dry deposition according to the CBM, multi- and single-layered artificial foliage and air concentration measurements for the artificial foliage perod. For Cl^-, NO_y, K^+, Ca^{2+}, and WA, the dry deposition according to the multi-layer artificial foliage ($DD_{AF,multi}$) was on average 16, 63, 70, 43 and 9 % lower, respectively, than the DD_{CBM}, while for Mg^{2+} it was 22 % higher. $DD_{AF,multi}$ of K^+ was lower than DD_{CBM} in the upper canopy (15-36 m), while in the lowest canopy layer (1.5-15 m) this was reversed. The multi-layer approach yielded very low DD values for H^+, NH_x and SO_x. Dry deposition measured by the single-layer artificial foliage ($DD_{AF,single}$) was 13 % lower than the DD_{CBM} for Cl^-, 32 % for Ca^{2+} and NO_3^- and 22 % higher for Mg^{2+}. Values for the other ions amounted to less than 50 % of those by the CBM.

$DD_{vd,avg}$ of NH_x was on average 76 % higher than the DD_{CBM}. Especially in the upper canopy (22-36 m) this method yielded a more than double DD value, but for the whole canopy (0-36 m) it was only 58 % higher. For NO_y, $DD_{vd,avg}$ was on average 36 % higher than DD_{CBM}, but for the whole canopy (0-36 m) it was 50 % higher. In contrast to the DD of N compounds, $DD_{vd,avg}$ of SO_x was 48 % lower than DD_{CBM}.

Total (wet + dry) N deposition ($mmol_c\ m^{-2}$) for this period was highest for the air concentration based method and 25 % lower for the CBM (Table 6.2). For total N+S deposition, the air concentration based method still yielded the highest values, but here the CBM was only 8 % lower. Total N and N+S deposition obtained by the multi-layer and single-layer approach was less than 50 % of the values derived by both other methods.

6.4.4 Influence of local canopy structure on throughfall variability and dry deposition

Net throughfall fluxes of NH_4^+, Cl^-, Na^+, SO_4^{2-}, K^+, Mg^{2+}, Ca^{2+} and WA ($mmol_c\ m^2\ period^{-1}$) were significantly correlated ($p < 0.01$) with the number of filled voxels above the collector during the leafed period (Fig. 6.5, Table 6.3). Correlations were significant for cuboids with a base of 1, 4 and 9 m^2, but the highest coefficients were obtained for cuboids with a base of 4 m^2. No significant correlation was observed for NTF volume and NTF of H^+ and NO_3^- in the leafed period. The DD_{CBM} showed the same trends (Table 6.3).

Table 6.2 Comparison of dry deposition, total (wet+ dry) inorganic nitrogen (N) and total inorganic N and sulphur (S) deposition (mmol$_c$ m^{-2} 5 months^{-1}; 7 May 2009 – 9 October 2009, i.e., the artificial foliage period) onto canopy layers estimated by (1) precipitation and throughfall measurements combined with the canopy budget model (CBM), (2) throughfall of multi- and single-layer artificial foliage (AF) and (3) measured air concentrations multiplied by an average deposition velocity (Table 6.1) (v_d,avg). Negative values indicate a lower throughfall deposition on the throughfall collectors with artificial trees than on those without. Values differing more than 50 % from those of the CBM are put in bold

	Layer (m)	H^+	NH_x	NO_y	Cl^-	Na^+	SO_x	K^+	Ca^{2+}	Mg^{2+}	WA	Total N	Total N+S
CBM	28-36	3.72	19.16	10.69	8.91	5.46	20.57	1.32	7.42	2.05	0.48		
	22-36	1.61	15.9	5	6.4	4.3	11.32	1.04	5.84	1.61	5.8		
	15-36	2.84	26.7	8.34	9.61	5.88	17.44	1.42	7.98	2.2	8.69		
	0-36	2.18	30.82	6.74	13.33	8.53	24.07	2.06	11.59	3.2	9.66	64.4	97.7
AF, multi	28-36	**0.01**	**0.61**	**1.67**	5.21	4.45	**0.41**	**-0.64**	**2.11**	1.38	0.58		
	22-36	**-0.01**	**0.1**	**1.32**	5.21	3.36	**-0.49**	-0.65	3.77	1.88	**2.49**		
	15-36	**-0.02**	**-0.7**	**2.53**	8.83	5.87	**-1.4**	**0.31**	6.8	**3.33**	5.91		
	0-36	**-0.01**	**1.81**	5.02	13.64	9.37	**-0.89**	**4.31**	10.26	**4.92**	12.76	33.6	**42**
AF, single	0-36	**-0.01**	**1.71**	**11.53**	**4.54**	**2.8**	**0.65**	**8.7**	7.88	3.91	**3.85**	33.1	**45.1**
v_d,avg	22-36		**36.62**	7.32			7.85						
	15-36		38.71	8.05			**8.46**						
	0-36		**48.49**	**10.95**			**10.11**					86.3	105.7

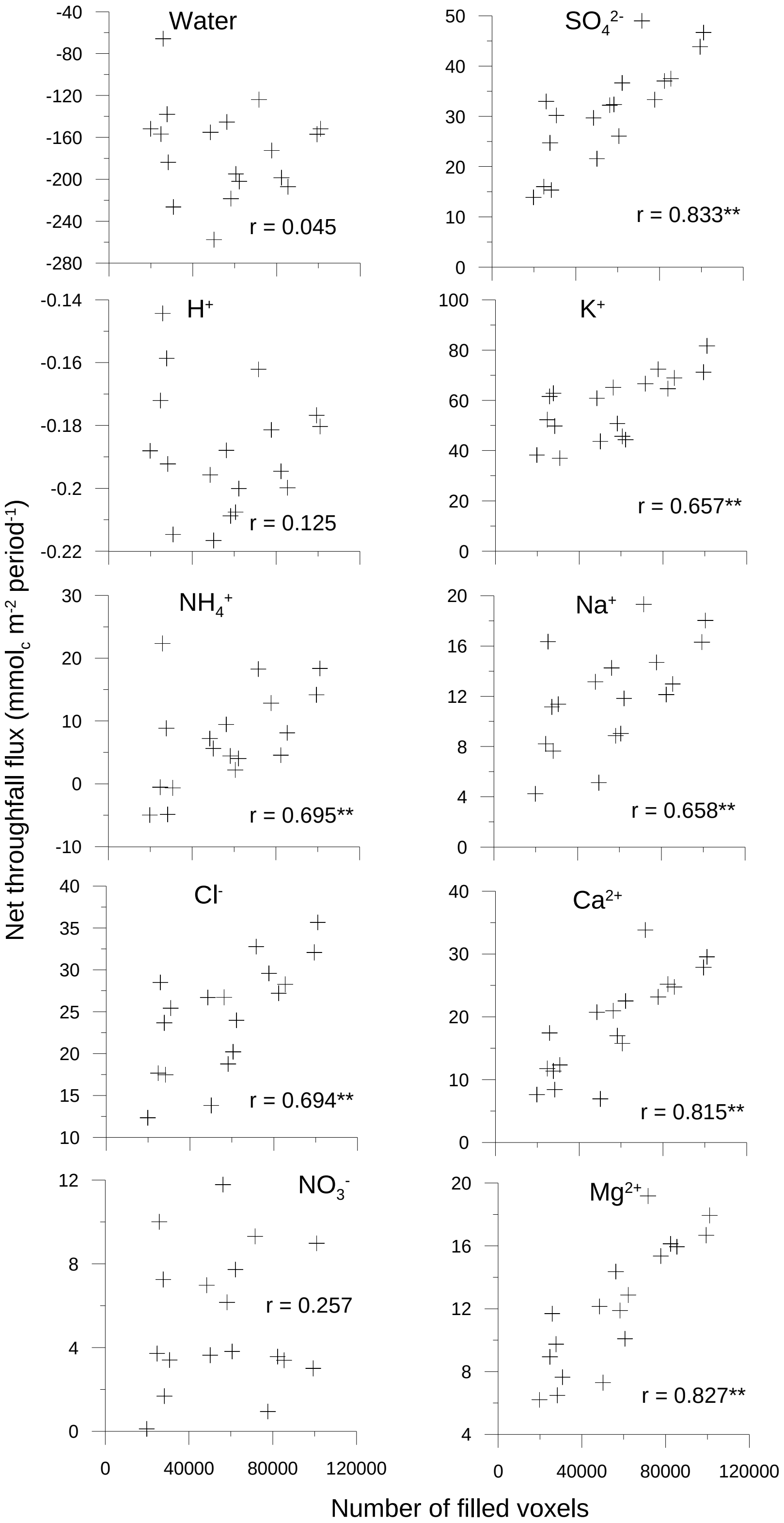

Fig. 6.5 Relationship between net throughfall volume (l m^{-2} period^{-1}) or ion deposition (mmol$_c$ m^{-2} period^{-1}) and the number of filled voxels above a throughfall collector in the leafed period. The number of filled voxels is calculated from a cuboid with a ground surface of 4 m^2 and a resolution of 0.05 m extracted from Terrestrial Laser Scanning data. Spearman rank correlation coefficient r_s is given

Table 6.3 Pearson correlation coefficients between net throughfall and dry deposition ($mmol_c\ m^{-2}\ period^{-1}$) and the number of filled voxels above a throughfall collector during the leafed period. The number of filled voxels is calculated from a cuboid with a ground surface of 4 m^2 and a resolution of 0.05 m extracted from Terrestrial Laser Scanning data. Bold values are significant at $p < 0.01$, underlined values at $p < 0.05$

Variable	Net throughfall	Dry deposition
Volume	0.045	/
H^+	0.123	-0.015
NH_4^+	**0.695**	**0.854**
NO_3^-	0.257	0.220
Cl^-	**0.694**	**0.694**
Na^+	**0.658**	**0.658**
SO_4^{2-}	**0.833**	**0.833**
K^+	**0.657**	**0.658**
Ca^{2+}	**0.815**	**0.658**
Mg^{2+}	**0.827**	**0.658**
Weak acids	**0.624**	/

6.5 Discussion

6.5.1 Vertical profiles of NH_3, NO_2 and SO_2

Remarkably few studies have reported gaseous concentration profiles within tree canopies. Andersen et al. (1999) reported lower NH_3 concentrations within than above a spruce canopy, which is in agreement with our observations. For SO_2, concentrations also decreased with increasing canopy depth, in agreement with decreasing SO_4^{2-} concentrations on filter papers with canopy depth reported by Lovett and Lindberg (1992), although the latter could be due to a decrease in either SO_2 or pSO_4^{2-} deposition. The absence of a clear vertical profile for NO_2 can be attributed to the gaseous emission of NO from forest soils, which then reacts with O_3 in the trunk space and within the canopy to form NO_2 beneath the canopy (Duyzer et al. 2004). As NO_2 rises through the canopy, radiation levels increase and NO_2 converts into NO through photolysis. Higher NO_2 emission can be expected at lower radiation levels and higher wind speed. However, at high ambient NO_2 concentrations (20 ppb_v) the flux is completely downward (Duyzer et al. 2004), which may explain why during the leafless period, when above canopy NO_2 concentrations were 24.5 ppb_v, concentrations within the canopy slightly decreased.

Gaseous concentration profiles in this study were obtained by means of passive samplers, which are considered as an inexpensive, accurate, easy to use method that is also applicable for remote locations (Cox 2003; Salem et al. 2009). On the negative side, this method quantifies cumulative exposure and cannot identify short-term pollutant episodes nor can it account for the stochasticity and dynamics of air pollutant exposure and plant response (Krupa and Legge 2000). Yet, our passive sampler measurements clearly indicated a concentration profile along the studied canopy for NH_3 and SO_2, but not for NO_2. Conclusions on forest exposure based on air concentration measurements made at ground level may therefore underestimate the actual exposure to NH_3 and SO_2.

6.5.2 Dry deposition measurements

6.5.2.1 Single-layer versus multi-layer approach

The results of the single-layer and multi-layer approach differed significantly, with a higher DD to the single-layer artificial foliage for NO_y and K^+, but a lower DD for Na^+, Cl^-, Ca^{2+}, Mg^{2+} and WA. The single-layer approach was used by Ignatova and Dambrine (2000), Stachurski and Zimka (2002) and Kram (2008, 2010) but has never been compared to other dry deposition measurements. In this study, the results for the multi-layer approach agreed well with CBM calculations for ions deposited as aerosols (Na^+, Cl^-, Ca^{2+}, Mg^{2+}, NO_y and K^+), which indicates that the multi-layer approach likely yields more realistic results, assuming that the CBM accurately quantifies DD of aerosols. Turbulence patterns and atmospheric concentrations decrease significantly with canopy depth (Merony 1968; Lovett and Lindberg 1992; Hofschreuder et al. 1997), and consequently, DD of aerosols and gases also decreases (Lovett and Lindberg 1992; Hofschreuder et al. 1997; Chapter 5). Our study showed that this vertical variability can be better integrated by a multi-layer approach, which is therefore recommended.

6.5.2.2 Aerosol deposition: base cations, sodium and chloride

The deposition of Na^+, Cl^-, K^+, Mg^{2+} and Ca^{2+} increased little from 28 to 15 m for both the CBM and the multi-layer approach. However, from 15 to 1.5 m, DD for all ions increased substantially according to both methods. In Chapter 5 the increased DD_{CBM} was attributed to an additional stemflow input in the throughfall collectors due to a low hanging branch. However, since the $DD_{AF,multi}$ was also high between 15 and 1.5 m, two additional explanations can be given. First, Fritsche et al. (1989) observed increased dry deposition of Na^+, Mg^{2+}, Ca^{2+} and NO_3^- to surrogate surfaces in the lower canopy of a Norway spruce tree and attributed this to the wind speed profile, which increased slightly from the middle to the lower canopy. A slight increase in wind speed between 18 and 12 m has also been measured in the studied beech tree (Samson 2001) due the lower leaf area density. Second, and this only holds true for Ca^{2+}, Mg^{2+} and particularly K^+, Lovett and Lindberg (1992) observed an increasing deposition with canopy depth for K^+ aerosols within a mixed oak forest and attributed this to an important in-canopy source of K^+ salt crystals exuded from leaves. This was confirmed by the absence of such profile during the leafless period (Chapter 5).

Artificial foliage generally captures well coarse particles that settle gravitationally, but it is less efficient in capturing smaller sized particles that are deposited by impaction (Rodrigo and Avila 2002). No deposition of K^+ particles, which have a mean mass diameter of approximately 32 % of those of Na^+ particles (Ruijgrok et al. 1997), was found on the artificial foliage at the top of the canopy. However, K^+ leached by leaves resides on coarse particles (Lindberg et al. 1986) and will be more easily deposited on the artificial foliage. This indicates that the assumption of equal deposition efficiency as used in the CBM will largely overestimate DD of K^+. The same conclusion was made by Ruijgrok et al. (1997), who found a good agreement between DD of particles on artificial branches and DD calculated by the model of Slinn (1982), except for K^+.

6.5.2.3 Sulphur

In the CBM, the net throughfall flux of SO_4^{2-} is assumed to be a good estimate of dry deposited SO_x (SO_2 and pSO_4^{2-}) because stomatal uptake of SO_2 is balanced by foliar leaching of SO_4^{2-} (Draaijers and Erisman 1995). This holds true in most studies (Cape et al. 1992; Draaijers et al. 1997), although significantly higher leaching than uptake also has been observed (Neary and Gizyn 1994; Cappellato et al. 1998). In this study, DD_{CBM} was significantly higher than DD_{vd}, especially during the leafless period. This difference could be attributed to DD of pSO_4^{2-}, but negligible DD was observed to the artificial foliage during for the artificial foliage period. However, these low values may also be due to the fact that pSO_4^{2-} generally consists of fine particles (Ruijgrok et al. 1997) that are not deposited well on artificial foliage (see 6.5.2.2.). So, the observed differences between the methods implicate that (i) significant SO_4^{2-} leaching occurs from the beech canopy, (ii) the contribution of pSO_4^{2-} to total DD exceeds 30 % (see 6.4.2), and/or (iii) a higher $v_d(SO_2)$ needs to be used. From this study we cannot distinguish between these factors. However, since current deposition estimates are overestimated by the CBM (as used in the ICP network) or underestimated by the air concentration based method (as used in the regional environmental monitoring network by the Flemish Environmental Network), causes for this discrepancy between both methods should be assessed in further research.

6.5.2.4 Nitrogen compounds

The estimated DD of nitrogen compounds based on throughfall measurements and the CBM is less precise than the estimated DD of other elements due to the complexity of the processes determining the nitrogen composition of throughfall water and due to the possible propagation of errors through the model (Staelens et al. 2008). Dry deposition of NH_x and NO_y may occur through gaseous and particulate deposition and substantial canopy uptake of both gaseous and dissolved compounds occurs in the canopy. Furthermore, stomatal uptake of gases cannot be retrieved via throughfall data. The air concentration based method and the artificial foliage used in this study can aid the understanding of total dry deposition of nitrogen, although both methods also have drawbacks. As is also the case for K^+ and SO_4^{2-}, fine particles (±0.6 µm) of NO_3^- and especially of NH_4^+ will not be captured well by the artificial foliage (Ruijgrok et al. 1997), while HNO_3 may also be deposited to the artificial foliage (Lovett and Lindberg 1992; Ferm and Hultberg 1999). Furthermore, the passive sampler measurements are cumulative measurements multiplied by a fixed v_d, hereby ignoring regional differences in, e.g., plant N status that may influence the deposition process.

In the leafed period, the DD_{CBM} of NH_x was slightly lower than $DD_{vd,avg}$, and particulate NH_x (pNH_x) deposition to the artificial foliage was rather low, indicating that DD consisted mainly of NH_3. Assuming that the uncertainty is 40 % on DD estimates by the CBM (Draaijers et al. 1996) and 30-50 % on DD estimates by an inferential model (Erisman et al. 1994), differences between the results of both methods can be considered negligible. The dominance of NH_3 in the DD of NH_x during the leafed period is confirmed by Neirynck et al. (2007), although in this study pNH_4^+ amounted to 30 % of gaseous DD. The ratio of gaseous and particulate NH_x deposition largely depends on the distance to the nearest emission source (Asman et al. 1998). In the leafless period, DD_{vd} decreased significantly due to lower NH_3 air concentrations, while DD_{CBM} remained constant. Since pNH_4^+ was not measured in the leafless period, we cannot distinguish if a higher pNH_4^+ then occurred or if the CBM yields inaccurate estimates for this period. The ^{15}N-labelling study in Chapter 4 showed that during the leafless period, canopy uptake of

NH_4^+ is not related to base cation leaching, hereby suggesting that the CBM might not be valid in this period and that DD_{CBM} is not correctly estimated. However, this remains to be investigated.

For NO_y, DD_{CBM} decreased with canopy depth, which is not possible and may point to inconsistencies in the model. Despite this, the sum of the $DD_{AF,multi}$ measuring particulate NO_3^- (pNO_3^-) and gaseous HNO_3 and DD_{vd} measuring NO_2 was 1.5 to 2 times higher than DD_{CBM}. This difference can be explained by the stomatal uptake of NO_2 or HNO_3 by the beech canopy. The DD of NO_2 in particular is driven by stomatal characteristics (Chaparro-Suarez et al. 2011) while HNO_3 deposition was found to be either surface (Ferm and Hultberg 1999) or stomata deposited (Garten et al. 1998) or both (Hanson and Garten 1992). However, DD_{vd} of NO_2 may be significantly overestimated, since Neirynck et al. (2007) observed a net upward flux of NO_2 for a mixed coniferous forest nearby. Net throughfall of NO_3^- at 1.5 m amounts to 4.5 $mmol_c$ m^2 for the artificial foliage period, which is slightly smaller than the $DD_{AF,multi}$ and the DD_{CBM} and could be due to canopy uptake. The same was observed by Ferm and Hultberg (1999) for a coniferous forest. This indicates that a combination of NTF measurements and artificial foliage may be a more suitable method to estimate DD of NO_y. During the leafless period, DD_{CBM} was lower than DD_{vd} of NO_2. However, DD_{vd} of NO_2 was likely lower than modelled due to the absence of stomatal conductance.

6.5.3 Role of canopy structure

Within-canopy variability of dry deposition can be attributed to physiological leaf characteristics, including surface wetness chemistry, external leaf wetness and stomatal opening, atmospheric concentrations and local canopy structure (Erisman and Draaijers 2003). As stomatal conductance (Van Wittenberghe 2009) and atmospheric concentrations (Fig. 6.3; Lovett and Lindberg 1992) decrease with canopy depth for most pollutants, the lower canopy may contribute little to dry deposition. Due to the complexity of measuring the 3D forest canopy structure, most studies have explained spatial throughfall variability, dry deposition and canopy exchange fluxes using a surrogate for the 2D or 3D structure, such as LAI measured by hemispherical photography (Whelan et al. 1998; Staelens et al. 2006) or aboveground biomass measured by litter fall (Potter et al. 1991). However, these methods cannot quantify the contribution of different canopy layers. The present study meets the need for a better characterisation of canopy structure to improve our understanding of its role in throughfall variability (Levia and Frost 2006).

Net throughfall and DD_{CBM} of all variables except throughfall volume, H^+ and NO_3^- were significantly correlated to the number of filled voxels above each collector, with the highest correlation for a cuboid with a base of 4 m^2. This indicates that all canopy layers contribute to DD, but also that within one layer, patterns are determined by the volume of canopy components vertically above the throughfall collector. Average net throughfall of H^+ and NO_3^- did not change significantly between the different canopy levels, and the average net throughfall volume only differed between 28 and 22 m (Chapter 5), which can explain the absence of a significant correlation for these elements.

6.6 Conclusion

In this study, the DD estimated by the canopy budget model was compared with the DD determined by an air-concentration-based method, for NH_3, NO_2 and SO_2, and with the DD of aerosols on artificial foliage. A good agreement was found between the CBM and the multi-layer artificial foliage for Na^+, Cl^-, Ca^{2+} and Mg^{2+}, at least after correction, while the CBM likely overestimates the DD of K^+. Passive sampler measurements showed a profile of decreasing NH_3 and SO_2 concentrations with canopy depth, but no profile for NO_2. This difference in concentrations between the atmosphere, the canopy and the trunk space can serve as input data for multi-layer deposition models, although additional measurements within tree canopies of other species are recommended to confirm the observed profiles. Estimated S deposition differed significantly between the air-concentration-based method and the CBM, and no particle deposition on the artificial foliage was detected. Causes for this discrepancy should further be assessed, as both methods are currently used to measure DD on a local and regional scale. Estimated DD of N was comparable for the air-concentration-based method and the CBM during the leafed period, but for NO_2 it should be assessed whether deposition or emission occurs. Furthermore, for the first time a relationship was found between net throughfall or DD and volume of canopy components obtained by Terrestrial Laser Scanning on a collector basis. This finding can be strengthened by improved processing of the multiple scans to canopy structure parameters, such as leaf area density, and a comparison with data in the leafless period.

7 Atmospheric deposition to forests based on throughfall measurements: effect of canopy budget model approaches on stand deposition, forest type effect and time trend analysis

After: Adriaenssens S, Staelens J, Baeten L, Verstraeten A, Boeckx P, Samson R, Verheyen K. Atmospheric deposition to forests based on throughfall measurements: effect of canopy budget model approaches on stand deposition, forest type effect and time trend analysis. To be submitted to an international peer reviewed journal

7.1 Abstract

Throughfall measurements are frequently used to quantify total atmospheric deposition to forest ecosystems. The canopy budget model is then used to calculate ion-exchange fluxes between the forest canopy and incident rainfall water. Various approaches for each model step have been reported and compared, but combinations of different approaches were not yet assessed. Therefore, the present study estimated the range of estimated dry and total deposition obtained by combining canopy budget model approaches for three typical case studies: (i) total nitrogen and potentially acidifying deposition onto a forest canopy, (ii) the ratio of these deposition variables between a coniferous and a deciduous stand and (iii) the parameters of a deposition time trend analysis. Furthermore, the effect of each step in the canopy budget model on the obtained range was assessed. The time step, type of precipitation data and tracer ion used in the model had a significant effect on the derived deposition in the three case studies. In addition, including or excluding canopy leaching of weak acids and canopy uptake of ammonium and nitrate during the leafless season largely affected the results, while including or excluding canopy uptake of nitrate generally showed no effect. We conclude that further research should focus on the assumptions of inertness of the tracer ion and the equal deposition efficiency of base cations and the tracer ion and on the quantification of weak acids in rainfall and throughfall water. Since local or tree-species specific effects might influence the results obtained in this study, a similar analysis is recommended for other tree species and regions when using the canopy budget model.

7.2 Introduction

The quantification of total deposition (TD) of sulphur (S) and nitrogen (N) is a key issue in determining potential effects of these pollutants on the diversity and functioning of forests. Furthermore, it allows to assess temporal and spatial trends and to evaluate the effect of emission abatement strategies (Erisman et al. 1994). Wet deposition can be measured with reasonable accuracy using wet-only or bulk collectors (Staelens et al. 2005), whereas for dry deposition a trade-off needs to be made between measurement accuracy and monitoring effort/costs (see Chapter 6 for an overview). The choice of a certain dry deposition method depends on the purpose of the study and throughfall measurements are considered to be more suitable for long-term monitoring purposes and large scale monitoring networks than micrometeorological measurements (Erisman et al. 1994; de Vries et al. 2003). Several studies report the use of throughfall measurements to evaluate trends in atmospheric deposition on forest ecosystems (Vanguelova et al. 2010; Pannatier et al. 2011). Furthermore, the method has often been used to compare deposition on different forest types, and deciduous vs coniferous forest stands in particular (de Vries et al. 2007; De Schrijver et al. 2007). An additional advantage of the throughfall method is that it also gives information on the internal nutrient cycle in forest ecosystems (Parker 1983; Friedland et al. 1991; Emmet et al. 1998; Ferm and Hultberg 1999; Neirynck et al. 2008).

When incident precipitation passes through the canopy it is altered by wash-off of gases and particles deposited in dry periods prior to the precipitation event and by ion exchange, e.g. uptake or leaching, between canopy surfaces and the solutions passing over them (Draaijers et al. 1997). Therefore, to quantify total atmospheric deposition with throughfall measurements it is necessary to distinguish dry deposition (DD) from canopy exchange (CE) (Parker 1983). This distinction is made by the canopy budget model of Draaijers and Erisman (1995), in which ion-exchange processes between the canopy and throughfall water are estimated. However, various approaches of this model with respect to the time step, type of open-field precipitation data, tracer ion and the ion exchange processes have been reported, of which an overview was given by Staelens et al. (2008). This review also assessed the sensitivity of atmospheric deposition onto two deciduous canopies to each approach compared to the reference model of Draaijers and Erisman (1995). Dry deposition of base cations was meaningfully affected by the type of precipitation data and the tracer ion used, while canopy uptake of ammonium (NH_4^+) and protons (H^+) was influenced by accounting for canopy leaching of weak acids. However, the authors suggested that future applications of the model could benefit from combining different approaches with each other to quantify the range of estimated DD and CE. This would also allow testing the relative influence of each approach on total N and potentially acidifying deposition.

Furthermore, Staelens et al. (2008) indicated that assuming no canopy uptake of nitrate (NO_3^-) (Harrison et al. 2000) had little effect on the total N deposition when the relative uptake efficiency of NO_3^- was considered to be low, but that more research was needed with regard to the uptake efficiency of NH_4^+ compared to NO_3^- for varying tree species and environmental conditions. In Chapter 4, NH_4^+/NO_3^- retention ratios from wet deposition were established by means of ^{15}N-labelled sources for four different tree species and phenological stadia. In this chapter, it was also observed that inorganic N compounds are merely adsorbed to woody plant surfaces instead of actually assimilated and that no relationship between $^{15}NH_4^+$ uptake and net throughfall of base cations was observed during the leafless season. This indicates that other processes than ion-exchange processes might play a role during the leafless season of deciduous species and that the ion-exchange assumptions made in the model might not be valid.

Therefore, in this study we applied a combination of the various assumptions that can be made at each step in the canopy budget model (Draaijers and Erisman 1995) to the rainfall and throughfall data of three case studies. The steps containing the approaches that showed a high impact on canopy exchange and dry deposition calculations according to Staelens et al. (2008) were included again. In addition, the effects of two new assumptions that emerged from the previous chapters in this thesis were tested. Our aim was to assess the effect of the different approaches and assumptions on (i) the range of canopy exchange, dry deposition and total N and acidifying deposition fluxes to a single forest stand (represented by data of an individual tree), (ii) the ratio of total N and acidifying deposition on a coniferous and deciduous plot and (iii) a time trend analysis of total N and acidifying deposition.

7.3 Materials and methods

7.3.1 Study sites

7.3.1.1 Individual beech canopy and time trend analysis

A full description of the Aelmoeseneie forest containing the mixed oak-beech stand of the time trend analysis and the studied beech tree is given in Section 5.3.1.

7.3.1.2 Deposition ratio between a coniferous and deciduous forest

The study site is located in the nature reserve ‘Heidebos’ in northern Belgium (Wachtebeke-Moerbeke) (51°11’N, 3°55’E, 11 m a.s.l.). The mean (1980–2007) annual precipitation is 873 mm year^{-1} and the mean annual temperature is 10.4°C (Royal Meteorological Institute of Belgium). Adjacent monospecific forest stands of pedunculate oak (*Quercus robur* L.) and Scots pine (*Pinus sylvestris* L.) were selected with the same soil type, stand history and tree age. The area has been continuously forested since at least 1775, and in 1947 the current tree species were planted. In 2007, tree density was similar in both stands (823 oaks ha^{-1} and 920 pines ha^{-1}). However, basal area was more than twice as large in the pine stand (42 m^2 ha^{-1}) as in the oak stand (20 m^2 ha^{-1}) due to the higher mean diameter at breast height of pines (23.4 cm) compared to oaks (17.1 cm). Mean tree height was 15.6 and 18.2 m for oaks and pines, respectively. The soil in both stands was a well-drained acid podzol (sandy texture) with a groundwater level below 1 m depth.

7.3.2 Sample collection and analysis

7.3.2.1 Individual beech canopy

A description of the throughfall sampling under the beech tree is given in Section 5.3.2.1. In the present chapter, only the results of the collectors at ground level are used. Samples were analyzed chemically as described in Section 4.3.2.3. Based on visual observations of the beech canopy, the following phenological periods were distinguished: leaf development (22 April to 20 May), fully leafed period (21 May to 24 September), leaf senescence (25 September to 19 November 2009) and leafless period (20 November 2009 to 22 April 2010).

7.3.2.2 Deposition ratio between a coniferous and deciduous forest

Bulk precipitation and throughfall water were sampled biweekly from 7 December 2007 until 3 December 2008. Bulk precipitation was measured in an open field 200 m from the forest stands using four collectors with a funnel diameter of 14.2 cm (158 cm^2). The funnels were set at 1.2 m height above the forest floor, had a sharp-edged rim, a slope of 45° and drained into polyethylene 2-L bottles placed below ground level to avoid the growth of algae and to keep the samples cool. A nylon 1-mm mesh placed in the funnels prevented contamination by large particles. Throughfall was sampled using similar devices as for bulk precipitation. Per stand 15 collectors were set randomly. Stemflow water was not sampled because of the relatively low contribution (< 1 %) to nutrient deposition to the forest floor reported for these rough-barked tree species (Genouw et al. 2005). Water volumes were measured biweekly in the field and aliquots were transported and stored in darkness at 5°C. For bulk precipitation, a composite sample was made in the field, while throughfall samples were analysed per individual collector. All funnels, wire meshes, and bottles were replaced biweekly by equipment rinsed by demineralised water. Biweekly water samples were pooled volume-weighted to fourweekly samples for chemical analysis. The same methods were used as for the individual beech tree (see 4.3.2.3). Based on visual observations of the oak canopy, the following phenological periods were distinguished for the oak throughfall data: leaf development (8 May to 5 June), fully leafed period (6 June to 8 October) and leaf senescence (9 October to 3 December 2008).

7.3.2.3 Trend analysis

Data of precipitation, throughfall and stemflow were collected according to the guidelines of the manual of the UNECE ICP Forests intensive monitoring programme (ICP Forests 2010). Precipitation, throughfall and stemflow were sampled biweekly from 1994 till 2010 in the oak-beech stand. Precipitation and throughfall were sampled using similar devices as described in Section 7.3.2.2, except for the fact that the funnels had a wider rim (4 mm). Bulk precipitation collectors (n = 4) were set up in an open field at less than 500 m from the forest. Throughfall was sampled with ten bulk collectors in a 0.25 ha plot, distributed on two crossing lines with a distance of 10 m between two collectors on the same line. Both for precipitation and throughfall, samples from all collectors were bulked together for chemical analysis at every sampling event. Stemflow sampling was conducted only for beech because stemflow amounts were negligible for oak trees during a preceding testing period. Stemflow was sampled for three beech trees that were selected according to stem diameter (mean dbh and mean ±1 times the standard deviation of the dbh of the trees in the plot in 1994). Stemflow collectors consisted of flexible polyvinylchloride collars/gutters (7 cm diameter) attached horizontally to the stem at 1 m height, draining to a series of 200-L polyethylene storage containers mounted in serial. Stemflow samples were taken from the container nearest to each tree, with sample volumes weighted to tree diameters, and bulked together for chemical analysis at every sampling event. The different phenological stages in this study were delineated based on K^+ throughfall deposition, which was shown to increase during leaf development and leaf senescence (Neary and Gizyn 1994; Houle et al. 1999; Staelens et al. 2007). Since this approach agreed well with visual observations made in the other two case studies, this is considered to be reliable.

Water samples were kept cool during transport and stored in darkness at 4°C after filtration (0.45 µm). Samples were treated and analysed as prescribed by ICP Forests (2010). Water pH (Multi 340i-glass electrode, WTW) and conductivity (Multi 340i-Tetracon®325, WTW) were measured before and after filtration within one week after sampling. Water concentrations of cations (Ca^{2+}, K^+, Mg^{2+}, Na^+, NH_4^+) and anions (NO_3^-, SO_4^{2-}, Cl^-) were measured with ion chromatography, (Dionex, Sunnyvale,USA). Alkalinity was determined by means of potentiometric titration. Quality control included analysis of blanks and participation to yearly ICP Forests water ring tests.

7.3.3 Canopy budget models

Although one specific version of the canopy budget model was already presented in Section 5.3.3.2, this section and Fig. 7.1 provide a more extensive overview of the canopy budget model and the assumptions tested in this chapter (Table 7.1).

The chemical composition of throughfall (TF) and stemflow (SF) water under a forest canopy is the result of incident precipitation, wash-off of dry deposited gases, particles or cloud droplets prior to the precipitation event, and the exchange between the canopy surfaces and the solutions passing over them (Lovett et al. 1996):

$$TF + SF = TD + CE = PD + DD + CE \tag{7.1}$$

where TD is the total deposition, PD the precipitation deposition, DD the dry deposition and CE the canopy exchange.

Total potentially acidifying deposition (TD_{ac}) can then be defined as the sum of TD of NO_3^- and NH_4^+ (TD_N), SO_4^{2-} (TD_S) and Cl^- (TD_{Cl}) corrected for the neutralizing effect of base cations (TD_{BC}; Na^+, K^+, Ca^{2+} and Mg^{2+}) (UBA 2004):

$$TD_{ac} = TD_N + TD_S + TD_{Cl} - TD_{BC} \tag{7.2}$$

The net throughfall (NTF) of an ion is defined as the difference between TF (+ SF; if available) and PD, and equals the sum of DD and CE.

$$NTF = TF(+SF) - PD = DD + CE \tag{7.3}$$

The aim of the canopy budget model is to distinguish DD from CE for all major ions. Positive values for CE represent canopy leaching (CL) and negative canopy uptake (CU). However, when we refer to CL or CU specifically, positive values are used. In the model, all fluxes are expressed on an equivalent basis (mol_c) per unit ground surface area and time. Further on, SF is not explicitly mentioned, but is included in TF for datasets where this flux is available. In the following subsections, the various approaches for each time step are discussed. Similar to Chapter 5 and 6, the term canopy uptake (CU) is used for N compounds, although the term canopy retention would be more appropriate (see Chapter 4).

7.3.3.1 Time step

The canopy budget model is usually applied at an annual time step, although some studies have used it on a semi-annual (Chapter 5-6), phenological (Staelens et al. 2007) or 1-week time step (Duchesne et al. 2001). Although the effect of the time step as such was not found to be very high for the two cases considered by Staelens et al. (2008), a higher temporal resolution allows to introduce different NH_4^+/NO_3^- uptake ratios and to exclude canopy uptake (CU) of NH_4^+ and NO_3^- during the leafless season (see below). Therefore, the model was applied on a phenological, semi-annual and annual basis.

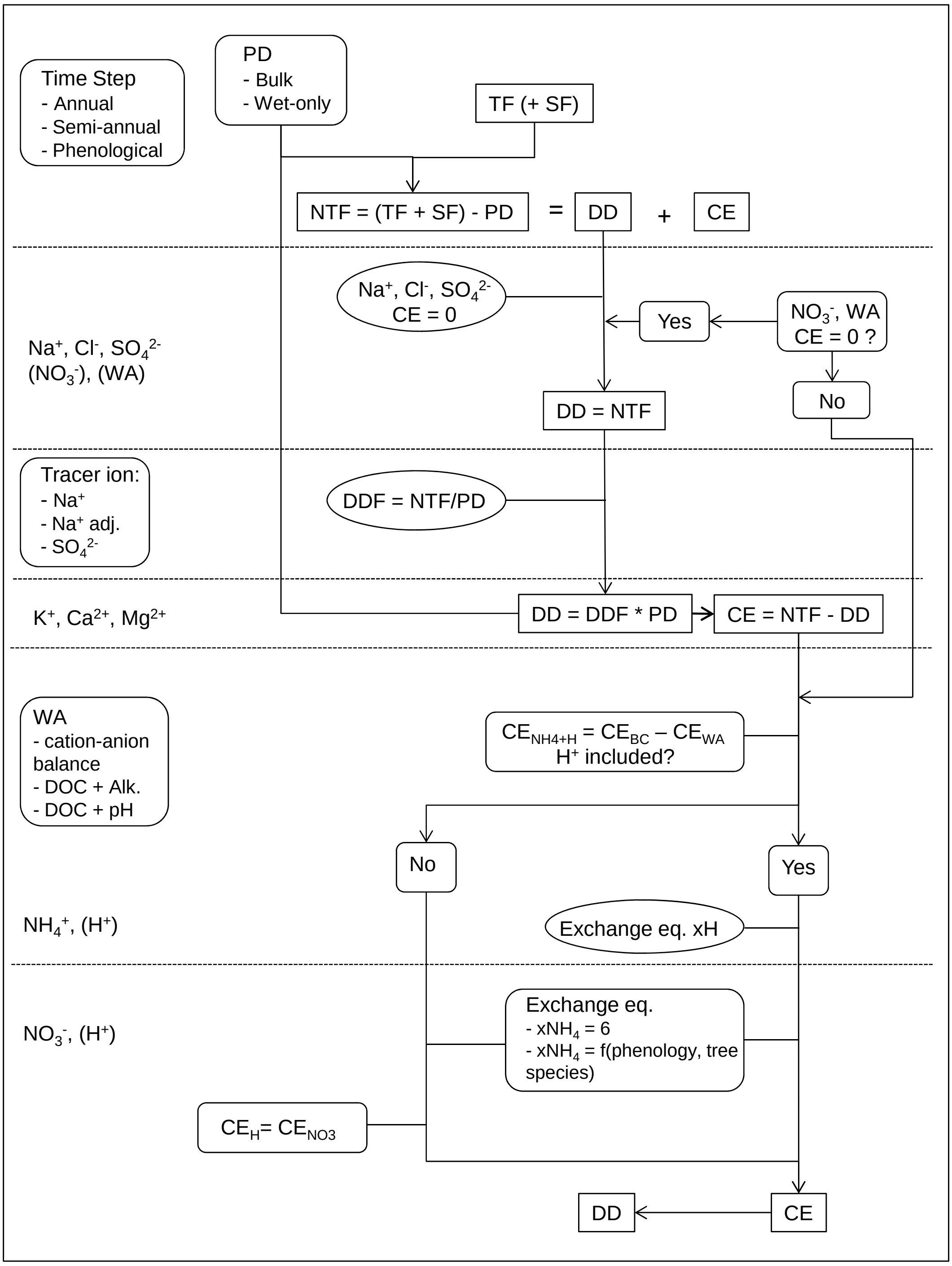

Fig. 7.1 Flow chart of the canopy budget model, extended from Staelens et al. (2008). The measured deposition of major ions in precipitation deposition (PD) and throughfall (+ stemflow) deposition (TF (+SF)) is used to calculate net throughfall deposition (NTF), which consists of dry deposition (DD) and canopy exchange (CE). Ellipses are used to indicate model assumptions about the deposition factor (DDF) and on CE processes. Rounded rectangles indicate model assumptions for which various approaches were tested. Dotted horizontal lines separate equations used for different ions or ion groups. WA: weak acids; Na^+ adj.: Na^+ as tracer ion with adjusted dry deposition of K^+; BC: base cations; DOC: dissolved organic carbon; xH: exchange efficiency of H^+ relative to NH_4^+; xNH_4: exchange efficiency of NH_4^+ relative to NO_3^-

Table 7.1 Overview of the different tested approaches at each step of the canopy budget model (see also Fig. 7.1). The number of approaches at each step is given in parentheses, which resulted in a total of 600 different canopy budget models

Step	Tested approaches
Time step (3)	Phenological
	Semi-annual
	Annual
Precipitation deposition (2)	Wet-only data derived from bulk deposition measurements
	Bulk deposition measurements
Tracer ion (3)	Na^+
	Na^+ with adjustment for a lower dry deposition rate of K^+
	SO_4^{2-}
Canopy leaching of weak acids (4)	Not included
	Weak acids estimated based on cation-anion balance
	Weak acids estimated based on DOC measurements and alkalinity
	Weak acids estimated based on DOC measurements and pH
Canopy uptake of NO_3^- (5)	Not included
	$CU(NH_4+H)=CL(BC)$; $xNH_4=6$
	$xNH_4=f$(phenology, tree species) (Table 7.1)
	$CU(NH_4)=CL(BC)$; $xNH_4=6$
	$xNH_4=f$(phenology, tree species) (Table 7.1)
Canopy uptake of NH_4^+ and NO_3^- in the leafless season (2)*	Included
	Not included

*Not applicable to coniferous species. CU: canopy uptake, BC: base cations, xNH_4: relative uptake efficiency of NH_4^+ compared to NO_3^-

7.3.3.2 Precipitation deposition

Precipitation deposition can be measured by wet-only and bulk deposition devices. In general, bulk deposition measurements are less accurate due to dry deposition of particles and gases onto the collecting surfaces, hereby overestimating PD and underestimating NTF (see Eq. 7.3). In this chapter, wet-only to bulk ratios determined at the beech site in 2003 (Staelens et al. 2005) were used to correct bulk precipitation data at the beech and oak-pine sites: 0.68 (Na^+), 0.66 (Cl^-), 0.44 (K^+), 0.54 (Ca^{2+}), 0.67 (Mg^{2+}), 0.76 (SO_4^{2-}-S), 0.79 (NO_3^--N), 2.70 (H^+) and 0.78 (NH_4^+-N).

7.3.3.3 Tracer ion

A filtering approach is used to estimate the DD of base cations K^+, Ca^{2+} and Mg^{2+} (Ulrich 1983). In this approach, aerosols containing base cations are assumed to be deposited with equal efficiency to the forest canopy as particles containing an inert tracer ion. This tracer ion is assumed not to be subject to canopy leaching (CE = 0). The DD of a base cation X (DD_X) is then calculated as:

$$DD_X = BD_X \cdot \frac{NTF_{tracer\ ion}}{BD_{tracer\ ion}} \tag{7.4}$$

Na^+ is considered most suitable as tracer ion but also Cl^- (Bouya et al. 1999) and SO_4^{2-} (Ignatova and Dambrine 2000; Ukonmaanaho and Starr 2002; Berger et al. 2008; Chapter 5) have been used, although Cl^- and SO_4^{2-} may overestimate DD_X if significant gaseous deposition of HCl or SO_2 occurs (Staelens et al. 2008). In Chapter 5, Na^+ was used for the beech site and SO_4^{2-} for a Danish spruce site. Therefore, the effect of these two ions as tracer ion was tested. In addition, it has been suggested (Draaijers and Erisman 1995; Ruijgrok et al. 1997; Staelens et al. 2008) that the deposition efficiency of K^+, Ca^{2+}, Mg^{2+} and Na^+ may differ according to their mean mass diameter (MMD). Especially for K^+ this may hold since its MMD amounts to only 30 % of that of Na^+. Results of DD measurements of K^+ on artificial foliage deviated from calculated DD by the canopy budget model on the beech site, in contrast to Ca^{2+} and Mg^{2+} (Chapter 6). Therefore, in a third approach the DD of K^+ was adjusted by the ratio of the MMD of K^+ to that of Na^+, measured to be 0.32 in the Netherlands in 1993 (Ruijgrok et al. 1997). Because of the assumed negligible CE, the DD of Na^+, Cl^- and SO_4^{2-} equals their NTF. Canopy leaching of K^+, Ca^{2+} and Mg^{2+} (CL_X) was obtained from the difference between NTF_X and DD_X (Eq. 7.3).

7.3.3.4 Canopy leaching of weak acids

Ion uptake of NH_4^+ and H^+ is modelled based on the assumption that ion exchange is the main process at the plant-water interface (Staelens et al. 2008). Several studies have indicated a relationship between the loss of NH_4^+ (Stachurski and Zimka 2002; Umana and Wanek 2010) or H^+ (Schaefer et al. 1988; Lovett et al. 1996; Chiwa et al. 2004) and the leaching of base cations in throughfall water. This is also supported by a relationship between $^{15}NH_4^+$ uptake and NTF of base cations in Chapter 4. In the reference approach, CU of NH_4^+ (CU_{NH4}) and H^+ (CU_H) is estimated from the CL of base cations (CL_{BC}):

$$CU_{NH_4^+ + H^+} = CL_{BC} \tag{7.5}$$

However, several studies have indicated that CL_{BC} cannot only be attributed to CU of NH_4^+ and H^+, but also to CL of weak organic acids (CL_{WA}) (Chiwa et al. 2004; Zhang et al. 2006; Staelens et al. 2007), which was also observed in Chapter 4. Eq. 7.5 then becomes:

$$CU_{NH_4^+ + H^+} = CL_{BC} - CL_{WA} \tag{7.6}$$

The inclusion of weak acid leaching also requires an estimate of the concentration of weak acids (WA), i.e. the sum of organic acids and bicarbonate, in both precipitation and throughfall water. De Vries et al. (2001) suggested that a calculation based on pH and DOC measurements is the most reliable approach. The measured pH of a water sample is then used to calculate its HCO_3^- concentration. When no DOC data are available, a mixed approach including the calculation of HCO_3^- based on pH and of DOC based on the charge balance has been recommended (de Vries et al. 2001). Alternatively, the concentration of HCO_3^- can be estimated from the measured alkalinity. In this study, these three approaches of estimating weak acids were tested, along with the approach of excluding CL_{WA}. Canopy leaching of weak acids is obtained by subtracting weak acid dry deposition (DD_{WA}) from NTF. Dry deposition is generally assumed to equal the PD of WA (Staelens et al. 2008).

To separate the uptake of NH_4^+ and H^+, a relative uptake efficiency factor is used. Based on a laboratory experiment for Douglas fir, it is assumed that H^+ has per mol an exchange capacity that is six times larger than NH_4^+ (Van der Maas et al. 1991).

$$CU_{NH_4^+} = \frac{TF_{NH_4^+}}{TF_{NH_4^+} + xH.TF_{H^+}}.CL_{BC} \tag{7.7}$$

Canopy uptake of H^+ (CU_H) is then calculated by subtracting the CU_{NH4} from the CU_{NH4+H}.

7.3.3.5 Canopy uptake of NO_3^-

Several field (Stachurski and Zimka 2002; Dail et al. 2009) and laboratory studies (Bowden et al. 1989; Chapter 4) have demonstrated that forest canopies can incorporate NO_3^-, but that preferentially NH_4^+ is retained. However, in the reference canopy budget model, no CU of NO_3^- (CU_{NO3}) is generally included so that the DD of NO_3^- equals its NTF. De Vries et al. (2001) suggested to calculate the CU of NH_4^+ and NO_3^- based on the throughfall fluxes of NH_4^+ and NO_3^-, using an efficiency factor of NH_4^+ vs NO_3^- canopy uptake (xNH_4) with a proposed value of six:

$$CU_{\left(NH_4^+ + NO_3^-\right)} = \frac{xNH_4^+ . TF_{NH_4^+} + TF_{NO_3^-}}{xNH_4^+ . TF_{NH_4^+}} \tag{7.8}$$

However, in Chapter 4 a highly variable NH_4^+/NO_3^- uptake ratio among tree species and phenological stages was observed. Therefore, a tree species specific xNH_4, which also varied for the different phenological stages, was tested in this study (Table 7.2). In case of a semi-annual or annual time step, a time-weighted average of xNH_4 was used in the calculations.

A disadvantage of including CU_{NO3} in the model is that the charge balance of the canopy is not maintained (Staelens et al. 2008). A modification of the model could be to set CU_{NO3} equal to the CU_H, in accordance with previous observations for beech (Stachurski and Zimka 2002). Eq. 7.6 then becomes:

$$CU_{NH_4^+} = CL_{BC} - CL_{WA} \tag{7.9}$$

The CU_{NO3} can then again be calculated by Eq. 7.8 and will result in a higher value, as for NH_4^+. This leads to five possible variations to calculate CU_{NO3}. Dry deposition of NH_4^+, NO_3^- and H^+ is obtained by subtracting the CU from the NTF.

Table 7.2 Ammonium to nitrate uptake ratios used for the calculation of the different canopy budget model versions, based on the results of Chapter 4. For semi-annual and annual time steps a time-weighted average of the ratios was used. For the oak-beech stand a weighted average of values for beech and pendunculate oak was used, based on basal area reported by De Couck. (2011)

	Beech	Pedunculate oak	Scots pine	Oak-beech
Leaf development	2.1	2.9	93.6	2.6
Fully leafed	4.7	4.1	4	4.3
Leaf senescence	7.7	3.9	9.2	5.3
Leafless	4.3	5.1	39.6	4.8
Leafed	5	4.2	16.7	4.5*
Annual	4.8	3.7	49.2	4.1*

*approximate value, ratios used in the time trend analysis vary between years depending on the length of each period

7.3.3.6 Canopy uptake of NH_4^+ and NO_3^- during the leafless period

Several studies have demonstrated retention of $^{15}NH_4^+$ and $^{15}NO_3^-$ by the woody plant surface (Bowden et al. 1989; Boyce et al. 1996; Wilson and Tiley 1998; Dail et al. 2009; see also Chapter 4). However, Chapter 4 also indicated that inorganic N retention by woody surfaces could be due to physicochemical adsorption rather than to actual assimilation. Moreover, in Chapter 4 no relationship between NTF of K^+, Ca^{2+} and Mg^{2+} and $^{15}NH_4^+$ uptake was observed during the leafless season, in contrast to the fully leafed and leaf development periods. This indicates that likely no ion-exchange reaction occurs between plant and atmosphere during the leafless season for deciduous species (CU = 0) and hence that Eq. 7.5, 7.6 and 7.9 are not valid. Since N adsorbed to the plant surface cannot be measured from throughfall measurements, but was generally low compared to retention by leaves (Chapter 4), adsorbed N was assumed to be zero during the leafless season for deciduous species (beech and pedunculate oak).

7.3.4 Data analysis

The combination of all approaches listed above (Table 7.2) resulted in 720 model versions. Since the approach of no canopy uptake of NH_4^+ and NO_3^- during the leafless period could not be calculated with an annual time step, 600 models remained. For the case studies on the beech tree and the coniferous vs. deciduous forest stands no DOC data were available, hereby resulting in 300 different models. For the comparison of forest types, deposition ratios between the coniferous and deciduous stand were evaluated. Time trends in annual deposition fluxes on the oak-beech stand were investigated through Kendall tests (Kendall 1975) to detect monotonically increasing or decreasing trends. This non-parametric regression method is based on the ranks of the observations and does not assume a fixed relationship in the time trend. Kendall's tau statistic represents the non-parametric correlation between a time series of data and time. When a linear trend can be assumed, the median slope and trend line is calculated non-parametrically.

The effect of different model versions was evaluated for two summarizing variables, i.e. the total N deposition ($NH_4^+ + NO_3^-$) (TD_N) and the potentially acidifying deposition (TD_{ac}; Eq. 7.2 in Section 7.3.3). Per considered time step (Table 7.2), the effect of different steps in the model, i.e. the type of precipitation data, the tracer ion, the CL of weak acids, the NO_3^- uptake and the CU of N during the leafless season (Table 7.2) was assessed by means of a multi-way Analysis of Variance (ANOVA). To evaluate model steps for the time trend analysis, a nonparametric Permanova test was performed on Kendall's tau and the median slope (Anderson 2001). The effect of time step was assessed by a one-way ANOVA. All calculations and statistical analyses were performed with R version 2.13.0 (R Development Core Team 2011). The obtained range in TD_N and TD_{ac} of the different stands in this study was compared with site-specific critical loads of acidity and nutrient N (eutrophication), determined with the widely-used simple mass balance model (UBA, 2004). Input data were as much as possible derived from site-specific measurements or regional data, as described by Staelens et al. (2009).

7.4 Results

7.4.1 Deposition onto an individual beech canopy

Application of the 300 different canopy budget models on the data for the beech canopy resulted in a canopy exchange (CE) ranging from -20 to 0 kg N ha^{-1} yr^{-1} and a dry deposition (DD) ranging from 6 to 28 kg N ha^{-1} yr^{-1} (Fig. 7.2). The highest canopy uptake (CU; first frequency class in Fig. 7.2) was calculated using Na^+ as a tracer (without adjustment for a different dry deposition rate of K^+), excluding CL of weak acids (WA) and including CU of N during the leafless season. The lowest CU (last frequency class in Fig. 7.2) was obtained with SO_4^{2-} as a tracer ion, including CL of WA, using bulk precipitation data and with a phenological time step. This lower CU of N was caused by occasionally negative canopy leaching (CL) of base cations or a high value for the estimated CL of WA, especially during leaf development. Lowest DD of N was calculated using SO_4^{2-} as a tracer ion, including CL of WA and using bulk precipitation data, while the highest DD was obtained with Na^+ as a tracer ion (with or without adjustment for K^+), excluding CL of WA, using wet-only precipitation data and including CU of N during the leafless season. When using a phenological time step, calculated DD of N during the leaf development period was negative when CL of WA was included.

The highest variance in total N deposition (TD_N) could be explained by CL of WA, followed by CU of N during the leafless season (in case of a phenological or semi-annual time step), the tracer ion used and the precipitation data. For total acidifying deposition (TD_{ac}) the highest variance could be explained by the tracer ion used, CL of WA and CU of N during the leafless season (Table 7.3). For TD_N, all factors were found to be significant, while for TD_{ac} the effect of NO_3^- uptake was not significant in the semi-annual and annual time step. Time step itself also had no significant effect both on TD_N ($p = 0.838$) and TD_{ac} ($p = 0.998$).

Including CL of WA significantly lowered CU of NH_4^+ and consequently lowered DD_N, TD_N and TD_{ac} (Fig. 7.3). The use of SO_4^{2-} as a tracer ion increased estimated DD of base cations compared to Na^+, hereby decreasing CL of these base cations and CU and DD of NH_4^+. This decreased TD_N but increased TD of base cations resulted in a greatly reduced TD_{ac}. Using bulk precipitation data decreased TD_N and TD_{ac} estimates. The CU of NO_3^- explained the least variance in the model results. A significant difference between excluding and including CU of NO_3^- was only found for TD_N.

Table 7.3 Degrees of freedom (df), F values, Sum of Squares and p values of the multi-way ANOVA analysis assessing the effect of different steps in the canopy budget model on total nitrogen (N) and total acidifying deposition to an individual beech tree. Bold values are significant at $p < 0.05$

		Total N deposition			Total acidifying deposition		
Source of variation	Df	Sum of Squares	F	p	Sum of Squares	F	p
a) Phenological time step							
Precipitation	1	215	142.9	< **0.001**	1237285	97.2	< **0.001**
Tracer ion	2	749	248.6	< **0.001**	18150635	712.7	< **0.001**
Weak acids	1	2308	1532.1	< **0.001**	11760662	923.6	< **0.001**
NO_3^- uptake	4	29	4.9	**0.001**	150065	3.0	**0.023**
Canopy uptake leafless	1	507	336.5	< **0.001**	2581627	202.7	< **0.001**
b) Semi-annual time step							
Precipitation	1	237	153.9	< **0.001**	1447603	8.3	< **0.001**
Tracer ion	2	798	258.7	< **0.001**	19108368	14.4	< **0.00**
Weak acids	1	1472	954.4	< **0.001**	7499000	60.8	< **0.001**
NO_3^- uptake	4	22	3.6	**0.008**	113115	2.1	0.084
Canopy uptake leafless	1	507	328.5	< **0.001**	2583094	93.2	< **0.001**
c) Annual time step							
Precipitation	1	147	12.5	< **0.001**	906756	3.0	< **0.001**
Tracer ion	2	864	46.1	< **0.001**	15011633	74.2	< **0.001**
Weak acids	1	576	54.9	< **0.001**	2936651	26.4	< **0.001**
NO_3^- uptake	4	17	4.9	**0.002**	84886	1.7	0.163

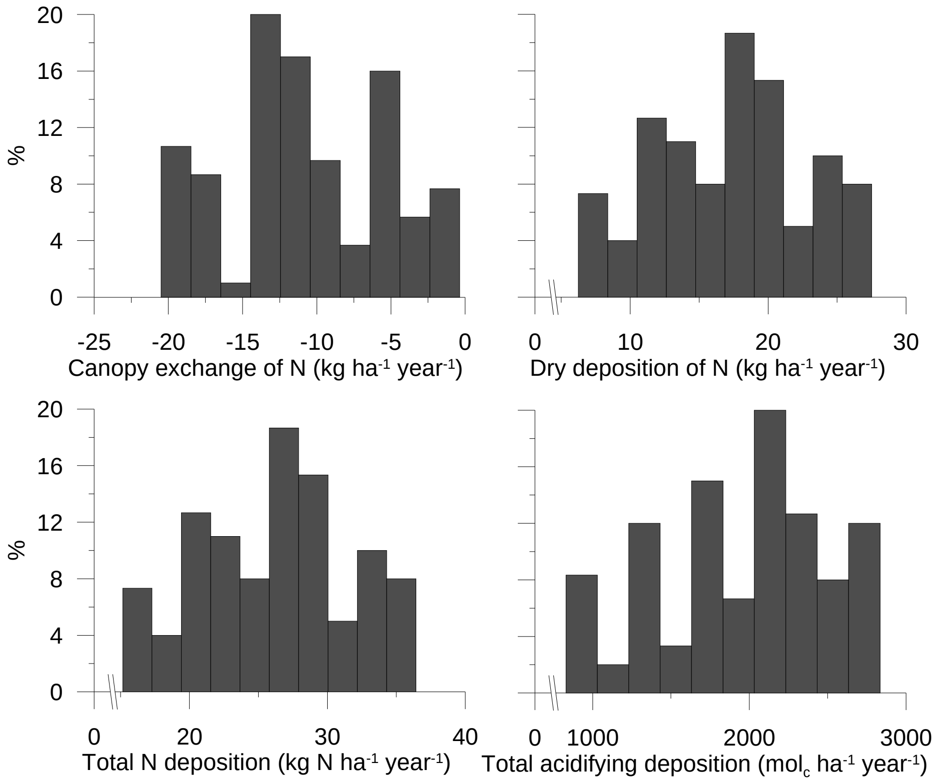

Fig. 7.2 Distribution (%) of calculated canopy exchange, dry and total nitrogen (N) deposition (kg ha^{-1} yr^{-1}) and total potentially acidifying deposition (mol$_c$ ha^{-1} yr^{-1}) on the individual beech tree calculated by 300 different canopy budget model versions

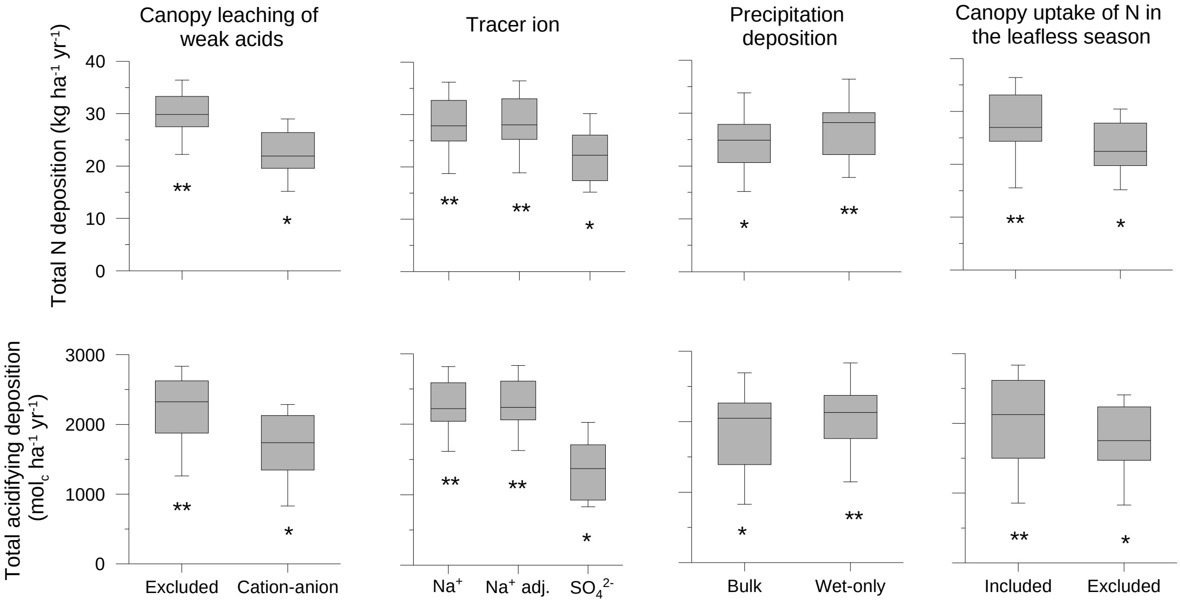

Fig. 7.3 Boxplots showing the effect of each step in the canopy budget model on total nitrogen (N) deposition (kg ha^{-1} yr^{-1}) and total potentially acidifying (mol$_c$ ha^{-1} yr^{-1}) deposition on the individual beech tree. Stars indicate significant differences between different levels of each step

7.4.2 Deposition ratio between a coniferous and a deciduous stand

Application of the 300 canopy budget models resulted in a ratio of TD_N (coniferous to deciduous forest stand) between 1.02 and 1.75 and a ratio of TD_{ac} (coniferous to deciduous) between 0.68 and 1.46 (Fig. 7.4). The highest variance on the ratios of TD_N and TD_{ac} could be explained by CU of N in the leafless season followed by the CL of WA the tracer ion and the (Table 7.4). No significant effect of NO_3^- uptake was found (except with an annual time step), and the type of precipitation data only significantly affected TD_N deposition ratios for a annual time step. Time step had a significant effect on TD_{ac} ($p < 0.001$) but not on TD_N ($p = 0.128$). Including CL of WA increased the coniferous to deciduous ratio of TD_N and TD_{ac} because TD_N of pedunculate oak decreased more relative to Scots pine (Fig. 7.5). The use of SO_4^{2-} also increased the ratio of TD_N and TD_{ac} because the deposition ratio of SO_4^{2-} was lower than of Na^+. Excluding CU of N during the leafless season significantly decreased TD_N on the pedunculate oak stand and consequently increased the ratio of TD_N and TD_{ac}. Wet-only precipitation data yielded a higher TD_{ac} ratio than bulk precipitation data and a phenological time step significantly decreased the TD_{ac} ratio compared to an annual or semi-annual time step.

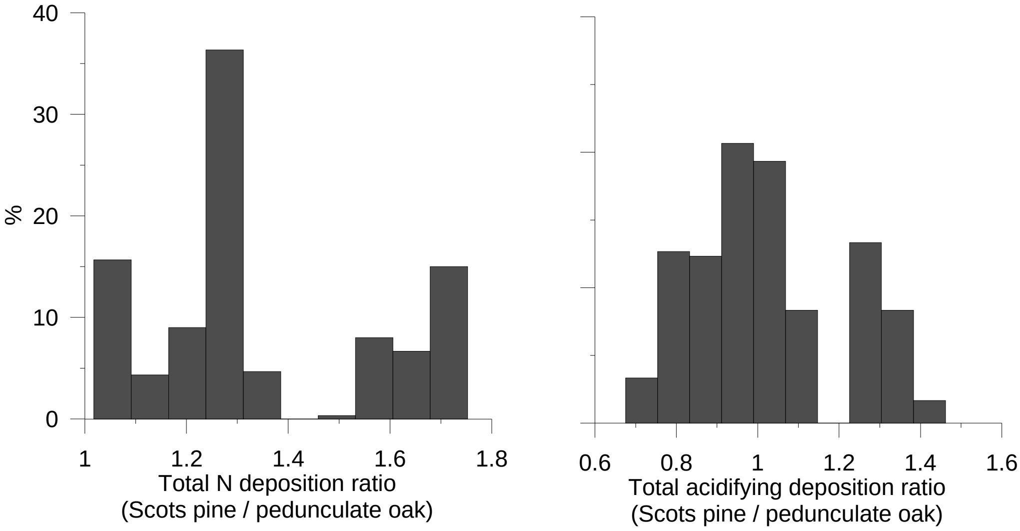

Fig. 7.4 Distribution (%) of the ratio of total nitrogen (N) deposition and total potentially acidifying deposition between a Scots pine and a pedunculate oak stand calculated by 300 different canopy budget model versions

It needs to be mentioned that in the pedunculate oak stand calculated DD values of N during leaf development were negative when CL of WA was included. For the Scots pine stand, CE of Ca^{2+} and/or Mg^{2+} was always negative during leaf development and senescence and CE of NO_3^-, NH_4^+ and H^+ was always positive during leaf development and when CL of WA was included also during other phenological periods.

Table 7.4 Degrees of freedom (df), F values, Sum of Squares and p values of the multi-way ANOVA analysis assessing the effect of different steps in the canopy budget model on the ratio of total N and total acidifying deposition to a coniferous and a deciduous stand. Bold values are significant at $p < 0.05$

		Total N deposition			Total acidifying deposition		
Source of variation	Df	Sum of Squares	F	p	Sum of Squares	F	P
a) Phenological time step							
Precipitation	1	0.00	0.5	0.479	0.19	208.0	< **0.001**
Tracer ion	2	0.02	7.4	< **0.001**	0.97	525.0	< **0.001**
Weak acids	1	1.69	1067.8	< **0.001**	0.53	577.8	< **0.001**
NO_3^- uptake	4	0.01	1.8	0.143	0.01	1.5	0.216
Canopy uptake leafless	1	2.22	1398.9	< **0.001**	1.20	1304.3	< **0.001**
b) Semi-annual time step							
Precipitation	1	0.00	0.7	0.418	0.16	133.02	< **0.001**
Tracer ion	2	0.01	2.3	0.103	0.08	35.4	< **0.001**
Weak acids	1	2.82	1155.8	< **0.001**	1.23	1034.7	< **0.001**
NO_3^- uptake	4	0.00	0.4	0.780	0.00	0.5	0.705
Canopy uptake leafless	1	2.47	1009.9	< **0.001**	1.53	1282.9	< **0.001**
c) Annual time step							
Precipitation	1	0.01	28.9	< **0.001**	0.18	1270.3	< **0.001**
Tracer ion	2	0.01	13.0	< **0.001**	0.09	308.6	< **0.001**
Weak acids	1	3.55	1244.5	< **0.001**	0.79	12775.2	< **0.001**
NO_3^- uptake	4	0.00	2.8	**0.038**	0.00	3.6	**0.011**

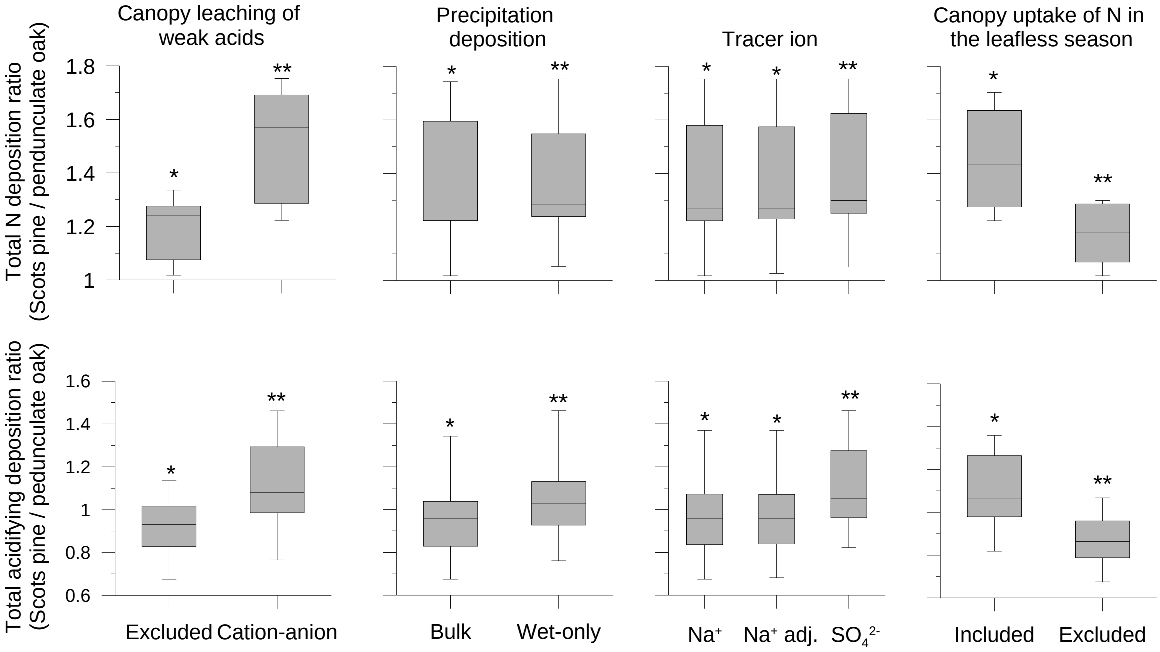

Fig. 7.5 Boxplots showing the effect of each step in the canopy budget model on the ratio of total nitrogen (N) deposition and total potentially acidifying deposition between a Scots pine and a pedunculate oak stand. Stars indicate significant differences between different levels of each step

7.4.3 Determination of trends in deposition

According to the Kendall tests, yearly TD_N on the beech site calculated by the 600 different canopy budget models showed a significant ($p < 0.05$) time trend over 1999-2010 for 66 % of the models (Fig. 7.6). Kendall's tau value varied from -0.76 for the highly significant time trends to -0.03 for non-significant time trends. The median slope varied from -1.75 to -0.05 mol_c N ha^{-1} yr^{-1} (Fig. 7.6). Yearly TD_{ac} showed a significant ($p < 0.05$) time trend for 58 % of the tested models. Kendall's tau value varied from -0.70 to -0.12 and the median slope from -197 to -26 mol_c N ha^{-1} yr^{-1} (Fig. 7.6).

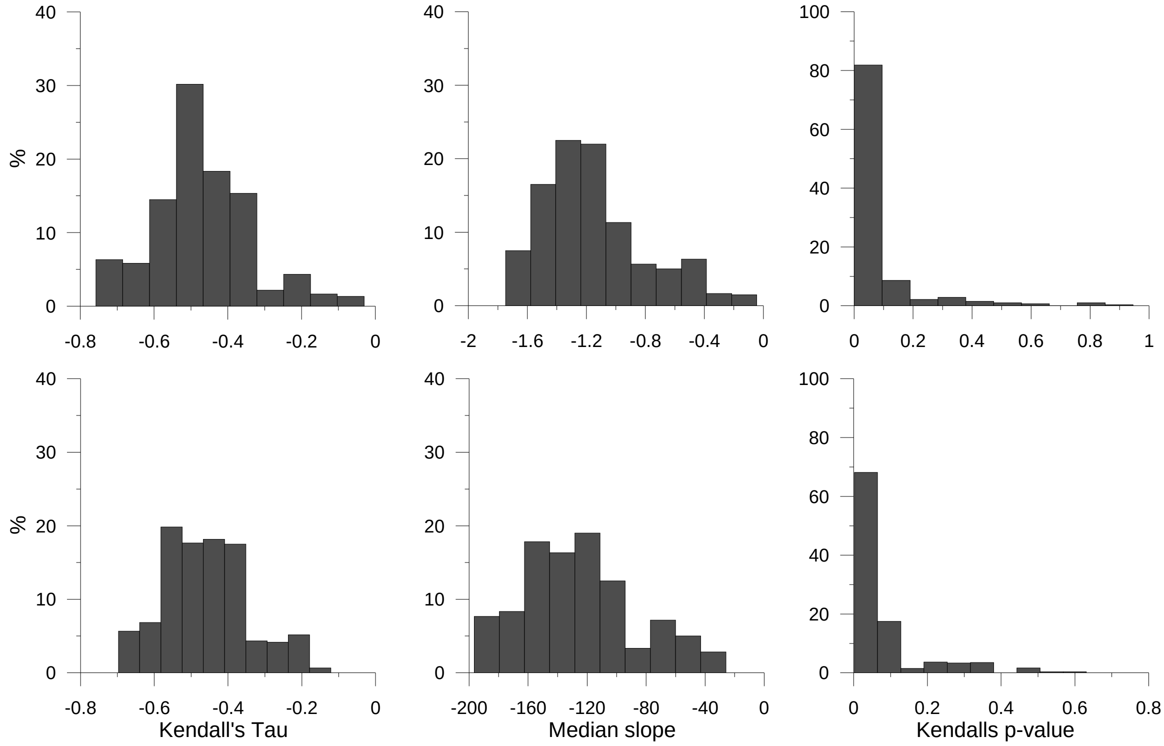

Fig. 7.6 Distribution (%) of Kendalls tau, estimated median slope (a: kg N ha^{-1} yr^{-1}; b: mol_c ha^{-1} yr^{-1}) and p values obtained by applying Kendall tests on time series of (a) total nitrogen (N) deposition (kg ha^{-1} yr^{-1}) and (b) total potentially acidifying deposition (mol_c ha^{-1} yr^{-1}) calculated by 600 canopy budget model versions

The variation in Kendall's tau values for TD_N and TD_{ac} could mainly be explained by the CL of WA followed by the type of precipitation data, the tracer ion and the CU of N in the leafless season, although the last factor was not significant for a semi-annual time step (Table 7.5). Choices regarding the CU of NO_3^- had no significant effect on the tau value of TD_N and TD_{ac}. Using wet-only precipitation data decreased TD_N by 13 % from 1999 to 2010, while TD_N reduced by 11 % with bulk precipitation data. Similarly, TD_{ac} decreased by 22 and 19 % using wet-only and bulk data, respectively (Fig. 7.7). Using SO_4^{2-} as a tracer reduced TD_N and TD_{ac} with 21 and 34 %, respectively, compared to 8 and 13 %, respectively, for Na^+. No significant difference was observed between Na^+ and Na^+ adjusted to reduce dry K^+ deposition. Furthermore, no significant trend was found for TD_N and TD_{ac} when CL of weak acids was estimated from the difference between cations and anions (−15 % TD_N and −3.5 % TD_{ac}; in 12 years). In contrast, this trend was highly significant when WA were estimated from DOC and alkalinity measurements (−25 % TD_N and −30 % TD_{ac}). Excluding CL of WA and including WA estimated from DOC and pH measurements showed intermediate but significant trends. Estimated WA in TF are approximately the same for the cation-anion balance and DOC combined with alkalinity measurements, while in PD estimated WA from DOC combined with alkalinity measurements were approximately four times higher. Excluding CU of N during the leafless season reduced TD_N and TD_{ac} with 12 and 20 %, respectively, and including with 14 and 22 %, respectively.

Table 7.5 Degrees of freedom (df), F values, Sum of Squares and P values of the multi-way ANOVA analysis assessing the effect of different steps in the canopy budget model on Kendalls tau of total N and total acidifying deposition on a deciduous oak-beech stand. Bold values are significant at $p < 0.05$

Source of variation	Df	Total N deposition Sum of Squares	 F	 p	Total acidifying deposition Sum of Squares	 F	 P
a) Phenological time step							
Precipitation	1	0.04	21.0	< **0.001**	0.04	35.6	< **0.001**
Tracer ion	2	0.43	107.1	< **0.001**	0.71	285.5	< **0.001**
Weak acids	1	1.92	316.4	< **0.001**	1.40	375.8	< **0.001**
NO_3^- uptake	4	0.01	1.0	0.374	0.00	0.5	0.777
Canopy uptake leafless	1	0.38	185.8	< **0.001**	0.11	89.0	< **0.001**
b) Semi-annual time step							
Precipitation	1	1.03	180.2	< **0.001**	0.06	58.9	< **0.001**
Tracer ion	2	5.02	440.2	< **0.001**	1.02	485.6	< **0.001**
Weak acids	1	22.55	1317.9	< **0.001**	2.47	784.7	< **0.001**
NO_3^- uptake	4	0.03	1.2	0.316	0.01	1.4	0.220
Canopy uptake leafless	1	0.01	0.9	0.320	0.09	89.9	< **0.001**
c) Annual time step							
Precipitation	1	0.97	172.2	< **0.001**	0.03	34.7	< **0.001**
Tracer ion	2	3.81	337.2	< **0.001**	0.54	308.8	< **0.001**
Weak acids	1	18.00	1060.7	< **0.001**	1.33	505.4	< **0.00**
NO_3^- uptake	4	0.05	2.1	0.084	0.00	0.9	0.495

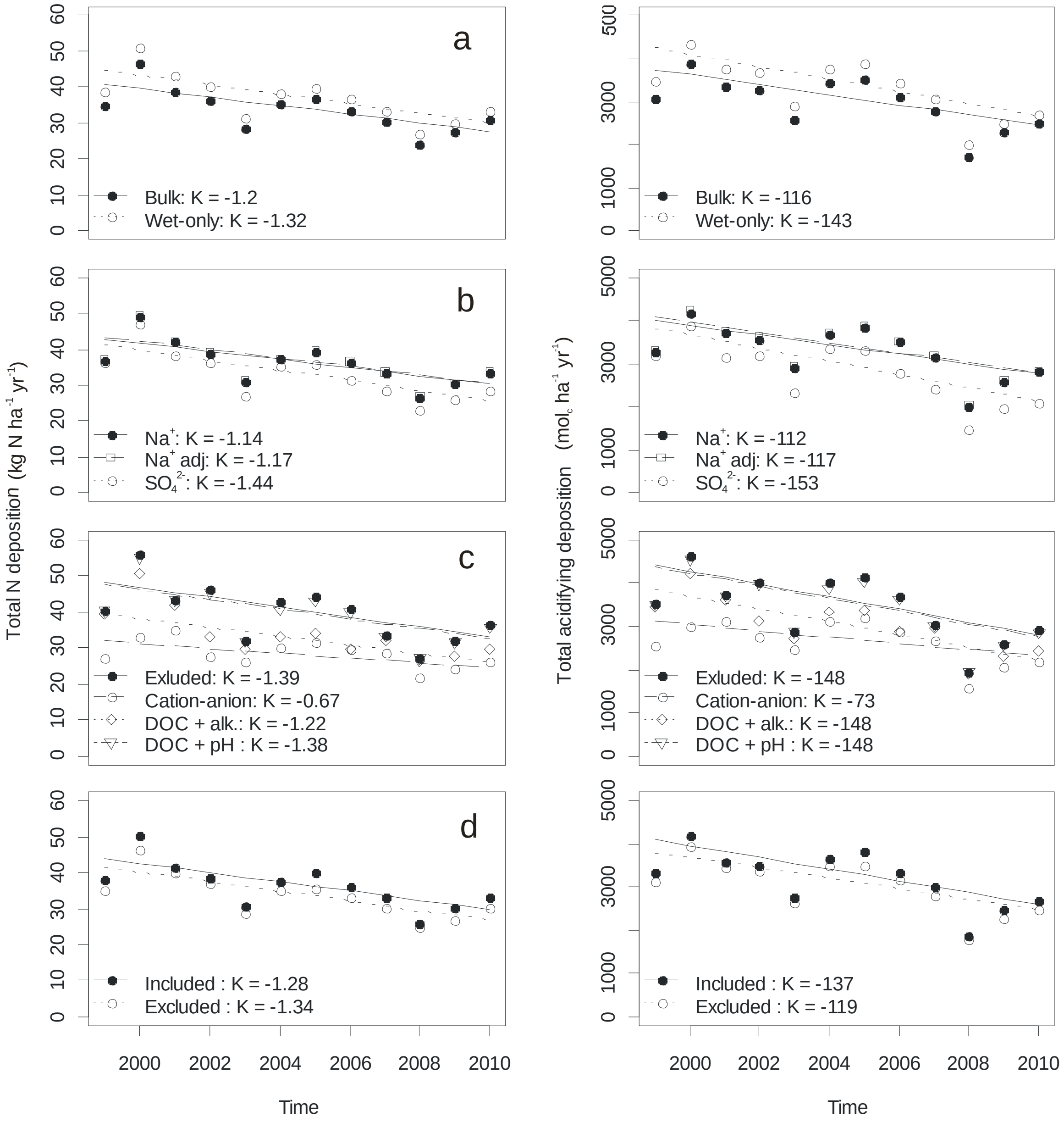

Fig. 7.7 Time series of total nitrogen (N) deposition and total potentially acidifying deposition calculated with canopy budget models using varying (a) precipitation data, (b) tracer ion, (c) canopy leaching of weak acids and (d) canopy uptake of N during the leafless season. The estimated median slope K (kg N ha^{-1} yr^{-1}) of each approach is given

7.5 Discussion

7.5.1 Effect of time step, type of precipitation data and tracer ion

Using different time steps in the canopy budget model allowed introducing different canopy exchange (CE) approaches, which are discussed further in this paragraph. However, there was also an effect of time step as such, with phenological or semi-annual time steps generally resulting in lower nitrogen and potentially acidifying deposition

(TD_N and TD_{ac}, respectively), lower TD_{ac} ratios between a coniferous and deciduous stand and in steeper decreasing deposition time trends, i.e. lower Kendall tau values and median slopes. The negative DD values of NO_3^-, NH_4^+ and H^+ calculated during the period of leaf development may be caused by 1) underestimated CL of base cations which is in turn caused by overestimated DD of base cations or 2) overestimated canopy leaching (CL) of weak acids (WA) by the cation-anion balance. The first cause was confirmed by negative CE values of Ca^{2+} and Mg^{2+} during the period of leaf development. This indicates that both Na^+ and SO_4^{2-} may be subject to leaching during leaf development (Reiners and Olson 1984; Staelens et al. 2007), which makes them less suitable as tracer ion. Staelens et al. (2007, 2008) suggested solving this by using the DDF of Na^+ calculated for the fully leafed period in the leaf development and senescence period. However, for the Scots pine stand this will not be sufficient as CE of Ca^{2+} and Mg^{2+} was also negative during the leafless period. Hence, more research is needed on the assumption of inertness for the tracer ion and the deposition efficiency of different particles.

Although the type of precipitation data was generally not the main source of variance between model versions, it did have a significant effect on the calculated TD_N and TD_{ac} in the three case studies. In particular for Na^+, Cl^-, K^+, Ca^{2+} and Mg^{2+}, which have high wet-only to bulk ratios, the NTF was significantly reduced when bulk instead of wet-only precipitation deposition was used. This in turn reduced CE of base cations and consequently CE of DD of N compounds. In general, the difference in TD_N or TD_{ac} between bulk and wet-only precipitation data increased when throughfall deposition increased, i.e. throughfall of Scots pine was higher than of pedunculate oak and higher in 1999 compared to 2010.

Wet-only to bulk deposition ratios used in this study were measured during nine months in 2003 on the beech site. As this ratio between wet-only and bulk deposition measurements depends on local gas and aerosol concentrations, turbulence intensities and the collection efficiency of the samplers (Staelens et al. 2005), they could have changed in 2010 due to e.g. decreasing base cation deposition (Verstraeten et al. 2010) or different meteorological conditions. For the trend analysis, wet-only to bulk ratios may also have varied throughout the years. This will reduce the effect of the type of precipitation data on TD_N and TD_{ac} for the individual beech tree. However, the aim of this study was to simulate the effect of using bulk deposition measurements instead of wet-only deposition measurements. As the ratios used in this study were generally well in range with other studies (Table 1 in Staelens et al. 2005), they may serve as a valuable input for simulation.

As indicated in Section 7.3.3.3, using SO_4^{2-} as tracer ion may significantly overestimate DD of base cations if significant gaseous SO_2 deposition occurs, and consequently underestimate DD of N. This is confirmed by the present study: TD_N to the beech canopy was significantly lower with SO_4^{2-} than with Na^+ as a tracer ion; and SO_2 deposition at this site was estimated to be 50 $mmol_c$ m^2 yr^{-1}, while almost no particulate SO_4^{2-} was deposited on artificial foliage (see Chapter 6). The ratio of TD_N and TD_{ac} between a coniferous and deciduous stand were significantly affected by the tracer ion due to the fact that in this case the throughfall deposition ratio of SO_4^{2-} was lower than of Na^+. An opposite effect is expected if the throughfall deposition ratio of SO_4^{2-} would be higher than of Na^+. In the time trend analysis of the oak-beech stand, steeper decreasing trends were observed with SO_4^{2-} as tracer ion since both the precipitation and throughfall deposition of SO_4^{2-} decreased more significantly from 1999 to 2010 than for Na^+. It is clear that for regions with high SO_2 deposition, Na^+ can be considered to be a more reliable tracer ion. In some cases, such as in coastal regions (Chapter 5) the use of SO_4^{2-} may be justified if particle deposition dominates DD of S, but then still the generally finer SO_4^{2-} particles (Lindberg et al. 1986; Ruijgrok et al. 1997) will be deposited with lower deposition efficiency (Slinn 1982) and results need to be handled with care.

We did not observe a significant effect between using Na^+ as a tracer ion and using Na^+ adjusted for a lower DD of K^+ in the three case studies. This was mainly due to the fact that atmospheric K^+ deposition is generally low, so that the impact of a reduced DD of K^+ was not detectable in the output of the canopy budget model (here summarized by TD_N and TD_{ac}). However, TD_{ac} for the individual beech tree and the ratio of TD_{ac} between the coniferous and deciduous stand were slightly increased when using Na^+_{adj}. We only adjusted the DDF of K^+ as this was suggested by several authors (Ruijgrok et al. 1997; Staelens et al. 2008) and also concluded from Chapter 6. Furthermore, the ratio between the MMD of K^+ and Na^+ was based on data of Ruijgrok et al. (1997) assuming a linear relationship between particle diameter and deposition efficiency for particles larger than 0.5 μm (Slinn 1982). However, this relationship may be inadequate under some circumstances, e.g. particular meteorological conditions or different geometry of the surface (Garland 2001). Further research could help to reveal these questions and maybe suggest an adjusted DDF for Ca^{2+} and Mg^{2+} too, which will increase the impact on TD_N and particularly TD_{ac}.

7.5.2 Effect of canopy exchange calculations: canopy leaching of weak acids, NO_3^- uptake and canopy uptake of N during the leafless season

In the three case studies, a strong effect of including or excluding CL of WA estimated by the cation-anion balance was observed. Especially for beech and oak significant CL of WA was calculated, while this was less important for the pine stand, i.e. leaching of WA was on average 37 % higher in the oak stand than in the pine stand. This is in agreement with base cation leaching in the studied pair of coniferous-deciduous stands, i.e. 53 % higher in the oak than in the pine stand, and in literature (Rothe et al. 2002; De Schrijver et al. 2007). In the trend analysis, CL of WA decreased significantly from 1999 to 2010, hereby decreasing the downward trend of TD_N. This high impact of including WA in the canopy budget model illustrates its importance, especially in deciduous stands where CL of WA contributed on average 50 % to CL of base cations. Estimating WA by means of the cation-anion balance was considered to be a reliable approach by de Vries et al. (2001), given that all other major ions are analysed precisely and accurately (Staelens et al. 2008). However, in the trend analysis the cation-anion approach differed significantly from the two DOC-based approaches. Using DOC measurements combined with HCO_3^- estimated from pH yielded very low WA estimates and resulted in similar TD_N and TD_{ac} values as excluding WA from the canopy budget model. In contrast, DOC measurements combined with HCO_3^- estimated from measured alkalinity decreased TD_N and TD_{ac}, but to a lesser extent than by using the cation-anion balance for WA. Weak acids in throughfall and PD estimated by the DOC approaches showed no time trend and consequently the trend analysis of TD_N and TD_{ac} estimated by this approach did not differ from the approach where CL of WA was excluded. In general, measuring WA can be preferred above estimating them. However, estimated HCO_3^- from alkalinity may contain some organic acids and calculated HCO_3^- from pH may be less accurate due to an uncertainty in pH measurements on weakly buffered water samples like precipitation samples (INBO, personal communication). Hence, more research is needed with regard to the estimation of WA in precipitation and throughfall samples.

In Chapter 2 and 5, a low but significant uptake of wet deposited NO_3^- by tree leaves was observed, particularly during the period of leaf development. Incorporating this NO_3^-

uptake in the canopy budget model increased the calculated TD_N on the individual beech tree significantly and is recommended given the clear experimental evidence. Nevertheless, this factor had neither effect on the comparison between a coniferous and a deciduous stand nor on the trend analysis and might be less important here. Also, TD_N did not differ between the different calculation approaches of NO_3^- uptake, suggesting that the proposed xNH_4 efficiency factor of six by de Vries et al. (2001) may also be suitable and that no differentiation according to leaf phenology is necessary. However, the studied tree species in this thesis were all shown to preferentially retain NH_4^+ compared to NO_3^-. The effect of the different calculation approaches of NO_3^- uptake will likely increase for species that have been shown to preferentially retain NO_3^- in their foliage instead of NH_4^+, such as red spruce (*Picea rubens* Sarg.) (Gómez-Guerrero et al. 2008; Dail et al. 2009). Therefore, it is still suggested to use a tree species specific xNH_4 at a phenological time step when available. Furthermore, calculating CE of H^+ by Eq. 7.5 or 7.6, often resulted in (impossible) negative DD values for the individual beech tree and the trend analysis, while this was generally not the case when CE of H^+ was determined via the CE of NO_3^-.

In this chapter we introduced the new concept of excluding the CU of NH_4^+ and NO_3^- during the leafless season for deciduous species, based on previous observations (Chapter 4). As expected, this approach significantly reduced TD_N and TD_{ac} for the individual beech tree (Fig. 7.3) and the ratios of TD_N and TD_{ac} between a coniferous and deciduous stand (Fig. 7.5). It also decreased the estimated tau value and estimated slope in the trend analysis for TD_N (Fig. 7.7). This approach may be suitable for deciduous trees that were found to retain almost no N in the twigs and stems (Chapter 4). Still, it should be taken into account that in mature temperate deciduous stands, some N can be retained by lichens and microbial assimilation processes (Reiners and Olson 1984), which were shown to occur mainly on older trees (Fritz et al. 2008; Ranius et al. 2008). Several studies have reported the significant contribution of N assimilation by epiphytic lichens to total CU in coniferous (Lang et al. 1976; Johansson et al. 2010) and tropical forests (Clark et al. 2005; Umana and Wanek 2010), but not in deciduous stands. If the latter is also significant, which needs to be investigated, attributing all NTF of N to DD during the leafless period will likely underestimate TD_N. For the Scots pine stand used in this study, CU of N in winter was not excluded since needles are then still present and since significant amounts of ^{15}N were found to be adsorbed to the bark surface, as was suggested by Wilson and Tiley (1998) and Dail et al. (2009). This retained N is likely not subject to ion exchange processes with K^+, Ca^{2+} and Mg^{2+} as assumed in the canopy budget model. However, this remains to be investigated.

7.5.3 Comparison with literature data

Based on this thesis and other literature data (Ignatova and Dambrine 2000; Staelens et al. 2008; Zhang et al. 2006), some canopy budget model approaches can be considered more reliable than others, e.g. Na^+ is a more suitable tracer ion than SO_4^{2-}, and the use of wet-only precipitation data and including CL of WA is recommended. If we only consider these models, DD to the beech canopy was 10-20 kg N ha^{-1} yr^{-1},TD_N was 19-29 kg N ha^{-1} yr^{-1} and TD_{ac} 1664-2285 mol$_c$ ha^{-1} yr^{-1}. This was comparable to the range at the Heidebos site for the oak stand (DD_N: 8-16 kg N ha^{-1} yr^{-1}; TD_N: 20-28 kg N ha^{-1} yr^{-1}; TD_{ac}: 1357-1902 mol$_c$ ha^{-1} yr^{-1}) but higher for the pine stand with regard to N (DD_N: 23-24 kg N ha^{-1} yr^{-1}; TD_N: 34-35 kg N ha^{-1} yr^{-1}; TD_{ac}: 1612-1895 mol$_c$ ha^{-1} yr^{-1}). For the oak-beech stand at the Aelmoeseneie forest in 2010 this was 15-31 kg N ha^{-1} yr^{-1}, 25-41 kg N ha^{-1} yr^{-1} and 2228-3382 mol$_c$ ha^{-1} yr^{-1}, respectively, for DD_N, TD_N and TD_{ac}, respectively. Neirynck et al. (2008) reported much higher DD_N and TD_N for a Scots pine stand in the same region, however, this stand was 70 years old and consequently had a higher collecting surface for dry deposition. The ratio of TD_N between a coniferous and deciduous stand was also within the range reported in a meta-analysis on this topic (Rothe et al. 2002; De Schrijver et al. 2007). To our knowledge, no such comparison has been done for TD_{ac}.

The modelled critical load of acidification was 1960-2285 mol$_c$ ha^{-1} yr^{-1} for the deciduous species at the two sites and 1294 eq ha^{-1} yr^{-1} for pine at the Heidebos site. For eutrophication, the critical loads amounted to 13-14 (deciduous species) and 8 (pine) kg N ha^{-1} yr^{-1}. The above-mentioned ranges for TD_N, all exceed the critical loads for eutrophication, while critical loads for acidification are not all exceeded by the ranges of TD_{ac}, i.e. for the Heidebos site the obtained ranges are below the critical loads and for the beech canopy approximately around the critical load values. This highlights again the importance of the canopy budget model approach used to calculate total atmospheric deposition on forest ecosystems and the need to verify some of the assumptions made.

7.5.4 Local effects

The three case studies for which the different canopy exchange approaches were tested are all situated in the region of Flanders. In the majority of the forests in this region, critical loads for acidifying and eutrophying deposition are exceeded (Craenen et al. 2000; Staelens et al. 2009). It is therefore certainly relevant to quantify total deposition, evaluate trends or compare different forest types for mitigation strategies in this region and this study can be considered as an important contribution to this aim. However, different effects of the canopy exchange calculations might be observed in regions such as China or India, where N and particularly S deposition have more recently started to rise than in Western Europe (Fowler et al. 2009) or in unpolluted boreal forests as Northern Europe (Ukonmaanaho and Starr 2002). The canopy budget model has not been properly evaluated for subtropical and tropical forest types, where large amounts of N are shown to be assimilated by epiphytic and bryophytic lichens (Umana and Wanek 2010). Furthermore, even within temperate forests different results might be obtained for different tree species, e.g. when NO_3^- is retained preferentially compared to NH_4^+ (see 7.5.2).

7.6 Conclusion

In this study varying approaches to parameterize the canopy budget model were used. As such we obtained a range in total and potentially acidifying deposition onto a deciduous forest canopy, deposition ratios between a coniferous and deciduous forest stand and the characteristics (Kendall's tau and median slope) of a deposition time trend analysis. The time step, type of precipitation data, tracer ion, canopy leaching of weak acids and canopy uptake of N during the leafless season all had a significant effect, whereas the canopy uptake of NO_3^- generally had no influence. A correction of bulk to wet-only precipitation data is recommended when available. Sodium can be considered as the most suitable tracer ion, but more research is needed on the assumption of inertness during the period of leaf development for different tree species. Furthermore, an adjustment according to the mean mass diameter for each base cation could improve the estimated DD of these elements. Including canopy leaching of weak acids in the canopy budget model had usually the highest impact in all three case studies, but this could mainly be attributed to the fact that weak acid concentrations in water samples were estimated from a cation-anion balance. However, the strong effect of including weak acid leaching disappeared when weak acids were calculated from DOC and bicarbonate measurements, indicating that the accuracy of analytical measurements is of high importance, especially for rainfall where ion concentrations are generally low. This study presents evidence of the importance of canopy exchange calculations for three relevant case studies in Flanders, a region with enhanced N deposition due to anthropogenic emissions. Since local and tree species-specific effects might also play a role here, similar studies in other regions are recommended. Some indications with regard to the most suitable approach were derived, but it is important to compare this approach to results obtained by other methods to estimate atmospheric deposition.

8 General discussion and conclusions

Human-induced atmospheric deposition of nitrogen (N) and sulphur (S) compounds has significantly altered nutrient cycling in temperate forest ecosystems and caused soil acidification and eutrophication (Chapter 1). As a consequence, the quantification of atmospheric deposition has gained importance to establish cause-effect relationships and evaluate abatement measures and mitigation strategies (Erisman et al. 1994; de Vries et al. 2003; Driscoll et al. 2003). Unfortunately, atmospheric deposition processes of gases and aerosols to forest ecosystems are highly complex and are further complicated by exchange reactions within the canopy (Lovett 1994). Different methods to estimate dry deposition and canopy exchange have been developed the last decades, which are applicable at different spatio-temporal scales. However, it is still a challenge to reconcile differences among existing methods, and a better understanding of the effect of canopy structure, species composition and retention processes is needed (Lovett 1994). Since atmospheric deposition data are aimed to have a direct influence on public policy (Shaver et al. 1994) and since the planetary boundaries for human perturbation of the N cycle are significantly exceeded, research addressing these issues remains important (Rockström et al. 2009; Sutton et al. 2011).

This thesis aimed to address existing knowledge gaps on dry deposition and canopy exchange processes, in particular of N, and to gain a better understanding on the influence the forest canopy exerts on these processes. To this aim, different methods were used to quantify dry deposition and canopy exchange response variables at the branch-, canopy- and stand-level and the effect of the forest canopy on these response variables was assessed. The study focussed on five important tree species, i.e. European beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.), silver birch (*Betula pendula* Roth), Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.). The first four tree species cover a range of sandy to more loamy soils and are widespread in Flanders and North-West Europe (Brus et al. 2011). Norway spruce has been planted throughout North-West and Central Europe since the 19th century to reforest devastated forest land (Berger et al. 2008). In Flanders, much research has been devoted to the conversion of homogeneous Scots pine stands to the closer-to-natural silver birch or pedunculate oak stands to mitigate the effects of atmospheric deposition (De Schrijver

2007; Wuyts 2009). In the surrounding regions, research has often focused on the conversion of homogeneous Norway spruce stands that are vulnerable to drought, insect attacks, storm events and other impacts of climate change to closer-to-natural European beech stands or mixed spruce-beech stands (Berger et al. 2008; Hojjati et al. 2009). Therefore, the considered tree species were chosen with the aim of providing results relevant to forest ecology and management in Flanders and its surrounding regions.

As a second aim, this thesis intended to evaluate the canopy budget model by Draaijers and Erisman (1995) which is used to calculate total atmospheric deposition from throughfall and precipitation measurements. This method is indirect, less costly and less intensive than other deposition methods (see Chapter 6 for an overview) and has the advantage of giving information on internal nutrient cycling as well (Erisman et al. 1994). This canopy budget model was evaluated by using the model at different spatio-temporal scales, comparing it with other methods and conducting a sensitivity analysis.

The results of this thesis led to i) methodological considerations on the quantification of canopy exchange and dry deposition of N, with a focus on the canopy budget model, ii) more insight into the control of the forest canopy on dry deposition and canopy exchange in general and iii) a better understanding of the control of the atmospheric N concentration on dry deposition and canopy exchange. After discussing these three topics, suggestions for further research are provided at the end of this concluding chapter.

8.1 Quantifying canopy exchange and dry deposition of N: methodological considerations

In this thesis, several methods at different spatial scales were used to quantify canopy exchange and dry deposition of inorganic N. This section aims to summarize and discuss the results obtained by these different methods. It needs to be mentioned that the distinction between dry deposition and canopy exchange differs somewhat between the different methods. In Chapter 3 and 4, we refer to “retention” as the sum of actual ^{15}N uptake through the cuticle, bark or stomata on the one hand and ^{15}N adsorption to the plant material on the other hand. This adsorbed ^{15}N is dry deposited $^{15}NH_3$ (Chapter 3) or resulting $^{15}NH_4^+$ of $^{15}NO_3^-$ from a dried water film. The rinsing experiment in Chapter 4 revealed that ^{15}N measured in leaves and needles represents actual uptake, while ^{15}N in twigs mainly consists of ^{15}N adsorbed to the bark surface. Therefore, in Chapter 2, which only considers foliage, the term “uptake” is used. In Chapter 3, foliage and twigs were not rinsed and thus no distinction could be made between uptake and dry deposition. Canopy uptake calculated from the canopy budget model in Chapter 5-7 represents the ion-exchange reaction between the water layer on the forest canopy and the canopy itself, while dry deposition is the sum of particulate and gaseous compounds deposited to the canopy surface. The “canopy” here consists of foliage, twigs and stems, microbiota and epiphytic lichens. The water layer on the forest canopy contains both wet deposited N and dissolved dry deposited N. Dry deposition and immediate stomatal uptake of gaseous compounds is not included in the model. Dry deposition measured by air concentration measurements multiplied with an average deposition velocity (Chapter 6) comprises both surface deposition and direct stomatal uptake.

In Chapter 2-4, the retention of dissolved NH_4^+ and NO_3^- and gaseous NH_3 by foliage and twigs of different tree species was accurately quantified with stable N isotope (^{15}N) tracing. The observed retention in foliage and twigs of dissolved $^{15}NH_4^+$ and $^{15}NO_3^-$ from wet deposition agreed well with other studies using the same method (Brumme et al. 1992; Eilers et al. 1992; Garten et al. 1998; Wilson and Tiley 1998; Dail et al. 2009). When these results were extrapolated to a mature stand, the obtained range of 0.08-2.23 kg N ha^{-1} yr^{-1} was generally below the dissolved inorganic N retention extrapolated from ^{15}N measurements of 9 kg N ha^{-1} yr^{-1} for Norway spruce (Eilers et al. 1992) and 3-10 kg N ha^{-1} yr^{-1} for beech (Brumme et al. 1992), and at the lower edge of the 1-5 kg N ha^{-1} yr^{-1} reported by Dail et al. (2009). However, N deposition levels used in these studies were generally higher than ambient levels. Based on net throughfall (NTF) measurements of $^{15}NH_4^+$ and $^{15}NO_3^-$, canopy retention of dissolved N ranged from 2 to 19 kg N ha^{-1} yr^{-1} (Chapter 4). The large difference between ^{15}N retrieved in foliage and twigs and ^{15}N calculated from throughfall was mainly due to a loss of NH_4^+, which could have been re-emitted to the atmosphere, transported to other plant parts, washed off with the rinsing water or converted to dissolved organic N (DON). Total canopy retention, i.e. from dry and wet deposition, has been calculated to range from 1-57 kg N ha^{-1} yr^{-1} (Lovett and Lindberg 1993; Harrison et al. 2000; Sievering et al. 2007; Neirynck et al. 2008; Kreutzer et al. 2009). Only few studies have compared retention from dry and wet deposition and concluded that foliar retention from the liquid phase is much more important than from the gaseous phase (Harrison et al. 2000; Sievering et al. 2007), although dry deposited N may significantly contribute to canopy retention (Horvath 2004). Dry deposited N during the antecedent dry period prior to a wet deposition event is mobilized by canopy wetness, after which the dissolved N is available to canopy foliage for use in photosynthesis (Sievering et al. 2007). Since no detailed data on stomatal conductance, meteorological conditions and turbulence were available in the present study, the observed ^{15}N retention of gaseous NH_3 in Chapter 3 could not be extrapolated to mature stands. Nevertheless, a significant cuticular deposition and stomatal uptake of NH_3 was observed, as found in other ^{15}N studies (Bruckner et al. 1993; Pérez-Soba and Van der Eerden 1993; Gessler et al. 2002; Jones et al. 2008) and gas flux experiments (Gessler et al. 2000, 2002; Neirynck et al. 2005; and many others). For a Scots pine stand in the study region, Neirynck et al. (2008) concluded that direct stomatal uptake of NH_3 contributed to a lesser extent to the observed canopy retention of dissolved inorganic N, which was estimated to be 7 kg N ha^{-1} yr^{-1}. Net fluxes of NO_2 were found to be mainly upward (Neirynck et al. 2007). The various canopy budget models in Chapter 7 yielded a canopy N retention by the studied individual beech tree of 4-14 kg N ha^{-1} yr^{-1}.

Based on the observations made in this thesis and from literature, we may conclude that the canopy budget model is a suitable method to calculate canopy retention of N, given that direct stomatal uptake of gaseous compounds does not contribute to a great extent to total canopy retention. However, canopy N retention calculated by the canopy budget model is generally higher than the N which is actually taken up or adsorbed to foliage and twigs, as measured by ^{15}N labelling experiments. This may be due to additional assimilation by epiphytic lichens, re-emission of N from drying water layers or conversion of inorganic N to DON. Since ^{15}N labelling experiments are generally conducted on saplings or young trees, it is possible that tree age might also have an influence on these results, although this seems unlikely. Until now, ^{15}N-labelled rainwater has only been applied to a mature forest in the experiment of Gaige et al. (2007) and Dail et al. (2009), but also here a discrepancy between ^{15}N in the different plant parts and NTF has been observed.

In Chapter 6 a comparison was made between three different dry deposition methods, i.e. the canopy budget model by Draaijers and Erisman (1995), single layer and multi-layer artificial foliage (method adjusted from Stachurski and Zimka 2000) and measured air concentrations multiplied by an average deposition velocity. According to the latter approach, annual dry deposition of NH_3 and NO_2, which may be partly taken up through the stomata, was estimated to be 14.9 and 5.0 kg N ha^{-1} yr^{-1}, respectively (Table 8.1). If dry deposition on the multi-layer artificial foliage, which mainly originates from particulate deposition, was extrapolated to a whole year and added to the NH_3 and NO_2 deposition to obtain total dry deposition of reduced and oxidised N, this amounted to 15.5 and 6.7 kg N ha^{-1} yr^{-1}, respectively (Table 8.1). However, as indicated in Chapter 6, the calculated NO_2 deposition might be a significant overestimation since net upward fluxes were observed for a forest in the same region (Neirynck et al. 2007). Consequently, dry deposition of oxidised N might be lower, and in the range of 1.7 kg ha^{-1} yr^{-1} as measured by the artificial foliage or 2.0-4.6 kg ha^{-1} yr^{-1} as calculated from the canopy budget model. Dry deposition on the beech canopy calculated from the range of canopy budget models in Chapter 7 amounted to 10-20 kg N ha^{-1} yr^{-1} (Table 8.1). This range covers well the dry deposition fluxes according to the other two methods and suggests that the canopy budget model might also be suitable to estimate dry deposition. However, the discussion in Chapter 7 still addressed some important inconsistencies in this model with regard to the assumption of inertness of the tracer ion, the equal deposition efficiency of aerosols and estimated canopy leaching of weak acids. These factors may significantly underestimate or overestimate dry deposition of N compounds and need to be clarified further. Additionally, Chapter 6 revealed a discrepancy between the dry deposition of SO_4^{2-} calculated by the canopy budget model and estimated from air concentration measurements (Chapter 6). Therefore, it is suggested 1) to combine the canopy budget model with other dry deposition methods, if possible, and 2) to conduct a similar sensitivity analysis as in Chapter 7 when using the canopy budget model in different regions or for different tree species.

Table 8.1 Summary of dry deposition (kg N ha^{-1} yr^{-1}) obtained by different dry deposition methods in this thesis, i.e. measured air concentrations multiplied by an average deposition velocity (vd_{avg}), dry deposition measured on multi-layered artificial foliage (AF_{multi}) and the various approaches of the canopy budget model

Method	Measured N compound	Dry deposition	Chapter
vd_{avg}	NH_3	14.9	6
	NO_2	5.0	6
AF_{multi}	pNH_4, (NH_3)	0.6	6
	pNO_3, (HNO_3)	1.7	6
Canopy budget model	NH_3^*, pNH_4	4.2 - 22.9	7
	NO_2^*, pNO_3	2.0 – 4.6	7

*not taken up by stomata

8.2 Forest canopy control on dry deposition and canopy exchange processes

Forest canopies affect dry deposition and canopy exchange processes in different ways. We first discuss the impact of leaf wettability, water storage capacity and bark roughness, then chemical and morphological leaf properties, and finally the physical structure of the canopy itself.

8.2.1 Leaf wettability, water storage capacity and bark roughness

The retention mechanism of dissolved inorganic N (DIN) through plant cuticles is an existing knowledge gap (Sparks 2009). With this thesis we attempted to partly fill this gap by assessing the role of leaf characteristics such as leaf wettability and water storage capacity on DIN retention. A significant correlation between leaf wettability, measured as the contact angle between a droplet on the leaf surface and the surface itself, and foliar $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake was observed (Chapter 2). Consequently, variations in leaf wettability could partly explain the observed differences in DIN uptake between tree species and phenological stages. No relationship with water storage capacity was found, suggesting that the contact time is less important for DIN uptake than the contact surface. As in other studies (Holloway 1969; Hall and Burke 1974; Neinhuis and Barthlott 1998), the differences in leaf wettability between tree species could partly be explained by visual differences in epicuticular wax structure, which was assessed by Scanning Electron Microscopy. On a within-canopy level, leaf wettability was found to increase from the top to the bottom of beech (Van Wittenberghe 2009) and Norway spruce canopies (Boyce et al. 1991). This increased wettability in the lower canopy could have contributed to the leaching of base cations that was observed (Chapter 5). However, no higher canopy uptake of N was observed at the bottom than at the top of the beech and spruce canopies, indicating that other factors than wettability explain differences in dissolved N uptake along a canopy gradient.

In Chapter 4, a high retention of $^{15}NH_4^+$ by twigs of Scots pine was observed, while this was less apparent for the three deciduous species. The rinsing experiment revealed that this retained N was adsorbed to the bark surface of pine and not assimilated. Many studies have suggested this mechanism (Bowden et al. 1989; Boyce et al. 1996; Wilson and Tiley 1998; Dail et al. 2009) but could not provide experimental evidence. Although bark roughness was not explicitly quantified in this thesis, the higher $^{15}NH_4^+$ retention by pine twigs can likely be attributed to the more rough bark surface that provides a lot of potential adsorption places. This implicates that especially tree species with a rough bark surface, such as Scots pine and Norway spruce, can temporarily store meaningful amounts of N that are not assimilated by the tree.

8.2.2 Leaf chemical and physiological characteristics

In Chapter 2-3, the observed retention of dissolved and gaseous ^{15}N was related to the plant N status, measured as the N content of leaves and twigs. In both chapters, a significant positive relationship between plant N and N retention was found, although not for all concentration levels of NH_3. However, this relationship was rather weak and could not fully explain the variation in N retention. Also within the beech canopy, the variation in leaf N content along the vertical gradient could not be linked directly to higher canopy N uptake (Chapter 5). A difficulty with the parameters leaf and twig N content is that they may reflect both plant physiological activity, i.e. a higher N content is associated with higher chlorophyll content and photosynthetic capacity (Evans et al. 2001; Niinnemets 2007) as well as plant N status with regard to passive diffusion-driven N uptake from the atmosphere. These two viewpoints may cause opposite effects on N retention. On the one hand, leaves with a higher physiological activity will be able to convert more inorganic N into amino acids. This is confirmed by the higher retention of the sunlit birch leaves having a higher photosynthetic capacity compared to shadow leaves of beech and oak during leaf emergence for both dissolved and gaseous N (Hikosaka 2005). Also the higher canopy uptake of N during the growing season compared to the dormant season and of the upper canopy compared to the lower canopy (Chapter 5) confirms this viewpoint, as well as the weak but significant correlation between $^{13}CO_2$ and $^{15}NH_3$ uptake in Chapter 3. On the other hand, a lower leaf N status may represent lower leaf mineral N (Schjoerring et al. 1998), which can enhance passive diffusion of N and hence increase N retention. However, during leaf senescence of the deciduous tree species, apoplastic NH_4^+ concentrations are generally high (Wang et al. 2011) but leaf N content low (Chapter 2 and 3). An increased retention of NH_4^+ and NO_3^- was observed but not of NH_3, indicating that DIN retention is not controlled by foliar apoplast concentrations. Based on the observations in this thesis, we may conclude that both dissolved and gaseous N uptake are controlled by leaf physiological activity during the fully leafed period, but that during leaf development and leaf senescence passive diffusion may become more important.

Many studies have linked stomatal conductance to NH_3 uptake (Van Hove et al. 1987; Gessler et al. 2000; and many others). In Chapter 3 an attempt was made to link easy-to-measure stomatal characteristics, i.e. stomatal density and pore surface and a theoretically derived maximum stomatal conductance g_{smax}, to the observed $^{15}NH_3$ retention to explain differences between tree species and phenological stages, but no relationship was found. Thus, these stomatal characteristics are not suitable parameters to explain variation in NH_3 uptake. However, in the beech canopy they may explain canopy exchange processes to some extent, since stomatal pore surface significantly decreased from the top to the bottom of the canopy (Van Wittenberghe 2009) along with canopy uptake of N.

8.2.3 Canopy structure

Canopy structure can be described at several spatial scales by several variables (Sinoquet et al. 2009), such as leaf area density, branch inclination angle, leaf and plant area index, leaf shape and orientation (Fleck 2001; Van der Zande et al. 2006). Several of these variables have been shown to affect dry deposition processes (Erisman and Draaijers 2003) and throughfall variability both in space and time (see Levia and Frost 2006 for an overview). In Chapter 5, canopy structure of beech and spruce was not explicitly measured, but the clear differences in canopy structure between the two tree species could explain the vertical patterns in dry deposition of aerosols containing Na^+, Cl^-, K^+, Ca^{2+} and Mg^{2+} (Fig. 5.6 and 5.7; Section 5.4.3). A large difference in dry deposition of these aerosols was expected between the leafed and the leafless season for the beech tree (Chapter 5) due to a decrease in plant area index, however, this was not the case. The high dry deposition of these aerosols during the leafless season can likely be attributed to increased atmospheric concentrations and a high capture efficiency of fine twigs. In Chapter 6, a 3D image of the beech tree was taken during the leafed season (Fig. 6.1) by means of the Light Detection and Ranging (LiDAR) Technology of terrestrial laser scanners. This LiDAR technology provides a new tool to generate a 3D description of canopy structure (Lefsky et al. 2005; Van der Zande et al. 2006), which allowed relating dry deposition and net throughfall fluxes within the beech canopy on a collector basis to the canopy structure. A significant positive relationship between the number of filled voxels above a throughfall collector, which may serve as a proxy for the volume of canopy elements, and fluxes of all elements except H^+, NO_3^- and net throughfall volume was found. This strengthens previous observations on the relationship between throughfall deposition and canopy structure estimated by less precise methods, such as hemispherical photographs (Staelens et al. 2006).

8.3 Control of atmospheric N concentration on dry deposition and canopy exchange

Several studies have reported increased fluxes of dissolved NH_4^+ and NO_3^- and gaseous NH_3 to the canopy with increasing N concentration of the solution or gas (Van Hove et al. 1987; Schaefer et al. 1988; Brumme et al. 1992; Eilers et al. 1992; Lumme and Smolander 1996; Gessler et al. 2000; Gessler et al. 2002). In Chapter 2 and 4 a 1.3-fold higher $^{15}NO_3^-$ retention was observed for a 3-fold higher $^{15}NO_3^-$ concentration available in the applied solution, hereby confirming literature observations. This effect might have been larger if the $^{15}NH_4^+$ availability in the solution was increased, since all tree species studied showed a higher affinity for NH_4^+ uptake compared to NO_3^-. In contrast, the short-term $^{15}NH_3$ experiment (Chapter 3) differed in this regard from previously conducted experiments since $^{15}NH_3$ deposition was not found to increase with increasing atmospheric NH_3 concentration. This observation was explained by the short-term exposure of the foliated twigs to $^{15}NH_3$ and subsequent exposure to ambient air which could have caused a temporal storage (Grundmann et al. 1993; Stulen et al. 1998) or significant re-emission of NH_3 at increasing initial NH_3 levels (Rennenberg and Gessler 1999). Despite the fact that the cause is unknown, the results of Chapter 3 clearly indicated that all tree species were not able to instantaneously assimilate NH_3 at increased availability. Since no different NH_4^+ levels were applied (Chapter 2), we cannot verify whether this also holds true for dissolved NH_4^+. However, based on previous studies (Brumme et al. 1992; Eilers et al. 1992; Chávez-Aguilar et al. 2006) and a recent experiment (unpublished results), it is likely that more NH_4^+ is retained at increased availability. On the within-canopy level, no higher canopy N retention was observed at the bottom of the beech and spruce canopy, where DIN concentrations in throughfall were higher compared to the top of the canopy (Chapter 5), indicating that the atmospheric N concentration is not the only driving factor for canopy retention of N (see also Section 8.2.1).

8.4 Suggestions for further research

This thesis has contributed to current knowledge on dry deposition and canopy exchange processes for temperate tree species and has formulated some important recommendations on the use of the canopy budget model. Nevertheless, there remain issues that could be addressed in future research, in addition to the research suggestions mentioned in the previous chapters.

Firstly, a shortcoming of this thesis was that no dissolved organic N (DON) was measured in the rainfall and throughfall samples, mainly due to the difficulty to accurately measure this fraction (Vandenbruwane et al. 2007). A significant fraction of DON in throughfall deposition and bulk deposition is ubiquitous (Michalzik et al. 2001; Gaige et al. 2007; Neirynck et al. 2007; Benitez et al. 2009; Wu et al. 2010), particularly in unpolluted regions. Several studies have indicated that canopy processes may convert atmospheric deposition of DIN to DON (Stadler et al. 2001; Gaige et al. 2007; Cape et al. 2010), but the exact processes are still largely unknown. Furthermore, it is still a challenge to incorporate DON into the canopy budget model (Neirynck et al. 2007; Staelens et al. 2008).

Secondly, stable N isotopes have been proven a highly valuable tool to gain more insight into dissolved and gaseous N exchange processes (Chapter 2-4). Likewise, tree labelling with stable isotopes of K^+, Ca^{2+} and Mg^{2+} could improve the understanding of 1) ion exchange between N and base cations, especially during the leafless season and 2) dry deposition of these base cations. This approach has recently been initiated by Augusto et al. (2011) and can still be further developed.

Thirdly, in Section 1.2., the interaction between shoot and root uptake of N was highlighted. In this thesis soil N availability was kept constant in the pot experiments of Chapter 2-4. An increasing N availability via the soil will likely increase foliar N (Berger and Glatzel 2001) and hence increase the influence of plant N status on canopy N retention (see 8.2.2.). It also remains to be investigated whether such increased soil N availability will be affect the leaching of base cations.

Finally, further development of the LiDAR technology could gain new insights in dry deposition and canopy exchange of atmospheric pollutants. Using this innovative technique, the effect of canopy structure on turbulence patterns within tree canopies can be assessed for real and complex canopies within a forest instead of saplings or model canopies in wind tunnels (Beckett et al. 2000; Ould-Dada 2002; Freer-Smith et al. 2004). Furthermore, the high spatial resolution can be used to scale up measurements from the leaf level to a canopy level, as has been done for transpiration measurements (Van der Zande et al. 2009).

Summary

Increased deposition of atmospheric nitrogen (N) and sulphur (S) on forest ecosystems has caused changes in biogeochemical processes, which may have adverse effects on forest structure and functioning. Therefore, an accurate quantification of total atmospheric deposition is required to establish cause-effect relationships and evaluate abatement measures and mitigation strategies. However, measurements of dry deposition, which is a major fraction of total deposition on forests, are still subject to several problems and are complicated by canopy exchange processes in tree canopies. This study aimed to address existing knowledge gaps on dry deposition and canopy exchange, in particular of N, and to gain a better understanding of the influence the forest canopy exerts on these processes. Next to this, we aimed to evaluate the often-used canopy budget model to calculate total atmospheric deposition from throughfall measurements. Dry deposition and canopy exchange processes were examined at different spatio-temporal scales for five temperate tree species widespread in Western Europe, a region with a high N deposition load. The effect of forest canopy characteristics, i.e. leaf characteristics and canopy structure, on the measured response variable was examined.

Retention of inorganic N from wet (ammonium (NH_4^+), nitrate (NO_3^-)) and dry (ammonia (NH_3)) deposition by foliage and twigs was quantified for saplings of European beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.), silver birch (*Betula pendula* Roth.) and Scots pine (*Pinus sylvestris* L.) by means of stable N isotope (^{15}N) tracing. Retention patterns for dissolved inorganic N were mainly determined by foliar uptake, except for Scots pine. In general, retention rates were 3-10 times higher for $^{15}NH_4^+$ than for $^{15}NO_3^-$, 2-3 times higher for deciduous species than for Scots pine and lower for developing leaves, although this was tree species dependent. The observed effects of tree species and phenological stage in foliar retention could be related to differences in leaf wettability, and not to water storage capacity. The small $^{15}NH_4^+$ retention by twigs was mainly due to physicochemical adsorption to the woody plant surface. Retention of $^{15}NH_4^+$ calculated from throughfall water was, on average, 20 times higher than retention by the plant material, indicating that a large part of the applied $^{15}NH_4^+$ could not be accounted for. The $^{15}NH_3$ retention by leaves and twigs was affected by tree species, treatment date, applied NH_3 concentration and the interaction between these factors.

Maximum $^{15}NH_3$ retention occurred at 5, 20 or 50 ppb, depending on the treatment date, but never at the highest NH_3 concentration level. Birch, beech and oak leaves showed the highest $^{15}NH_3$ retention in August, while for pine needles this was in June, and was generally higher for the deciduous species than for pine. Both leaf characteristics as well as measured $^{13}CO_2$ retention did not provide a strong explanation for the observed differences in $^{15}NH_3$ retention.

Throughfall deposition, dry deposition and canopy exchange of major ions calculated by the canopy budget model were studied along a vertical gradient within a beech and two Norway spruce (*Picea abies* Karst.) canopies, located in forests in Belgium and Denmark, respectively. Throughfall and net throughfall deposition of all ions other than H^+ increased significantly with canopy depth in the middle and lower canopy of the beech tree and in the whole canopy of the spruce trees. Dry deposition of all ions and canopy uptake of inorganic N and H^+ occurred mainly in the upper canopy, while canopy leaching of K^+, Ca^{2+} and Mg^{2+} was observed at all canopy levels. Canopy exchange was always higher during the growing season compared to the dormant season. This observational study illustrated that biogeochemical deposition models would benefit from a multilayer approach for shade-tolerant tree species such as beech and spruce.

For the beech canopy, a comparison of dry deposition calculated by the canopy budget model was made with dry deposition calculated from air concentration measurements and dry deposition onto multi- and single-layered artificial foliage. The multi-layered artificial foliage showed a good agreement with the canopy budget model for coarse aerosol deposition. For NH_x, the canopy budget model and the air concentration measurements yielded similar results, however, for SO_x the canopy budget model yielded higher dry deposition than estimated from air concentration measurements and for NO_y the model resulted in lower estimates. Combining throughfall measurements with multi-layered artificial foliage could aid to quantify dry deposition of NO_y. Net throughfall and dry deposition of all variables except throughfall volume, H^+ and NO_3^-, were significantly correlated with the volume of canopy elements above the throughfall collectors, which was measured by terrestrial laser scanning.

To determine the effect of different canopy budget model approaches on atmospheric deposition estimates, we calculated the range of atmospheric deposition obtained by combining canopy budget model approaches for three typical case studies: (i) total N and potentially acidifying deposition onto a forest canopy, (ii) the ratio of these deposition variables between a coniferous and a deciduous stand and (iii) the parameters of a deposition time trend analysis. The effect of each step in the canopy budget model on the obtained range was assessed. The time step, type of precipitation data and tracer ion used in the model had a significant effect on the derived deposition in the three case studies. In addition, including or excluding canopy leaching of weak acids and canopy uptake of NH_4^+ and NO_3^- during the leafless season largely affected the results, while including or excluding canopy uptake of NO_3^- generally showed no effect.

Our results provided more insight into the role of the forest canopy on dry deposition and canopy exchange of major ions. Furthermore, canopy retention of N was accurately quantified and methodological recommendations with regard to the quantification of dry deposition and canopy exchange for N compounds from the canopy budget model could be formulated.

Samenvatting

Bosecosystemen zijn onderhevig aan een verhoogde atmosferische depositie van stikstof- (N) en zwavel-componenten (S), die veranderingen in biogeochemische processen veroorzaken. Deze veranderingen kunnen negatieve effecten hebben op het functioneren van bosecosytemen. Een accurate kwantificering van de totale atmosferische depositie van deze polluenten is dan ook noodzakelijk om oorzaak-gevolg relaties vast te kunnen stellen en het effect van emissiereducerende en mitigerende maatregelen te evalueren. Metingen van droge depositie zijn echter nog steeds onderhevig aan verschillende problemen. Daarnaast wordt de kwantificering van de totale depositie bemoeilijkt door kroonuitwisselingsprocessen in boomkronen. Deze studie had als eerste doel om meer inzicht te verkrijgen in droge depositie en kroonuitwisselingsprocessen, met de focus op N, en in de rol van boomkronen op deze processen. Het tweede doel was om het vaak gebruikte kroonbudgetmodel te evalueren dat de totale atmosferische depositie op basis van doorvalmetingen schat. Droge depositie en kroonuitwisselingsprocessen werden onderzocht op verschillende spatio-temporele niveaus bij vijf gematigde boomsoorten die wijdverspreid zijn in West-Europa, een regio met een hoge stikstofdepositielast. Het effect van verschillende kroonkarakteristieken, i.e. bladkarakteristieken en kroonstructuur, op de gemeten responsvariabelen werd onderzocht.

De retentie van anorganische N uit natte (ammonium (NH_4^+), nitraat (NO_3^-)) en droge (ammoniak (NH_3)) depositie door bladeren en twijgen werd gekwantificeerd d.m.v. stabiele isotopen (^{15}N) voor jonge beuken (*Fagus sylvatica* L.), zomereiken (*Quercus robur* L.), ruwe berken (*Betula pendula* Roth.) en grove dennen (*Pinus sylvestris* L.). Verschillen in retentie van opgeloste anorganische N tussen soorten en fenologische stadia werden vooral bepaald door bladopname, behalve voor grove den. Algemeen was de retentie 3-10 keer hoger voor NO_3^- dan voor NH_4^+, 2-3 keer hoger voor de loofboomsoorten dan voor grove den en steeds lager voor ontluikende bladeren, hoewel dit laatste boomsoortafhankelijk was. Verschillen in NH_4^+ en NO_3^- retentie tussen boomsoorten en fenologische stadia konden gerelateerd worden aan verschillen in bladbevochtigbaarheid. De lage NH_4^+ retentie door twijgen vond plaats door fysico-chemische adsorptie aan het plantoppervlak. Ammoniumretentie, berekend op basis van doorvalmetingen, was gemiddeld 20 keer hoger dan deze gemeten in het plantmateriaal.

Een aanzienlijk deel van de NH_4^+ in de natte depositie kon dus niet teruggevonden worden. De $^{15}NH_3$ retentie door bladeren en twijgen werd significant beïnvloed door de factoren boomsoort, tijdstip, toegepaste NH_3 concentratie en de interactie tussen deze factoren. Optimale retentie vond plaats bij 5, 20 or 50 ppb_v, afhankelijk van het tijdstip in het groeiseizoen, maar nooit bij 100 ppb_v. Bladeren van berk, beuk en eik vertoonden de hoogste retentie in augustus, terwijl dit voor naalden van grove den in juni was. Ammoniakretentie was meestal hoger voor de loofboomsoorten dan voor grove den. Zowel de bladkarakteristieken als de simultaan gemeten $^{13}CO_2$ opname vormden geen verklaring voor de geobserveerde verschillen in $^{15}NH_3$ retentie.

In een observationele studie bestudeerden we doorvaldepositie, droge depositie en kroonuitwisseling van ionen berekend door het kroonbudgetmodel langsheen een vertikale gradiënt in de kroon van één beuk en twee fijnsparren (*Picea abies* Karst.). Doorval- en netto-doorvaldepositie van alle ionen behalve H^+ namen significant toe met de diepte in de kroon in de middelste en onderste laag van de beukenkroon en in alle kroonlagen van fijnspar. Droge depositie voor alle ionen en kroonopname voor anorganische N en H^+ vond vooral in de bovenste kroonlaag plaats, terwijl kroonuitloging van K^+, Ca^{2+} and Mg^{2+} in alle kroonlagen werd geobserveerd. Kroonuitwisseling was steeds hoger tijdens het groeiseizoen dan tijdens het dormant seizoen. Deze studie illustreert dat biogeochemische depositiemodellen gebaat zouden zijn bij een meerlagige benadering voor schaduwtolerante soorten zoals beuk en fijnspar.

Voor de beukenkroon werd een vergelijking gemaakt tussen de droge depositie berekend door het kroonbudgetmodel en deze berekend via luchtconcentratiemetingen en via droge depositie op één- en meerlagig artificiële boomkronen. De meerlagige artificiële boomkronen vertoonden een goed overeenkomst met het kroonbudgetmodel voor grove aerosoldepositie. Voor NH_y leverden het kroonbudgetmodel en de luchtconcentratiemetingen gelijkaardige resultaten, maar voor SO_x leverde het kroonbudgetmodel een hogere droge depositie dan deze geschat via luchtconcentratiemetingen terwijl het model lagere droge depositie voor NO_y schatte. Een combinatie van doorvalmetingen met meerlagige artificiële boomkronen kan helpen om de droge depositie van NO_y nauwkeurig te kwantificeren. De netto-doorval en droge depositie van alle variabelen, behalve het doorvalvolume, H^+ en NO_3^-, was significant gecorreleerd met het volume van kroonelementen boven elke doorvalcollector gemeten via grondgebaseerde laser scanning.

Om het effect van verschillende benaderingen van het kroonbudgetmodel op de schatting van atmosferische depositie te bepalen, werd de grootteorde van atmosferische depositie bepaald d.m.v. het combineren van alle verschillende kroonbudgetmodelbenaderingen. Dit werd uitgevoerd voor drie typische case studies: (i) totale N en potentieel verzurende depositie op een boomkroon, (ii) de ratio van deze depositie variabelen tussen een naald- en een loofbestand en (iii) de parameters van een trendanalyse op deze depositievariabelen. Voor elke stap in het kroonbudgetmodel werd het effect op de berekende grootteorde nagegaan. In alle drie de case studies was er een significant effect van de gebruikte tijdstap, het type neerslagdata en het gebruikte tracer ion. Daarnaast beïnvloedde het in- of uitsluiten van kroonuitloging van zwakke zuren en van kroonopname van NH_4^+ en NO_3^- (enkel tijdens de onbebladerde periode) de resultaten, terwijl meestal geen effect werd teruggevonden van het in- of uitsluiten van kroonopname van NO_3^- (over het hele jaar).

De resultaten van deze studie hebben meer inzicht verschaft in de rol van boomkronen in de droge depositie en kroonuitwisseling van ionen. Daarnaast konden enkele methodologische beschouwingen geformuleerd worden met betrekking tot de kwantificering van droge depositie en kroonuitwisseling van N componenten.

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Curriculum vitae

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2007	12-day course in Vegetation Science, Inverde Institute, Groenendaal
2001 – 2006	MSc in Bioscience engineering, Land and Forest Management Faculty of Bioscience Engineering, Ghent University
1995 – 2001	Secondary School Sciences – Mathematics Berkenboom Humaniora Sint-Niklaas

Professional experience

Jan 2012 – Present	Scientific worker at the Belgian Interregional Environment Agency (IRCEL) on the Interreg IV-B project Joaquin
Jan 2008 – Dec 2011	PhD-research at Ghent University, Faculty of Bioscience Engineering, Laboratory of Forestry; financed by the Research Foundation - Flanders (FWO)
Nov 2006 – Dec 2007	Project manager of the section Water and Ecology in the firm of consulting engineers Libost-Groep NV
Aug 2006 – Sept 2006	Scientific worker at Ghent University, Faculty of Bioscience Engineering, Laboratory of Forestry on a project financed by the province of Limburg
August 2004	Fieldworker for the Flemish Institute of Nature Conservation, Brussels, Belgium

Scientific publications

Publications in international journals with peer review cited in the Science Citation Index (IF = impact factor for 2010)

Adriaenssens S, Staelens J, Van der Zande D, Coppin P, Boeckx P, Samson R, Verheyen K. Dry deposition along a vertical gradient within a beech (*Fagus sylvatica* L.) canopy: comparison of methods and dependence on canopy structure. Submitted to Journal of Geophysical Research-Biogeosciences (IF = 3.303)

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Adriaenssens S, Staelens J, Van Wittenberghe S, Samson R, Boeckx P, Verheyen K (2010) Seasonal canopy uptake of dry deposited ^{15}N-NH$_3$ under different N loads and the interaction with leaf physical properties. In: Wuyts K, Samson R, De Maerschalck B, Kardel F, Janssen S, Engelen M (eds) Proceedings of the international conference on “Local air quality and it’s interactions with vegetation”, 21 – 22 January 2010, CCC Elzenveld, Antwerp, Belgium, pp 131-134

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Abstracts of presentations at scientific congresses

Adriaenssens S, Staelens J, Wuyts K, Samson R, Boeckx P, Verheyen K (2011) Opname van stikstof uit regenwater door boomkronen in relatie tot doorvalmetingen. Oral presentation in: Starters voor Bosonderzoek 2011, 17 March 2011, Brussels, Belgium, p 3

Vanden Broek A, Gruwez R, Cox K, Adriaenssens S, Verheyen K (2010) Integratie van genetische variatie in het soortbeschermingsplan Jeneverbes. Poster abstract in: Studiedag Genetica in Natuurbeleid en Beheer, 18 November 2010, Brussels, Belgium

Adriaenssens S, Staelens J, Samson R, Verheyen K, Boeckx P (2010) Canopy uptake of dissolved ^{15}N: a validation of the canopy budget model. Oral presentation in: BASIS annual meeting 2010, 15 – 16 April 2010, Arnhem, the Netherlands. Winner of the Young Scientist Award

Adriaenssens S, Staelens J, Samson R, Boeckx P, Verheyen K (2010) Spatial and temporal variability of throughfall water and dry deposition along a vertical gradient in a beech canopy. Oral presentation in: 24th IUFRO conference for specialists in air pollution and climate change effects on forest ecosystems “Adaption of forest ecosystems to air pollution and climate change”, 22 – 26 March 2010, Antalya, Turkey, p 23

Adriaenssens S, Staelens J, Van den Bilcke N, Boeckx P, Samson R, Verheyen K (2009) Quantifying aboveground nitrogen uptake from wet deposition: impact of tree species, N load and leaf phenology. Poster abstract in: Ukonmaanaho L, Nieminen TM, Starr M (eds) 6th International Symposium on Ecosystem Behaviour: Biogeomon Conference 2009, 29 June – 3 July 2009, Helsinki, Finland, p 192

Adriaenssens S, Staelens J, Van den Bilcke N, Boeckx P, Samson R, Verheyen K (2009) Quantifying aboveground N uptake from wet deposition: impact of tree species, N load and leaf phenology. Poster abstract in: BASIS annual meeting 2009, 2 – 3 April 2009, Bruges, Belgium

Adriaenssens S, Staelens J, Verheyen K, Boeckx P, Samson R (2009) Bepaling van de bovengrondse stikstofopname door bladeren: effect van boomsoort, stikstoflast en bladfenologie. Poster abstract in: Starters voor Bosonderzoek 2009, 19 March 2009, Brussels, Belgium

Scientific reports

Verheyen K, De Schrijver A, Staelens J, Baeten L, De Frenne P, Adriaenssens S, Verstraeten G, Ampoorter E, Van Nevel L, Demey A, Wuyts K, Gruwez G (2010) Pilootstudie naar kwantificering van de relaties tussen de achteruitgang van biodiversiteit en chronische overschrijding van kritische lasten. Studie in opdracht van het Instituut voor Natuur- en Bosonderzoek (Natuurrapportering), 76 p

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MSc thesis

Adriaenssens S (2006) Vergelijkend onderzoek naar de productie en kiemkracht van jeneverbeszaden in Vlaanderen en omliggende regio's. MSc thesis, Universiteit Gent, 134 p

Scientific activities

Participation in congresses, symposia or workshops

Participation with oral presentation

17 March 2011. Opname van stikstof uit regenwater door boomkronen in relatie tot doorvalmetingen. Starters voor Bosonderzoek 2011, Brussels, Belgium

15 – 16 April 2010. Canopy uptake of dissolved ^{15}N: a validation of the canopy budget model. BASIS annual meeting 2010, Arnhem, the Netherlands. Winner of the Young Scientist Award

22 – 26 March 2010. Spatial and temporal variability of throughfall water and dry deposition along a vertical gradient in a beech canopy. 24th IUFRO conference for specialists in air pollution and climate change effects on forest ecosystems "Adaption of forest ecosystems to air pollution and climate change", Antalya, Turkey

Participation with poster presentation

21 – 22 January 2010. Seasonal canopy uptake of dry deposited $^{15}N\text{-}NH_3$ under different N loads and the interaction with leaf physical properties. CLIMAQS workshop "Local air quality and it's interactions with vegetation", CCC Elzenveld, Antwerp, Belgium

5 – 8 October 2009. Seasonal canopy uptake of dry deposited $^{15}N\text{-}NH_3$ under different N loads and the interaction with leaf physical properties. International conference of "Atmospheric transport and chemistry in forest ecosystems", Castle of Thurnau, Germany

29 June – 3 July 2009. Quantifying aboveground nitrogen uptake from wet deposition: impact of tree species, N load and leaf phenology. 6th International Symposium on Ecosystem Behaviour: Biogeomon Conference 2009, Helsinki, Finland

2 – 3 April 2009. Quantifying aboveground N uptake from wet deposition: impact of tree species, N load and leaf phenology. BASIS annual meeting 2009, Bruges, Belgium

19 March 2009. Bepaling van de bovengrondse stikstofopname door bladeren: effect van boomsoort, stikstoflast en bladfenologie. Starters voor Bosonderzoek 2009, Brussels, Belgium

Supervision of M.Sc.thesis students

2009-2009	Van Wittenberghe Shari. Karakterisatie van anatomische en fysiologische bladeigenschappen bij loof- en naaldboomsoorten en langsheen een verticale gradiënt bij beuk. Supervisors: Prof. dr. ir. Kris Verheyen and Prof. dr. ir. Roeland Samson

Participation in scientific projects

2006	Evolutie (1985-2006) en toekomst van de jeneverbes (*Juniperus communis* L.) in de provincie Limburg
2010	Pilootstudie naar kwantificering van de relaties tussen de achteruitgang van biodiversiteit en chronische overschrijding van kritische lasten

Review tasks for international journals

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