



Isotopic evidence for the occurrence of biological nitrification and nitrogen deposition processing in forest canopies

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Abstract:	This study examines the role of tree canopies in processing atmospheric nitrogen (Ndep) for four forests in the UK subjected to different Ndep loads: Scots pine and beech stands under high Ndep (HN, 13 and 19 kg N ha ⁻¹ yr ⁻¹ , respectively), compared to Scots pine and beech stands under low Ndep (LN, 9 kg N ha ⁻¹ yr ⁻¹). Changes of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations in rainfall (RF) and throughfall (TF) together with a quadruple isotope approach, which combines $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$ and $\delta^{15}\text{N}$ in NO_3^- and $\delta^{15}\text{N}$ in NH_4^+ , were used to assess N transformations within the canopies. Generally, HN sites showed higher $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations in RF compared to the LN sites. Similar values of $\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ in RF suggested similar source of atmospheric NO_3^- (e.g., local traffic), while more positive values for $\delta^{15}\text{N}$ - NH_4^+ at HN compared to LN likely reflected the contribution of dry Ndep from intensive local farming. The isotopic signatures of the N-forms changed during canopy processing, although changes in ion concentrations between RF and TF were significant only at the HN sites. ^{15}N -enriched NH_4^+ in TF compared to RF at all sites suggested canopies played an important role in buffering dry Ndep also at the low atmospheric N load. By using $\Delta^{17}\text{O}$, we could prove for the first time that up to 60% of NO_3^- in TF at the HN sites derived from nitrification occurring in tree canopies, with a higher fraction in beech vs. Scots pine. Our study suggests that tree canopies do not play a passive role in the N cycling within forest ecosystems. Processing of Ndep within canopies should not be neglected and needs further exploration,

	with the combination of powerful tracers, i.e., $\Delta^{17}O$, in order to better quantify the role of forests as sinks but also sources of N.

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1 **Isotopic evidence for the occurrence of biological nitrification and nitrogen deposition**
2 **processing in forest canopies**

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1 Abstract

2 This study examines the role of tree canopies in processing atmospheric nitrogen (N_{dep}) for
3 four forests in the UK subjected to different N_{dep} loads: Scots pine and beech stands under
4 high N_{dep} (HN, 13 and 19 kg N ha⁻¹ yr⁻¹, respectively), compared to Scots pine and beech
5 stands under low N_{dep} (LN, 9 kg N ha⁻¹ yr⁻¹). Changes of NO₃-N and NH₄-N concentrations in
6 rainfall (RF) and throughfall (TF) together with a quadruple isotope approach, which
7 combines $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$ and $\delta^{15}\text{N}$ in NO₃⁻ and $\delta^{15}\text{N}$ in NH₄⁺, were used to assess N
8 transformations within the canopies. Generally, HN sites showed higher NH₄-N and NO₃-N
9 concentrations in RF compared to the LN sites. Similar values of $\delta^{15}\text{N}$ -NO₃⁻ and $\delta^{18}\text{O}$ in RF
10 suggested similar source of atmospheric NO₃⁻ (e.g., local traffic), while more positive values
11 for $\delta^{15}\text{N}$ -NH₄⁺ at HN compared to LN likely reflected the contribution of dry N_{dep} from
12 intensive local farming. The isotopic signatures of the N-forms changed during canopy
13 processing, although changes in ion concentrations between RF and TF were significant only
14 at the HN sites. ¹⁵N-enriched NH₄⁺ in TF compared to RF at all sites suggested canopies
15 played an important role in buffering dry N_{dep} also at the low atmospheric N load. By using
16 $\Delta^{17}\text{O}$, we could prove for the first time that up to 60% of NO₃⁻ in TF at the HN sites derived
17 from nitrification occurring in tree canopies, with a higher fraction in beech vs. Scots pine.
18 Our study suggests that tree canopies do not play a passive role in the N cycling within forest
19 ecosystems. Processing of N_{dep} within canopies should not be neglected and needs further
20 exploration, with the combination of powerful tracers, i.e., $\Delta^{17}\text{O}$, in order to better quantify the
21 role of forests as sinks but also sources of N.

22

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24 Key words: Nitrogen deposition, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$, NH₄, NO₃, forest canopy interception,
25 canopy nitrification, Scots pine, beech

1

2 **1. Introduction**

3 Forest canopies play a significant role in regulating carbon and water exchanges with the
4 atmosphere, with profound effects on climate. On one hand, through photosynthesis in the
5 canopy, forests remove from the atmosphere approximately 30% of anthropogenically
6 produced carbon dioxide (CO₂) annually (Schulze, 2006; Canadell et al., 2007), thus
7 contributing to the mitigation of climate change. On the other hand, CO₂ uptake occurs at the
8 expense of water taken up from the soil and subsequently lost through stomata during
9 transpiration, with both having significant effects on the terrestrial hydrological cycle (Bonan,
10 2008).

11 The contribution of tree canopies in altering the chemical composition of precipitation and,
12 consequently, the nutrient cycling within a forest has been less investigated. In particular, it is
13 unclear whether the deposition of reactive nitrogen species (N_{dep}) to canopies is retained, re-
14 emitted and/or altered by chemical or biological reactions, and what a portion and chemical
15 form of deposited N eventually reaches the soil as washed out N-compounds. Interception of
16 N_{dep} by forest canopies contributes to the cycling of N in the terrestrial biosphere, thereby
17 affecting plant health, community structure and biodiversity, nutrient cycling, greenhouse gas
18 balance, soil pH and water quality (Cape and Percy, 1998; Galloway et al., 2004; Lindberg et
19 al., 1986; Pitcairn et al. 1998; Pitman et al., 2010; Prescott, 2002; Rennenberg and Gessler,
20 1999; Vanguelova et al., 2011; Vitousek et al., 1997).

21 Understanding all the interactions taking place between atmospheric N and forest canopies,
22 under different environmental conditions, for various forest types (*e.g.*, conifer vs. broadleaf
23 forests), for contrasting N loadings, chemical forms and species remains complex. Systematic
24 monitoring of the main N chemical species (*i.e.*, NH₄⁺, NO₃⁻, dissolved organic N) in rainfall
25 (RF) and throughfall (TF) has now been carried out for almost two decades in a network of

1 experimental European forests (*i.e.*, Level II network of ICP plots <http://icp-forests.net/>).

2 While these measurements quantify the atmospheric N inputs to forests and soils, they have

3 not been useful for assessing in-canopy processes that may be affecting changes in N

4 compounds.

5 Forests are particularly efficient at scavenging pollutants via dry and occult deposition due to

6 their aerodynamically rough canopies (Fowler et al. 1989). As a consequence, the total N

7 speciation and N concentration in RF differ from those in TF. Fluxes of N in TF reflect a

8 mixture of wet, occult (fog/cloud), and dry deposition, that may also be chemically or

9 biologically modified during canopy exchange and uptake. Commonly, TF has a higher N-

10 compounds concentration compared with RF, particularly in area subjected to high N input

11 from the atmosphere (Vanguelova et al., 2010), due to the washing out of dry N_{dep} through

12 forest canopies. Occult deposition can also be marked in areas where seasonal fogs and N

13 pollution sources coincide. This has resulted in very large N inputs ($25\text{--}45 \text{ kg ha}^{-1} \text{ yr}^{-1}$) to the

14 most highly exposed forests of the Los Angeles air basin (Bytnerowicz and Fenn, 1996).

15 Foliar uptake of aqueous N was recently proved to occur in beech and birch, with NH_4^+ more

16 readily taken up than the NO_3^- (Wuyts et al., 2015). Ammonia is readily absorbed directly

17 onto foliage and TF N fluxes are enhanced in forests that are near NH_3 sources such as

18 agricultural areas (Vanguelova and Pitman, 2009). Moreover, in very low N_{dep} areas (*e.g.*,

19 total N_{dep} of $2\text{--}3 \text{ kg ha}^{-1} \text{ yr}^{-1}$), such as in Finland, tree canopies tend to retain much of the N

20 they capture by dry deposition due to uptake by epiphytic lichens, microbial immobilization

21 within the canopy, N absorption into foliage and assimilation by leaves and stems (Mustajärvi

22 et al., 2008). A recent study conducted in forests within the Italian national long-term

23 monitoring network CONECOFOR reported a canopy consumption of N for sites at low N_{dep} ,

24 *i.e.*, $< 4\text{--}6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Ferretti et al., 2014). Similarly, in a study conducted in three

25 National Park in Washington State (USA) subjected to low N_{dep} was found that up to 90% of

1 the atmospheric N, mostly in the form of $\text{NO}_3\text{-N}$, was consumed by the forest canopies (Fenn
2 et al., 2013).

3 The stable nitrogen isotope composition ($\delta^{15}\text{N}$) of wet N_{dep} has helped to characterize the
4 sources of atmospheric N (Freyer, 1991; Heaton, 1987, Kendall et al. 2007 and references
5 therein) and its transformations when interacting with the biosphere, as assessed through
6 measurements of $\delta^{15}\text{N}$ in plants and soil (Ammann et al., 1999; Guerrieri et al., 2009, 2011;
7 Nadelhoffer et al., 1999; Saurer et al., 2004; Savard et al., 2009). In addition, observations
8 have been made of changes in the $\delta^{15}\text{N}$ of NO_3^- in TF that suggested the occurrence of
9 nitrification processes in the canopy (*i.e.*, from NH_4^+ to NO_3^-) of Norway spruce of central
10 Europe (Sah and Brumme, 2003) and of montane rain forest in Ecuador (Schwarz et al.,
11 2011). Teuber et al. (2007) found evidence that autotrophic nitrifiers were present in the
12 needles of a spruce forest exposed to high levels of N_{dep} (but not in needles of tree canopies
13 exposed to low levels of N_{dep}), and proposed that canopy N transformations may partly be
14 bacterial. However, a broad range of processes can lead to similar alterations of TF isotopic
15 composition, so distinguishing between various processes using a single-isotope approach is
16 challenging.

17 The application of the dual isotope approach, *i.e.*, the combined measurement of $\delta^{15}\text{N}$ and
18 $\delta^{18}\text{O}$ in NO_3^- in bulk precipitation and stream water has provided another important step
19 towards a better understanding of the importance of N_{dep} and of its cycling in forests. For
20 example, $\delta^{18}\text{O}$ can help assess whether the NO_3^- in the soil solution derives from atmospheric
21 N or from nitrification processes. This is possible because of the large difference between the
22 isotopic signature of the atmospherically-derived NO_3^- (between 20 and 80 ‰) and the
23 signature for the NO_3^- derived from nitrification (between -10 and +10 ‰, Kendall, 1998;
24 Burns and Kendall, 2002).

1 An even more powerful approach has been proposed by Michalski et al. (2002, 2003) and
2 Costa et al., (2011) based on the measurements of $\delta^{17}\text{O}$, together with $\delta^{18}\text{O}$, to characterize
3 the sources of NO_3^- . Mass-dependent isotope fractionation leads to a consistent relationship
4 between $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$, *i.e.*: $\delta^{17}\text{O} \approx 0.52 \times \delta^{18}\text{O}$ (Matsuhisa et al., 1978; Miller, 2002; Young
5 et al., 2002). However, in the case of ozone-mediated nitrate formation in the atmosphere,
6 mass-independent oxygen isotope compositions are observed (Michalski et al., 2002). This
7 ‘excess’ of ^{17}O is quantified by $\Delta^{17}\text{O} = \delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O}$. This means that ozone-derived
8 NO_3^- has a $\Delta^{17}\text{O} > 0$, while mass-dependent nitrification produces NO_3^- with $\Delta^{17}\text{O} = 0$. These
9 new tools offer the possibility to test some of the hypotheses previously proposed in the
10 literature, in particular to determine the relative contribution of occult dry deposition and of
11 bacterial-mediated nitrification in tree canopies to the chemical composition of canopy TF
12 and the N input to the soil.

13 This study investigated whether N transformations occurred within the tree canopies of four
14 different forests in the UK subjected to different levels of N_{dep} . The $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$
15 concentrations in RF and TF were used to assess the role of canopy in filtering and changing
16 atmospheric N speciation. Furthermore, we used $\delta^{15}\text{N}$ - $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ in NO_3^- and the measure
17 of $\delta^{15}\text{N}$ in NH_4^+ , to assess if and how atmospheric N is processed within the canopy. In
18 particular we tested the following hypotheses: 1) In forests with low levels of N_{dep} (*i.e.*, 10 kg
19 $\text{ha}^{-1} \text{yr}^{-1}$) no differences exist between RF and TF for either ions concentrations or their
20 isotopic signature. Therefore, the composition and isotopic signatures of NO_3^- and NH_4^+ in TF
21 should reflect that of atmospheric N in RF, as a result of low canopy processing and canopy
22 uptake. 2) At high N_{dep} sites, exceeding critical N loads (*i.e.*, 20-30 kg $\text{ha}^{-1} \text{yr}^{-1}$), significant
23 differences exist between RF and TF for both ionic concentrations and their isotopic
24 signature, as a result of isotope fractionations during N processing within the canopy and
25 enhanced by the high input of wet and dry N_{dep} . Finally, we used $\Delta^{17}\text{O}$ to determine if a mass-

1 dependent bacterial nitrification from NH_4^+ to NO_3^- occurs in forest canopies at high N_{dep}
2 levels.

3

4 **2. Materials and Methods**

5 *2.1 Site description and sampling*

6 Two Scots pine (*Pinus sylvestris* L) stands and two beech (*Fagus sylvatica* L.) stands were
7 studied. The pine stands were within the UK Forest Monitoring network
8 (<http://www.forestry.gov.uk/fr/INFD-67MEVC>; Vanguelova et al., 2010), which is part of the
9 ICP European Forest Network. The two beech stands are part of long term experiments on
10 monitoring of the effects of N_{dep} on forest and soil biochemical cycling in the UK
11 (Vanguelova and Pitman, 2009, 2011). Two sites, one for each tree species, were situated at
12 Alice Holt and Rogate (6 km apart) in South East England and the remaining two sites were
13 at Thetford (< 8 km apart), East England. These sites were chosen on the basis of similarity in
14 stand (age, density, and management history), climate, and soil conditions, but at contrasting
15 levels of ambient N_{dep} (Table 1). In particular, the pine and beech stands at Thetford are
16 subjected to higher background levels of N_{dep} (13 kg N ha⁻¹ yr⁻¹ and 19 kg N ha⁻¹ yr⁻¹,
17 respectively) compared to forest stands at Alice Holt and Rogate (9-10 kg N ha⁻¹ yr⁻¹)
18 (Table1). Thetford in East Anglia, is known to be among the areas with highest atmospheric
19 N inputs in the UK (RoTAP report, 2012; Vanguelova et al., 2010), mostly as dry N_{dep} ,
20 coming mainly from the intensive livestock farms (in particular pigs and chickens). Therefore,
21 the two forest stands in Thetford will be referred to as HN (high nitrogen) and the forests in
22 Rogate and Alice Holt as LN (low nitrogen) sites. Rainfall (RF) and throughfall (TF)
23 sampling and analysis have been carried at the sites over a number of years by means of two
24 bulk RF collectors and 10 TF collectors per site. Sampling and analytical procedures followed
25 the level II protocols described in detail in the ICP Forests manual (2010). In this study only

1 samples collected during the 2011 growing season, from June until November, were
2 considered.

3

4 *2.2 Chemical and isotope analyses of water samples*

5 After collection, RF and TF water samples were filtered through a 0.45 μm membrane filter
6 and then analysed for $\text{NH}_4\text{-N}$, colorimetrically, for dissolved organic carbon (DOC) and total
7 N by Carbon analyser (Shimadzu 5000, Osaka, Japan) and for $\text{NO}_3\text{-N}$ by Ion Chromatography
8 (Dionex DX-500). Dissolved organic nitrogen (DON) was calculated from measured total and
9 inorganic nitrogen forms. Other chemical components were measured as described in
10 Vanguelova et al. (2010), but were not included in this investigation. The RF and TF
11 elemental fluxes were calculated using measured water volumes at the sites and measured
12 elemental concentrations. Dry N_{dep} values were estimated as difference between RF and TF
13 for each of the N-form according to European ICP forest monitoring manual, which assumed
14 zero canopy exchange (ICP, 2010) (Table 1).

15 A sub-sample of the water analysed for ion concentrations was used for stable isotope
16 measurements. Water collected from June until August and then from September until
17 November was combined to obtain a minimum of 0.5 mg of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ for the
18 isotope analyses. This was also necessary for RF water samples collected at the two LN and
19 the two HN sites. Pooling RF water samples within each level of N_{dep} are not likely to have
20 impacted the results. There was no significant differences in the amount of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-}$
21 N in RF at either of the two sites, except at Thetford where the $\text{NH}_4\text{-N}$ was significantly
22 ($p < 0.05$) higher in the beech relative to the pine stand. This was likely the result of the beech
23 site being located only a few hundred meters away from a chicken farm that generates NH_3
24 concentrations as high as $\sim 73 \mu\text{g}/\text{m}^3$ (Vanguelova and Pitman, 2009, 2011).

1 Each RF and TF sample was composited as described above and then passed through cation
2 and anion exchange resins. Ammonium from the cation resin was eluted with hydrochloric
3 acid and converted to ammonium sulfate on a quartz filter paper using an alkaline diffusion
4 method (Heaton, 2001). Nitrate from the anion resin was eluted with hydrobromic acid, and
5 processed to silver nitrate (Chang et al., 1999; Heaton et al., 2004). The $^{15}\text{N}/^{14}\text{N}$ ratios of the
6 ammonium sulfate and the silver nitrate were analysed by combustion in a Flash EA on-line
7 to a Delta Plus XL mass spectrometer (ThermoFinnigan, Bremen, Germany), with $\delta^{15}\text{N}$
8 values versus air (atmospheric N_2) calculated by comparison with standards calibrated against
9 IAEA N 1 and N 2 assuming these had values of +0.4‰ and +20.3‰, respectively. $^{18}\text{O}/^{16}\text{O}$
10 ratios of the silver nitrate were analysed by thermal conversion to CO gas at 1400°C in a TC-
11 EA on-line to a Delta Plus XL mass spectrometer (ThermoFinnigan, Bremen, Germany), with
12 $\delta^{18}\text{O}$ values calculated versus SMOW by comparison with IAEA- NO_3 assuming it had a value
13 of +25.6‰. Analytical precisions (1 SD) were typically <0.3‰ for $\delta^{15}\text{N}$ and <0.6‰ for
14 $\delta^{18}\text{O}$. Finally, a sub-sample of the composite RF and TF water as described above was used
15 for $\delta^{17}\text{O}$ measurements by Delta V Plus ratio mass spectrometer. The NO_3^- was converted to
16 O_2 and N_2 using the denitrifier method (Casciotti et al., 2002; Kaiser et al., 2009). Analytical
17 precisions (1 SD) for $\Delta^{17}\text{O}$ were <1.0‰ based on replicate analysis of the reference material
18 USGS35.

19

20 *2.3 Data analyses*

21 *2.3.1 Statistical analyses*

22 Concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ were log-transformed to account for non-normality and
23 variance heterogeneity, as assessed through Shapiro and Levene test, respectively.
24 Independent sample *t*-tests were employed to test for differences between deposition levels
25 (e.g. HN and LN) and water samples (i.e., RF and TF) for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$, while, within

1 each water sample, differences between concentrations of different compounds were tested
 2 through paired-samples *t*-test (*t*). The non-parametric Wilcoxon test (*W*) was employed when
 3 log-transformed data did not conform to a normal distribution. Given the small sample size
 4 available for the isotopic data, we calculated the difference in isotopic fractionation between
 5 TF and RF without separating beech and pine stands and used a *t*-test to test the significance
 6 of the difference between LN and HN stands. The level of significance of all statistical tests
 7 was set as $p \leq 0.05$. R project statistical computing (vers. 3.0.2; R Core Development Team,
 8 2014) was used for all the analyses.

9

10 2.3.2 Mass balance calculation

11 A mass balance approach, based on the use of $\Delta^{17}\text{O}$, was employed to assess the sources of
 12 NO_3^- collected in the TF at the HN sites by using end-members analyses using the following
 13 equations:

$$14 \quad \Delta^{17}\text{O}_{\text{TF}} = f_{\text{Bio}}(\Delta^{17}\text{O}_{\text{Bio}}) + f_{\text{Atm}}(\Delta^{17}\text{O}_{\text{Atm}}) \quad (1)$$

15 where $\Delta^{17}\text{O}_{\text{TF}}$ is the measured isotopic composition of NO_3^- in TF, while $\Delta^{17}\text{O}_{\text{Bio}}$ and $\Delta^{17}\text{O}_{\text{Atm}}$
 16 indicate the isotopic signatures of the biologically and atmospherically-derived NO_3^- ,
 17 respectively. f_{Bio} and f_{Atm} are the unknown NO_3^- mole fractions from the two different sources,
 18 the sum of which is 1. f_{Atm} included both wet (f_{wet}) and the dry (f_{dry}) NO_3^- deposition washed
 19 out from the canopy and not retained and/or taken up by the canopies (f_U), i.e., $f_{\text{Atm}} = f_{\text{wet}} + f_{\text{dry}} -$
 20 f_U . Assuming that $\Delta^{17}\text{O}_{\text{Bio}} = 0$ (Michalski et al. 2003), equation 1 can be reduced to:

$$21 \quad f_{\text{Atm}} = (\Delta^{17}\text{O}_{\text{TF}} / \Delta^{17}\text{O}_{\text{Atm}}) \quad (2)$$

22 and

$$23 \quad f_{\text{Bio}} = 1 - f_{\text{Atm}} \quad (3)$$

24

25

1

2 **3. Results**3 *3.1 Concentrations of NH₄-N and NO₃-N in RF and TF*

4 The concentration of N compounds in RF and TF at the two sites were significantly different.
5 At the two LN sites the concentrations of ions in RF were not significantly different (Fig. 1)
6 and the RF and TF had similar NH₄-N and NO₃-N concentrations, (Scots pine: $t=1.78, 7.97$
7 and $p=0.11, 0.56$, respectively; beech: $W=163, 125$ and $p=0.73, 0.48$, respectively). In
8 contrast, at the HN sites, the NH₄-N and NO₃-N concentrations were significantly higher in
9 TF compared to RF, for both Scots pine ($t=6.42, 6.26, 4.45$, respectively; all $p<0.001$) and
10 beech ($W=265, 250$, respectively; all $p<0.001$) (Fig. 1). Ion concentrations in both RF and TF
11 were significantly higher at the HN (**Scots pine** – RF: NO₃-N = 0.58 ± 0.09 , NH₄-N = $0.72 \pm$
12 0.09 ; TF: NO₃-N = 3.04 ± 0.69 , NH₄-N = 2.47 ± 0.38 ; **Beech** –RF: NH₄-N = 2.09 ± 0.18 ; TF:
13 NO₃-N = 1.53 ± 0.17 , NH₄-N = 7.18 ± 0.9) than LN sites (**Scots pine** – RF: NO₃-N = 0.31 ± 0.05 ,
14 NH₄-N = 0.27 ± 0.03 ; TF: NO₃-N = 0.69 ± 0.30 , NH₄-N = 0.66 ± 0.21 ; **Beech** – RF: NH₄-N =
15 0.5 ± 0.12 ; TF: NO₃⁻ = 0.60 ± 0.06 , NH₄-N = 0.47 ± 0.06), with the exception of RF in the
16 beech stands, which had similar NO₃-N concentrations (LN = 0.47 ± 0.09 ; HN = 0.53 ± 0.05).
17 The mean of total N fluxes based on measured rainfall and measured during the 6 months we
18 considered in this study (*i.e.*, June to November 2011) are reported in Table 2. TF-N fluxes
19 were higher than RF fluxes at the HN sites, with particular reference to the NH₄⁺ at the beech
20 site. By contrast, at the LN sites RF N-fluxes were higher than TF-N fluxes for both species
21 (Table 2).

22

23 *3.2 Values of $\delta^{15}\text{N-NH}_4$*

24 Values of $\delta^{15}\text{N}$ of NH₄ in RF (Fig. 2A) ranged from positive at the HN site ($+1.49 \pm 3.5\text{‰}$) to
25 very negative at the LN site ($-9.14\text{‰} \pm 0.2$). Due to the limited number of measurements (*i.e.*,

1 n=2 per species and per water sample), statistical analyses of isotope data were performed per
2 level of N_{dep} , combining data for both tree species and focussing on the differences between
3 RF and TF. However, TF values measured separately for beech and Scots pine are presented
4 in Figure 2A, to show the species-specific changes in the isotope compositions in N
5 compounds collected below the canopies. More positive values were measured for $\delta^{15}\text{N-NH}_4$
6 in TF compared to RF at both HN ($t=-2.85$, $p<0.05$) and LN ($t=-15.16$, $p<0.001$) sites. The
7 TF-RF difference for $\delta^{15}\text{N}$ in NH_4 was much higher ($t=-2.65$, $p<0.05$) at the LN compared to
8 the HN site (Fig. 2B).

9

10 3.3 Values of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O-NO}_3^-$

11 The $\delta^{15}\text{N}$ in NO_3^- of RF (Fig. 3A) showed similar negative values at the HN ($-3.4\text{‰} \pm 1.4$)
12 and LN sites ($-2.8\text{‰} \pm 1.7$). Albeit lower, the $\delta^{15}\text{N-NO}_3^-$ values in TF at the HN sites (diff=
13 $4.9\text{‰} \pm 3.4$) were only slightly different ($t= -1.72$, $p=0.06$) compared to the LN sites (diff=
14 $+1.1\text{‰} \pm 0.54$) (Fig. 3D). Despite differences between RF and TF for $\delta^{15}\text{N}$ in NO_3^- not being
15 significant within each level of N_{dep} , it is worth mentioning that at the HN sites, $\delta^{15}\text{N}$ in NO_3^-
16 showed more negative values in TF than RF, especially for Scots pine (Figure 3A).

17 The $\delta^{18}\text{O}$ in NO_3^- of RF showed similar values at the two different levels of N_{dep} , i.e., LN =
18 $63.9\text{‰} \pm 0.88$; HN = $64.1\text{‰} \pm 3.2$ (Fig. 3B). Within each level of N_{dep} , $\delta^{18}\text{O}$ values did not
19 significantly differ between RF and TF. However, a significant contrast ($t=-2.34$, $p<0.05$) was
20 found in the difference between the $\delta^{18}\text{O}$ values of NO_3^- in TF compared with RF across
21 levels of N_{dep} (Figure 3E), with more negative $\delta^{18}\text{O-NO}_3^-$ values at HN than LN sites.

22 $\Delta^{17}\text{O}$ values measured in RF at our sites ranged from $23.14 (\pm 0.58)\text{‰}$ at the LN sites to 25.53
23 $(\pm 0.76)\text{‰}$ at the HN sites. A significant difference was found in the $\Delta^{17}\text{O}$ of NO_3^- in the TF
24 vs. RF at the HN sites ($W=16$, $p<0.05$), but not at the LN sites. Within individual species, it is

1 worth pointing out that beech showed lower $\Delta^{17}\text{O}$ values than Scots pine (Figure 3C). When
2 we considered the difference between RF and TF, $\Delta^{17}\text{O}$ values in NO_3^- had lower values at the
3 HN sites ($t = -1.86, p=0.05$) than LN sites (Fig. 3F).

4 Combined plots for the three isotopic species of NO_3^- at the Scots pine and beech sites are
5 given in Figure 4 as trajectories of change from RF to TF values, to emphasise the
6 consequences of canopy processing for the three tracers, with particular references to forests
7 at HN levels. For Scots pine (Fig. 4 A and B), only in the case of HN sites did $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and
8 to less extent $\Delta^{17}\text{O}$ values in TF diverge from those measured in RF. For beech (Fig. 4 C and
9 D), distinct changes in $\delta^{18}\text{O}$ vs. $\delta^{15}\text{N}$ were not observed, and only in the case of HN sites, did
10 $\Delta^{17}\text{O}$ become lower from RF to TF.

11

12 *3.4 Assessing the source of NO_3^- in the TF at the sites with high atmospheric N loads*

13 Mass balance calculations (Eq. 2 and 3 in the section 2.3.2) were used to estimate the relative
14 contribution of atmospheric vs. nitrification-derived NO_3^- collected underneath tree canopies.

15 Using the two end-member mixing model with $\Delta^{17}\text{O}$ values measured in TF and RF during
16 2011 growing season (Table 3), the fraction of NO_3^- in TF coming from nitrification (f_{bio})

17 ranged from 0.13 up to 0.6 (*i.e.*, 13 to 60%) at the two HN sites (Fig. 5A). Most of the NO_3^-
18 collected in the TF at the Scots pine stand derived from the atmosphere (mean of $f_{Atm} =$

19 0.83 ± 0.05), with only a minor contribution from nitrification (mean of $f_{Bio} = 0.17 \pm 0.05$). By

20 contrast, biologically-derived NO_3^- seemed to be the dominant fraction of the NO_3^- in TF of
21 the beech stand ($f_{Bio} = 0.59 \pm 0.04$), at least for the time period considered in this study (Fig. 5

22 A). Interestingly, the f_{Bio} seems to show a species specific seasonal trend, although the few
23 point measurements did not allow us to test it statistically (Fig. 5B). Nevertheless, the mass

24 balance calculations did not help assessing the source of NO_3^- in TF at the LN sites. When we

25 used eq. 2 with $\Delta^{17}\text{O}$ values measured at the LN sites, we obtained values of $f_{Atm} > 1$ for beech

1 ($f_{Atm} = 1.1$ in June-August and 1.2 in September-November), which then held to negative
2 values for f_{Bio} . Whereas in the case of the Scots pine f_{Atm} was > 1 (*i.e.*, 1.2) for the late
3 summer/fall months, while in the most active period of the growing season we were able to
4 detect that f_{Atm} and f_{Bio} were 0.51 and 0.49, respectively. However, the two end-member
5 mixing model with $\Delta^{17}O$ should be used with caution at the LN sites. Indeed, in this case
6 using $\Delta^{17}O$ as the end-member of the mixing model can be sensitive to errors, due to the low
7 NO_3-N concentrations in RF and similar NO_3-N values in RF and TF coupled with the lack of
8 difference in $\Delta^{17}O-NO_3^-$ between RF and TF.

9

10 **4. Discussion**

11

12 *4.1 Characterizing atmospheric N and its isotopic signatures at the contrasting N_{dep} levels*

13 Both tree species at HN sites were subjected to air masses with high NH_3 concentrations and
14 had higher NH_4-N deposition relative to the LN sites. The HN beech site, which is right next
15 to an intensive chicken farm, is trapping the farms high NH_3 emissions along a very distinct
16 200 m long N gradient where concentrations decrease to levels similar to those in the Scots
17 pine site or the unpolluted Alice Holt site (Vanguelova and Pitman, 2009). This is showed by
18 the higher NH_4-N concentrations at the beech site than the HN Scots pine site, while no
19 difference was found for NO_3-N concentrations (Fig. 1). These results are in line with the
20 results from long-term monitoring within the ICP forest network, which showed that Thetford
21 is among the sites receiving highest N deposition in the UK (RoTAP report, 2012;
22 Vanguelova et al., 2010), due to the high N_{dep} , mostly as dry N_{dep} and in the reduced form,
23 coming mainly from the intensive livestock farms (in particular pigs and chickens).
24 Most of the N deposited as NO_3-N came from the wet portion and NO_3-N deposition was
25 similar at both HN and LN sites (*e.g.*, 3.3 and 3.2 kg N ha⁻¹ yr⁻¹, respectively; Table 1). This

1 supports the evidence that in the comparisons of HN with LN areas, amount and chemical
2 form of dry deposition play important roles in canopy N filtering and in the amount reaching
3 the soils. Records over more than 10 years also suggest the overall total N_{dep} at the Thetford
4 pine site has decreased due to reductions in wet (in both forms $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) rather than
5 dry deposition (Vanguelova et al., 2010), confirming the national trend (RoTAP, 2012). The
6 different contribution of dry vs. wet N_{dep} at the site-level is in agreement with deposition rates
7 obtained from 5x5 km scale modelled deposition map (RoTAP report, 2012). The modelled
8 (5 x 5 km) data suggested similar values of the total oxidized N forms (NO_3 , NO_2 and HNO_3)
9 between HN ($17.92 \text{ kg ha}^{-1} \text{ yr}^{-1}$) and LN ($18.68 \text{ kg ha}^{-1} \text{ yr}^{-1}$) sites. Whereas, the HN
10 and LN sites had different rates of measured NH_3/NH_4 deposition ($30 \text{ kg ha}^{-1} \text{ yr}^{-1}$ and 16 kg
11 $\text{ha}^{-1} \text{ yr}^{-1}$, respectively) with the dry NH_4/NH_3 deposition contributing the most to the total
12 (HN= 25.5 versus LN= $11.31 \text{ kg ha}^{-1} \text{ yr}^{-1}$). In contrast there were similar rates of NH_4^+ wet
13 deposition at both the HN ($5.7 \text{ kg ha}^{-1} \text{ yr}^{-1}$) and LN ($5.3 \text{ kg ha}^{-1} \text{ yr}^{-1}$) sites. This suggests that
14 capturing small scale variability in N_{dep} , and especially as dry deposited NH_3 is vital to
15 understanding and assessing the impact on the environment.

16 Isotopic signatures measured in NO_3^- and NH_4^+ in RF (Fig. 2, 3A, B) at our sites were in the
17 same range of values found in previous analyses of monthly rainfall samples from a range of
18 sites in the UK (Heaton et al, 1997; Curtis et al., 2012; Heaton, unpublished data; Table 4).
19 Overall, $\delta^{15}\text{N}$ values in NH_4^+ measured across the UK ranged from negative to positive values
20 (-12.6‰ to $+2.8\text{‰}$), with a mean of -4.3‰ . The positive values observed at the Thetford sites
21 are likely reflecting the contribution of NH_4/NH_3 emissions coming from the chicken
22 intensive farms in the area. Indeed, Heaton et al. (1997) reported that the $\delta^{15}\text{N}$ value of TF
23 ammonium in part of a Scots pine plantation artificially fumigated with ammonia gas was
24 17‰ higher than the value for TF in the non-fumigated part of the plantation. Moreover, in a
25 recent study Yeatman et al. (2001) measured $\delta^{15}\text{N}$ values of $+13.5\text{‰}$ in aerosol- NH_4 sampled

1 near chicken, cow and pig livestock enterprises and positive $\delta^{15}\text{N}$ values in bulk precipitation
2 were also reported by Emmett et al. (1998) for two conifer stands near livestock feed lots in
3 the Netherland.

4 The $\delta^{15}\text{N}$ values of NO_3^- were in the same range as those reported in the study by Heaton et
5 al. (1997). However, a high range of values was measured across the UK (-8.2‰ to +4.3‰)
6 (Table 4), with a mean $\delta^{15}\text{N}$ - NO_3^- values of -2‰. A similar range of $\delta^{15}\text{N}$ values in NO_3^- from
7 -11 ‰ to +3.5 ‰ was reported in studies across the USA (Kendall et al., 1998; Kendall et al.,
8 2007; Elliott et al., 2007), while Tobarí et al. (2010) measured $\delta^{15}\text{N}$ values in bulk
9 precipitation ranging from -7 to +15.4 ‰ across different watersheds in Japan. Moreover, a
10 number of studies in the literature used $\delta^{15}\text{N}$ to assess the anthropogenic NO_x source. For
11 instance, very negative (-13‰ to -2‰) $\delta^{15}\text{N}$ - NO_x values were reported in the case of
12 emissions coming from traffic, while positive values (between 4 ‰ and 16 ‰) were measured
13 for emissions from coal-fired power plants (Heaton, 1990). Similar values of $\delta^{15}\text{N}$ - NO_3^- in RF
14 at HN and LN sites in our study suggest a similar anthropogenic NO_x source, most likely
15 emissions coming from local road traffic. This is confirmed also by the similar values we
16 measured for $\delta^{18}\text{O}$ - NO_3^- in RF, irrespective of the N_{dep} levels (i.e., LN=63.9 ‰ \pm 0.88 and
17 HN=64.1 ‰ \pm 3.2). Moreover, $\Delta^{17}\text{O}$ in RF at the HN sites was 2‰ higher than that measured
18 at the LN sites, suggesting that NO_x went through different oxidation processes (Michlaski et
19 al., 2003). $\Delta^{17}\text{O}$ values measured at our sites (ranging from 22 ‰ to 26 ‰) were similar to
20 those reported by Costa et al. (2011) for NO_3^- in rain samples (23.1 ‰ \pm 1.8) collected in
21 Michigan and by Michalski et al. (2004) in aerosol (26 ‰ \pm 3) sampled in Southern
22 California.

23

24 *4.2 Canopy N processes as detected through stable isotopes*

1 Our analyses showed that only at HN sites there were significant increases in $\text{NH}_4\text{-N}$ and
2 $\text{NO}_3\text{-N}$ concentrations in TF relative to RF, irrespective of tree species (Fig. 1), confirmed
3 also when we looked at the N fluxes (Table 2). This result is in line with previous studies in
4 the literature (Vanguelova et al., 2010; De Vries et al., 2014) and it suggests that at areas with
5 high dry N_{dep} , canopy filtering and rain washing will contribute to increasing the N input to
6 the soils compared to low dry N_{dep} areas. By contrast, at the LN sites, TF-N fluxes were lower
7 than RF N-fluxes, suggesting that most of the atmospheric N is retained by tree canopies, as
8 observed also in other studies in the literature (Fenn et al., 2013, Ferretti et al., 2014).
9 However, is it only the contribution of dry N_{dep} that helps explaining the higher NH_4^+ and
10 NO_3^- concentrations in TF at the HN sites? To answer to this question, we used a quadruple
11 isotope approach, namely the $\delta^{15}\text{N}$ in NO_3^- and NH_4^+ , $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ in NO_3^- .
12 The pathway through the canopies did affect the isotopic signature of both NH_4^+ and NO_3^- ,
13 with TF generally showing ^{15}N -enriched NH_4^+ and ^{15}N -depleted NO_3^- .
14 The more positive values for $\delta^{15}\text{N}$ in NH_4^+ collected in TF are consistent with the overall
15 increase of $\text{NH}_4\text{-N}$ in TF at the Thetford site (Fig. 1-2). In addition, the $\delta^{15}\text{N}$ values of NH_4^+
16 in dry deposition tend to be higher than those measured in bulk precipitation (Heaton, 1997),
17 suggesting that a fraction of the measured TF originated from dry N_{dep} . The more positive
18 values $\delta^{15}\text{N}$ in $\text{NH}_4\text{-N}$ are also consistent with the occurrence of nitrification in the canopy,
19 which should result in the accumulation of ^{15}N -enriched NH_4^+ (Högberg 1997), as a residual
20 product. Interestingly, while at the LN sites the $\text{NH}_4\text{-N}$ concentration did not vary
21 significantly from RF to TF, a fingerprint of dry N_{dep} was still detected at these sites by the
22 ^{15}N enrichment in NH_4^+ underneath the canopies.
23 $\text{NO}_3\text{-N}$ concentrations in TF were higher at HN forest stands than at the LN stands, and it was
24 expected that this difference would be reflected also in the isotopic signature of both N and O
25 in nitrate. The higher $\text{NO}_3\text{-N}$ at the HN sites for both Scots pine and beech in TF, could in

1 principle also result from a combination of dry deposition and canopy nitrification processes.
2 As in the case of NH_4^+ , higher values of $\delta^{15}\text{N}$ of NO_3^- in TF compared to RF could be
3 expected (Heaton, 1997), but were not found at these sites (Fig. 3A). Nitrification of NH_4^+
4 leads to the production of ^{15}N depleted NO_3^- leaving behind more ^{15}N enriched NH_4^+
5 (Högberg, 1997). Indeed, we did measure more negative (but not significantly so) $\delta^{15}\text{N}\text{-NO}_3^-$
6 values in TF at the HN site (diff= $-4.9\text{‰} \pm 3.4$) compared to the LN (diff= $+1.1\text{‰} \pm 0.54$).
7 The ^{15}N depletion of NO_3^- in TF was particularly detected for Scots pine at the HN site. (Fig.
8 3A, 4A) A decrease in $\delta^{15}\text{N}$ in NO_3^- from RF to TF was reported in studies in a spruce forest
9 in Germany by Sah and Brumme (2003) and in a montane rain forest in Ecuador by Schwarz
10 et al. (2011), explained in both cases by isotope fractionation during nitrification of NH_4^+ to
11 NO_3^- in the canopy leaves. However, none of these previous studies could unequivocally
12 attribute the shifts in $^{15}\text{N}\text{-NO}_3^-$ to biological NH_4^+ nitrification. In our study, evidence of
13 nitrification occurring within the canopy was clearly provided by $\Delta^{17}\text{O}$, since there is not
14 likely to be a big $\Delta^{17}\text{O}$ difference in dry deposited NO_3^- compared to wet NO_3^- . Indeed, using
15 a mass balance approach, we found that at the HN sites nitrification can contribute up to 60%
16 to the NO_3^- recovered underneath the tree canopies (Fig. 5A, B). Interestingly, for beech at the
17 HN, the $\delta^{18}\text{O}\text{-NO}_3^-$ signals in TF vs. RF could not clearly provide an indication of nitrification,
18 while $\Delta^{17}\text{O}$ proved that the fractional contribution of biologically-derived NO_3^- was as much
19 as that from the atmospherically-derived NO_3^- (Fig. 5A). Interestingly, the seasonal trend of
20 f_{Bio} goes in the opposite direction for the two investigated species. Indeed the beech showed a
21 reduction in f_{Bio} towards in the late summer/fall, likely associated to the phenology and
22 therefore the changes in the leaf physiology.

23 One could ask: is it so relevant to determine the sources of the NO_3^- reaching the soil?
24 Beyond the effect of canopy nitrification on potential N uptake by trees, the fact that canopies
25 can process some of the atmospheric NH_x before even reaching the soil has important

1 implications on the different biogeochemical processes occurring in the soil. In particular, the
2 proportion of NH_x vs. NO_x exchanged by canopies will affect the role of soil as sink (through
3 ammonification and nitrification) vs. source (by denitrification) of N.

4 The dramatic reduction of $\delta^{15}\text{N}$ and to less extent of $\delta^{18}\text{O}\text{-NO}_3$ in TF vs. RF for the Scots pine
5 at HN (Fig. 4A) could not be attributed solely to atmospheric dry deposition or canopy
6 exchange. This result suggests that also other processes might take place at this site. One
7 possibility might be that NO_x emissions from the soil, likely related to denitrification
8 processes, react with tree canopies before reaching the atmosphere. A number of studies have
9 shown that part of the soil NO emission can be converted to NO_2 by reacting with ozone,
10 which in turn can be either trapped in the stem space below the canopy or processed again
11 within the canopy (Rennenberg et al., 1998; Dorsey et al., 2004; Duyzer et al., 2004;
12 Neiryneck et al., 2007). Among the level II plots in the UK, symptoms of N saturation have
13 been observed at Thetford, with high mean annual NO_3^- concentrations in soil solution at the
14 Scots pine stand of up to 40 mg/l (Vanguelova et al., 2009, 2011). A preliminary study also
15 suggested significantly higher soil respiration at the HN compared to LN pine sites, which
16 may imply that the NO_x emission could be higher too (Vanguelova, personal communication).
17 Under these conditions, denitrification might take place at this site, although no direct
18 measurements are currently available to support this hypothesis. Such denitrification is likely
19 to produce NO and NO_2 depleted in both ^{15}N and ^{18}O relative to soil nitrate, with very low,
20 negative $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values (Li and Wang, 2008; Felix and Elliot, 2014). Subsequent
21 reactions with ozone would increase $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values, but not $\delta^{15}\text{N}$. Thus, nitrate formed
22 by this mechanism might have $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values similar to atmospheric nitrate, but with
23 very low $\delta^{15}\text{N}$ values compared to RF.

24

25 **5. Conclusion**

1 To our knowledge, this is the first study that combined measurements of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$
2 fluxes together with their relative isotope signatures, i.e., $\delta^{15}\text{N}$ in NO_3^- and NH_4^+ and $\delta^{18}\text{O}$ and
3 $\Delta^{17}\text{O}$ in NO_3^- to determine the role of canopy filtering of atmospherically-derived N_{dep} .
4 Specifically, changes in $\Delta^{17}\text{O}$ in NO_3^- as N passes through forest canopies had not previously
5 been determined. Oxygen isotopes have been mostly considered at the watershed level, to
6 characterize the source of NO_3^- in the stream water, but have not previously been applied to
7 the investigation of the role of canopy in processing atmospheric N. Our results partially
8 confirm our initial hypotheses that:

9 1) At the LN sites, ion concentrations in TF and their respective isotopic signatures
10 reflected the input of atmospheric N as derived from RF. However, isotope data revealed
11 that even with a low atmospheric N load, canopies played an important role in
12 intercepting and retaining dry N_{dep} (with particular reference to the reduced N-form),
13 which represents an additional (but often overlooked) N source relative to wet N_{dep} as
14 assessed through RF.

15 2) At the HN sites, the passing of atmospheric N through canopies affected both ion
16 concentrations and their isotopic signature. The occurrence of dry deposition probably
17 explains the higher NH_4^+ concentrations and ^{15}N enrichment in NH_4^+ measured below the
18 canopy in TF water vs. RF. As for the higher NO_3^- in TF vs. RF, the isotopes $\delta^{15}\text{N}$ and
19 $\delta^{18}\text{O}$ could not provide clear indications of its origin, even though for Scots pine $\delta^{15}\text{N}$ -
20 NO_3^- provided some indications of biologically-derived NO_3^- . The unambiguous response
21 came however from $\Delta^{17}\text{O}$, which allowed to determine that a consistent fraction of the
22 NO_3^- recovered underneath the canopies derived from biological nitrification, with an
23 unexpected higher magnitude at the beech stand. Moreover, the very negative values of
24 $\delta^{15}\text{N}$ in $\text{NO}_3\text{-N}$ for Scots pine at the N-saturated site, suggested that nitrate could derive

1 also from soil NO_x emissions being re-processed and deposited again in the canopy before
2 mixing in TF water.

3 This study is part of a broader project, aiming to look at the effect of ambient N_{dep} on tree
4 growth, water-use efficiency and $\delta^{15}\text{N}$ in tree rings. Hence, the characterization of the short-
5 term N input from the atmosphere is a crucial step toward a better understanding of the long-
6 term effect of N_{dep} to be assessed through stable isotopes in tree rings (Guerrieri et al., in
7 preparation).

8 We acknowledge that the conclusions of this study rely on a limited number of isotope
9 measurements at each site and a limited selection of forest stands, which did not allow
10 detailed investigations of the tree species-specific pattern of canopy N transformations.
11 However, the study identified canopy processing of atmospheric deposition (and especially
12 canopy biological nitrification) as a major process that should not be neglected and needs
13 further exploration, to better quantify the role of forests as sinks but also sources of N. This
14 has important implications for policy-related emission abatement strategies, which aim to
15 manage forests and landscape not only for enhancing C-sequestration, but also for
16 atmospheric N capture.

17

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3

4

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1 **Table 1.** Site, climatic and atmospheric N_{dep} information of the four forest stands included in the study. Climate data are mean values calculated
 2 over the years 1960-2010 and deposition data are mean values over a number of years (*e.g.*, Alice Holt - Beech site- 2006-2008; Rogate-Scots
 3 pine site - 2010-2012; Thetford Scots pine site - 1995-2010; Thetford-Beech site - 2006-2008).

Site	Species	Stand age	Soil type (WRB, 2006)	Precipitation (mm)	T (° C)	NH ₄ ⁺ /NO ₃ ⁻ Dry (kg ha ⁻¹ yr ⁻¹)	NH ₄ ⁺ /NO ₃ ⁻ Wet (kg ha ⁻¹ yr ⁻¹)	totN dep Dry/Wet (kg ha ⁻¹ yr ⁻¹)	Tot N_{dep} (kg ha ⁻¹ yr ⁻¹)
Alice Holt	Beech	70	Cambisol	800	11.6	2.7/0.2	3.7/3.2	2.9/6.9	9.8
Rogate	Scots pine	60	Cambisol	800	11.6	4.1/0.6	3.1/2.9	4.8/5.9	10.7
Thetford	Beech	70	Arenosol	600	11.3	4.9/4.6	7.5/2.7	9.5/10.2	19.7
	Scots pine	45				3.2/1.8	5/3.3	5.0/8.4	13.4

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Table 2. Mean values of the NH_4^+ and NO_3^- fluxes measured over the 6 months considered in this study, *i.e.*, June to November 2011) at the two LN sites (e.g. Alice Holt - Beech stand; Rogate-Scots pine stand) and HN sites (Thetford Scots pine stand; Thetford-Beech stand).

Site	Species	$\text{NH}_4^+/\text{NO}_3^-$ RF ($\text{kg ha}^{-1} \text{ yr}^{-1}$)	$\text{NH}_4^+/\text{NO}_3^-$ TF ($\text{kg ha}^{-1} \text{ yr}^{-1}$)
Alice Holt	Beech	0.8/0.7	0.2/0.3
Rogate	Scots pine	0.5/0.5	0.3/0.3
Thetford	Beech	1.6/0.5	4.9/1.0
	Scots pine	0.6/0.5	1.8/1.9

Table 3. $\Delta^{17}\text{O}$ values for NO_3^- in rainfall (RF) and throughfall (TF) water samples collected during 2011 growing season at the HN sites. Water sampled in June-August and September-November was combined for isotope analyses. This was necessary also for RF water samples collected at the Scots pine and beech stands (see Materials and Methods for more details).

Site	Forest stand	Months	$\Delta^{17}\text{O}$ (‰)	
			TF	RF
Thetford (HN)	Scots pine	June-August	21.72	24.99
		September-November	20.71	26.06
	Beech	June-August	10.06	26.06
		September-November	10.98	24.99

Table 4. Range of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values for NO_3^- and NH_4^+ in monthly rainfall samples from mainly remote upland areas in north and west mainland Britain (Heaton et al, 1997; Curtis et al., 2012; Heaton, unpublished data)

Isotope	Total range	Mean	Interquartile range	N
$\delta^{15}\text{N}\text{-NO}_3$	-8.2‰ to +4.3‰	-2.0‰	-3.8‰ to -0.5‰	117
$\delta^{18}\text{O}\text{-NO}_3$	+50‰ to +82‰	+69‰	+65‰ to +73‰	117
$\delta^{15}\text{N}\text{-NH}_4$	-12.6‰ to +2.8‰	-4.3‰	-6.2‰ to -2.8‰	86

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Figure 1. $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ in Rainfall (RF) and Throughfall (TF) Scots pine and beech forests at the LN (*i.e.*, Alice and Holt and Rogate, respectively) and HN (*i.e.*, Thetford). Each symbol represents the mean (\pm SE) for ions concentrations measured in water samples collected from June until November 2011.

Figure 2. A) $\delta^{15}\text{N}$ values of N-NH_4^+ in Rainfall (RF) and Throughfall (TF) Scots pine and beech at the LN (*i.e.*, Alice and Holt and Rogate, respectively) and HN (*i.e.*, Thetford). Each symbol represents the mean (\pm SE) for isotope measurements carried out in water samples collected from June-August and September- November 2011. **B)** Differences (mean \pm CI) between TF and RF for $\delta^{15}\text{N-NH}_4^+$ values measured at the LN and HN sites, without distinguishing between tree species. Please note that the two symbols for the $\delta^{15}\text{N-NO}_3^-$ values measured in TF for the two species overlap (*i.e.*, Scots pine = $-1.76 \pm 0.17 \text{‰}$; beech = $-1.72 \pm 1.25 \text{‰}$).

Figure 3. A) $\delta^{15}\text{N}$, **B)** $\delta^{18}\text{O}$ and **C)** $\Delta^{17}\text{O}$ values of N-NO_3 in rainfall (RF) and throughfall (TF) for Scots pine and beech at the LN (*i.e.*, Alice and Holt and Rogate, respectively) and HN (*i.e.*, Thetford). Each symbol represents the mean (\pm SE) for isotope measurements carried out in water samples collected from June-August and September-November 2011. Differences (diff., mean \pm CI) between TF and RF for **D)** $\delta^{15}\text{N-NO}_3^-$, **E)** $\delta^{18}\text{O-NO}_3^-$ and **F)** $\Delta^{17}\text{O}$ values measured at the LN and HN sites, without distinguishing between tree species.

Figure 4. $\delta^{15}\text{N}$ vs. $\delta^{18}\text{O}$ and $\delta^{18}\text{O}$ vs. $\Delta^{17}\text{O}$ for Scots pine (**A** and **B**, respectively) and beech (**C** and **D**, respectively) measured in RF and TF. Each symbol represents the mean (\pm SE) for isotope measurements carried out in water samples collected from June-August and September- November 2011 at the LN (Rogate and Alice Holt for the Scots pine and Beech,

respectively) and HN (Thetford, for both tree species). Arrows depict dramatic changes from RF to TF for the isotope values.

Figure 5. A) Mean (\pm SD) of the NO_3^- fraction derived from the atmosphere (f_{Atm}) and nitrification (f_{Bio}) measured for the June-August and September-November months at the two HN forest stands. **B)** Fraction of NO_3^- derived from nitrification (f_{Bio}) for the two sampling periods (*i.e.*, June-August and September-November) at the HN forest sites.

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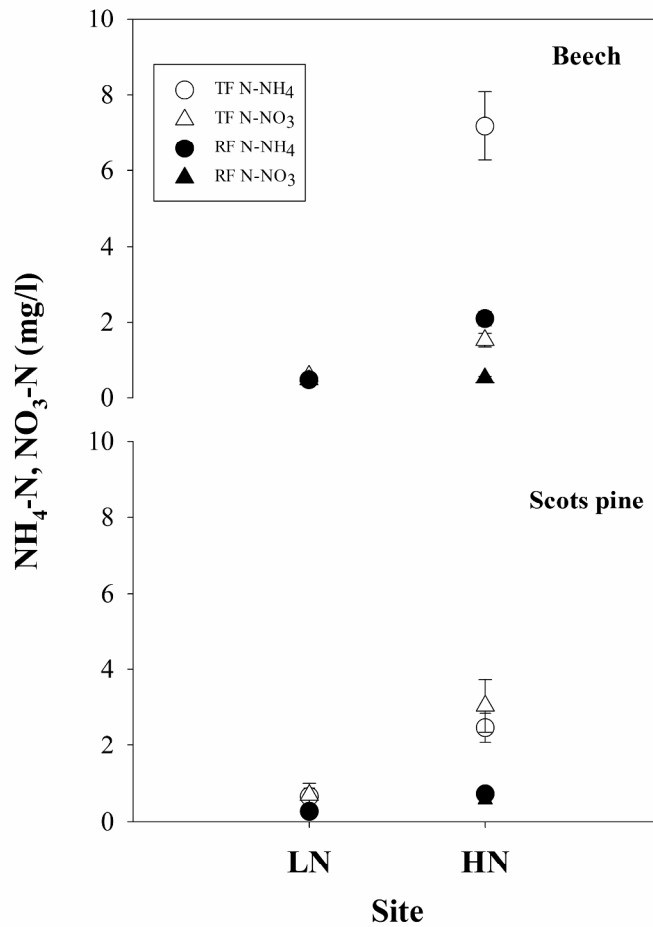


Figure 1. $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ in Rainfall (RF) and Throughfall (TF) Scots pine and beech forests at the LN (i.e., Alice and Holt and Rogate, respectively) and HN (i.e., Thetford). Each symbol represents the mean (\pm SE) for ions concentrations measured in water samples collected from June until November 2011.
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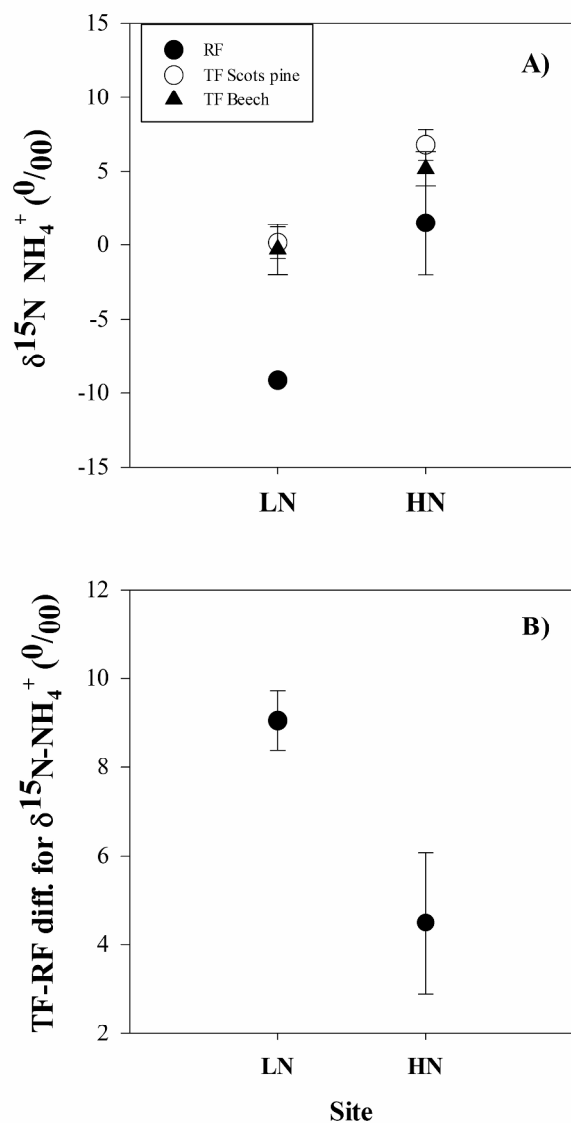


Figure 2. A) $\delta^{15}\text{N}$ values of N-NH_4^+ in Rainfall (RF) and Throughfall (TF) Scots pine and beech at the LN (i.e., Alice and Holt and Rogate, respectively) and HN (i.e., Thetford). Each symbol represents the mean (\pm SE) for isotope measurements carried out in water samples collected from June-August and September-November 2011. B) Differences (mean \pm CI) between TF and RF for $\delta^{15}\text{N-NH}_4^+$ values measured at the LN and HN sites, without distinguishing between tree species. Please note that the two symbols for the $\delta^{15}\text{N-NO}_3^-$ values measured in TF for the two species overlap (i.e., Scots pine = -1.76 ± 0.17 ‰; beech = -1.72 ± 1.25 ‰).

309x506mm (300 x 300 DPI)

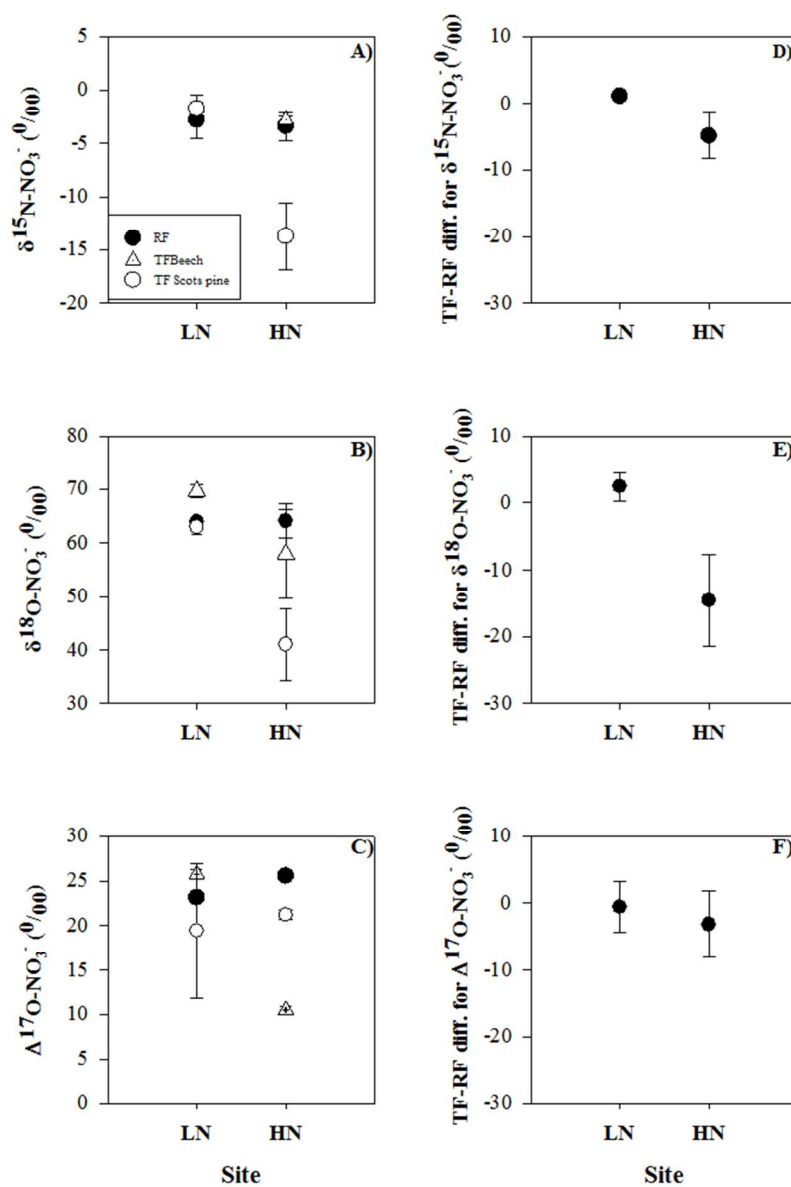


Figure 3. A) $\delta^{15}\text{N}$, B) $\delta^{18}\text{O}$ and C) $\Delta^{17}\text{O}$ values of N-NO₃ in rainfall (RF) and throughfall (TF) for Scots pine and beech at the LN (i.e., Alice and Holt and Rogate, respectively) and HN (i.e., Thetford). Each symbol represents the mean (± SE) for isotope measurements carried out in water samples collected from June-August and September-November 2011. Differences (diff., mean ± CI) between TF and RF for D) $\delta^{15}\text{N}-\text{NO}_3$ -, E) $\delta^{18}\text{O}-\text{NO}_3$ - and F) $\Delta^{17}\text{O}$ values measured at the LN and HN sites, without distinguishing between tree species.

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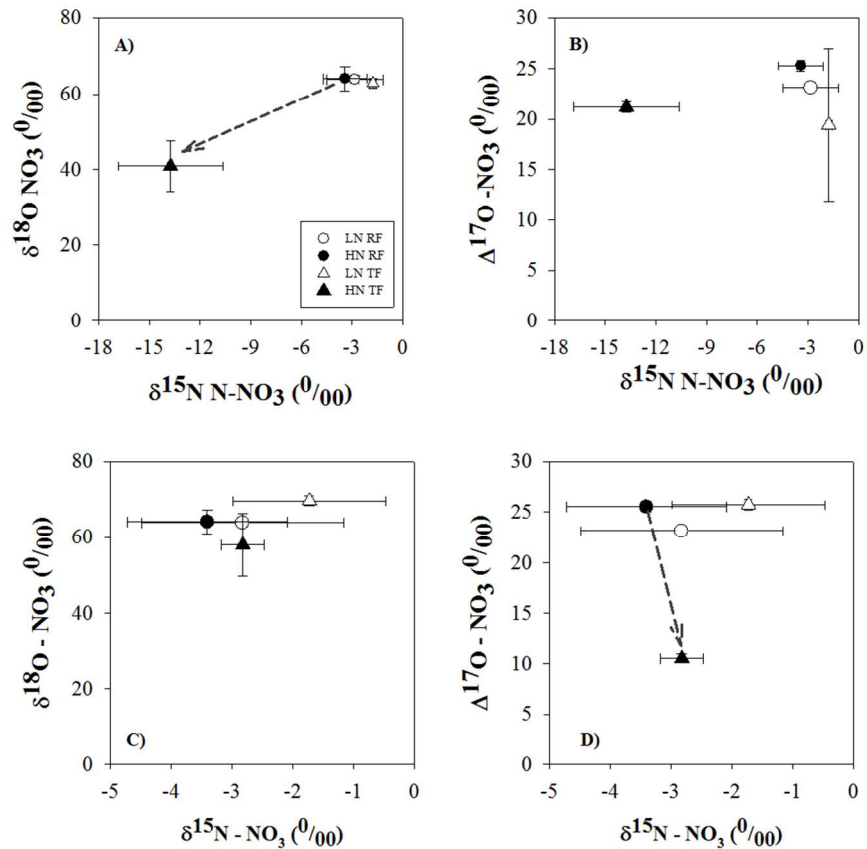


Figure 4. $\delta^{15}\text{N}$ vs. $\delta^{18}\text{O}$ and $\delta^{18}\text{O}$ vs. $\Delta^{17}\text{O}$ for Scots pine (A and B, respectively) and beech (C and D, respectively) measured in RF and TF. Each symbol represents the mean (\pm SE) for isotope measurements carried out in water samples collected from June-August and September- November 2011 at the LN (Rogate and Alice Holt for the Scots pine and Beech, respectively) and HN (Thetford, for both tree species). Arrows depict dramatic changes from RF to TF for the isotope values.

266x355mm (96 x 96 DPI)

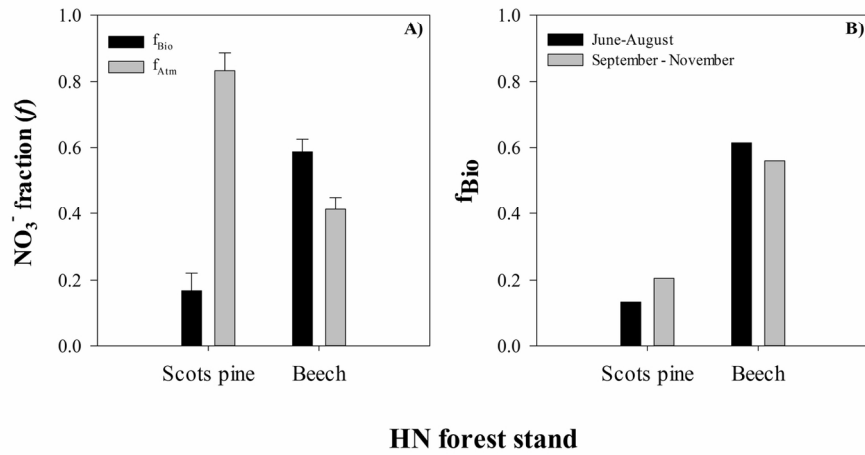


Figure 5. A) Mean (\pm SD) of the NO_3^- fraction derived from the atmosphere (f_{Atm}) and nitrification (f_{Bio}) measured for the June-August and September-November months at the two HN forest stands. B) Fraction of NO_3^- derived from nitrification (f_{Bio}) for the two sampling periods (i.e., June-August and September-November) at the HN forest sites.
148x81mm (300 x 300 DPI)