THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

Forest canopy nitrogen uptake can supply entire foliar demand

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Functional Ecology

OPEN ACCESS

RESEARCH ARTICLE

Functional Ecology | BRITISH ECOLOGICAL SOCIETY

Forest canopy nitrogen uptake can supply entire foliar demand

Daniele Ferraretto[1] | **Richard Nair[2]** 🅾 | **Nadeem W. Shah[3]** | **Dave Reay[1]** 🅾 |
Maurizio Mencuccini[4,5] 🅾 | **Michael Spencer[1]** 🅾 | **Kate V. Heal[1]** 🅾

[1]School of GeoSciences, University of Edinburgh, Edinburgh, UK

[2]Department Biogeochemical Integration, Max Planck Institute for Biogeochemistry, Jena, Germany

[3]Forest Research, Northern Research Station, Roslin, UK

[4]CREAF, Bellaterra, Spain

[5]ICREA, Barcelona, Spain

Correspondence
Richard Nair
Email: rnair@bgc-jena.mpg.de

Abstract

1. Plant canopies intercept, process and potentially assimilate atmospheric nitrogen (N) additions, but the forest-scale effects of canopy processes on N cycling and plant nutrition are not clear. Substantial method artefacts and scaling issues exist in previous experimental studies which measure relevant N fluxes either at (a) natural abundance, (b) via a ^{15}N tracer or (c) by incorporation of additional ^{15}N into measured biomass, meaning these processes are often overlooked or discounted.

2. Here, working in a mature Sitka spruce plantation and under ambient conditions, we used all three of these methods independently at stand, tree and branch scale to assess the effects of canopy interception on capture, assimilation and incorporation of inorganic N deposition by canopy biomass.

3. We consistently found that above 70% of N deposition was unaccounted for (i.e. assimilated, processed beyond detectability, volatilised or retained) when passing through the canopy, independent of the assessment method. Using short-term ^{15}N tracers, we found that this unaccounted N was retained in canopy tissues. Apparent uptake from monitoring of ecohydrological fluxes was sustained and consistent, suggesting that the tree canopies were a sink for N deposition. Seasonal variation in NO_3^- recovery also suggested biological activities influenced fluxes and transformations of this ion within the canopy.

4. The total amount of this canopy N uptake was greater than the mean annual N in litterfall, implying that this flux was supporting the turnover of the canopy. When we used ^{15}N to directly assess uptake into branch biomass we also did not find evidence for immediate relocation to other parts of the tree.

5. Our findings suggest that at our N-limited site, canopy uptake of N deposition is likely to be as important as root uptake of N but may be limited to supplying N to the canopy rather than the whole tree. Further research in this area is crucial to better understand the interactions of future changes in N deposition on primary production and carbon storage in forests.

Daniele Ferraretto and Richard Nair should be considered joint first authors.

1 | INTRODUCTION

Anthropogenic nitrogen (N) deposition is a major driver of the increasing temperate forest carbon (C) sink (de Vries et al., 2014; Etzold et al., 2020; Fernández-Martínez et al., 2017). However, major questions remain about the mechanisms of forest growth enhancement and extra C storage due to N, and, consequently, the future relevance of N deposition. One uncertainty is that, despite a high capacity for interception of deposition (Pahl et al., 1994) and N constraints to productivity (Etzold et al., 2020; LeBauer & Treseder, 2008; Schulte-Uebbing & de Vries, 2018), canopy interactions with atmospheric N deposition are not commonly represented by experimental approaches, resulting in ambiguity in the role of these processes in forest N and C cycles.

There are several potential mechanisms for N uptake by canopies which may involve uptake over leaves via stomata, or via leaf or branch surfaces by cuticular diffusion (Hu et al., 2014; Krupa, 2003; Sparks, 2009). There is also considerable evidence of canopy water uptake from many biomes (Schreel & Steppe, 2020) and foliar N treatments are common in agriculture (Fageria et al., 2009). Forest canopies also play an important general role in N cycling (Bortolazzi et al., 2021; Guerrieri et al., 2021), but actual rates, mechanisms and effects of canopy nitrogen uptake (CNU) in forest ecosystems are difficult to generalise or scale. A key question relates to differences between experimental approaches. There is a need to understand an organ-level process at the scale of ecosystems, and so scientists may measure fluxes across the canopy, inferring CNU from a difference (e.g. N deposition minus N in throughfall and stemflow), or use recovery of ^{15}N tracers at the canopy, organ or mesocosm level and scale up.

Typically, incorporation of reactive N into plants from CNU in sapling or branch-level studies is 0%–20% of total N deposition (Adriaenssens et al., 2011; Bowden et al., 1989; Garten et al., 1998; Wang et al., 2021), but such studies are prone to pot artefacts (Poorter et al., 2012) and limited by differences between saplings and mature trees (Schindler, 1998). These experiments allow mechanistic insight difficult at stand scale, for example disproportionate CNU-^{15}N tracer distribution to high C:N, woody pools (Nair et al., 2016) or effects on photosynthetic performance without such tracer-allometry effects (Wang et al., 2021) but may not represent real systems.

When canopy ^{15}N tracer applications were used in a mature stand, N recovery was primarily in woody branch biomass (Dail et al., 2009). However, it is not clear if this is because uptake is highest in these organs, implying a potential relocation of this N within branches, or if there is a sink in or on these organs (coarse surfaces or branch-dwelling micro-organisms) not necessarily contributing to plant growth. On the whole-tree scale, manipulative methods

such as artificial misting and wet N applications (both labelled and unlabelled) often report a higher CNU, ~20%–70% of N (Cape et al., 2010; Chiwa et al., 2004; Dail et al., 2009; Gaige et al., 2007; Li et al., 2020). Field 'ecohydrological' approaches which quantify CNU via budgets of rainfall (RF), throughfall and stemflow often implicitly find an even higher N retention, although site differences are substantial (Table 1). This is also true for different methods at the same site (compare Dail et al., 2009; Gaige et al., 2007; Sievering et al., 2000). Natural abundance ecohydrological approaches may also include allometric effects of N deposition which occur over longer time-scales than experiments and are also stronger aboveground (Li et al., 2020). However, information such as this allometric effect often come from N treatments applied to soil, and changes induced by root uptake may not apply to N obtained by leaves or branches (e.g. Nair et al., 2016). Consequently, it is not clear which of these methods represents actual processes best, and how important CNU may be for N budgets and ecosystem function.

There is also uncertainty regarding species, and environment controls on the size and relevancy of a CNU flux (Zhang et al., 2015). Seasonal variations in CNU (Klopatek et al., 2006) could be due to endogenous (plant activity during the main growing season) or exogenous (weather conditions in some seasons favouring CNU) drivers, which may explain both the range observed and CNU relevance with changing deposition rates and forest cover. Ecohydrological approaches implicitly incorporate seasonal variation and stand structure and rely on accurately assessing fluxes in and out of the canopy and vary considerably between sites (Table 1). However, such measurements do not allow the finer tracing of processes possible with isotope tracers, which is sometimes possible in manipulative studies. This means that distinctions such as separating N leached from the canopy from new N in throughfall may not be possible. In addition, many studies have not managed to incorporate all fluxes, in particular inclusion of dry N deposition, and even sometimes not distinguishing between 'rainfall' and 'throughflow' fluxes because of the placement of collectors, inherently discounting canopy processes. In order to understand the bias between methods, comparisons are needed between such 'ecohydrological' approaches and manipulative methods, keeping other factors, such as site choice, the same.

A further complication is that field research into the global N cycle has taken place over more than half a century, during which regional N deposition levels have changed. Many sites in Europe and North America were substantially N saturated in the industrialised 20th century due to heavy N and S deposition, which has both lessened and changed in composition due to emission control regulations in the 1980s and 1990s (Du, 2016; Verstraeten et al., 2017). In other less regulated regions, N deposition is currently high. Historically, N cycle concerns in Europe and North America centred around negative effects of eutrophication and acidification, but later interest

TABLE 1 Comparison of field/plot-scale CNU estimates. Reporting of CNU is variable in terms of estimates, method and detail. Here, for consistency and comparison, mean percentage uptake estimates are presented for total CNU and for separate nitrogen species and seasons when available. Tree species are max. four dominant species at site if described. Nitrogen deposition (N_{dep}) is wet plus dry deposition if available. N_{dep} applied is total amount per year regardless of application frequency. We do not include 'total' CNU estimates unless reported but do calculate percentage of N inputs if possible. We consider 'Canopy N retention' as equivalent to 'Canopy N Uptake', even if studies herein do not (Gaige et al, 2007). We include the range of deposition for Houle et al. (2014), which shows a long-term declining deposition trend, between the initial and end deposition values (*)

Reference	Location	Tree species	N_{dep} (kg ha^{-1} year^{-1})	Methodology to obtain CNU	N_{dep} applied (kg ha^{-1} year^{-1})	NO_3-CNU (%NO_3-N_{dep})	NH_4-CNU (%NH_4-N_{dep})	Total CNU (% N_{dep})
Chiwa et al. (2004)	Scotland	*Picea sitchensis*	NA	Canopy fertilisation (misting)	NA	34%	31%	
Klopatek et al. (2006)	Washington State, USA	*Pseudotsuga menziesii*	8–10	Ecohydrological monitoring(3 years)	—	<80% (winter) 67%–79% (summer)	0 (winter) 86%–90% (summer)	
Sievering et al. (2000)	Maine, USA	*Abies balsamea, Picea rubens, Tsuga canadensis*	4	Ecohydrological monitoring (7 years)	—			89%
Gaige et al. (2007)			18	Canopy fertilisation (wet)	18	66%	78%	
Dail et al. (2009)				Canopy ^{15}N trace		38%	67%	67%
Tomaszewski et al. (2003)	Colorado, USA	*Picea engelmannii, Abies lasiocarpa, Pinus contorta*	4–8	Ecohydrological monitoring (2 years)	—	75%	90%	
Mustajärvi et al. (2008)	Finland	*Pinus sylvestris, Picea abies*	1–4	Ecohydrological monitoring (7 years)	—	45%	23%	
Cape et al. (2010)	Scotland	*Pinus sylvestris*	1.2–2.2	Canopy fertilisation (misting)	50	8%	40%	
Wortman et al. (2012)	Switzerland	*Quercus cerris, Quercus pubescens, Castanea sativa, Betula pendula*	19–37	Ecohydrological monitoring and canopy budget model	- (only for CNU est.)			20%–25%
Fenn et al. (2013)	Washington State, USA	*Tsuga heterophylla, Pseudotsuga menziesii*	1.3–1.4	Ecohydrological monitoring (2 years) multiple sites	—	90%	Net negative	
Schwarz et al. (2014)	Germany	Spruce forests	10.1–11.8	Ecohydrological monitoring (multiple sites)	—			45%
		Beech forests	10.1–11.8	Ecohydrological monitoring (multiple sites)	—			67%
Houle et al. (1999)	Quebec, Canada	*Abies balsamea, Picea rubens, Betula papyrifera*	7.5	Ecohydrological monitoring (8 years)	—	Net negative	85%	
		Acer saccharum, Betula alleghaniensis, Fagus grandifolia			—	77%	41%	
Houle et al. (2014)	Quebec, Canada	*Picea mariana, Pinus banksia*	6.6–4.4*	Ecohydrological monitoring (13 years)	—	45%	60%	
		Abies balsamea, Picea rubens, Betula papyrifera	2.8–1.87*	Ecohydrological monitoring (15 years)	—	54%	67%	
Liu et al. (2020)	Central China	Mixed deciduous forest	19.6	Canopy fertilisation (wet)	25; 50	52%	44%	

in other drivers of vegetation growth (e.g. increased CO_2 concentrations, warming) has shifted attention towards oligotrophication (e.g. Craine et al., 2018). Hence past N deposition experiments often used gross N inputs substantially above ambient deposition (Table 1) which may be less relevant in the future conditions. CNU potentially depends on N availability, both edaphic and in deposition, so legacy effects of historic high N deposition rates (Wuyts et al., 2015), or elevated treatment levels of N in experiments, could saturate N uptake capacity or release trees from competition with soil microbes, promoting downregulation of CNU if under active control.

To understand CNU under field conditions and low-medium deposition inputs, we performed a series of field monitoring and manipulation experiments at different scales to examine how a mature Sitka spruce plantation forest processes N in the canopy. A mesocosm study on saplings of this species under very low, chronic treatment inputs found a high (60%) CNU from ^{15}N-labelled N applications (Nair et al., 2016), including 15% recovery in stems, and thus a disproportionate importance of CNU for carbon budgets. Critically, we used three independent methods aiming to quantify apparent CNU at the same site, and approximately the same time. First, in an 'ecohydrological' approach, we measured N in forest hydrological fluxes (N deposition and throughfall, stemflow) to estimate CNU indirectly by the difference between fluxes into the canopy, and fluxes out. Secondly, in 'canopy fertilisation', we applied a ^{15}N tracer over the ecosystem in simulated N deposition and recovered this in throughfall and stemflow. Thirdly, in 'branch fertiliser addition', we applied a ^{15}N tracer directly to individual branches quantifying the precise ^{15}N input and measuring the recovery in the branch tissue. We assessed uptake into the rest of the tree by girdling the branch (inactivating the phloem transport vessels). Both the ecohydrological and canopy fertilisation approaches allowed an implicit assessment of CNU, henceforth iCNU, as the missing N from the difference in input and output budgets. The branch addition measured actual incorporation of N added into biomass, henceforth explicit CNU, eCNU. Comparing these would allow a robust assessment of how important CNU is in our system, minimising uncertainty caused by use of a single method. As far as possible, we also worked at almost natural abundance conditions to maximise transferability to unmanipulated, natural ecosystems. We hypothesised:

Hypothesis 1 *Both ecohydrological and canopy fertilisation approaches would provide similar estimates of iCNU. Estimated eCNU from direct application of ^{15}N tracer to the branches will be comparable to iCNU from both methods despite the different scales of all three approaches. If CNU only replaces other N leached from the canopy, iCNU from the canopy fertilisation approach would be lower than the ecohydrological approach. If iCNU was negative (i.e. net N losses from the canopy occur), N losses would be reflected in ecosystem-scale N saturation losses in streamflow.*

Hypothesis 2 *Any CNU (in proportion to N inputs) would be maximised in summer, driven by biological activity in the canopy.*

Hypothesis 3 *^{15}N added directly to the branch surface (branch addition) will be disproportionately recovered in branches rather*

than leaves, and will be higher in girdled compared to ungirdled branches, due to translocation to the rest of the tree.

2 | MATERIALS AND METHODS

2.1 | Study site

We worked at Griffin Forest in Perthshire, Scotland (56°36'23.59" N, 3°47'48.55"W), a Sitka spruce (*Picea sitchensis* (Bong.) Carr.) plantation established in 1980–1981 at 2,770 trees/ha on a stagno-humic gley soil (Clement et al., 2012), with no understorey vegetation apart from occasional moss patches. During the study period, tree height exceeded 20 m. Monthly mean air temperatures (1981–2010, data source: Met Office UK) range from 3.4 to 11.9°C, mean total annual RF is 1,106 mm, mostly between October and January, although precipitation occurs year-round. The plantation was prepared by burning the native heather *Calluna vulgaris* ((L.) Hull), ploughing and 375 kg/ha phosphorus fertilisation (Clement et al., 2012). A single application of 350 kg/ha urea (~163 kg N/ha) was made in 1997 to suppress heather growth. Thinning was conducted in 2004, reducing tree density to ~1,750 trees/ha by removing every fifth row and cutting every third tree on the row either side of the thinned row. Nitrogen deposition is relatively low; during the 5-year study period, inorganic N deposition in rain and cloudwater (CW) was mean 7.1 kg ha^{-1} year^{-1} (min 5.3 kg N/ha, max 8.8 kg N/ha). There are no substantial known local emission sources of mineral or organic N deposition. Total soil content as measured in 2017 was ~5 mg/g in the top 5 cm and ~3 mg/g in soil at 5–15 cm depth (Supporting Information S1).

2.2 | Ecohydrological approach

We measured throughfall, stemflow, RF, streamflow and litterfall fluxes at the study site from January 2012 to May 2017. Two 30-ha plots ('A' and 'B') were established, treated identically, but located ~650 m apart on opposite sides of a valley (56°36'22"N, 3°47'41"W and 56°36'38"N, 3°47'40"W, Figure 1). Collection of water fluxes was conducted monthly and following standard methods (Clarke et al., 2016; Dämmgen et al., 2005). Rainfall was collected from two bulk RF collectors in the scarce open areas above (440 m a.s.l.) and below the plots (286 m a.s.l.) to represent the range of altitudes, sufficiently far from the plantation to minimise any turbulence from the presence of tall obstacles. At the upper station, CW was collected using a passive harp wire device (Crossley et al., 1992), located a short distance from the RF collector (to minimise interference). Rainfall and CW were collected using 15-cm diameter borosilicate glass funnels into black 2.7 L (RF) and 5.7 L (CW) HDPE bottles. Glass wool in the mouth of each bottle filtered debris. 2.5 ml (for RF) and 5.7 ml (for CW) orthophosphoric acid was added to bottles as a biocide before exposure in the field. We refer to bulk inorganic N deposition (N_{dep}), as the sum of RF and CW. Dry deposition was
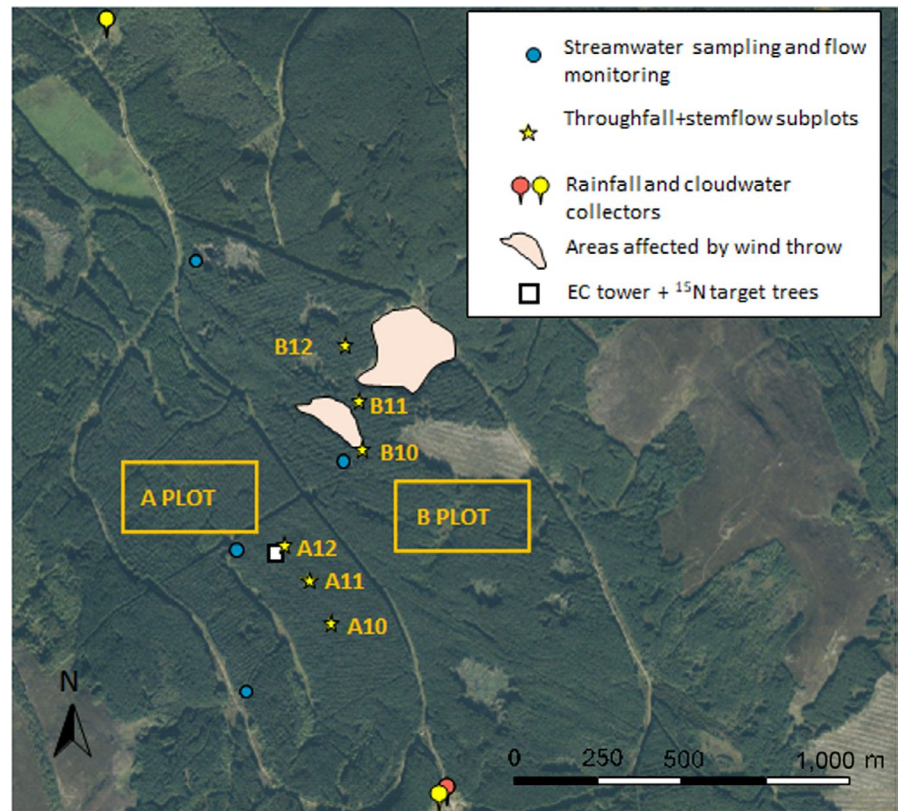
not measured independently but was collected by deposition on the surfaces of the RF and CW collectors.

Throughfall was measured at three subplots in each plot, selected as representative following a site-level inventory of 3,859 stems conducted in 2010. We placed throughfall collectors at three locations per subplot in a stratified manner, with one each per thinned, un-thinned and partially thinned row. Due to the uneven distribution of thinned areas, this may have slightly overestimated throughfall since thinned areas cover ~20% of the site and intercept less deposition. In each subplot, throughfall was collected through two inclined gutters (4.02 × 0.234 m) draining into a 120-L HDPE barrel with a strainer in the lid to capture debris. The gutter angle ranged from 12° to 17° (median 16°), to maximise collection surface without causing any ponding and ensuring ready drainage to minimise evaporative loss. On each measurement occasion, the depth of the water in the barrel was measured to the nearest centimetre with a plastic rule and converted to volume through the relevant calibration equation (Figure S1). Any relevant observations relating to the samplers (e.g. gutters not fully aligned with the strainer, presence of debris, bird droppings on gutters or strainer, slugs, worms or insects in the barrels, foam or anomalous turbidity) were recorded together with the throughfall depth measurement. Mean throughfall depth (mm) was calculated by dividing each water volume by the total surface projection of the gutters and the strainer. For details of calibration and scaling equations used, refer to Supporting Information S2.

Stemflow was collected from 22 stemflow samplers across the plots. Stemflow samplers were not directly located in throughfall sampling locations but attached to trees representing eight DBH

size classes from the 2010 survey. Stemflow was collected in a 30-L covered barrel, fed by a length of cut-away 5 cm external/4 cm internal diameter flexible vinyl tubing (Parkland Engineering, Glasgow) tubing wrapped around the trunk, held in place by sealant and small nails. No biocide was used in the throughfall and stemflow collectors due to constant cool conditions under the forest canopy and to avoid disposal on site. Disposable nitrile gloves were worn at all times during measurements and sample collection. Before TF/SF sample collection, the sample bottle was rinsed three times with water from the barrel, then filled to the brim, capped and stored in a cool box for transport to the laboratory. There, water samples were filtered (0.45 μm EMD Millipore Millex™ sterile syringe filters) within 24–36 hr, and stored at 4°C until analysis, which was performed within a week of filtration.

Litterfall was measured by collection from the gutters and strainers of the throughfall samplers and oven-dried at 70°C to constant mass before weighing. Litter N content was estimated for all samples based on measurement of litter N content in one sample per litter collector (18 in total) selected from months representing different seasons (January, May and September 2012). Analysis of three replicate samples from each collector in September showed consistency in the N contents, so only one sample per collector was analysed for the months of January and May 2012. Samples were finely ground using a Retsch MM-200 ball mill, and total N content was measured by colorimetric analysis following digestion of the ground litter using concentrated sulfuric acid and hydrogen peroxide.

Streamwater N fluxes per ha were estimated from concentrations of N ions in streamwater samples collected on each monthly

sampling occasion from four 90° V-notch thin-plate weirs, one up-stream and one downstream of each plot. For further details, see Supporting Information S2.

Nitrogen (NH_4^+ and NO_3^-) concentrations in all samples were measured by automated colorimetric analysis on dedicated autoanalysers (AA3 Bran & Luebbe, Norderstedt, Germany). To determine the NH_4^+ concentration, the sample was reacted with salicylate and dichloroisocyanuric acid to produce a blue compound with absorbance measured at 660 nm, using nitroprusside as a catalyst. To determine the NO_3^- concentration, NO_3^- in the sample was reduced to NO_2^- by hydrazine in alkaline solution and then reacted with sulfanilamide and N-(1-Naphthyl) ethylenediamine to form a pink compound with absorbance measured at 550 nm. This method yields oxidised dissolved inorganic nitrogen (DIN) (NO_2^- as well as NO_3). Five standards (0.5, 1, 2, 4 and 8 mg/L NO_3/NH_4) were used to calibrate the instruments. For quality control, one of the standards was analysed as a sample to verify the calibration at the beginning, middle (after 20–25 samples) and the end of each run.

Throughfall and stemflow were scaled to the whole site by considering the representativeness of the collectors in terms of site area and the total population of stems respectively. Litterfall was scaled to the site level in the same way as throughfall.

2.3 | Canopy fertilisation

Recovery of experimentally added N 'deposition' was assessed by applying ^{15}N as double-labelled ammonium nitrate solution over the canopy of three trees. This was applied with a hand sprayer with 5.4-m extension lance to three adjacent trees from a 20 m tall walk-up tower up previously used for micrometeorological measurements ('EC tower' Figure 1). The three trees had similar DBH (26.5, 28.0 and 30.0 cm) and were 1–2 m taller than the tower.

We sprayed each tree crown twice (5 August 2016 and 26 February 2017, hereafter 'summer' and 'winter') with 3.5 L of a 98% atom% ^{15}N double-labelled NH_4NO_3 solution. The total applied N (~7.6 g NH_4NO_4 per tree crown or 4.66 kg N/ha) was similar to the largest monthly N deposition event (April–May 2014, 3.2 kg N/ha). Dates were representative of the growing (summer) and dormant (winter) seasons, but constrained by calm, dry conditions necessary for mist-based application. The volume of solution selected was sufficient for full canopy wetting but with minimal throughfall (Supporting Information S3).

We collected throughfall and stemflow below the three study trees using similar gutters and small barrels as the ecohydrological monitoring (four throughfall and one stemflow collector per tree, accounting for ~20% of the crown projection). Sample collection was as soon as possible after major RF following the application. At least 5 L of throughfall/stemflow (where available) was collected to ensure 50–150 µg N for stable isotope analyses. The entire volume of throughfall/stemflow per sampling occasion was transferred into a 10-L HDPE bottle (Thermo Scientific Nalgene) in the field to create one throughfall or stemflow composite sample per tree. When

the total volume of throughfall exceeded 10 L, a composite sample of 5–10 L was obtained in proportion to per-collector water depth. After each collection, any remaining water was emptied. Control (no additional N/^{15}N) throughfall and stemflow samples were taken at subplot A12 (~100 m from the tower, Figure 1), using the same design, and consisted of one composite throughfall sample and one stemflow sample from mixing a 0.5 L sample from each of the collectors at A12.

We collected samples over 17 weeks (six collections) in summer, but reduced to 4 weeks (three collections) in winter, due to the decline in enhanced N concentrations in throughfall and stemflow. Samples were filtered and stored at 4°C until N concentration results were available (usually within 5 days). Once we knew the per sample NH_4 and NO_3^- concentration, we took a subsample of 50–150 µg N which we trapped in acidified filters via pH adjustment using ammonia diffusion (Sebilo et al., 2004). We modified this method volumetrically for low N concentrations at natural abundance, and volumes of up to 1 L. The filter papers containing the concentrated sample were analysed for $\delta^{15}N$ (^{15}N stable isotope composition of the total N) on both NH_4^+ and NO_3^- at the NERC Life Sciences Mass Spectrometry Facility (LSMSF) in an automated Carlo Erba NA1500 elemental analyser coupled to the isotope ratio mass spectrometer (Dennis Leigh Technologies).

2.4 | Branch fertiliser addition

We applied a 98% atom% double-labelled ^{15}N-NH_4NO_3 solution directly to branches, similar to sapling studies (Boyce et al., 1996; Nair et al., 2016). We applied a low dose of N (9.73 mg in a 0.5 L application), equivalent to additional 'deposition' of 0.048 kg N/ha, with a clean brush in two events per branch (afternoon of 6 May 2017 and morning of 7 May 2017). Due to extraordinarily dry conditions in May 2017, we rewetted the branches using deionised (DI) water following the first application to create conditions closer to those normally found in situ.

Ten branches were selected from two different trees, both easily accessible from the tower. The branches were assigned to three height classes, between 17 and 20 m above the ground. One of the selected trees was part of the previous experiment, although tissue enrichment was only minor. We split each branch into 'treatment' and 'control' sub-branches, the latter receiving no additional N nor DI water application, and treated controls separately per tree. To estimate contribution to whole-tree nutrition, we girdled five branches (with separate girdled control and treatment sections) to stop transport of ^{15}N in metabolic products through the phloem. Girdling was conducted 2 hr after the first application on 6 May 2017, as practised in other studies (e.g. Högberg et al., 2001).

The branches were removed in the late afternoon of 7 May 2017 with a lopper and wrapped in separate plastic bags, then immediately transported to the laboratory. New twigs and needles (<2 years) were separated from old twigs and needles (2+ years). We assessed N uptake in four 'compartments': new twigs, old twigs,

new needles and old needles, each containing 10 treatment and 10 control samples. We estimated total branch surface area by measuring all twigs to calculate a kite-shaped geometric surface area, and calculated the mean ^{15}N applied to calculate ^{15}N recovery. All sampled twigs were washed and rinsed three times in DI water and dried to constant weight in an oven at 70°C. The dry mass per cm of needles and twigs in both age classes was calculated. Dried samples were ground to a fine powder in a Retsch MM-200 ball mill and a subsample analysed for δ^{15}N using the instruments and facilities as the canopy fertilisation.

2.5 | Data processing and statistical analysis

We define CNU in two ways based either on missing N (iCNU), or on N incorporation into biomass (eCNU).

Implicit CNU was the 'missing' N from transit of N deposition through the canopy, that is the difference between N or ^{15}N input to the canopy and subcanopy throughfall and stemflow. Input was RF and CW N deposition in the ecohydrological monitoring, or our experimental N and ^{15}N additions (considered separately). This means iCNU includes N missing due to canopy processing of N by microbes in the phyllosphere (Guerrieri et al., 2015; Papen et al., 2002), if this produces N in throughfall which is not NH_4^+ or NO_3^-, such as organic N. Likewise, this includes recovery of ambient deposition in throughfall and stemflow in the canopy fertilisation. We adjusted our calculations to take account of N in deposition but not additional ^{15}N (i.e. assuming deposition was at natural abundance) as ^{15}N in deposition was expected to be a very small fraction of the total enrichment in the treatment. We define iCNU as.

$$iCNU (\%) = \frac{((RF + CW) - (TF + SF))}{100}. \qquad (1)$$

As we focus on canopy uptake, our iCNU is the inverse of TF-RF as commonly used to assess N balance in canopies (i.e. ICP forests, long-term monitoring sites in the United States).

We compared iCNU for the ecohydrological approach, treating each month as independent (assuming minor differences in sampling period affected total recovery randomly). We split deposition into reduced (NH_4^+) and oxidised (NO_3^-) deposition between two seasons, defining 'summer' months as May–September and 'winter' months as November–March, removing the months between each season. We used regression models to examine relationships between N inputs, N recoveries and seasonal periods, checking residuals met assumptions of constant variance, normal distribution and independence. Upon visual detection of a step change, we also looked at changes between two adjacent 3-month periods of the year (March–May and June–August). This subset of data also satisfied assumptions.

To calculate eCNU from the controlled branch addition, we calculated N recovery from ^{15}N concentration, N concentration and mass of the pools as an excess compared to the mean concentrations for the same pools in unlabelled portions of the same branch. We discounted any additional input from atmospheric deposition as

this would be the same for both control and treatment branches. We then calculated the percentage recovery of the total applied tracer in the pool (Equation 2). Full details are found in Supporting Information S4.

$$eCNU = \frac{^{15}N \, (\text{recovered})}{^{15}N \, (\text{applied})} \times 100. \qquad (2)$$

To analyse the branch addition, we used linear mixed effects models (lme4 in R (Bates, 2010)), implementing models with the predictors ^{15}N treatment (control vs. treated), girdling (control vs. girdled), twig/needle age class, biomass age class and height position. We used a random slope of treatment within branch within tree, to account for the spatial structure. We modelled both δ^{15}N (including a treatment effect) and ^{15}N recovery (without a treatment effect), transforming both to normality by power transformation before analysis. We selected from candidate models, beginning with all possible two-way interactions, selecting models using the dredge function (MuMIn package, Barton, 2020), ranking models by AICc (small-sample corrected Akaike information criterion) with a cut-off value of 4 AICc which resulted in a single model in both cases. We considered this reasonable given the limit to interactions we could interpret biologically. We calculated statistical significance using a Holm–Bonferroni correction for multiple comparisons using the package MULTCOMP (Hothorn et al., 2008). All statistical analyses were conducted using R 4.0.3 (R Core Team, 2018) with a significance level of $p < 0.05$ unless otherwise stated.

3 | RESULTS

3.1 | Nitrogen budget from ecohydrological monitoring

Through the ecohydrological approach (Figure 2), we found a mean annual N deposition of 7.1 kg ha^{-1} year^{-1}, throughfall of 2.0 kg ha^{-1} year^{-1} and stemflow of 0.1 kg ha^{-1} year^{-1} and hence a mean annual implicit CNU of 5 kg ha^{-1} year^{-1} or 70.1% of total deposition. Between years, iCNU ranged from 61.7% of total N deposition in 2014 to 78.1% in 2012. iCNU could be predicted from N deposition in RF using a second-order polynomial (adj. $R^2 = 0.9$, $p < 0.001$, Figure 3), which differed significantly from a best fit linear relationship ($p < 0.001$, chi.sq. test) with a lower adj. R^2. This was similar for NO_3^- alone (adj. $R^2 = 0.9$, $p < 0.001$) but NH_4^+ could be described with a linear slope instead (adj. $R^2 = 0.9$, $p < 0.001$). There was no similar relationship (linear or polynomial) between monthly iCNU and either monthly RF or CW depth (Supporting Information S6).

Overall, contribution of the different N species to throughfall and stemflow fluxes also changed through the year. NH_4^+-N was frequently the dominant ion form in throughfall (Figure 2) but was also the species with the occasional negative (i.e. throughfall + stemflow > RF + CW) iCNU outlier (Figure 4). Generally, throughfall and stemflow NO_3^--N were positively correlated ($r^2 = 0.577$, $p < 0.001$) together and with RF ($r^2 = 0.494$, $p < 0.001$). This was unlike NH_4-N
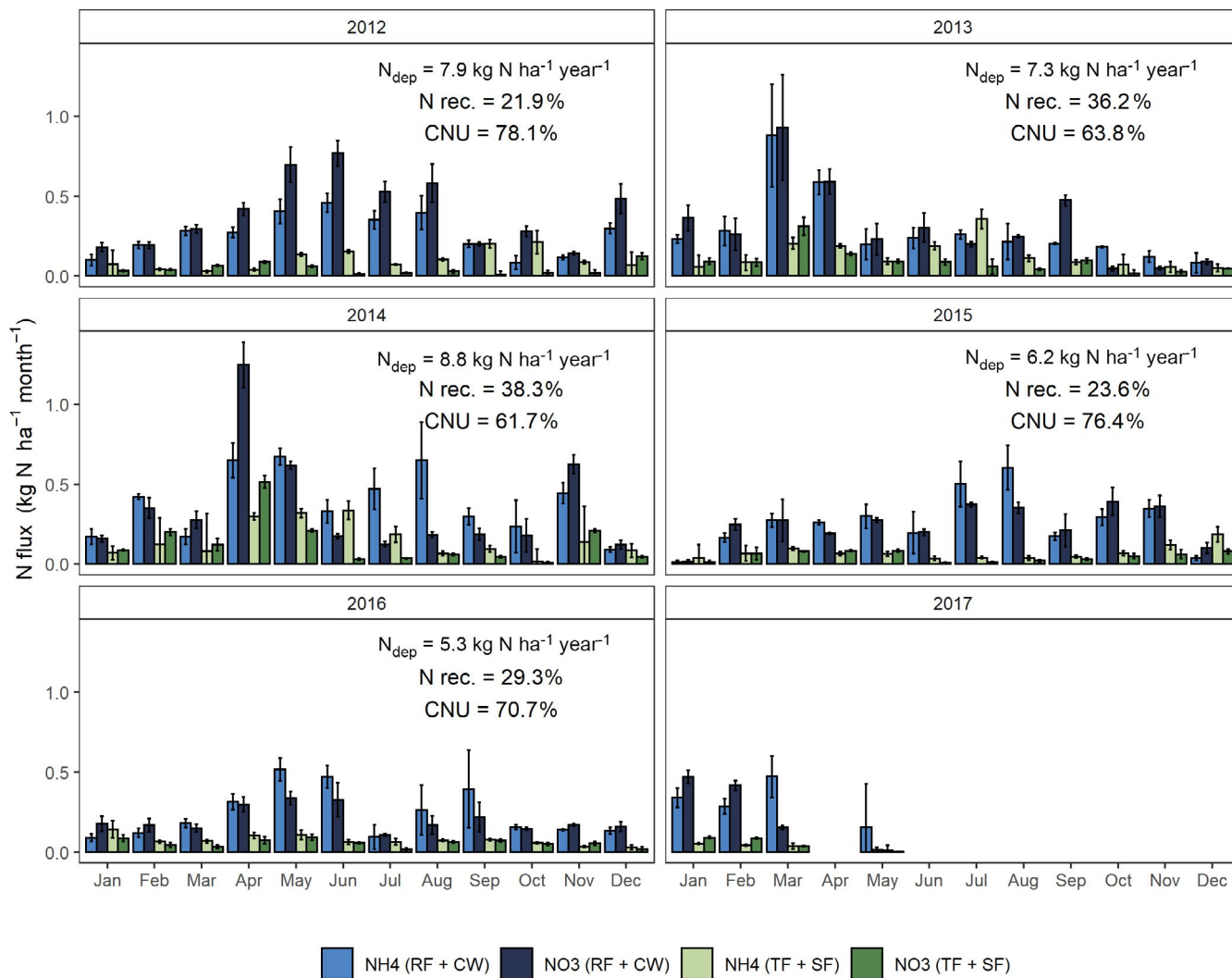
FIGURE 2 Ecohydrological N fluxes above (Rainfall (RF) + Cloudwater, (CW)) and below (Throughfall (TF) + Stemflow (SF)) canopies for both NH_4 and NO_3^- ions. Also shown is total annual measured wet N deposition (N_{dep}), N recovery (N_{rec}) and calculated CNU (100 – N_{rec}) of (RF + CW) N in (TF + SF) collectors; ~70 % of wet N deposition is attributable to CNU. Error bars are ± SE and reflect variability among collectors, for TF + SF this includes variation in volumes and concentrations between 6 TF locations plus the average of SF (which was ~5 % of total fluxes, and not directly paired). For 2017, data are shown to May to provide the context for the canopy and branch addition experiments, after which monitoring stopped. The incomplete 2017 year is also missing a measurement in April due to extremely dry conditions leading to almost no water captured by collectors

fluxes where throughfall and stemflow positively correlated with each other ($r^2 = 0.408$, $p = 0.001$) but not with RF NH_4^+-N.

Mean litter transfer of N from the canopy to the forest floor was 4.4 ± 1.4 kg ha^{-1} year^{-1}, indicating a net surplus of 0.6 kg ha^{-1} year^{-1} from iCNU-LF. Streamwater fluxes leaving the plots were ~0.4 kg ha^{-1} year^{-1} (mean of both fluxes, N concentrations were often below the limit of detection for analyses), so most N entering the ecosystem via RF and CW was remaining. In other words, the system was not N saturated.

We found differences in iCNU between reduced and oxidised N in deposition and within the year (Figure 4). The highest iCNU was in summer (May–September, 74 ± 9%) compared to November–March (67 ± 9%) but the difference was not significant. This difference was stronger for oxidised (NO_3^-) ions (83 ± 7%, 71 ± 11%, $p < 0.01$), compared to reduced (NH_4^+) ions (65 ± 16%, 61 ± 13%, n.s.). We observed a

consistent change between the March–May and June-August periods (compared in Figure 4), where the mean iCNU of NO_3^- was significantly higher in the later period than before ($p < 0.01$). Treating N recovery within each month as independent, we found, in general, that NO_3^- recovery was greater than NH_4^+ recovery from deposition ($p < 0.01$).

3.2 | Canopy fertilisation

In total 88.8 ± 1.1 (mean of three trees $\pm SE$) of $^{15}NO_3^-$ and 83.0 ± 3.0% of the $^{15}NH_4^+$-N were attributable to iCNU recovered 17 weeks after the ^{15}N-NH_4NO_3 application (Figure 5). This iCNU was lower in February: 34.1 ± 7.1 (NO_3^--N) and 64.9 ± 6.2% (NH_4^+-N) respectively. The missing ^{15}N was greater than the same calculation for unlabelled N added in the same treatments (Figure 5), and
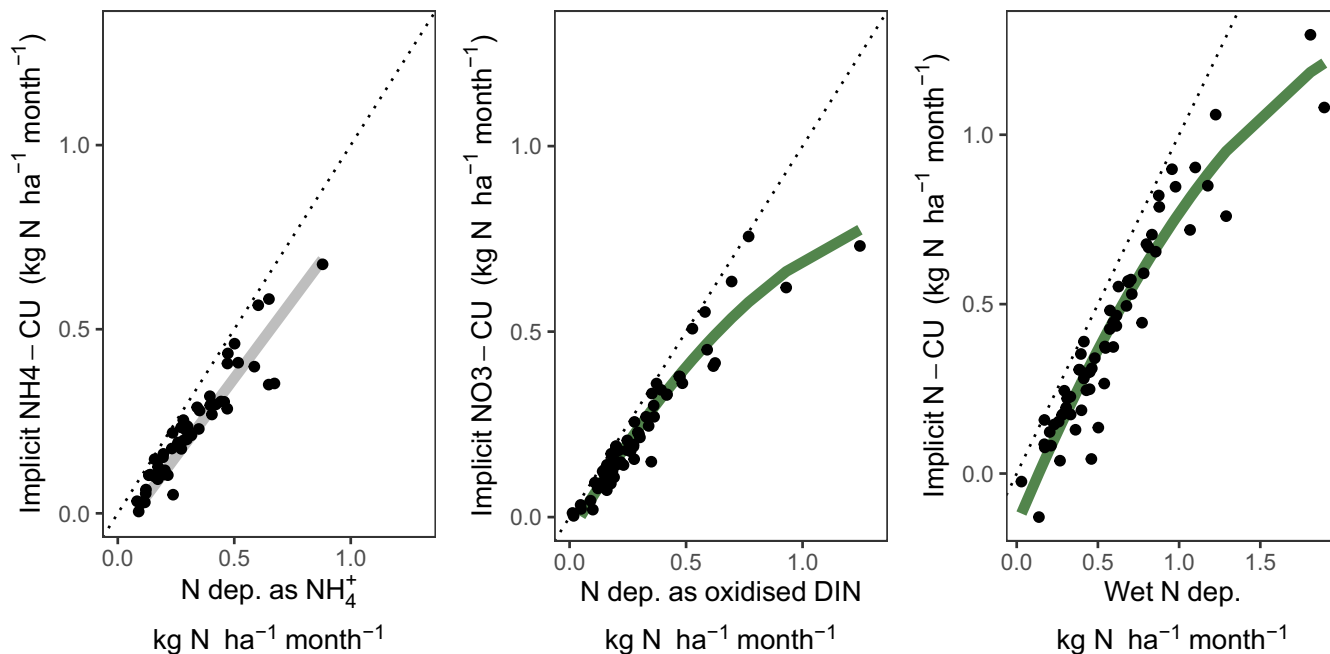
FIGURE 3 Relationship between total N inputs missing from throughfall and stemflow (Implicit CNU) and N deposition in rain inputs for NH_4+, oxidised DIN N (NO_3- and NO_2-) and total inorganic N. Total wet N deposition is the sum of NH_4+ and NO_3-. Net negative CNU occurs in two instances when more N is found below the canopy than in deposition, either because of leaching from the canopy or retention between observational periods. Green lines indicate a second order polynomial model if this fits the data better than a linear relationship, represented by a grey line
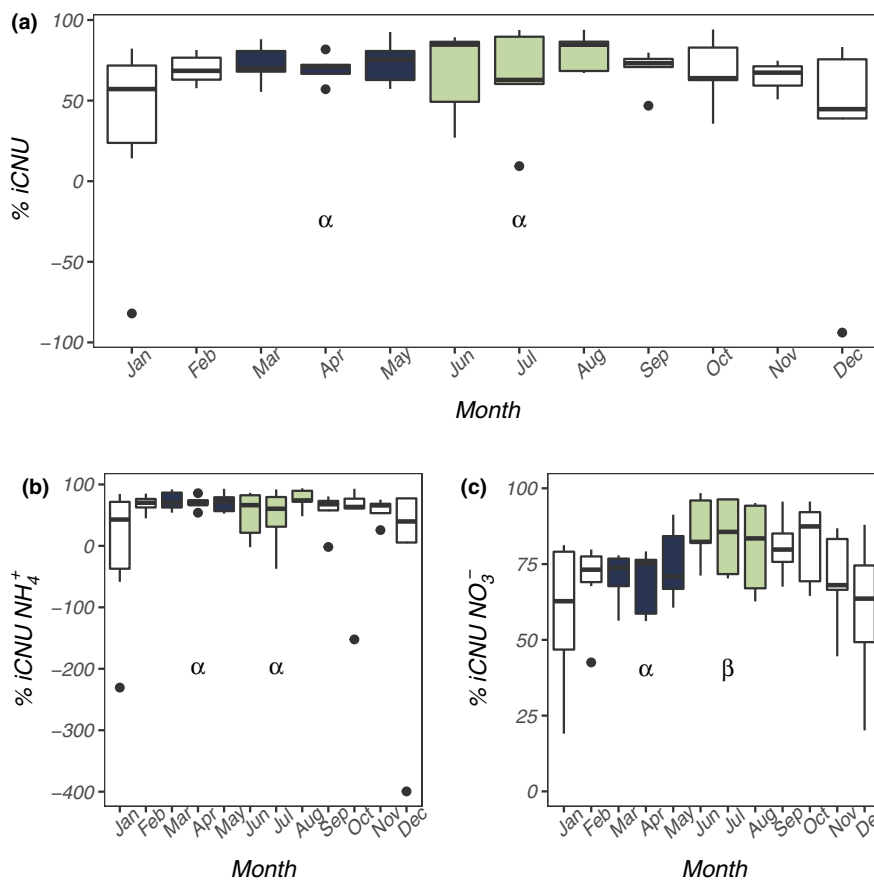

FIGURE 4 Monthly box plots of implicit CNU over the 5 complete years of our time series (2012–2016) for (a) total N, (b) NH_4+ and (c) NO_3-. Generally, iCNU is higher for NO_3- than NH_4+, driven by occasional net negative iCNU (i.e. more N lost from the tree canopy in throughfall/stemflow than entering the ecosystem in deposition) for NH_4+ (a). There is a significant ($p < 0.05$) difference between iCNU for NO_3- , indicated by Tukey HSD groupings (α and β) between March–May (dark blue) and Jun–Aug (green) for NO_3- (b). The horizontal line inside each box represents the median and the lower and upper hinges correspond to the first and third quartiles. The upper and lower whiskers depict the largest and smallest values respectively within 1.5 * the interquartile range (IQR). Dots represent outliers
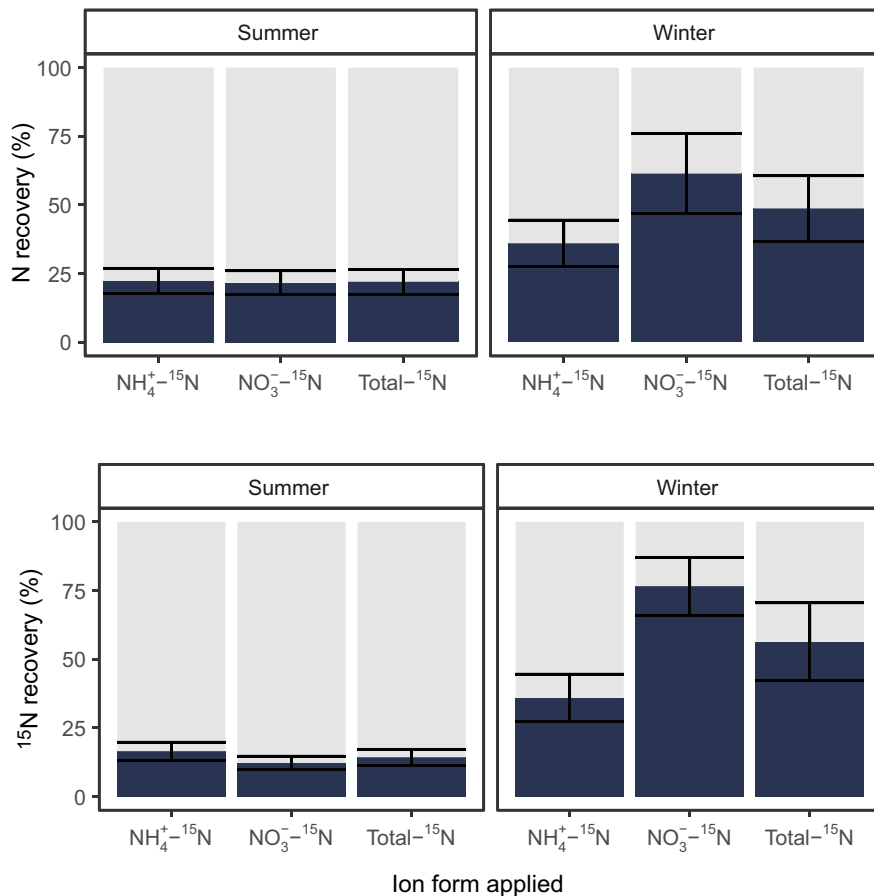
FIGURE 5 N and ^{15}N recovery below the canopy as % of measured throughfal plus stemflow from applications above the canopy in summer 2016 and winter 2017. Light green shaded areas show 'missing' N / ^{15}N, iCNU. Bars show means ± propagated standard error from crown projection, N addition amount and collection volumes; these were several orders of magnitude larger than errors in the measurements of N and ^{15}N. Small white circles show individual per-tree recovery (n = 3 trees)

the plot-scale ecohydrological monitoring within the period (summer 2016 and spring 2017) in which the experiment was conducted (Figure 2), despite our experimental treatment slightly raising the short-term N status of the canopy.

These recoveries differed between seasons and, in winter, between ions. Similar to the ecohydrological approach, we recovered more of the N applied as NH_4^+ than NO_3^- below the canopy, implying a greater uptake or processing of NO_3^- than NH_4^+. We observed a similar seasonal pattern for NO_3^- and NH_4^+ (more uptake in summer, less in winter) although the differences were much larger for NO_3^-.

3.3 | Branch fertiliser addition

We recovered more ^{15}N (eCNU) in twigs (10.8 ± 2.1% (mean + SE)) compared to needles (3.45 ± 0.6%). When we compared δ^{15}N of biomass, the significant terms biomass class (twigs vs. needles; $p < 0.001$), height ($p < 0.05$), ^{15}N application ($p < 0.001$) and an interaction between ^{15}N application and biomass class ($p < 0.001$) remained in our most parsimonious model (Table S2), indicating treatment was affecting ^{15}N content. The weak height effect was driven by a higher δ^{15}N in the lower canopy branches but offset by scaling from both N content and total branch biomass. The alternate model based on total ^{15}N recovery (Table S3) did not contain height as a significant factor, and this only remained in the model due to its interaction terms with tissue age and biomass class in the δ^{15}N model.

TABLE 2 Percentage recovery of ^{15}N double-labelled NH_4NO_3 tracer solution directly applied to entire branches (n = 5 per compartment) at 17–20 m tree height. More ^{15}N was recovered in twig than leaf tissue but there was no effect of girdling. Values are means ± SE. Total recovery is not the exact sum of compartments due to rounding

Biomass compartment	^{15}N recovery (%)	
	Girdled	**Ungirdled**
New needles (<2 years)	1.4 ± 0.2	1.8 ± 0.2
New twigs (<2 years)	5.7 ± 1.0	5.3 ± 0.9
Old needles (2–3 years)	1.8 ± 0.3	1.9 ± 0.5
Old twigs (2–3 years)	5.8 ± 1.4	4.7 ± 0.7
Total recovery (eCNU)	14.4 ± 2.6	14.4 ± 1.8

Otherwise, this model had similar effects of biomass class ($p < 0.001$), tissue age ($p < 0.05$) and interactions age:height ($p < 0.05$) and biomass class:height ($p < 0.001$). Branch height thus had a much weaker effect on the ^{15}N recovery than tissue type. Girdling treatments notably did not have a significant effect on ^{15}N recovery (Table 2).

4 | DISCUSSION

From our ecohydrological approach (Figure 2), >70% of inorganic N deposition did not reach the forest floor, implying a substantial

iCNU. We found similar results from our additions of ^{15}N at *close to ambient levels* in the canopy fertilisation (Figure 5) as 86% of the total ^{15}N label did not appear in TF+SF in summer, and appear ~50% in winter. On the other hand, in the branch addition approach (eCNU, Table 2), only 14.4% was retained of the total ^{15}N applied to individual branches.

4.1 | Magnitude of CNU across different scales and comparison to other estimates

The magnitudes of iCNU derived from our ecohydrological and canopy addition approaches were similar to those reported for canopy budgets from the US Pacific coast (Fenn et al., 2013) and Finland (Mustajärvi et al., 2008). While our CNU from the canopy ^{15}N addition (Figure 4) was slightly higher than inferred from the ecohydrological measurements (Figure 5), when calculated from total N (not just ^{15}N) from the canopy fertilisation, they were much more closely aligned (largely supporting H1). We attribute the difference between ^{15}N tracer and N budget (otherwise performed at the same time, in the same treatment) to dry conditions during the canopy fertilisation. Dry conditions would promote retention of (close to natural abundance) N deposition in the canopy from before the experimental period, which may have been washed out of the canopy by our liquid treatment. This would contribute to total N, but not ^{15}N recovery in throughfall and SF. No other significant or seasonally variable ^{15}N enrichment was expected from other sources at our site.

Our ecohydrological and canopy fertilisation CNU estimates were similar (supporting H1) and closer to higher estimates from N budget studies (e.g. Fenn et al., 2013; Tomaszewski et al., 2003) usually under lower N deposition conditions (Table 1) than N additions over canopies. Manipulations targeting low deposition CNU are rare, but a high recovery was reported in a study combining saplings and low deposition (Nair et al., 2016), suggesting saturation of a naturally limited uptake capacity in the canopy may be the primary cause of low CNU in manipulations. In general, low deposition sites where NO_3^--N deposition exceeds NH_4^+-N deposition have very high CNU (Table 1). In mixed conifer forests in Washington State (USA) with around four times as much NO_3^--N compared to NH_4^+-N deposition, NO_3^--N is consumed by canopy interactions while NH_4^+-N is produced (Fenn et al., 2013), suggesting substantial net N reduction is occurring. However, several other studies report higher NH_4^+ canopy uptake than NO_3^- (Table 1), and indeed, conifers generally prefer ammonium over nitrate for root uptake (Buchmann et al., 1995; Kronzucker et al., 1997). Seasonally, from helicopter-applied fertilisers in Dail et al. (2009), maximum NH_4^+-N canopy uptake was in summer, as occurred for our study for the seasonal variation in NO_3^--N. Conversely, in old-growth stands of Douglas fir, more iCNU appears to occur in winter than in summer (Klopatek et al., 2006), although this is mostly due to seasonal variation in NH_4^+ interception by the canopy. Our site was considerably drier and had less seasonal variability in precipitation than

in many other studies, which may affect the dynamics of the highly soluble NH_4^+ ion. Thus our NO_3^--driven uptake could also reflect a greater biological processing of this ion in summer. In a Catalan site (Spain), with high dry deposition, canopy nitrification influences throughfall NO_3^--N fluxes, with the abundance and activity of different phyllosphere microbes also changings subannually (Guerrieri et al., 2020). However, canopy conditions in our cool temperate site are likely very different from this Mediterranean forest. Ecological, environmental or seasonal differences in canopy interactions between the two N ion forms may also affect CNU; mechanistically understanding this is a critical further step in understanding canopy processes.

So why did we find a much smaller eCNU when we were able to directly assess N uptake via the branch addition? Other studies (Boyce et al., 1996; Nair et al., 2016; Wang et al., 2021) tend to report higher levels of CNU from branch-level additions on saplings, although both the method (misting or brushing) and CNU observed differ. We discounted translocation into the rest of the tree (as shown in saplings in Nair et al., 2016) because of the lack of a difference between girdled and ungirdled branches (rejecting H3) but can offer several alternate, non-exclusive explanations. Firstly, as already mentioned, the site was atypically dry in spring 2017 and CNU may be affected by either leaf wetness (Burkhardt & Hunsche, 2013) or by soil drought [which reduces both cuticle hydraulic conductance (Binks et al., 2020) and transpiration (Magnani et al., 2002)]. Dry conditions may also enhance ammonium losses if dew is a night-time reservoir and volatilisation source (Wentworth et al., 2016). Secondly, we only examined uptake over a short 24-hr time-scale and ^{15}N may have remained on leaf and branch surfaces only to be washed away in cleaning. Notably the sapling study (Nair et al., 2016) using similar methods examined recovery after 16 months and found an uptake much more in line with our iCNU. Thirdly, our eCNU estimate is at the scale of an individual branch. As we found only very limited differences between branches dependent on their position in the canopy, eCNU may be expected to progressively accumulate downwards, as N deposition washes from one branch to another. Thus, if the average eCNU was representative of all CNU across a single branch regardless of its position in the canopy, interception by between five and eight branches with living foliage would be required to match the implicit ~70% 'missing' CNU from the ecohydrological approach (S7). Wet deposition N interception by multiple branches may result in total uptake three to eight times greater than observed via a single interception event on an individual branch (Boyce et al., 1996). While the forest leaf area index (LAI) was not measured during this study, site-level LAI from 2008 was 5.1 (Dengel et al., 2015), in line with remote-sensed values for evergreen forests (Asner et al., 2003). We therefore consider our single branch ^{15}N recovery, scaled to the whole canopy, plausible alongside total observed uptake from our other methodologies and in agreement with the strong role of the forest canopy in obtaining N from deposition in our system. Because of the correspondence between this scaled short-term ^{15}N recovery and the consistent ecohydrological fluxes, we consider that the missing N in

the canopy from all three methods was most likely being taken up by the trees.

Seasonally, iCNU from our ecohydrological approach was higher in summer than in winter (Figure 4), although N deposition tended to peak earlier in the year (Figure 2). Likewise, in the canopy fertilisation treatment, there was a seasonal pattern in NO_3^--N and NO_3^--^{15}N tracer (Figure 5). These findings agreed with H2. Here seasonal (February vs. August) variability in recovery was also greater for NO_3^--N than for NH_4^+-N, indicating a stronger canopy sink for NO_3^--N in summer. iCNU from unlabelled N in the tracer addition agreed even more with the ecohydrological approach in summer 2016 (71%). This canopy N sink is likely related to plant or canopy community phenology. Budburst in Sitka spruce in Scotland occurs in early-mid May (Dewar & Watt, 1992), when we observed high iCNU and a consistent increase in NO_3^--N iCNU during the period of rapid shoot expansion (Figure 4). NO_3^- is the most common N compound used for plant growth (Bertoni, 2012), with nitrate transporters present in plant leaves (Hu et al., 2014), and smaller scale experiments showing nitrate assimilation across foliar surfaces (Uscola et al., 2014).

The monthly iCNU from the 5-year ecohydrological approach increased with increasing RF N fluxes (Figure 3), showing signs of saturation at high NO_3^- and total N deposition. This was not evident in the (less abundant) NH_4^+ deposition, suggesting a limited capacity for iCNU, at least in the NO_3^- form. This saturation threshold for iCNU is not found in other studies which consider multiple sites and species (e.g. Houle et al., 2014; Lovett & Lindberg, 1984; Schwarz et al., 2014), suggesting that it may be a dynamic property related to species, ecosystem structure or edaphic N availability. Furthermore, saturation of assimilation capacity may explain why substantial CNU is not inferred in experiments which raise N above background levels (Gaige et al., 2007), or when ambient N deposition is high. Under low N loading, canopy N uptake may contribute to both canopy and whole plant nutrition, complementing root uptake. This would be otherwise facilitated by increased soil N as conditions tend towards ecosystem saturation and high N concentrations in the canopy, which push N interactions towards deleterious effects (Galloway & Cowling, 2002; Schulte-Uebbing & de Vries, 2018). Consequently, only results from whole ecosystem N flux monitoring (e.g. Figure 6) and those tracer experiments performed at close to natural N inputs should be used to measure CNU under ambient conditions.

4.2 | Uncertainties in the measurements

Minor differences in CNU estimates derived from our three methods were expected due to errors in scaling of canopy projection to the ground, from uneven application of ^{15}N-enriched mist, or from interaction of the canopies with neighbouring trees which all affected the two fertiliser application treatments but not the ecohydrological approach. The numerous methods to assess CNU also all have
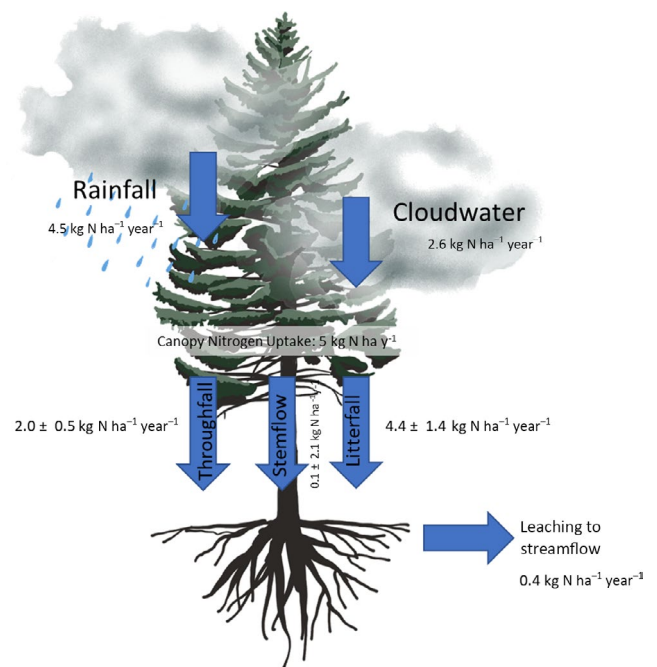

FIGURE 6 Summary of N fluxes measured in our ecohydrological approach. Fluxes are means across the complete years (2012–2016) in the study. The other two measurement approaches (canopy fertilisation and branch addition) yielded similar estimates of canopy nitrogen uptake

different uncertainties which we have attempted to address via the comparison of methods in this study. All methods discounted a major error due to canopy volatilisation of N deposition. This flux is rarely discussed, usually discounted, and likely to be small. While ammonia volatilisation from dry deposition can occur (Hanson & Lindberg, 1991), we expected our site to experience low dry inputs due to its isolated location. More of the added ^{15}N-ammonium than ^{15}N-nitrate ions were also recovered in TF/SF and, in our cool temperate forest, evaporation from canopy surfaces would also be limited for most of the year. Furthermore, while we did not measure dry deposition, our subcanopy measurements were throughfall and stemflow combined which would contain both dry and wet deposition, so missing N inputs from dry deposition would reduce overall CNU.

We also only considered inorganic N fluxes. Worldwide, organic N (ON) is around 25% N deposition (Jickells et al., 2013) but we expected low ON deposition, far from agricultural areas with intensive fertiliser applications or livestock. In southern Scotland, <10% throughfall N is ON in similar Sitka spruce plantations (Cape et al., 2010), even after potential canopy transformations. Assuming a similar amount of mineral N transformed to ON by the canopy could be missed (i.e. total N in throughfall and stemflow could be 10% higher), observed iCNU of inorganic deposition reduces to 67%. The lack of data for ON highlights a shortcoming of budget-based measurements (including our ecohydrological

monitoring) for elemental partitioning which often assume that unmeasured fluxes are unimportant, resulting in incomplete tracking of inputs. For example, a substantial transformation of inorganic N to organic N in the canopy could remove N from throughfall and stemflow without any actual CNU. The composition of total N deposition between these forms may explain some of the differences between studies if these components are processed differently by the canopy.

Because of these uncertainties, the N sink we attribute to CNU may be plant N uptake, but could also be nitrification by phyllosphere microbes (Guerrieri et al., 2015, 2020) or retention on canopy surfaces (Dail et al., 2009). Indeed, the few months when net NH_4^+ fluxes were negative in the ecohydrological approach (Figure 4b) probably indicate canopy retention of N inputs from 1 month to the next. N fluxes in RF and throughfall are influenced by RF mobilising accumulated dry N deposition in canopies (Klopatek et al., 2006; Lovett, 1994; Vanguelova et al., 2010), which may have affected the difference between N and ^{15}N additions in both winter and summer (as the former could be diluted by unlabelled N already held in the canopy). Overall, we assumed that N remaining on leaf surfaces was removed by washing with deionised water. It is possible that some fraction of the missing N was retained by epiphytic phyllosphere organisms not removed by this method (sometimes, chemical compounds are required to specifically extract microbes from leaves (e.g. Kembel et al., 2014)).

Perhaps the greatest uncertainty in generalising our results is our study species. The foliar and canopy traits of trees differ, which result in different rates of CNU across species (Adriaenssens et al., 2011).

Sitka spruce is the most common forestry species in the British Isles (Forestry Commission, 2020), but is native to the northern Pacific coast of North America, and its adaptions for damp climates may influence uptake. Notably, the highest other estimates of CNU come primarily from NW America (e.g. Fenn et al., 2013). Compared to other field estimates in similar species outside the Pacific Northwest, our CNU is high. It is also higher than conifers in the branch-level study by Adriaenssens et al. (2011), although, as previously discussed, our branch-level estimates could be scaled to match the whole-tree measurements. Our experiments also occurred with lower total N additions (treatment + ambient deposition) over the canopy than most other studies, especially in Europe (e.g. Schwarz et al., 2014), and showed signs of saturation (Figure 3) in months with higher deposition. This is notably different than a 13- to 15-year time series of generally declining N deposition (Houle et al., 2014), which seemingly suppressed overall 'ecohydrological' CNU. However, this other study featured a change in the NH_4^+/NO_3^- ratio in deposition across both time and multiple sites. Generalising obscures different trends between ion forms and sites, which suggest that underlying factors such as edaphic N availability or species traits drove responses. At this stage, we cannot clearly separate Sitka spruce's ecological niche from other factors, but our multiple validated approach at ambient conditions does suggest the total canopy uptake of around 70% N is not an artefact of measurement. Scaling to the whole canopy when assessing CNU and considering species traits and actual ambient conditions, as opposed to artificial high treatments or historic periods, is critical to generalise understanding for large-scale forest function.

4.3 | Relevance of CNU for whole-tree nutrition

Nair et al. (2016) found a very high recovery of CNU-^{15}N in high C:N stem biomass in saplings of the same species of this study. We did not find differences in CNU between girdled and ungirdled branches in the branch addition nor between age classes (Table 2), indicating the total uptake was not translocated over 24 hr to the rest of the tree. Nonetheless, the recovery within branches was biased towards wood, as in other studies (e.g. Dail et al., 2009). Evergreen conifers may 'store' N in the youngest needles (Millard & Grelet, 2010) and we worked in spring, when conifers accumulate N in the previous year's needles (Wyka et al., 2016). This may explain the lack of short-term translocation. Due to detection limits and the cost of tracers, assessing whole-tree recovery using tracers is difficult, necessitating the short-term girdling. We however note the short (24 hr) time-scale of the branch addition. The processes which relocate this canopy acquired N may occur on a longer timescale or be dependent on seasonal/phenological conditions outside the experiment period.

Our study site was not likely N saturated due to extremely low N concentrations in surface water outflow, suggesting the high CNU observed may be of relevance to plant functioning. While root uptake of N is both difficult to measure and not available for our site, the low soil available N concentrations also indicate N limitation, so CNU potentially satisfied canopy N demand. Indeed, assuming canopy mass was constant in the mature plantation, the total ecohydrological iCNU (5 kg N ha^{-1} year^{-1}) was greater than the mean annual litterfall N (4.4 kg ha^{-1} year^{-1}). Hence CNU could supply the entire annual demand for regrowth of foliage. A small surplus (0.6 kg N ha^{-1} year^{-1} or 12% of our mean iCNU) could contribute to growth and maintenance of other organs if translocated within the trees, potentially supporting allometric shifts under chronic deposition (Ibáñez et al., 2016). A key aspect of future studies should be quantifying the medium- to long-term fate of CNU-N, and the potential for supplementation or the replacement of root N uptake.

Canopies have an important role in the forest N cycle (Bortolazzi et al., 2021). We found that direct uptake of N was occurring at our study site, potentially enough to satisfy the entire leaf turnover. Saturating N conditions (either at the canopy or whole system level) may influence results in CNU experiments which we avoided by working close to natural abundance. Nitrogen assignment within trees also obeys phenological cycles, probably related to N conservation; short-term experiments are difficult to scale and generalise but these issues are avoided by long-term monitoring approaches.

At our current state of knowledge, these coarse-scale ecosystem N budgets, which are unsuitable for diagnosing particular processes, were as suitable as isotope tracers. Some of the differences between studies may be also attributable to species habit, deposition, climate and soil regimes, so large uncertainties remain for this flux, which potentially affects the responsiveness of forests to N deposition. This is important when expanding tree cover for C cycle management, as N in the soil may be processed differently from new N inputs passing through the canopy. A thorough understanding of CNU, and general canopy N interactions, needs experiments in real contexts (both in terms of forest structure and N addition simulation), while improved understanding of the complex processes involved in canopy interactions necessitates experiments that include consideration of canopy and root uptake together.

CONFLICT OF INTEREST
No conflict of interest is known.

AUTHORS' CONTRIBUTIONS
D.F., K.V.H. and M.M. conceived the ideas and designed the methodology; D.F. and M.S. performed all fieldwork and data collection; D.F. and R.N. performed the data analysis and R.N. led the writing of the manuscript based on a PhD thesis produced by D.F. All authors contributed critically to the drafts and interpretation of the results and gave final approval for publication.

FIELDWORK PERMISSIONS
No fieldwork permissions were needed beyond site access in acknowledgements.

DATA AVAILABILITY STATEMENT
The data used in this paper are available at https://doi.org/10.7488/ds/3272 (Ferraretto & Nair, 2022).

ORCID
Richard Nair https://orcid.org/0000-0002-6293-3610
Dave Reay https://orcid.org/0000-0001-5829-9007
Maurizio Mencuccini https://orcid.org/0000-0003-0840-1477
Michael Spencer https://orcid.org/0000-0003-1472-2082
Kate V. Heal https://orcid.org/0000-0002-1019-748X

REFERENCES
Adriaenssens, S., Staelens, J., Wuyts, K., Schrijver, A., 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, & Soil Pollution, 219*(1–4), 43–57. https://doi.org/10.1007/s11270-010-0682-8

Asner, G. P., Scurlock, J. M. O., & Hicke, J. A. (2003). Global synthesis of leaf area index observations: Implications for ecological and remote sensing studies. *Global Ecology and Biogeography, 12*(3), 191–205. https://doi.org/10.1046/j.1466-822X.2003.00026.x

Barton, K. (2020). *MuMIn: Multi-model inference*. R package (1.43.17.) [Computer software]. https://CRAN.R-project.org/package=MuMIn

Bates, D. M. (2010). *Lme4: Mixed-effects modeling with R*. http://Lme4.r-Forge.r-Project.Org/Book

Bertoni, G. (2012). A nitrate transporter for both roots and shoots. *The Plant Cell, 24*(1), 1. https://doi.org/10.1105/tpc.112.240110

Binks, O., Coughlin, I., Mencuccini, M., & Meir, P. (2020). Equivalence of foliar water uptake and stomatal conductance? *Plant, Cell & Environment, 43*(2), 524–528. https://doi.org/10.1111/pce.13663

Bortolazzi, A., Da Ros, L., Rodeghiero, M., Tognetti, R., Tonon, G., & Ventura, M. (2021). The canopy layer, a biogeochemical actor in the forest N-cycle. *Science of the Total Environment*, 146024. https://doi.org/10.1016/j.scitotenv.2021.146024

Bowden, R. D., Geballe, G. T., & Bowden, W. B. (1989). Foliar uptake of ^{15}N from simulated cloud water by red spruce (*Picea rubens*) Seedlings. *Canadian Journal of Forest Research, 19*, 382–386.

Boyce, R. L., Friedland, A. J., Chamberlain, C. P., & Poulson, S. R. (1996). Direct canopy nitrogen uptake from ^{15}N-labelled wet deposition by mature red spruce. *Canadian Journal of Forest Research, 26*, 1539–1547.

Buchmann, N., Schulze, E. D., & Gebauer, G. (1995). ^{15}N-ammonium and ^{15}N-nitrate uptake of a 15-year-old *Picea abies* plantation. *Oecologia, 102*, 361–370. https://doi.org/10.1007/BF00329803

Burkhardt, J., & Hunsche, M. (2013). "Breath figures" on leaf surfaces—Formation and effects of microscopic leaf wetness. *Frontiers Plant Science, 4*(422). https://doi.org/10.3389/fpls.2013.00422

Cape, J. N., Sheppard, L. J., Crossley, A., van Dijk, N., & Tang, Y. S. (2010). Experimental field estimation of organic nitrogen formation in tree canopies. *Environmental Pollution, 158*(9), 2926–2933. https://doi.org/10.1016/j.envpol.2010.06.002

Chiwa, M., Crossley, A., Sheppard, L. J. J., Sakugawa, H., & Cape, J. N. N. (2004). Throughfall chemistry and canopy interactions in a Sitka spruce plantation sprayed with six different simulated polluted mist treatments. *Environmental Pollution, 127*(1), 57–64. https://doi.org/10.1016/S0269-7491(03)00259-8

Clarke, N., Žlindra, D., Erwin, U., Morello, R., Derome, J., Derome, K., König, N., Lövblad, G., Draaijers, G. P. J., Hansen, K., Thimonier, A., & Waldner, P. (2016). Part XIV sampling and analysis of deposition. In UNECE ICP Forests Programme Co-ordinating Centre (Ed.), *Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests* (p. 32). Thünen Institute of Forest Ecosystems. http://www.icp-forests.org/Manual.htm

Clement, R. J., Jarvis, P. G., & Moncrieff, J. B. (2012). Carbon dioxide exchange of a Sitka spruce plantation in Scotland over five years.

Agricultural and Forest Meteorology, *153*, 106–123. https://doi.org/10.1016/j.agrformet.2011.07.012

Craine, J. M., Elmore, A. J., Wang, L., Aranibar, J., Bauters, M., Boeckx, P., Crowley, B. E., Dawes, M. A., Delzon, S., Fajardo, A., Fang, Y., Fujiyoshi, L., Gray, A., Guerrieri, R., Gundale, M. J., Hawke, D. J., Hietz, P., Jonard, M., Kearsley, E., … Zmudczyńska-Skarbek, K. (2018). Isotopic evidence for oligotrophication of terrestrial ecosystems. *Nature Ecology & Evolution*, *2*(11), 1735–1744. https://doi.org/10.1038/s41559-018-0694-0

Crossley, A., Wilson, D. B., & Milne, R. (1992). Pollution in the upland environment. *Environmental Pollution*, *75*(1), 81–87. https://doi.org/10.1016/0269-7491(92)90060-N

Dail, D. B., Hollinger, D. Y., Davidson, E. A., Fernandez, I., Sievering, H. C., Scott, N. A., & Gaige, E. (2009). Distribution of nitrogen-15 tracers applied to the canopy of a mature spruce-hemlock stand, Howland, Maine, USA. *Oecologia*, *160*(3), 589–599. https://doi.org/10.1007/s00442-009-1325-x

Dämmgen, U., Erisman, J. W., Cape, J. N., Grünhage, L., & Fowler, D. (2005). Practical considerations for addressing uncertainties in monitoring bulk deposition. *Environmental Pollution*, *134*(3), 535–548. https://doi.org/10.1016/j.envpol.2004.08.013

de Vries, W., Du, E., & Butterbach-Bahl, K. (2014). Short and long-term impacts of nitrogen deposition on carbon sequestration by forest ecosystems. *Current Opinion in Environmental Sustainability*, *9–10*(September), 90–104. https://doi.org/10.1016/j.cosust.2014.09.001

Dengel, S., Grace, J., & MacArthur, A. (2015). Transmissivity of solar radiation within a *Picea sitchensis* stand under various sky conditions. *Biogeosciences*, *12*(14), 4195–4207. https://doi.org/10.5194/bg-12-4195-2015

Dewar, R. C., & Watt, A. D. (1992). Predicted changes in the synchrony of larval emergence and budburst under climatic warming. *Oecologia*, *89*(4), 557–559. https://doi.org/10.1007/BF00317163

Du, E. (2016). Rise and fall of nitrogen deposition in the United States. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(26), E3594–E3595. https://doi.org/10.1073/pnas.1607543113

Etzold, S., Ferretti, M., Reinds, G. J., Solberg, S., Gessler, A., Waldner, P., Schaub, M., Simpson, D., Benham, S., Hansen, K., Ingerslev, M., Jonard, M., Karlsson, P. E., Lindroos, A.-J., Marchetto, A., Manninger, M., Meesenburg, H., Merilä, P., Nöjd, P., … de Vries, W. (2020). Nitrogen deposition is the most important environmental driver of growth of pure, even-aged and managed European forests. *Forest Ecology and Management*, *458*, 117762. https://doi.org/10.1016/j.foreco.2019.117762

Fageria, N. K., Filho, M. P. B., Moreira, A., & Guimarães, C. M. (2009). Foliar fertilization of crop plants. *Journal of Plant Nutrition*, *32*(6), 1044–1064. https://doi.org/10.1080/01904160902872826

Fenn, M. E., Ross, C. S., Schilling, S. L., Baccus, W. D., Larrabee, M. A., & Lofgren, R. A. (2013). Atmospheric deposition of nitrogen and sulfur and preferential canopy consumption of nitrate in forests of the Pacific Northwest, USA. *Forest Ecology and Management*, *302*, 240–253. https://doi.org/10.1016/j.foreco.2013.03.042

Fernández-Martínez, M., Vicca, S., Janssens, I. A., Ciais, P., Obersteiner, M., Bartrons, M., Sardans, J., Verger, A., Canadell, J. G., Chevallier, F., Wang, X., Bernhofer, C., Curtis, P. S., Gianelle, D., Grünwald, T., Heinesch, B., Ibrom, A., Knohl, A., Laurila, T., … Peñuelas, J. (2017). Atmospheric deposition, CO2, and change in the land carbon sink. *Scientific Reports*, *7*(1), 9632. https://doi.org/10.1038/s41598-017-08755-8

Ferraretto, D., & Nair, R. (2022). *Forest canopy nitrogen uptake experiments data, 2012–2017*. University of Edinburgh, School of GeoSciences.

Forestry Commission. (2020). *Forestry Statistics 2020: A compendium of statistics about woodland, forestry and primary wood processing in the United Kingdom*. Forest Research.

Gaige, E., Dail, D. B., Hollinger, D. Y., Davidson, E. A., Fernandez, I. J., Sievering, H., White, A., & Halteman, W. (2007). Changes in canopy processes following whole-forest canopy nitrogen fertilization of a mature spruce-hemlock forest. *Ecosystems*, *10*(7), 1133–1147. https://doi.org/10.1007/s10021-007-9081-4

Galloway, J. N. J. N., & Cowling, E. B. E. B. (2002). Reactive nitrogen and the world: 200 years of change. *Ambio*, *31*(2), 64–71. https://doi.org/10.1579/0044-7447-31.2.64

Garten, C. T., Schwab, A. B., & Shirshac, T. L. (1998). Foliar retention of ^{15}N tracers: Implications for net canopy exchange in low- and high-elevation forest ecosystems. *Forest Ecology and Management*, *103*(2–3), 211–216. https://doi.org/10.1016/S0378-1127(97)00185-0

Guerrieri, R., Lecha, L., Mattana, S., Cáliz, J., Casamayor, E. O., Barceló, A., Michalski, G., Peñuelas, J., Avila, A., & Mencuccini, M. (2020). Partitioning between atmospheric deposition and canopy microbial nitrification into throughfall nitrate fluxes in a Mediterranean forest. *Journal of Ecology*, *108*(2), 626–640. https://doi.org/10.1111/1365-2745.13288

Guerrieri, R., Templer, P., & Magnani, F. (2021). Canopy exchange and modification of nitrogen fluxes in forest ecosystems. *Current Forestry Reports*, *7*(3), 115–137. https://doi.org/10.1007/s40725-021-00141-y

Guerrieri, R., Vanguelova, E. I., Michalski, G., Heaton, T. H. E., & Mencuccini, M. (2015). Isotopic evidence for the occurrence of biological nitrification and nitrogen deposition processing in forest canopies. *Global Change Biology*, *21*(12), 4613–4626. https://doi.org/10.1111/gcb.13018

Hanson, P. J., & Lindberg, S. E. (1991). Dry deposition of reactive nitrogen compounds: A review of leaf, canopy and non-foliar measurements. *Atmospheric Environment. Part A. General Topics*, *25*(8), 1615–1634. https://doi.org/10.1016/0960-1686(91)90020-8

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A. F., Ekblad, A., Högberg, M. N., Nyberg, G., Ottosson-Löfvenius, M., & Read, D. J. (2001). Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, *411*(6839), 789–792. https://doi.org/10.1038/35081058

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Houle, D., Marty, C., & Duchesne, L. (2014). Response of canopy nitrogen uptake to a rapid decrease in bulk nitrate deposition in two eastern Canadian Boreal Forests. *Oecologia*, *177*, 29–37. https://doi.org/10.1007/s00442-014-3118-0

Houle, D., Ouimet, R., Paquin, R., & Laflamme, J.-G. (1999). Interactions of atmospheric deposition with a mixed hardwood and a coniferous forest canopy at the Lake Clair Watershed (Duchesnay, Quebec). *Canadian Journal of Forest Research*, *29*(12), 1944–1957. https://doi.org/10.1139/cjfr-29-12-1944

Hu, Y., Fernández, V., & Ma, L. (2014). Nitrate transporters in leaves and their potential roles in foliar uptake of nitrogen dioxide. *Frontiers Plant Science*, *5*(360). https://doi.org/10.3389/fpls.2014.00360

Ibáñez, I., Zak, D. R., Burton, A. J., & Pregitzer, K. S. (2016). Chronic nitrogen deposition alters tree allometric relationships: Implications for biomass production and carbon storage. *Ecological Applications*, *26*(3), 913–925. https://doi.org/10.1890/15-0883

Jickells, T., Baker, A. R., Cape, J. N., Cornell, S. E., & Nemitz, E. (2013). The cycling of organic nitrogen through the atmosphere. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1621), 20130115. https://doi.org/10.1098/rstb.2013.0115

Kembel, S. W., O'Connor, T. K., Arnold, H. K., Hubbell, S. P., Wright, S. J., & Green, J. L. (2014). Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(38), 13715–13720. https://doi.org/10.1073/pnas.1216057111

Klopatek, J. M., Barry, M. J., & Johnson, D. W. (2006). Potential canopy interception of nitrogen in the Pacific Northwest, USA. *Forest Ecology and Management*, *234*, 44–354. https://doi.org/10.1016/j.foreco.2006.07.019

Kronzucker, H. J., Siddiqi, M. Y., & Glass, A. D. M. (1997). Conifer root discrimination against soil nitrate and the ecology of forest succession. *Nature*, *385*, 59–61. https://doi.org/10.1038/385059a0

Krupa, S. (2003). Effects of atmospheric ammonia (NH3) on terrestrial vegetation: A review. *Environmental Pollution*, *124*(2), 179–221. https://doi.org/10.1016/S0269-7491(02)00434-7

LeBauer, D. S., & Treseder, K. K. (2008). Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, *89*(2), 371–379. https://doi.org/10.1890/06-2057.1

Li, W., Zhang, H., Huang, G., Liu, R., Wu, H., Zhao, C., & McDowell, N. G. (2020). Effects of nitrogen enrichment on tree carbon allocation: A global synthesis. *Global Ecology and Biogeography*, *29*(3), 573–589. https://doi.org/10.1111/geb.13042

Liu, T., Mao, P., Shi, L., Eisenhauer, N., Liu, S., Wang, X., He, X., Wang, Z., Zhang, W., Liu, Z., Zhou, L., Shao, Y., & Fu, S. (2020). Forest canopy maintains the soil community composition under elevated nitrogen deposition. *Soil Biology and Biochemistry*, *143*, 107733. https://doi.org/10.1016/j.soilbio.2020.107733

Lovett, G. M. (1994). Atmospheric deposition of nutrients and pollutants in North America: An ecological perspective. *Ecological Applications*, *4*(4), 629–650. https://doi.org/10.2307/1941997

Lovett, G. M., & Lindberg, S. E. (1984). Dry deposition and canopy exchange in a mixed oak forest as determined by analysis of throughfall. *Journal of Applied Ecology*, *21*, 1013–1027. https://doi.org/10.2307/2405064

Magnani, F., Grace, J., & Borghetti, M. (2002). Adjustment of tree structure in response to the environment under hydraulic constraints. *Functional Ecology*, *16*(3), 385–393. https://doi.org/10.1046/j.1365-2435.2002.00630.x

Millard, P., & Grelet, G.-A. (2010). Nitrogen storage and remobilization by trees: Ecophysiological relevance in a changing world. *Tree Physiology*, *30*(9), 1083–1095. https://doi.org/10.1093/treephys/tpq042

Mustajärvi, K., Merilä, P., Derome, J., Lindroos, A.-J., Helmisaari, H.-S., Nöjd, P., & Ukonmaanaho, L. (2008). Fluxes of dissolved organic and inorganic nitrogen in relation to stand characteristics and latitude in Scots pine and Norway spruce stands in Finland. *Boreal Environment Research*, *13*(suppl. B), 3–21.

Nair, R. K. F., Perks, M. P., Weatherall, A., Baggs, E. M., & Mencuccini, M. (2016). Does canopy nitrogen uptake enhance carbon sequestration by trees? *Global Change Biology*, *22*(2), 875–888. https://doi.org/10.1111/gcb.13096

Pahl, S., Winkler, P., Schneider, T., Arends, B., Schell, D., Maser, R., & Wobrock, W. (1994). Deposition of trace substances via cloud interception on a coniferous forest at Kleiner Feldberg. *Journal of Atmospheric Chemistry*, *92*(19), 231–252. https://doi.org/10.1007/BF00696590

Papen, H., Geβler, A., Zumbusch, E., & Rennenberg, H. (2002). Chemolithoautotrophic nitrifiers in the phyllosphere of a spruce ecosystem receiving high atmospheric nitrogen input. *Current Microbiology*, *44*(1), 56–60. https://doi.org/10.1007/s00284-001-0074-9

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J., & Postma, J. A. (2012). Pot size matters: A meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology*, *39*, 839–850. https://doi.org/10.1071/FP12049

R Core Team. (2018). *R: A language and environment for statistical computing.*

Schindler, D. W. (1998). Whole-ecosystem experiments: Replication versus realism: The need for ecosystem-scale experiments. *Ecosystems*, *1*(4), 323–334. https://doi.org/10.1007/s100219900026

Schreel, J. D. M., & Steppe, K. (2020). Foliar water uptake in trees: Negligible or necessary? *Trends in Plant Science*, *25*(6), 590–603. https://doi.org/10.1016/j.tplants.2020.01.003

Schulte-Uebbing, L., & de Vries, W. (2018). Global-scale impacts of nitrogen deposition on tree carbon sequestration in tropical, temperate, and boreal forests: A meta-analysis. *Global Change Biology*, *24*(2), e416–e431. https://doi.org/10.1111/gcb.13862

Schwarz, M. T., Bischoff, S., Blaser, S., Boch, S., Schmitt, B., Thieme, L., Fischer, M., Michalzik, B., Schulze, E.-D., Siemens, J., & Wilcke, W. (2014). More efficient aboveground nitrogen use in more diverse central European forest canopies. *Forest Ecology and Management*, *313*, 274–282. https://doi.org/10.1016/j.foreco.2013.11.021

Sebilo, M., Mayer, B., Grably, M., Billion, D., & Mariotti, A. (2004). The use of the "ammonium diffusion" method for δ15N-NH4+ and δ15N-NO3- measurements: Comparison with other techniques. *Environmental Chemistry*, *1*(2), 99–103. https://doi.org/10.1071/EN04037

Sievering, H., Fernandez, I., Lee, J., Hom, J., & Rustad, L. (2000). Forest canopy uptake of atmospheric nitrogen deposition at eastern U.S. conifer sites: Carbon storage implications? *Global Biogeochemical Cycles*, *14*(4), 1153–1159. https://doi.org/10.1029/2000GB001250

Sparks, J. P. (2009). Ecological ramifications of the direct foliar uptake of nitrogen. *Oecologia*, *159*(1), 1–13. https://doi.org/10.1007/s00442-008-1188-6

Tomaszewski, T., Boyce, R. L., & Sievering, H. (2003). Canopy uptake of atmospheric nitrogen and new growth nitrogen requirement at a Colorado subalpine forest. *Canadian Journal of Forest Research*, *33*(11), 2221–2227. https://doi.org/10.1139/X03-147

Uscola, M., Villar-Salvador, P., Oliet, J., & Warren, C. R. (2014). Foliar absorption and root translocation of nitrogen from different chemical forms in seedlings of two Mediterranean trees. *Environmental and Experimental Botany*, *104*, 34–43. https://doi.org/10.1016/j.envexpbot.2014.03.004

Vanguelova, E. I., Benham, S., Pitman, R., Moffat, A. J., Broadmeadow, M., Nisbet, T., Durrant, D., Barsoum, N., Wilkinson, M., Bochereau, F., Hutchings, T., Broadmeadow, S., Crow, P., Taylor, P., & Durrant Houston, T. (2010). Chemical fluxes in time through forest ecosystems in the UK - Soil response to pollution recovery. *Environmental Pollution*, *158*(5), 1857–1869. https://doi.org/10.1016/j.envpol.2009.10.044

Verstraeten, A., Neirynck, J., Cools, N., Roskams, P., Louette, G., De Neve, S., & Sleutel, S. (2017). Multiple nitrogen saturation indicators yield contradicting conclusions on improving nitrogen status of temperate forests. *Ecological Indicators*, *82*, 451–462. https://doi.org/10.1016/j.ecolind.2017.07.034

Wang, X., Wang, B., Wang, C., Wang, Z., Li, J., Jia, Z., Yang, S., Li, P., Wu, Y., Pan, S., & Liu, L. (2021). Canopy processing of N deposition increases short-term leaf N uptake and photosynthesis, but not long-term N retention for aspen seedlings. *New Phytologist*, *229*(5), 2601–2610. https://doi.org/10.1111/nph.17041

Wentworth, G. R., Murphy, J. G., Benedict, K. B., Bangs, E. J., & Collett Jr., J. L. (2016). The role of dew as a night-time reservoir and morning source for atmospheric ammonia. *Atmospheric Chemistry and Physics*, *16*(11), 7435–7449. https://doi.org/10.5194/acp-16-7435-2016

Wortman, E., Tomaszewski, T., Waldner, P., Schleppi, P., Thimonier, A., Eugster, W., Buchmann, N., & Sievering, H. (2012). Atmospheric nitrogen deposition and canopy retention influences on photosynthetic performance at two high nitrogen deposition Swiss forests. *Tellus B: Chemical and Physical Meteorology*, *64*(1), 17216. https://doi.org/10.3402/tellusb.v64i0.17216

Wuyts, K., Adriaenssens, S., Staelens, J., Wuytack, T., Van Wittenberghe, S., Boeckx, P., Samson, R., & Verheyen, K. (2015). Contributing factors in foliar uptake of dissolved inorganic nitrogen at leaf

level. *Science of the Total Environment*, *505*, 992–1002. https://doi.org/10.1016/j.scitotenv.2014.10.042

Wyka, T. P., Żytkowiak, R., & Oleksyn, J. (2016). Seasonal dynamics of nitrogen level and gas exchange in different cohorts of scots pine needles: A conflict between nitrogen mobilization and photosynthesis? *European Journal of Forest Research*, *135*, 483–493. https://doi.org/10.1007/s10342-016-0947-x

Zhang, W., Shen, W., Zhu, S., Wan, S., Luo, Y., Yan, J., Wang, K., Liu, L., Dai, H., Li, P., Dai, K., Zhang, W., Liu, Z., Wang, F., Kuang, Y., Li, Z., Lin, Y., Rao, X., Li, J., … Fu, S. (2015). CAN canopy addition of nitrogen better illustrate the effect of atmospheric nitrogen deposition on forest ecosystem? *Scientific Reports*, *5*, 11245. https://doi.org/10.1038/srep11245

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.