New Phytologist

Research

Tree water uptake enhances nitrogen acquisition in a fertilized boreal forest – but not under nitrogen-poor conditions

Nils Henriksson[1], Hyungwoo Lim[1], John Marshall[1,2], Oskar Franklin[1,3], Ross E. McMurtrie[4], Reimo Lutter[1,5], Ruth Magh[1], Tomas Lundmark[1] and Torgny Näsholm[1]

[1]Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå SE-90283, Sweden; [2]Global Change Research Institute CAS, Bělidla 986/4a, Brno 603 00, Czech Republic; [3]International Institute for Applied Systems Analysis, Schlossplatz 1, Laxenburg A-2361, Austria; [4]School of Biological, Earth and Environmental Sciences, The University of New South Wales, Sydney, NSW 2052, Australia; [5]Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Kreutzwaldi 5, Tartu EE-510 06, Estonia

Author for correspondence:
Nils Henriksson
Email: nils.henriksson@slu.se

Received: *12 March 2021*
Accepted: *17 June 2021*

New Phytologist (2021) **232:** 113–122
doi: 10.1111/nph.17578

Key words: ^{15}N, deuterium, diffusion, isotope, mass flow, nitrogen uptake, *Pinus sylvestris* (Scots pine), water uptake.

Summary

- Understanding how plant water uptake interacts with acquisition of soil nitrogen (N) and other nutrients is fundamental for predicting plant responses to a changing environment, but it is an area where models disagree.
- We present a novel isotopic labelling approach which reveals spatial patterns of water and N uptake, and their interaction, by trees. The stable isotopes ^{15}N and ^{2}H were applied to a small area of the forest floor in stands with high and low soil N availability. Uptake by surrounding trees was measured. The sensitivity of N acquisition to water uptake was quantified by statistical modelling.
- Trees in the high-N stand acquired twice as much ^{15}N as in the low-N stand and around half of their N uptake was dependent on water uptake (^{2}H enrichment). By contrast, in the low-N stand there was no positive effect of water uptake on N uptake.
- We conclude that tree N acquisition was only marginally dependent on water flux toward the root surface under low-N conditions whereas under high-N conditions, the water-associated N uptake was substantial. The results suggest a fundamental shift in N acquisition strategy under high-N conditions.

Introduction

Plant productivity depends on the acquisition and use of resources, including light, carbon dioxide (CO_2), water and nutrients. The first two are captured by the foliage but the last two, water and nutrients, depend on their availability in soils and the activity of roots and mycorrhiza. Water and nutrient uptake is less well understood than their aboveground counterparts, and is often poorly represented in Terrestrial Biosphere Models (Zaehle *et al.*, 2014; McCormack *et al.*, 2017), which underlines the urgent need for better understanding of water and nutrient uptake and their potential interaction.

Nutrients such as nitrogen (N) are acquired via two soil-transport processes, diffusion through the water and mass flow in water moving toward the root. Mass flow is the movement of a fluid, here soil water and its solutes, down a water potential gradient. In a plant, the water potential across the root surface is created by transpiration. Diffusion, by contrast, occurs along concentration gradients within the water, for example from high to low nitrate concentrations near a root. The final step of N acquisition involves active uptake across membranes via specialized transporters in the root, and is mainly constrained by the N concentration at the root epidermis (Lambers *et al.*, 1998; Oyewole *et al.*, 2014, 2016).

Because soil N is both carried by mass flow toward the root and it diffuses toward the low concentrations at the root surface, the interaction between uptake of water and uptake of N is not straightforward. Unravelling of the interaction requires partial differential equation models that simulate solute concentrations in the rhizosphere surrounding roots, and N uptake at the root surface (Barber & Cushman, 1981; Tinker & Nye, 2000). Comparisons of modelled N uptake with and without mass flow suggest that the rate of mass flow (i.e. the water flux toward the root) often has little impact on N uptake, but it can enhance N acquisition considerably in certain conditions (Nye & Marriott, 1969; Yanai, 1994; Barber, 1995; Williams & Yanai, 1996; BassiriRad *et al.*, 2008; McMurtrie & Näsholm, 2018). Although the effect of mass flow on root N uptake has been demonstrated by soil microdialysis measurements conducted when mass flow is and is not occurring (Oyewole *et al.*, 2016), and in glasshouse experiments (Cramer *et al.*, 2008; Matimati *et al.*, 2014), the effect has not been tested under field conditions. On the basis of the aforementioned models, certain physiological and anatomical characteristics can be expected to increase the sensitivity of tree N

acquisition to mass flow. These include low hydraulic resistance of fine and coarse roots as well as sapwood hydraulic resistance and a high transpiring area relative to water-absorbing area (Samuelson *et al.*, 2008). Collectively, these characteristics act to increase the potential for high rates of mass flow toward root surfaces in the soil. Notably these characteristics have also been associated with responses to inorganic N fertilization, which has been shown to increase the number and size of tracheids (Kalliokoski *et al.*, 2013; Makinen & Hynynen, 2014). Root diameter is also increased when inorganic N is encountered, mainly via larger radius of the water-conducting stele (Wang *et al.*, 2017). Nitrogen addition can also alter tree carbon (C) partitioning (Albaugh, 1998) and enhance whole-tree hydraulic conductance by increasing the ratio of sapwood area to leaf area, leading to higher transpiration per unit ground area (Samuelson *et al.*, 2008; Lim *et al.*, 2015).

McMurtrie & Näsholm (2018) published a model describing the roles of mass flow and diffusion in root nutrient uptake. Fig. 1 illustrates the hypothesized outcome of a comparison between low vs high N availability. It shows that daily root N uptake responds positively to daily root water uptake, and that the effect is exaggerated if soil N availability is high. McMurtrie & Näsholm's model, which is derived from the Barber–Cushman model of root-N uptake (Barber & Cushman, 1981; Tinker & Nye, 2000), represents the soil environment as a uniformly spaced, parallel array of cylindrical soil volumes with a root positioned at the centre of each cylinder. Solute moves radially by diffusion and mass flow within each cylinder until it reaches the root surface where plant uptake occurs. Typically, a high proportion of solute is taken up by soil microbes before reaching the root surface. Mass flow is important because it can hasten solute movement toward the root, and hence decrease the likelihood of microbial immobilization before solute reaches the root. At high rates of mass flow, potential N immobilization by soil microbes is reduced and a high N concentration can be maintained at the root surface, facilitating N uptake by roots. Under fertilization, when soil N is more available, root N uptake is modified by two additional factors. First, for a given water-uptake rate, if the probability of microbial immobilization is unaltered by fertilization, then soil N concentrations will be higher at the root surface, enhancing root N uptake as illustrated in Fig. 1. Second, when applying Fig. 1 to fertilized and nonfertilized stands, where tree physiology has been altered in response to N fertilization as discussed above, it is necessary to also consider that the rate of mass flow toward root surfaces might be greater in the fertilized forest. Therefore, roots of a fertilized tree would have higher *x*-values and hence higher *y*-values than shown in Fig. 1, further increasing the difference in N uptake between trees in the reference and fertilized stands. Thus, on the basis of McMurtrie & Näsholm (2018), transpirationally driven mass flow is hypothesized to enable significantly greater N uptake per unit water uptake in fertilized trees than in nonfertilized trees.

Stable isotope labelling techniques can quantify N uptake by trees and other plants, but in order to draw inferences about how N uptake is linked to water uptake, the water itself must also be isotopically labelled. A previous study showed which trees in a boreal forest acquired N from a specific patch of isotopically labelled soil (Göttlicher *et al.*, 2008). A study from the Brazilian Amazon showed analogous water uptake data (Sternberg *et al.*, 2002). The difficulty in merging these types of approaches is that the methods employed have been considered to be not compatible. Acquired N is incorporated into plant organs such as leaves, and is retained there to be sampled and quantified, whereas water-labelling studies in the field are generally designed as pulse-chase studies where the absorbed water is sampled as it passes through the xylem stream (Sternberg *et al.*, 2002; Kulmatiski *et al.*, 2017). This incompatibility has hampered the possibility to conduct dual-labelling experiments in the field, similar to the single-label examples stated above.

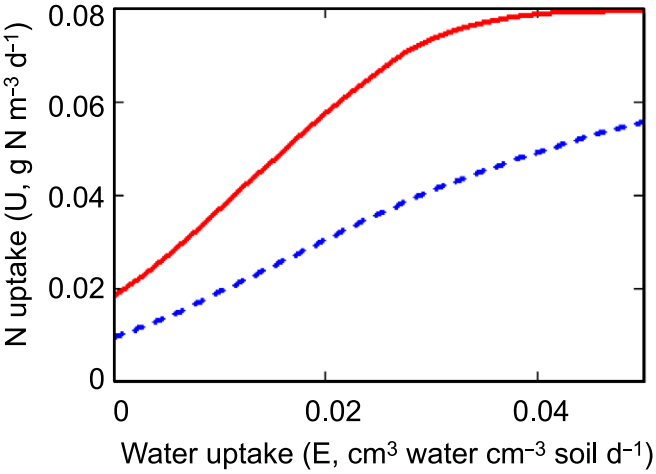

Fig. 1 Modified from McMurtrie & Näsholm (2018). The modelled nitrogen (N) uptake rate (*y*-axis) at variable water uptake rates (*x*-axis) under high N (red) and low N soil conditions (blue). In the absence of water uptake ($x = 0$), the higher N concentration in the soil solution under high N conditions leads to enhanced diffusional N uptake, compared to lower N conditions. Additionally, leaf/root mass is 1.68 times higher for trees in the fertilized stand than in the reference stand. Thus, reference trees would plot on the blue curve, and the fertilized trees would not only plot on the red curve, but also farther to the right along the *x*-axis.

We present a new approach that bridges this gap and can provide actual plant uptake measurements to compare with theoretically modelled predictions. In six boreal forest plots, we applied a label solution containing $K^{15}NO_3^-$ and ^2H_2O to 1 m^2 of forest soil, and after one growing season we assessed acquired ^{15}N in tree foliage and absorbed ^2H which had been incorporated into the sapwood of that year's growth ring. The current study is the first to show that ^2H labelling of the sapwood in tree stems can be used to quantify water uptake by trees. This new method enabled us to quantify the coordinated N and water uptake of trees growing at different distances from a labelled soil patch, without the confounding influence of transport velocities or varying path lengths.

The current study tests the hypothesized link between water uptake and N acquisition presented by McMurtrie & Näsholm (2018), employing the novel isotope labelling approach in two boreal forest stands of low and high N availability in northern Sweden. Based on the predictions from the model, we

hypothesized a stronger interaction between water and N uptake would be displayed for trees in the high N stand than in the low N reference stand.

Materials and Methods

The study site

The six plots used in the current study were located in a pair of adjacent boreal forest stands (i.e. three plots per site) in northern Sweden (Rosinedal, 64°10′N, 19°45′E, 145 m above sea level). The stands were *c.* 16 ha each and established as experimental field sites in 2006 (12 yr prior to the current study) for the purpose of studying ecosystem-level carbon fluxes using eddy covariance methods. Due to the requirements of such measurements, the fertilization and reference treatments were not replicated. The stands are both *Pinus sylvestris* monocultures, *c.* 100 yr of age, and are located 2 km apart. Both stands are on deep sandy sediment. The ground vegetation consists of lichens and ericaceaous dwarf shrubs, mostly *Vaccinium vitis-ideae* and *Calluna vulgaris* (Lim *et al.*, 2015; Hasselquist *et al.*, 2015).

The fertilized stand received annual doses of nitrogen (NH_4NO_3), 100 kg N ha^{-1} yr^{-1} for 6 yr, and thereafter 50–64 kg N ha^{-1} yr^{-1} for a further 7 yr until the time of the current study. In each stand, three permanent 0.1 ha mensuration plots have been monitored since the sites' establishment. The current isotopic labelling was performed in these six mensuration plots early in the growing season, and samples were then collected from the trees after the end of the growing season (see below).

The position of every tree in the six mensuration plots was mapped, using a total station theodolite (Trimble S5; Trimble Inc., Sunnyvale, CA, USA). Stand density in the mensuration plots was 1007 ± 131 stems ha^{-1} in the reference stand, and 847 ± 76 stems ha^{-1} in the fertilized stand (mean ± 1 SD).

Isotopic labelling

We applied isotopically labelled nitrogen (5 g ^{15}N; i.e. 36 g of KNO_3 99.99 at.% ^{15}N, Larodan Fine chemicals, Malmö, Sweden) and water (400 ml ^2H_2O, 99.5 at.% pure; Cambridge Isotope Laboratories, Tewksbury, MA, USA) to a central 1 m^2 in six boreal forest plots. Nitrate was chosen as the added N form because of its mobility in soil solution. This made it relevant to the hypotheses tested in the current study, which state that high mass flow rates enhance the flux of mobile solutes toward the roots (Nye & Marriott, 1969; McMurtrie & Näsholm, 2018). On day of the year (DOY) 155–158 (4–7 June 2018) the ^{15}N dose was applied with half of the deuterium (200 ml) dissolved in 25 l of tap water, giving the mixture a deuterium enrichment signature of $\delta^2H = 51\,270‰$ (VSMOW). To ensure continued uptake of labelled water during the second half of the season, the remaining half of the deuterium was applied on DOY 207 (26 July 2018), mixed with 25 l of tap water.

The label solution was applied underneath the moss layer to reduce evaporative loss of the label. This was done using syringes (50 ml, Plastipak; Sigma-Aldrich) with a plastic extension (15 cm long) attached that could be inserted into the moss and reach the surface of the mineral soil below. The 1 m^2 area to be labelled was covered by a grid frame and the label solution was injected into 256 points, in order to disperse the solution evenly across the area.

Sampling and analysis

All trees growing within a 14 m radius of the labelled area were sampled. Current-year needles were collected for ^{15}N analysis using pole scissors (all samples taken from midcanopy height, and facing the plot centre) and sapwood cores were taken from the tree stems, at breast height and facing the plot centre, using a hole puncher (10 mm diameter) for ^2H analysis. From each tree, one sample of needles and one sample of stem wood were collected. The signal from the most recent growing season was recorded in the outermost growth ring, which was extracted using a scalpel. Both needle and sapwood samples were dried for 48 h at 65°C, before being milled and analysed using isotope ratio mass spectroscopy (IRMS) at the Swedish University of Agricultural Sciences Stable Isotope Laboratories (SSIL, Umeå, Sweden). In both cases, bulk samples were analysed. To allow uptake of ^{15}N and ^2H in trees to proceed throughout the growing season, from early May to late September, samples were taken after the end of the growing season (DOY 288, 2018 – DOY 74, 2019).

Calculations and data analysis

All isotopic calculations were performed based on atom fractions. The natural abundance of ^{15}N in the foliage of the research sites was measured (−3.8 ± 1.8‰ in the reference stand, −1.8 ± 2‰ in the fertilized stand). This difference in natural abundance can be attributed to the fertilization treatment. In the current study, ^{15}N enrichment was calculated as an isotopic fraction in excess of site-specific mean natural abundance + 2 standard deviations.

For ^2H, natural abundance was defined by the trees most distant from the labelled area (>12 m). This corresponded to 0.014 at.% (or *c.* −99‰ on the VSMOW scale). As with the N label, trees were considered to have taken up the ^2H label if the isotopic enrichment of samples was above mean natural abundance + 2 standard deviations.

Within the six mensuration plots, all trees had been measured regularly (height, diameter at 1.3 m and base of the green crown). Sample trees had previously been harvested in each stand to produce allometric growth equations to calculate the biomass and growth increment of various tree compartments of each tree growing within the mensuration plots (see Supporting Information Methods S1; Lim *et al.*, 2015). These data were used to scale up isotopic enrichment data to the total amount of label taken up by each tree (Dataset S1). The scaling procedure was as follows: the isotopic enrichment was calculated (based on at.% in excess of natural abundance, and the total N or H content of the sample) and multiplied by a biomass factor for the sampled tissue type, as produced by the allometric equations (Table S1). Thus, needle excess ^{15}N concentration (μg g^{-1}) was multiplied by the current-year foliage biomass, and ^2H excess concentrations from

the latest growth ring were multiplied by the annual wood increment of stem, branches and coarse roots (see Eqn 1 in Methods S1). This scaling was only employed to estimate the label recovery in the plots. Isotopic concentration ($\mu g\, g^{-1}$) in excess of natural abundance was used in both correlation analyses (needle ^{15}N vs wood ^{2}H) and in the modelling exercises, as described below.

Statistical modelling of N acquisition and water uptake

Both needle ^{15}N and wood ^{2}H decline exponentially with distance between the tree and the label source, which simply reflects the decline in density of roots with increasing distance from a tree. We want to separate this pure spatial correlation between N and water uptake due to root distribution from the interaction of the uptake processes at the root level.

First, we estimated the root-level correlation based on the residuals against the exponential distance dependence for each variable, that is extracting the nondistance-dependent correlation between needle ^{15}N and wood ^{2}H concentrations. The R^2 of this correlation represents the fraction of variation in N uptake that is explained by water uptake ($N_{w\,all}$). This fraction includes both the direct effect of N dissolved in water accumulating at the root surface (the mass flow effect) and indirect effects, such as higher N availability in wetter than in drier spots in the soil.

In a second analysis we estimate the direct mass flow-related effect of water uptake on N uptake as a linear effect, which is combined with a distance effect in a nonlinear regression model of total N uptake:

$$N = a1 \cdot e^{(-\text{Distance} \cdot a2)} + a3 \cdot W + a4 \qquad \text{Eqn 1}$$

where $a1$, $a2$, $a3$ and $a4$ are constants estimated in the regression. In Eqn 1, N refers to the needle ^{15}N concentration ($\mu g\, g^{-1}$). 'Distance' refers to the distance, in metres, between the tree and the label source. W refers to the wood ^{2}H concentration of sampled tree rings ($\mu g\, g^{-1}$). We interpret $a1.e^{(-\text{Distance} \cdot a2)} + a4$ as the fraction of acquired ^{15}N that was not directly dependent upon water uptake, and $a3.W\,(=N_w)$ as the fraction of acquired ^{15}N that was directly dependent upon water uptake. Thus, the fraction of total N acquisition that was directly dependent upon water uptake (N_w) can be expressed as

$$N_w = a3 \cdot W / N \qquad \text{Eqn 2}$$

In contrast to the first approach where the water-dependent N uptake was based on correlation of residuals ($N_{w\,all}$), here the water-dependent uptake (N_w) does not include the indirect (including nonlinear) effects of water. Thus, the indirect effect of water uptake on N uptake can be estimated as $N_{w\,all} - N_w$. Modelling was performed using an *nls* function in R statistics software (R Core Team, v.4.0).

Results

The isotopic labeling signal ($\mu g\, g^{-1}$ in excess of natural abundance) was strongest in trees growing near the application point, and from there declined with distance. An exponential fit of the isotopic concentrations (needle ^{15}N and wood ^{2}H) across distance yielded an R^2 for ^{15}N of 0.43 and 0.71 (reference and fertilized stands, respectively) and an R^2 for ^{2}H of 0.49 and 0.58 (reference and fertilized stands, respectively) (Fig. 2). Excluding the distance effect by fitting the residuals against each other (^{15}N vs ^{2}H) yielded correlations with $R^2 = 0.58$ in the fertilized stand and $R^2 = 0.07$ in the reference (Fig. 3), indicating that 58% of the variation in total N uptake is explained by water uptake in the fertilized stand, whereas only 7% of the variation in N uptake is explained by water uptake in the reference stand.

The ^{2}H tracer was found in trees within a smaller radius around the labelled centre compared with ^{15}N. Thus, the trees growing closest to the labelled area were enriched in both tracers, and the ^{15}N signal was observed in a greater number of trees compared with the ^{2}H signal (Fig. 4; Table 1). In the reference stand, ^{15}N was detected in 14.7 ± 1.5 trees per plot, and ^{2}H was detected in 8.0 ± 2 trees per plot ($n = 3$, mean $\pm$ 1 SD, $P = 0.031$). In the fertilized stand, 9.7 ± 2.1 trees took up the applied ^{15}N and 6.0 ± 1.7 trees took up the ^{2}H label ($n = 3$, $P = 0.170$) (Table 1; Fig. S1).

Isotopic label recovery was estimated based on scaling isotopic concentration to total uptake via allometric biomass equations (Table S1, EQ S1). In the reference stand, an estimated 247 ± 34.4 mg ^{15}N was taken up, and the uptake of ^{2}H into the new tree ring was 17.3 ± 4.3 mg (mean $\pm$ 1 SE). The plots in the fertilized stand took up 505.8 ± 42 mg ^{15}N and 11.9 ± 1.9 mg ^{2}H. In other words, $10.1 \pm 0.8\%$ of the applied ^{15}N and $0.015 \pm 0.002\%$ of the applied ^{2}H was recovered in the fertilized plots, and the recovery in the reference plots was $4.9 \pm 0.7\%$ of applied ^{15}N and $0.022 \pm 0.005\%$ of applied ^{2}H, calculated on a molar basis (Table 2).

Needle ^{15}N concentration was significantly correlated with wood ^{2}H concentration in both stands, but the relationship was much stronger in the fertilized ($R^2 = 0.82$, $P < 0.0001$) compared to the reference stand ($R^2 = 0.14$, $P < 0.0001$) (Fig. 5). Excluding nonlabelled trees from the analysis altered the fit so that $R^2 = 0.7$ in the fertilized stand ($P < 0.0001$) and $R^2 = 0.01$ ($P = 0.63$) in the reference stand.

Our regression model, which aimed to quantify the fraction of ^{15}N uptake that was directly related to water uptake (Eqn 2), concluded that a significant fraction of needle ^{15}N was correlated with the isotopic water signal (^{2}H) in the fertilized stand, whereas the water signal did not significantly affect ^{15}N uptake in the reference stand. According to our model, an average of 48% of total ^{15}N acquisition was related to ^{2}H uptake in the fertilized stand (Fig. 6a). The model fit was $R^2 = 0.3$ and $R^2 = 0.85$ for the reference and fertilized stands, respectively (Fig. 6b).

The number of labelled trees provides the belowground overlap density for the two resources in each stand (trees m^{-2}, Table 1), and the distance between labelled trees and the source location reflects the lateral reach of uptake. However, we also observed that several trees failed to acquire the label (^{15}N or ^{2}H) despite growing within a radius of the source where other trees were labelled (Fig. 2), suggesting gaps in the root systems. Within 0–2 m from the label source, all trees acquired at least one of the

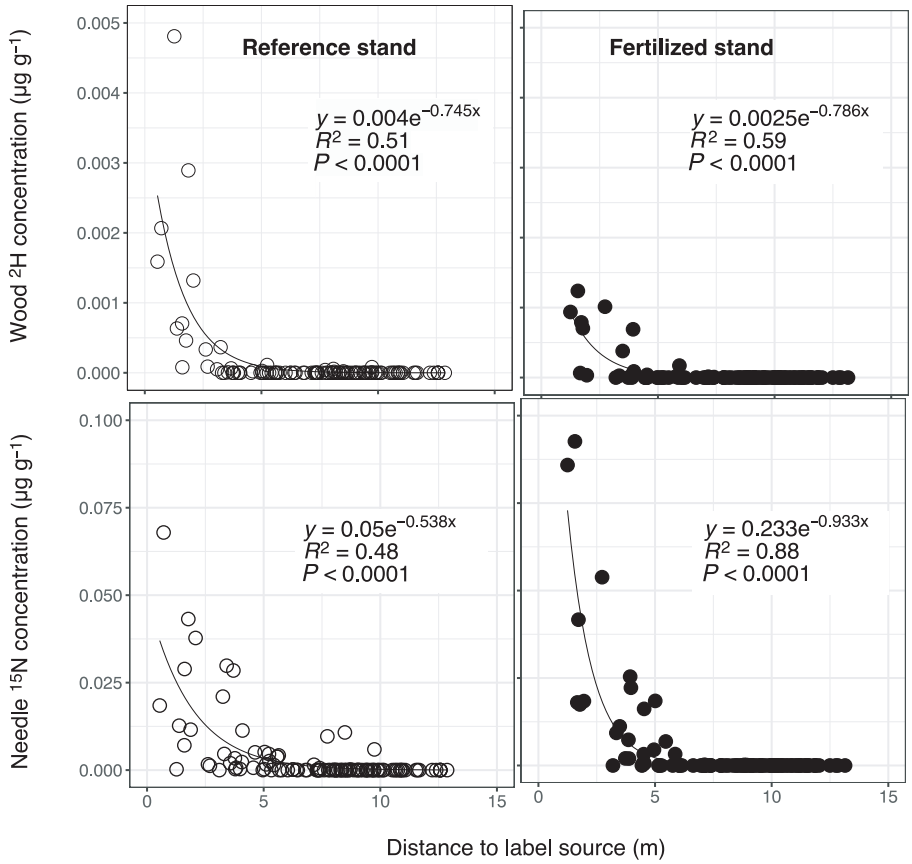

Fig. 2 The label concentration (µg g^{-1} excess) observed in *Pinus sylvestris* trees surrounding the labelled 1 m^2 of ground. Top panels show deuterium (^2H) signal and the bottom panels show the ^{15}N signal. Note that the labelling and sampling were replicated three times in each stand (open circles = reference, $n = 111$; filled circles = fertilized, $n = 109$), which have been combined to produce the current figure. An exponential decay curve is fitted to the data (in the reference stand R^2 for $^{15}N = 0.48$, and for $^2H = 0.51$; in the fertilized stand, R^2 for $^{15}N = 0.88$ and for $^2H = 0.59$).

isotopic tracers, but this fraction then dropped gradually with increasing distance, following a logistic decay curve (Fig. S1). The curve showed different inflection points for the two stands, such that 50% of trees were labelled by at least one of the isotopes at a distance of 7.7 ± 0.3 m in the reference stand and 6.8 ± 0.2 m in the fertilized stand.

Discussion

The current study provides the first demonstration of the sensitivity of tree N acquisition to water uptake in the field. We show that tree uptake of water and N are more closely connected to each other in a fertilized forest stand than in a nontreated reference stand (Figs 5, 6a).

Previous theoretical and experimental work has shown that root N uptake is constrained by the rate of transport toward the root surface (Leadley *et al.*, 1997; Cramer *et al.*, 2008; Matimati *et al.*, 2014; Oyewole *et al.*, 2016; McMurtrie & Näsholm, 2018). A high rate of water transport towards the root surface should, according to these reports, enhance root uptake both by reducing the opportunity for soil microbes to intercept dissolved N, which leads to increased N concentration at the root surface, and by steepening the gradient of N concentration in the rhizosphere adjacent to the root surface (Nye & Marriott, 1969; Oyewole *et al.*, 2016; McMurtrie & Näsholm, 2018). These experimental results and models conclude that the rate of water flow per root area is the key factor that enhances N uptake, rather than the volume of absorbed water at the whole plant level. While transpiration rates are important, the distribution of transpirationally driven water uptake over the root system is another key driver for N uptake. Thus, if a given volume of water is absorbed over a large root surface area, then uptake of N would be less enhanced than if the same volume of water were taken up via a smaller root surface area. Furthermore, previous work has subdivided fine roots into short-lived absorptive fine roots and longer lived transport fine roots (McCormack *et al.*, 2015; Iversen *et al.*, 2017), indicating that the proportion of roots in each category could affect mass flow rates. Models thereby predict that certain phenotypic characteristics should increase the sensitivity of N uptake to mass flow, causing a stronger interaction between the two.

Notably, N fertilization has been shown to alter tree physiology and biomass partitioning toward characteristics which are predicted to enhance mass flow-enabled N uptake (Kalliokoski *et al.*, 2013; Makinen & Hynynen, 2014; Lim *et al.*, 2015; Wang *et al.*, 2017). To that end, in the current study, trees in the

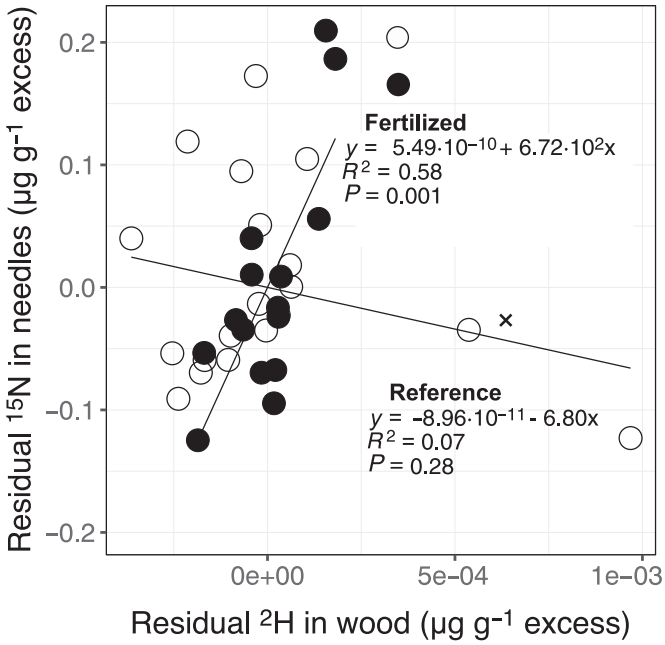

Fig. 3 Residuals of *Pinus sylvestris* needle ^{15}N concentration and wood ^{2}H concentration after an exponential fit against the distance between tree and label source (Fig. 2). Uptake of both isotopic labels (^{2}H and ^{15}N) was strongly correlated with distance. Each isotope signal was therefore fitted to an exponential regression against distance, and the residuals were then regressed against one another. This figure thus shows the fit of ^{15}N uptake vs ^{2}H uptake after removing the correlation with distance (in the equations, *x* and *y* correspond to the residuals as shown in the respective axis titles). Open and filled circles indicate trees in the reference stand (n = 19) and fertilized stand (n = 16), respectively.

Table 1 Number of *Pinsu sylvestris* trees in which isotopic labels (^{2}H and ^{15}N) were detected (mean ± 1 SD). N = 3 for each forest stand and the reported P values were produced from a matched pairs t-test, comparing the number of ^{15}N-labelled trees and ^{2}H-labelled trees within each stand (significant differences indicated by an asterisk).

Stand	Subplot	^{15}N-labelled trees per plot	^{2}H-labelled trees per plot	Trees per plot (total)	P
Reference	1	16	10	38	
	2	13	8	40	
	3	15	6	33	
	Mean (SD)	14.7 (1.5)	8.0 (2.0)	37.0 (7.7)	0.03*
Fertilized	1	8	5	36	
	2	12	5	35	
	3	9	8	38	
	Mean (SD)	9.7 (2.1)	6.0 (1.7)	36.3 (7.3)	0.17

fertilized stand had around 50% greater foliage mass than trees in the reference stand (5.9 and 3.9 kg m^{-2}, respectively), and 33% lower fine root mass (339 and 507 g m^{-2}, respectively), both changes contributing to a higher rate of water uptake per root surface area. N fertilization was also reported to reduce wood density while increasing coarse root biomass in the same study area (Lim *et al.*, 2015), changes that have been reported to correlate with increased hydraulic conductance (Samuelson *et al.*, 2008). Based on the theoretical framework described above, we predicted that roots of the two contrasting forest systems in our study would exhibit different rates of water uptake and consequently differ in the sensitivity of N acquisition to water uptake.

We found that a large fraction of tree N acquisition (58%) was correlated with water uptake in the fertilized stand, while our regression model did not identify any such dependency in the reference stand (Figs 3, 4); and trees in the fertilized stand acquired approximately twice as much ^{15}N as trees in the reference stand (Table 2). The first result supports the hypothesis that mass flow enables enhanced N uptake by roots under conditions conducive to high rates of water uptake per root area, as occurred in the fertilized stand. The second result demonstrates that this did in fact lead to higher N acquisition. Our study therefore corroborates the conclusion reached by McMurtrie & Näsholm (2018) that N

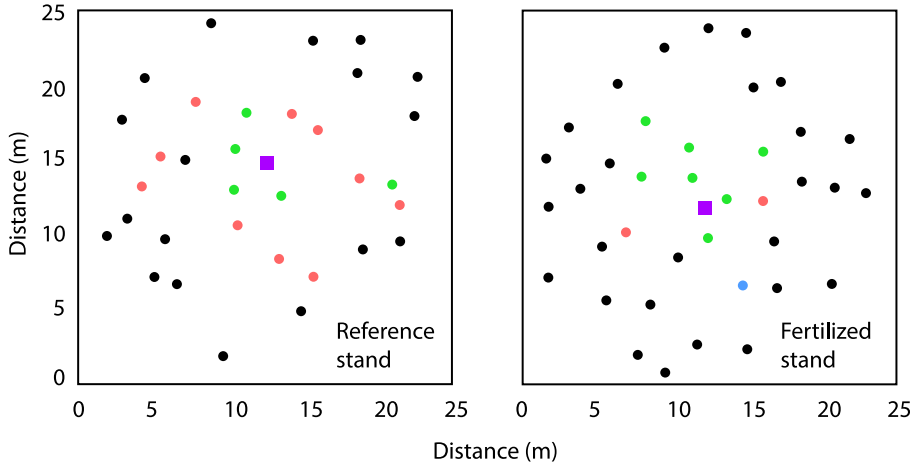

Fig. 4 Representations of two labelled plots. Coloured round markers identify isotopically labelled *P. sylvestris* trees as follows: green = both isotopes (^{15}N and ^{2}H); red = only ^{15}N; blue = only ^{2}H; black = no label uptake. The purple square indicates the location of the label source (1 m^{2} of ground to which the label solution was injected below the moss layer). Each panel depicts one out of the three plots located in each stand.

Table 2 Sum isotopic label recovered in *Pinus sylvestris* trees per labelled plot. The applied amount of label was 5 g of ^{15}N and 80 g of ^{2}H. The label was dissolved in 25 l of water and injected uniformly, below the moss layer, across an area of 1 m^2.

Stand	Subplot	Total ^{2}H uptake per plot (mg)	Total ^{15}N uptake per plot (mg)	Recovery of applied ^{2}H (%)	Recovery of applied ^{15}N (%)
Reference	1	9.0	315.3	0.011	6.3
Reference	2	19.8	205.0	0.025	4.1
Reference	3	23.2	220.6	0.029	4.4
	Mean (SE)	17.3 (4.3)	247 (34.4)	0.022 (0.005)	4.9 (0.7)
Fertilized	1	8.43	424.3	0.011	8.5
Fertilized	2	15.1	529.0	0.019	10.6
Fertilized	3	12.1	564.2	0.015	11.3
	Mean (SE)	11.9 (1.9)	505.8 (42)	0.015 (0.002)	10.1 (0.8)

uptake is more sensitive to water uptake when rooting density is low, and concurrently that N acquisition is enhanced. Other models have concluded that a high mass flow rate would reduce the diffusive flux of solutes, thus cancelling out any potential benefit in terms of N uptake (Yanai, 1994; BassiriRad *et al.*, 2008), but our study contradicts this hypothesis, as we observed an increased dependency of N acquisition on water uptake in the fertilized stand. The current study is thereby the first field experiment able to test the validity of these competing hypotheses on the basis of direct measurements of plant uptake.

Several trees failed to take up the ^{15}N label despite growing within the radial distance where label was taken up by other trees (Figs 2, S2). This observation, which is in line with previous findings (Ferrill & Woods, 1966; Sternberg *et al.*, 2002; Göttlicher *et al.*, 2008), suggests that root systems do not extend uniformly outwards (Bishop, 1962; Taskinen *et al.*, 2003). The finding that the proportion of labelled trees was higher in the reference stand may signify a greater root density at a given distance, or a more evenly distributed root system, thus improving the likelihood of encountering the label. Trees growing within this area should potentially reach the label but may by chance not have roots within the labelled area. Hypothetically, the trade-off for such an expanded root surface area would be a reduced inward water flux at the root surface, making soil microbes more competitive for soil N, which is in line with our model results. Previous field experiments have shown that immobilization of N by soil organisms, including ectomycorrhizal fungi, was alleviated under N fertilization (Näsholm *et al.*, 2013; Hasselquist *et al.*, 2015; Henriksson *et al.*, 2021). This observation is consistent with the current study, where total ^{15}N recovery per plot was twice as high in the fertilized stand as in the reference stand (Table 2). However, it has been shown that root absorbing activity throughout the root system can be modulated to target nutrient-rich patches of soil (Kiba & Krapp, 2016; Kulmatiski *et al.*, 2017), suggesting that a widely distributed root system does not necessarily lead to a proportionally large active absorbing area. It has also been demonstrated that new tree roots forming in soil patches rich in inorganic N can develop physiology that enhances their hydraulic conductivity (Wang *et al.*, 2017).

The current method of detecting ^{2}H tracers in tree rings provides a useful tool for investigations into tree water relationships. Due to stomatal evaporation, leaf water is naturally enriched in the heavier isotope (Roden & Ehleringer, 1999). However, the mechanisms by which cellulose reflects the isotopic composition

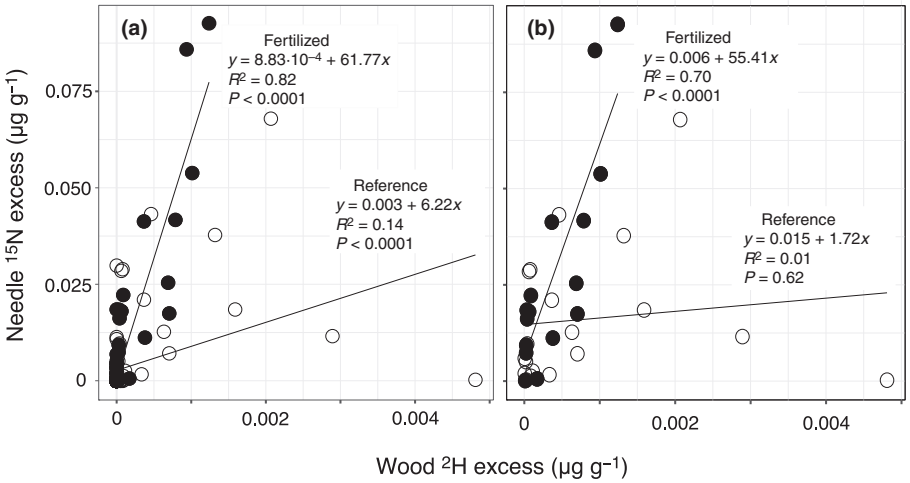

Fig. 5 Needle ^{15}N concentration vs wood ^{2}H concentration of *Pinus sylvestris* trees in fertilized (filled circles) and reference (open circles) stands. Linear regressions fitted without considering distance between trees and the label source. Isotopic concentration in excess of natural abundance is reported. (a) The correlation using the full dataset (n = 111 and n = 109 in the reference and fertilized stands, respectively). (b) The result of excluding nonlabelled trees (n = 19 and n = 16 in the reference and fertilized stands, respectively).

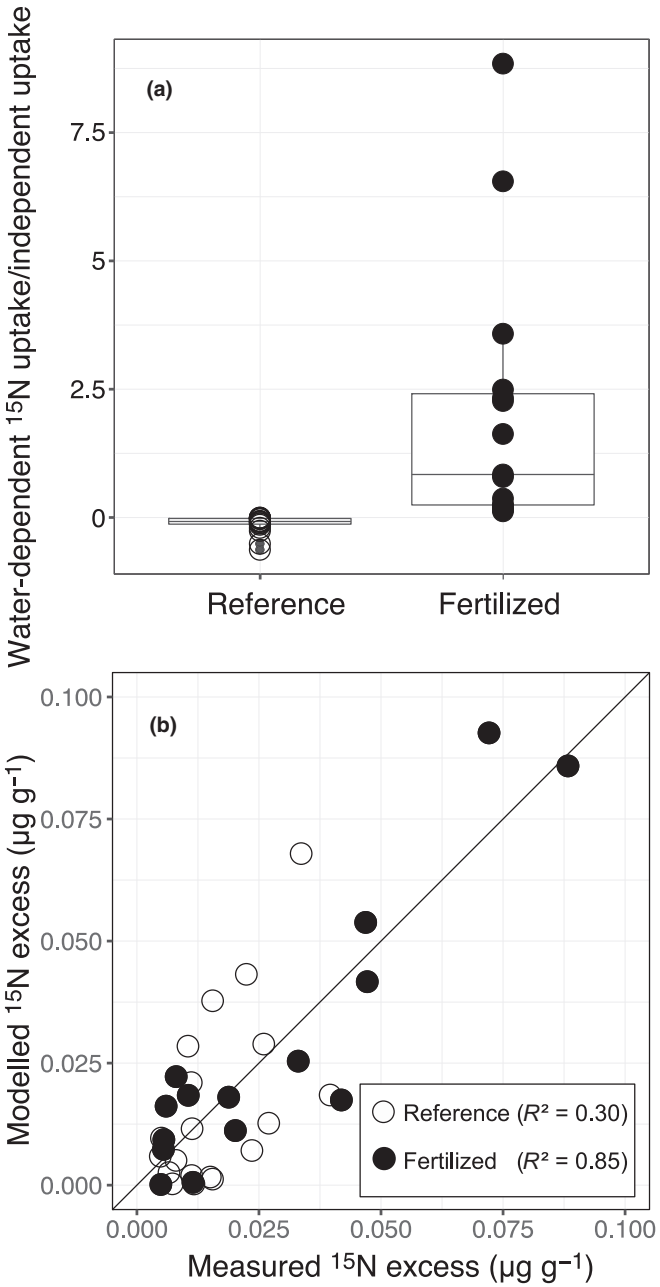

Fig. 6 Impact of water uptake on tree N acquisition by *Pinus sylvestris* trees in the reference and fertilized stands (open and filled markers, respectively). (a) The *y*-axis shows water-enabled N uptake, as a fraction of water-independent N uptake. The boxplots display the median value, and the 1st and 3rd quartiles. (b) Themodel fit (actual vs predicted). Model R^2 was 0.3 in the reference stand and 0.85 in the fertilized stand. Only trees which took up both isotopic labels (^{2}H and ^{15}N) were included in the analysis (n = 19 and n = 16 in the reference and fertilized stands, respectively).

of leaf water and source water are complicated because a fraction of hydrogens in cellulose molecules is exchangeable with the surrounding water (Cheesman & Cernusak, 2017), and the ^{2}H may be incorporated into existing cellulose via chemical exchange with the xylem sap. Additionally, the ^{2}H signature in tree ring cellulose reflects the proportional use of stored carbohydrates and new photosynthetic assimilates (Lehmann *et al.*, 2021). These factors can add to the uncertainty and variability of ^{2}H enrichment in cellulose. In the current study, however, the label intensity was so high (δ^2H = 51.270‰ (VSMOW), in the applied mixture) that it should have overcome the smaller effects described above.

In conclusion, the current study tested a previously theorized interaction between plant water uptake and N acquisition. Our experimental system provided rigorous test conditions and the isotopic approach we present allows all trees to be sampled at the end of the season, for both water uptake (^{2}H in tree rings) and N uptake (^{15}N in foliage). The integrated measurement of isotopic incorporation into biomass across an entire season's growth disclosed a strong N acquisition dependency on tree water uptake in a fertilized stand while no such dependency was found for an N-limited stand. The higher fraction of tracer N detected in fertilized trees compared to N-limited trees suggests mass flow-mediated N uptake to be an efficient means of N acquisition. Our results also suggest adding mineral N to a forest system in which soil N is dominated by organic N (Inselsbacher & Näsholm, 2012) does not simply alleviate N limitation but promotes a fundamental shift in the way trees acquire N.

Acknowledgements

We are grateful for the skilled work of Jonas Lundholm and Jenny Ekman at the SLU Stable Isotopes Laboraory (SSIL). J. Lundholm developed the protocols for ^{2}H analysis of tree ring samples and ran the analysis, and J. Ekman handled the ^{15}N analyses. We would also like to extend our appreciation to the anonymous reviewers for their rigorous and constructive input toward publishing this work. The current study was financed by research grants from the Knut and Alice Wallenberg foundation (nos. 2015.0047 and 2018.0259) and the experiment is part of the SITES (Swedish Infrastructure for Ecosystem Science) project. We acknowledge the staff at the Vindeln Experimental Forest for assisting with fieldwork. HL is partly sponsored by Formas (no. 2020-02319).

Author contributions

The experiment was designed and planned by NH, HL, JM, TN and TL. The field work and sample collection were performed by NH and HL, with help from RL. Data were analysed and the manuscript drafted by NH with assistance from HL, JM, OF, RE McM, RL, RM, TL and TN. Statistical models were developed by OF, and physiological models were provided by RE McM. All authors contributed to interpretation of the analysed data and to the final version of the manuscript.

ORCID

Oskar Franklin https://orcid.org/0000-0002-0376-4140
Nils Henriksson https://orcid.org/0000-0003-1088-9192
Hyungwoo Lim https://orcid.org/0000-0001-9457-7203
Tomas Lundmark https://orcid.org/0000-0003-2271-3469
Reimo Lutter https://orcid.org/0000-0001-5847-4282

Ruth Magh https://orcid.org/0000-0002-4695-0891
John Marshall https://orcid.org/0000-0002-3841-8942
Ross E. McMurtrie https://orcid.org/0000-0002-3140-1064
Torgny Näsholm https://orcid.org/0000-0002-2275-2030

References

Albaugh TJ. 1998. Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions. *Forest Science* **44**: 217–328.

Barber SA. 1995. *Soil nutrient bioavailability – a mechanistic approach.* New York, NY, USA: John Wiley & Sons.

Barber SA, Cushman JH. 1981. Nutrient uptake model for agronomic crops. In: Ilskander IK, ed. *Modelling wastewater renovation land treatment.* New York, NY, USA: John WIley & Sons, 382–409.

BassiriRad H, Gutchick V, Sehtiya H. 2008. Control of plant nitrogen uptake in native ecosystems by rhizopheric processes. In: Bruuslema T, Ma L, Ahuja L, eds. *Quantifying and understanding plant nitrogen uptake for systems modeling.* Boca Raton, FL, USA: CRC Press, 71–93.

Bishop DM. 1962. Lodgepole pine rooting habits in the blue mountains of northeastern Oregon. *Ecology* **43**: 140–142.

Cheesman AW, Cernusak LA. 2017. Infidelity in the outback: climate signal recorded in $\Delta^{18}O$ of leaf but not branch cellulose of eucalypts across an Australian aridity gradient. *Tree Physiology* **37**: 554–564.

Cramer MD, Hoffmann V, Verboom GA. 2008. Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist* **179**: 1048–1057.

Ferrill MD, Woods FW. 1966. Root extension in a longleaf pine plantation. *Ecology* **47**: 97–102.

Göttlicher SG, Taylor AFS, Grip H, Betson NR, Valinger E, Högberg MN, Högberg P. 2008. The lateral spread of tree root systems in boreal forests: estimates based on ^{15}N uptake and distribution of sporocarps of ectomycorrhizal fungi. *Forest Ecology and Management* **255**: 75–81.

Hasselquist NJ, Metcalfe DB, Inselsbacher E, Stangl Z, Oren R, Näsholm T, Högberg P. 2015. Greater carbon allocation to mycorrhizal fungi reduces tree nitrogen uptake in a boreal forest. *Ecology* 1012–1022.

Henriksson N, Franklin O, Tarvainen L, Marshall J, Lundberg-Felten J, Eilertsen L, Näsholm T. 2021. The mycorrhizal tragedy of the commons. *Ecology Letters* **24**: 1215–1224.

Inselsbacher E, Näsholm T. 2012. The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. *New Phytologist* **195**: 329–334.

Iversen CM, McCormack ML, Powell AS, Blackwood CB, Freschet GT, Kattge J, Roumet C, Stover DB, Soudzilovskaia NA, Valverde-Barrantes OJ *et al.* 2017. A global Fine-Root Ecology Database to address below-ground challenges in plant ecology. *New Phytologist* **215**: 15–26.

Kalliokoski T, Makinen H, Jyske T, Nojd P, Linder S. 2013. Effects of nutrient optimization on intra-annual wood formation in Norway spruce. *Tree Physiology* **33**: 1145–1155.

Kiba T, Krapp A. 2016. Plant nitrogen acquisition under low availability: regulation of uptake and root architecture. *Plant and Cell Physiology* **57**: 707–714.

Kulmatiski A, Adler PB, Stark JM, Tredennick AT. 2017. Water and nitrogen uptake are better associated with resource availability than root biomass. *Ecosphere* **8**: 1–10.

Lambers H, Chapin FS III, Pons T. 1998. *Plant physiological ecology.* New York, NY, USA: Springer.

Leadley PW, Reynolds JF, Chapin FS. 1997. A model of nitrogen uptake by *Eriophorum vaginatum* roots in the field: ecological implications. *Ecological Monographs* **67**: 22.

Lehmann MM, Vitali V, Schuler P, Leuenberger M, Saurer M. 2021. More than climate: hydrogen isotope ratios in tree rings as novel plant physiological indicator for stress conditions. *Dendrochronologia* **65**: 125788.

Lim H, Oren R, Palmroth S, Tor-ngern P, Mörling T, Näsholm T, Lundmark T, Helmisaari H-S, Leppälammi-Kujansuu J, Linder S. 2015. Inter-annual variability of precipitation constrains the production response of boreal *Pinus sylvestris* to nitrogen fertilization. *Forest Ecology and Management* **348**: 31–45.

Makinen H, Hynynen J. 2014. Wood density and tracheid properties of Scots pine: responses to repeated fertilization and timing of the first commercial thinning. *Forestry* **87**: 437–448.

Matimati I, Verboom GA, Cramer MD. 2014. Nitrogen regulation of transpiration controls mass-flow acquisition of nutrients. *Journal of Experimental Botany* **65**: 159–168.

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari H-S, Hobbie EA, Iversen CM, Jackson RB *et al.* 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist* **207**: 505–518.

McCormack ML, Guo D, Iversen CM, Chen W, Eissenstat DM, Fernandez CW, Li Le, Ma C, Ma Z, Poorter H *et al.* 2017. Building a better foundation: improving root-trait measurements to understand and model plant and ecosystem processes. *New Phytologist* **215**: 27–37.

McMurtrie RE, Näsholm T. 2018. Quantifying the contribution of mass flow to nitrogen acquisition by an individual plant root. *New Phytologist* **218**: 119–130.

Näsholm T, Högberg P, Franklin O, Metcalfe D, Keel SG, Campbell C, Hurry V, Linder S, Högberg MN. 2013. Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? *New Phytologist* **198**: 214–221.

Nye PH, Marriott FHC. 1969. A theoretical study of the distribution of substances around roots resulting from simultaneous diffusion and mass flow. *Plant and Soil* **30**: 459–472.

Oyewole OA, Inselsbacher E, Näsholm T. 2014. Direct estimation of mass flow and diffusion of nitrogen compounds in solution and soil. *New Phytologist* **201**: 1056–1064.

Oyewole OA, Jämtgård S, Gruffman L, Inselsbacher E, Näsholm T. 2016. Soil diffusive fluxes constitute the bottleneck to tree nitrogen nutrition in a Scots pine forest. *Plant and Soil* **399**: 109–120.

Roden JS, Ehleringer JR. 1999. Observations of hydrogen and oxygen isotopes in leaf water confirm the Craig-Gordon model under wide-ranging environmental conditions. *Plant Physiology* **120**: 1165–1174.

Samuelson LJ, Farris MG, Stokes TA, Coleman MD. 2008. Fertilization but not irrigation influences hydraulic traits in plantation-grown loblolly pine. *Forest Ecology and Management* **255**: 3331–3339.

Sternberg L da SL, Moreira MZ, Nepstad DC. 2002. Uptake of water by lateral roots of small trees in an Amazonian tropical forest. *Plant and Soil* **238**: 151–158.

Taskinen O, Ilvesniemi H, Kuuluvainen T, Leinonen K. 2003. Response of fine roots to an experimental gap in a boreal *Picea abies* forest. *Plant and Soil* **255**: 503–512.

Tinker PB, Nye PH. 2000. *Solute movement in the rhizosphere.* New York, NY, USA: Oxford University Press.

Wang G, Liu F, Xue S. 2017. Nitrogen addition enhanced water uptake by affecting fine root morphology and coarse root anatomy of Chinese pine seedlings. *Plant and Soil* **418**: 177–189.

Williams M, Yanai RD. 1996. Multi-dimensional sensitivity analysis and ecological implications of a nutrient uptake model. *Plant and Soil* **180**: 311–324.

Yanai RD. 1994. A steady-state model of nutrient uptake accounting for newly grown roots. *Soil Science Society of America Journal* **58**: 1562–1571.

Zaehle S, Medlyn BE, De Kauwe MG, Walker AP, Dietze MC, Hickler T, Luo Y, Wang Y, El-Masri B, Thornton P *et al.* 2014. Evaluation of 11 terrestrial carbon–nitrogen cycle models against observations from two temperate Free-Air CO_2 Enrichment studies. *New Phytologist* **202**: 803–822.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Isotopic enrichment of needles and stemwood from *Pinus sylvestris* trees growing at varying distances from the label application point.

Fig. S1 Lateral uptake profiles for each isotopic label in the two *Pinus sylvestris* stands.

Fig. S2 Proportion of *Pinus sylvestris* trees labelled at varying distance from the label source

Methods S1 Scaling of isotope concentration data to whole tree uptake, based on allometric functions.

Table S1 Allometric equations for each tree biomass component.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.