Global Change Biology

Global Change Biology (2010), doi: 10.1111/j.1365-2486.2010.02362.x

The legacy of enhanced N and S deposition as revealed by the combined analysis of δ^{13}C, δ^{18}O and δ^{15}N in tree rings

R. GUERRIERI*†, M. MENCUCCINI*, L. J. SHEPPARD‡, M. SAURER§, M.P. PERKS¶, P. LEVY‡, M. A. SUTTON‡, M. BORGHETTI† and J. GRACE*

School of GeoSciences, University of Edinburgh, Crew Building, West Mains Road, EH9 3JN Edinburgh, UK, ‡Centre of Ecology and Hydrology, CEH, Edinburgh, Bush Estate, Penicuik EH26 OQB Midlothian Scotland, UK, §Laboratory of Atmospheric Chemistry, Paul Scherrer Institute, CH-5232, Villigen PSI, Switzerland, ¶Forest Research, Centre for Forestry and Climate Change, NRS, Bush Estate, Roslin, Midlothian EH25 9SY, UK, †Department of Crop Systems, Forestry and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano 10, I-85100 Potenza, Italy

Abstract

This study aimed to evaluate the effects of long-term repeated aerial nitrogen (N) and sulphur (S) misting over tree canopies of a Sitka spruce plantation in Scotland. We combined δ^{13}C and δ^{18}O in tree rings to evaluate the changes in CO_2 assimilation (A) and stomatal conductance (g_s) and to assess their contribution to variations in the intrinsic water-use efficiency (WUE_i, i.e., the A/g_s ratio). Measurements of δ^{15}N enabled shifts in the ecosystem N cycling following misting to be assessed. We found that: (i) N applications, with or without S, increased the ratio between A and g_s in favour of A, thus supporting a fertilizer effect of added N. (ii) After the treatments ceased, the trees quickly adjusted to the reductions of N deposition, but not to the reduction in S deposition, which had a negative effect on WUE_i by reducing A. This indicates that the beneficial role of N deposition may be negated in forests that previously received a high load of acid rain. (iii) δ^{15}N in tree rings reflected the N dynamics caused by canopy retention, with the fingerprint also present in the litter, after the experiment stopped. (iv) Both our results (obtained using canopy applications) and a collection of published data (obtained using soil applications) showed that generally WUE_i increased in response to an increase of N applications, with the magnitude of the changes related to soil conditions and the availability of other nutrients. The shifts observed in δ^{15}N in tree rings also suggest that both the quantity of the applied N and its quality, mediated by processes occurring during canopy N retention, are important determinants of the interactions between N and C cycles. Stable isotopes are useful probes to understand these processes and to put the results of short-term experiments into context.

Keywords: acid mist, annual tree rings, CO_2 assimilation, nitrogen, Sitka spruce, stable C–O–N isotopes, stomatal conductance, sulphur, WUE_i

Received 30 July 2010 accepted 20 September 2010

Introduction

The extent to which forests mitigate climate change is influenced by the increase in nitrogen (N) deposition (N_{dep}) from the atmosphere, due to anthropogenic activities. Together with elevated atmospheric CO_2, N_{dep} has been recognized as one of the main drivers enhancing the terrestrial carbon 'sink' (Norby, 1998; Gruber & Galloway, 2008). However, the carbon (C) and N cycles interact in very complex ways, with mechanisms that can be grouped under two distinct scenarios. A beneficial role of N_{dep} has been hypothesized for N-limited systems (e.g., temperate forests), as N_{dep} can act as a low-level but sustained input of fertilizer, potentially increasing tree growth and C uptake (Högberg, 2007; Magnani *et al.*, 2007; Sutton *et al.*, 2008; Thomas *et al.*, 2009). However, high levels of N_{dep} may result in soil acidification (Vitousek *et al.*, 1997; Galloway *et al.*, 2002), increased tree mortality (Magill *et al.*, 2004) and declines in biodiversity (Phoenix *et al.*, 2006).

The extent of the enhancement in C uptake by N-limited forests that can be attributed to N_{dep} has been hotly debated, with variable estimates provided (Magnani *et al.*, 2008), which suggests that a deeper investigation of the crucial underlying mechanisms may be useful. One of the main issues relates to understanding whether the interactions between N_{dep} and tree canopies play an important role in linking the N and the C cycles. Most of our knowledge regarding the

Correspondence: Present address: R. Guerrieri, School of GeoSciences, University of Edinburgh, Crew Building, The King's Buildings, West Mains Road, Edinburgh EH9 3JN, UK. tel. +44 131 650 5096, fax +44 131 662 0478, e-mail: Rossella.Guerrieri@ed.ac.uk

effects of N_{dep} on forests relies upon experiments carried out in managed fertilized forests, where the additional N was supplied as fertilizer to the soil for 1–2 years (Elhani *et al.*, 2005; Brooks & Coulombe, 2009) or for more than 10 years (Magill *et al.*, 2004; Nadelhoffer *et al.*, 2004; Högberg *et al.*, 2006; Betson *et al.*, 2007; Feng *et al.*, 2008). The relevance of such investigations, which exclude the opportunity for canopy exchange and processing of N on the leaf surface, is debatable as it is well-known that canopy uptake of several ions occurs (Rennenberg & Gessler, 1999; Cape *et al.*, 2001; Gaige *et al.*, 2007; Sievering *et al.*, 2007). This uptake provides an important source of N readily available for plant metabolism (Sparks, 2009), whereas N applied to the soil may be rapidly incorporated into microbial biomass instead. Recently, Dezi *et al.* (2009) showed that inclusion of canopy uptake in an ecosystem level model significantly increased ecosystem C storage capacity.

Another relevant point is that deposition of N onto forest canopies occurs in combination with other pollutants, e.g., sulphur (S), which may negate the potential beneficial effects of N_{dep} and accelerate tree decline. Even though S emissions have been significantly reduced in Europe over recent years (ICP, 2009), Fowler *et al.* (2007) observed a nonlinearity in the relationship between emission and deposition changes, showing that S_{dep} in remote areas has not decreased at the same rate of areas closer to S sources.

Only a few experiments have examined the contribution of atmospheric N, by spraying or misting above tree canopies: the experiment at Deepsyke forest, in the United Kingdom (cf. Sheppard *et al.*, 2004, 2008) and at Howland Forest, in the USA (cf., Gaige *et al.*, 2007; Dail *et al.*, 2009). Between the two experimental sites, the Deepsyke experiment is unique in providing the opportunity to evaluate the effects of frequent (>50 applications during the growing season) aerial N and S misting onto the canopy of a Sitka spruce forest for a relatively long period (i.e., 5–8 years). At this experimental site, N treatments significantly increased tree stem area increment, irrespective of whether N was added with or without S (Sheppard *et al.*, 2004).

This study aimed to investigate how experimental applications of N_{dep} and S_{dep} affected the ratio between canopy CO_2 assimilation (A) and stomatal conductance (g_s), known as intrinsic water-use efficiency (WUE_i), for the Sitka spruce stand at Deepsyke. Sitka spruce is one of the most widely planted tree species in the United Kingdom, performing well on in nutrient-poor soils. This species is tolerant of low-N availability, and at the same time very responsive to small N additions (Chandler & Dale, 1993), so that a beneficial effect of N_{dep} on WUE_i, achieved through an increase in A, can be hypothesized.

Our approach combined the analysis of the stable isotopes of carbon ($\delta^{13}C$), oxygen ($\delta^{18}O$) and N ($\delta^{15}N$) in annual tree rings. Tree rings are very useful archives of information concerning changes in tree growth in response to environmental conditions. The combination of all three isotopes $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ in tree rings can provide more specific information on the underlying ecophysiological processes. The effect of different treatments on WUE_i, as assessed through $\delta^{13}C$ in tree rings, was investigated. Further, the combination of $\delta^{13}C$ and $\delta^{18}O$ allowed us to suggest which physiological traits, i.e., A and/or g_s, respectively, contributed most to the variations in WUE_i. Finally, $\delta^{15}N$ provided indications of changes in ecosystem N cycling in response to the different treatments.

We addressed the following main questions: (i) Do canopy N additions increase the balance between A and g_s (i.e., WUE_i) in favour of A, and does the addition of S counteract the effect of N? (ii) Does $\delta^{15}N$ in tree rings carry a signal of changes in the N cycle within the ecosystem in response to canopy N addition? (iii) When the experimental applications are terminated, how fast do trees adjust to a reduction in N and S deposition? (iv) Are the effects on tree physiological traits and N dynamics caused by canopy addition different from those predicted by studies that have used direct application of N to the soil?

Materials and methods

Stable isotope theory

The relative abundance (R) of the stable isotopes, i.e., $^{13}C/^{12}C$, $^{18}O/^{16}O$, $^{15}N/^{14}N$, in plant organic matter is expressed as the relative deviation from the international standards (V-PDB for $\delta^{13}C$, V-SMOW for $\delta^{18}O$ and atmospheric N_2 for $\delta^{15}N$), using delta (δ) notation, i.e., $\delta_{sample} = \left(\frac{R_{sample}}{R_{standard}} - 1\right)1000$.

$\delta^{13}C$ and its link to WUE_i

Plant material is generally depleted in the heavier isotope (^{13}C) compared with atmospheric CO_2, because of isotope fractionations taking place during the biochemical and diffusional processes leading to photosynthesis. Indeed, carbon isotope composition in tree rings ($\delta^{13}C$) reflects variation of the ratio between CO_2 concentration in leaf intercellular spaces and the atmosphere (i.e., c_i/c_a ratio) related either to changes of CO_2 assimilation (A) or stomatal conductance (g_s). According to the simplified version of the Farquhar equation, $\delta^{13}C$ in plant material ($\delta^{13}C_p$) is described as follows (Farquhar *et al.*, 1989):

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a)\frac{c_i}{c_a}, \quad (1)$$

where $\delta^{13}C_a$ is the isotopic signature of atmospheric CO_2, a is the fractionation for $^{13}CO_2$ as a result of diffusion through air (4.4‰), b is the fractionation during carboxylation (27‰) by the

CO_2-fixing enzyme Rubisco. Owing to their reciprocal link to the c_i/c_a ratio, $\delta^{13}C$ can be considered to infer changes in the WUE_i, which is defined as the ratio between A and stomatal conductance to water vapour (g_{H_2O}), according to Ehleringer *et al*. (1993):

$$WUE_i = \frac{A}{g_{H_2O}} = \frac{(c_a - c_i)}{1.6}. \quad (2)$$

From Eq. (1) c_i can be derived as follows:

$$c_i = c_a \frac{\delta^{13}C_a - \delta^{13}C_p - a}{b - a}, \quad (3)$$

$\delta^{13}C_a$ and c_a for each year can be estimated according to the equations proposed by Feng (1999):

$$\delta^{13}C_a = -6.429 - 0.0060 \exp[0.0217(t - 1740)], \quad (4)$$

$$c_a = 277.78 + 1.350 \exp[0.01572(t - 1740)], \quad (5)$$

where t is the calendar year.

Finally, Eq. (2) can be solved as:

$$WUE_i = \frac{c_a}{1.6} \frac{b - \delta^{13}C_a + \delta^{13}C_p}{b - a}. \quad (6)$$

Hence, $\delta^{13}C$ in tree rings allows changes in the A/g_s ratio to be assessed taking into account the long-term variation of atmospheric CO_2 concentration and its isotopic signature. Although postphotosynthetic fractionations (Cernusak *et al*., 2009) may partially reduce the strength by which the primary isotope signal imprinted in leaf organic matter is recorded in heterotrophic tissues, $\delta^{13}C$ in tree rings still contains long-term information on changes of leaf physiological processes (Saurer *et al*., 2004; Gessler *et al*., 2009).

Link between $\delta^{18}O$ in tree rings and stomatal conductance

Variation in $\delta^{13}C$ can be related to changes in either A or g_s whereas $\delta^{18}O$ in organic material is specifically related to changes of $\delta^{18}O$ in leaf water in response to transpiration. Hence, when $\delta^{18}O$ of source water and atmospheric water vapour, does not vary among study sites, $\delta^{18}O$ in plant organic matter records changes in g_s. In fact, during transpiration molecules of water containing the lighter isotopes, $H_2^{16}O$, tend to diffuse faster from the sites of evaporation to the atmosphere than $H_2^{18}O$, so that water at the evaporative site becomes more enriched in the heavier isotopes ^{18}O compared with water in the transpiration stream i.e., coming from the soil (source of water). Variations in the oxygen isotope signature at the evaporative site (i.e., $\delta^{18}O_e$) are linked to changes in the ratio between water vapour in the atmosphere and in the intercellular spaces ($e_a/e_i \approx$ Relative humidity, Rh), according to Craig & Gordon (1965):

$$\delta^{18}O_e = \delta^{18}O_s + \varepsilon^* + \varepsilon_k + (\delta^{18}O_v - \delta^{18}O_s - \varepsilon_k)\frac{e_a}{e_i} \quad (7)$$

where $\delta^{18}O_s$ and $\delta^{18}O_v$ are the oxygen isotopic compositions of soil water and atmospheric water vapour, respectively, ε_k is the kinetic fractionation during evaporative water diffusion through stomata and boundary layers, ε^* is the fractionation associated with the lower vapour pressure of $H_2^{18}O$ compared with that of $H_2^{16}O$. However, at increasing levels of transpiration, the primary ^{18}O enrichment at the evaporative site is modified by the Péclet effect (Farquhar & Lloyd, 1993; Barbour *et al*., 2000a), which describes the mixing of enriched water at the evaporative sites with unenriched water pools within leaves. The signal of $\delta^{18}O$ in the bulk leaf water is then imprinted in the leaf organic molecules, due to the isotopic exchange between carbonyl oxygen and water in which they are formed (Sternberg *et al*., 1986).

Several authors have reported a negative relationship between $\delta^{18}O$ in leaf organic matter and g_s, due to their reciprocal link to vapour pressure deficit, which, in turn depends on Rh (Barbour *et al*., 2000b; Grams *et al*., 2007; Sullivan & Welker, 2007; Ripullone *et al*., 2009). The signal of changes in the $\delta^{18}O$ of leaves may also be dampened in tree rings, due to isotope fractionation during translocation of sucrose and synthesis of cellulose in the tree stem (Sternberg *et al*., 1986; Saurer *et al*., 1997; Gessler *et al*., 2009). However, $\delta^{18}O$ in tree rings still carries information related to changes in g_s (McCarroll & Loader, 2004; Barbour, 2007).

Combining $\delta^{13}C$ and $\delta^{18}O$ in a conceptual model

Scheidegger *et al*. (2000) proposed a conceptual model, which helps to clarify whether changes in A, g_s or both accounted for the variations of WUE_i. The model inferred changes in c_i and therefore A from $\delta^{13}C$, assuming a negative relationship between the two parameters, according to Eq. (1). Further, changes in Rh were derived from $\delta^{18}O$ in tree organic matter, which, in turn, allows variations in g_s to be predicted. As a further extension of the model, Grams *et al*. (2007) showed that g_s can be inferred directly from $\delta^{18}O$ in leaf cellulose, which was confirmed also by comparing stable isotopes with gas exchange measurements. In both approaches, an important prerequisite is similar $\delta^{18}O$ of source water and vapour water for the investigated trees.

$\delta^{15}N$ and ecosystem N cycle

The theoretical understanding of $\delta^{15}N$ in tree pools is not as comprehensive as for $\delta^{13}C$ and $\delta^{18}O$. This is due to the complexity of the N cycle and the possibility for plants to use different forms of N, i.e., ammonium (NH_4^+), nitrate (NO_3^-) and dissolved organic N. Overall, the $\delta^{15}N$ in tree pools reflects the isotopic signature of the main N sources (soil and atmosphere) and isotope fractionations occurring during N uptake by roots and foliage, and assimilation within plant compartments (Robinson *et al*., 1998; Comstock, 2001). On the other hand, $\delta^{15}N$ in the soil reflects the relative contribution of different N compounds and is strongly affected by fractionations during biogeochemical processes. Indeed, nitrification produces ^{15}N-depleted NO_3^-, leaving ^{15}N-enriched NH_4^+ in the soil. Ammonia volatilization can also increase the ^{15}N enrichment of the NH_4^+ remaining in the soil and therefore, available for plants, whereas atmospheric deposition of NH_3 may correspondingly supply ^{15}N-depleted N (Tozer *et al*., 2005). NO_3^- can also be lost via leaching and denitrification,

leaving ^{15}N-enriched NO_3^- in the soil (Nadelhoffer & Fry, 1994; Högberg, 1997). Mineralization have been reported to reduce the δ^{15}N of the product NH_4^+ (Létolle, 1980), although isotopic fractionation associated with mineralization of the large pool of soil organic matter is slight relative to the other processes occurring in the soil (Högberg, 1997).

Atmospheric N can be a significant source of N for trees. Before reaching the soil, atmospheric N (e.g., NO_x and NH_x forms) interacts with tree canopies in such a way that a consistent part can be retained and taken up by canopies, leading to a δ^{15}N signal which depends on the relative contribution of the exchanged N compounds (NH_4^+ vs. NO_3^-) (Heaton *et al.*, 1997). As a consequence, the amount of atmospheric N reaching the soil as wet and dry deposition and its isotope signature are modified by the interaction with forest canopies.

Overall, it can be hypothesized that, at very low levels of N_{dep}, when mineralization is the chief source of available N, and provided canopies have also reached full closure, δ^{15}N in tree pools should be relatively stable and most closely related to soil δ^{15}N with little long-term variation. By contrast, when an increase in available N occurs, e.g., an increase of N_{dep} from the atmosphere or from fertilization, variations of δ^{15}N in tree pools should reflect: (i) the contribution of the added N, according to its δ^{15}N and (ii) the variations in soil δ^{15}N because of changes in the degree of closure of the N cycle (i.e., losses caused by NO_3^- leaching, nitrification, denitrification and NH_3 volatilization), the prevailing effect of which is to enrich the δ^{15}N signal of tree pools.

Experimental site

The study was carried out in a *Picea sitchensis* (Bong.) Carr. plantation at Deepsyke (290 m asl, latitude 55°46′N, longitude 3°18′W) in the Scottish Borders (Scotland, UK), with a low total (i.e. dry + wet) ambient N_{dep} (8–10 kg N ha^{-1} yr^{-1}). A mixture of provenances (identities unknown) was planted in 1986 at 2 m spacing on drained deep peat soil, on mounds formed from the inversion of the organic horizon removed to form the drainage ditches (Sheppard *et al.*, 1999).

Within the forest stand, an area of 1.5 ha was divided into four replicated blocks, each containing six different spraying treatments: N, S, NS (as a single dose), 2NS (as a double dose of both N and S), wet control (e.g., only water), no treatment (e.g., no spraying). Treatments increased the amount of precipitation by 20% (for N, NS and wet control) or 40% (for 2NS). In this study, we considered only N, NS, 2NS and wet control (Table 1, Fig. 1a and b). Acid mist was applied approximately twice weekly during the growing season (>50 applications from April until November) from 1996 to 2000, with four replicates for each treatment. In a second phase (2001–2003), two plots (selected at random, irrespective of the block) were maintained on the original treatment, whereas in the other two S or N addition of the original treatment was removed in order to simulate the recovery from reduced S and N (Table 1) (cf. Sheppard *et al.*, 2004, 2008).

Within each plot, five of the 10 trees were selected for sampling wood cores (two per tree) in June 2009, using a 0.5 cm diameter increment borer. Within each plot, we calculated for each of the 10 trees the diameter variation relative to the mean diameter in the wet control. The five trees for wood cores sampling were selected after excluding trees showing extreme growth rates (exceptionally low or high), which could have confused the detection of the treatment effect. The undecomposed litter and the organic soil (0–10 cm) were sampled from five locations in the furrows along the planting lines.

Sample preparation and isotope analyses

Before isotopic measurements, wood cores were subjected to a chemical pretreatment in order to remove N mobile compounds, which may affect the detection of the δ^{15}N signal. For this purpose, we used the rapid procedure proposed by Sheppard & Thompson (2000): 4 h in a mixture of toluene: ethanol; 4 h in ethanol; 4 h in distilled water. After drying, each wood core was dated and thereafter annual rings were identified and separated from bark to pith under a stereoscope. Along a chronology of 15 years (1995–2009), annual rings were split in to the following sections: (I) the year 1995 (before the treatments); (II) the single annual rings from 1996 to 2003 (during the treatments); (III) the group of annual rings from 2004 to 2009 (after the treatments, i.e., all six rings together with no annual separation). Within a plot, all the samples from the five trees were ground with a centrifugal mill and pooled by year, in order to average the isotopic signal on individual trees.

Soil and litter samples were dried in the oven for 12 h at 105 °C. After removing traces of fine root and litter, soil samples were ground with a ceramic mortar. For litter samples, a centrifugal mill was used to produce a fine powder for the analysis.

For δ^{13}C, δ^{15}N and δ^{18}O measurements, 0.6–0.8, 15–20, and 0.5–0.7 mg of wood powder, respectively, were weighed in tin capsules (silver capsules for oxygen). An amount of 4–5 mg of soil and litter samples was weighed into tin capsules. Further, 4–5 mg of the ammonium nitrate (NH_4NO_3) used for the spraying and stored from 1996 was weighed into tin capsules ($n = 6$) for the δ^{15}N analyses. We measured a δ^{15}N value of 1.6 ± 0.42‰.

For δ^{13}C and δ^{15}N analyses, each determined in a separate measurement, samples were combusted under an excess of oxygen with an elemental analyzer (EA-1100, Carlo Erba, Milano, Italy), connected to an isotope ratio mass spectrometer (Delta-S Finnigan MAT, Bremen, Germany) through a variable open split interface (ConFlo II, Finnigan MAT). For δ^{18}O, the weighed bulk organic material was decomposed to CO by thermal pyrolysis at 1080 °C in an elemental analyzer (EA-1108, Carlo Erba), which was connected to the continuous flow mass spectrometer (Delta-S Finnigan MAT).

Ground bulk samples obtained from 1-year-old needles collected in December 1997 and used in a previous investigation of nutrient content in needles (e.g., Sheppard *et al.*, 2004) were used for determinations of δ^{13}C and δ^{15}N. An amount of 3–4 mg of needle samples was weighed into tin capsules for combustion in the elemental analyzer (NA2500, Carlo Erba) and for determination of both δ^{13}C and δ^{15}N by VG Prism III Isotope ratio mass spectrometer (Fisons, VG, Micromass, Manchester, UK).

Table 1 Treatments of acid mist applied on a young Sitka spruce plantation at Deepsyke forest (Scotland, UK) from 1996 to 2003

Treatments	Number of plots	Components	Doses ($kg\,ha^{-1}\,yr^{-1}$) N	S	pH
I phase (1996–2000)					
NS acid	4	$NH_4NO_3 + H_2SO_4$	48	50	2.5
2NS acid	4	$NH_4NO_3 + H_2SO_4$	96	100	2.5
N	4	NH_4NO_3	48	–	4.5
Wet control	4	Rain water	–	–	4.5
II phase (2001–2003)					
NS acid	2	$NH_4NO_3 + H_2SO_4$	48	50	2.5
NS-recovery (N)	2	NH_4NO_3	48		4.5
2NS acid	2	$NH_4NO_3 + H_2SO_4$	96	100	2.5
2NS-recovery (2N)	2	NH_4NO_3	96		4.5
N	2	NH_4NO_3	48	–	4.5
N-recovery (wet control)	2	Rain water	–	–	4.5
Wet control	4	Rain water	–	–	4.5

In the first phase of the experiment (1996–2000), all treatments had four replicates, whereas in the second phase (2001–2003), two plots were maintained with the original treatments, whereas in the other two the recovery from acidity was started. Adapted from Sheppard *et al*. (2004).

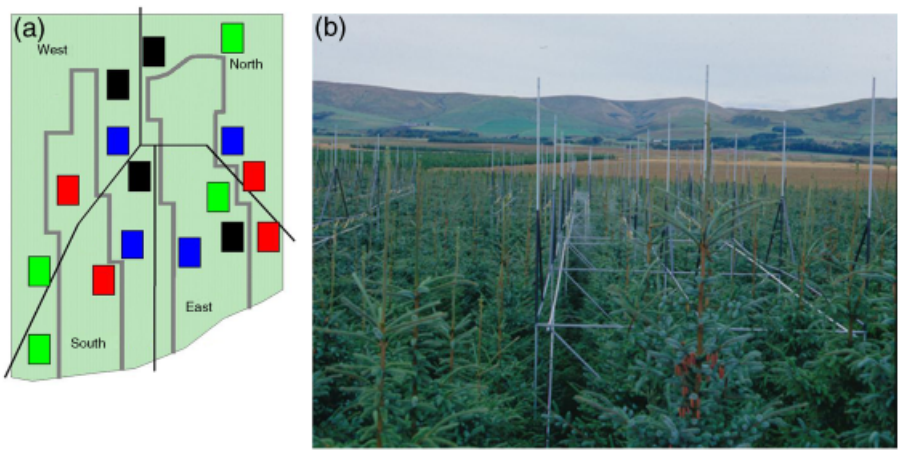

Fig. 1 Distribution of replicates for each treatment (a), modified from Chiwa *et al*. (2004). Picture of the experimental site with spraying system set up (b). The different colors visible in (a) refer to plots belonging to different treatments: black, 2NS; red, NS; green, N and blue, Wet control. Black lines delineate the four replicated blocks designated as South, West, North and East. Picture in (b) was provided by Dr Alan Crossley (CEH).

The isotope values were expressed in the δ-notation (in per mil; ‰) as relative deviation from the international standard (atmospheric N_2 for $\delta^{15}N$, V-PDB for $\delta^{13}C$ and V-SMOW for $\delta^{18}O$).

Meta-analysis

Among all the studies in the literature reporting on tree growth responses to increased N_{dep}, mainly simulated as ground fertilization, we selected those experiments that satisfied the following criteria: (i) were carried out on tree stands; (ii) did not involve a ^{15}N-labelling experiment; (iii) were conducted in the field; (iv) measured $\delta^{15}N$ in tree rings and $\delta^{13}C$ in either needles or tree rings. These studies are summarized in Table 2. Most of the sites considered in our meta-analysis were located in areas with low-level of N_{dep}. Elhani *et al*. (2005) reported values ranging between 9 and 13 $kg\,ha^{-1}\,yr^{-1}$, for the years after the fertilization experiment. The $\delta^{15}N$ signal of the mineral N-fertilizer supplied during the

Table 2 Studies included in the meta-analysis

Authors	Location	N_{dep} (kg ha^{-1} yr^{-1})	Species	Age (years)	Fertilizer	Application	Dose applied (kg ha^{-1} yr^{-1})	Duration of the experiment	Isotope
Balster *et al.* (2009)	24 sites across the Interior NW, USA	NA	*Pseudotsuga menziesii*	85	Urea	Soil	178 357	Dose applied twice over a 6-year interval	δ^{13}C and δ^{15}N in tree rings
Betson *et al.* (2007)	Norrliden, Sweden	3	*Pinus sylvestris*	~50	Ammonium nitrate	Soil	30 60 90	32 years: dose applied yearly from 1971 to 2002	δ^{13}C in needles
Brooks & Coulombe (2009)	Washington, USA	NA	*Pseudotsuga menziesii*	85	Ammonium nitrate	Soil	157 314 471	1 year: dose applied only once, in 1964	δ^{13}C, WUEi in tree rings
Choi *et al.* (2005)	Scotland County, NC, USA	NA	*Pinus taeda*	15	Urea	Soil	101	7 years: dose applied every year from 1992 to 1998	δ^{13}C in needles
Elhani *et al.* (2005)	Brittany, France	9–13	*Fagus sylvatica*	80	Ammonium nitrate	Soil	100 100with PKCa	2 years: dose applied once (e.g., in 1973 and 1974)	δ^{13}C, δ^{15}N in tree rings
Heaton & Crossley (1995)	Scotland, UK	10	*Picea sitchenisis*	20	Ammonium nitrate	Canopy	48	3 years: dose applied twice per week during the growing season from 1991 to 1993.	δ^{13}C in needles
Nadelhoffer *et al.* (2004)	Massachusetts, USA	8	Deciduous species	~80	Ammonium nitrate	Soil	58 158	16 years: 6 monthly additions during the growing season from 1988 to 2004	δ^{15}N in tree rings
Nadelhoffer *et al.* (2004)	Massachusetts, USA	8	*Pinus resinosa*	~80	Ammonium nitrate	Soil	58 158		

NA, not applicable.

experiments had values close to that of the atmospheric N_2 (i.e., 0‰) (Nadelhoffer *et al.*, 2004; Elhani *et al.*, 2005). We measured a $\delta^{15}N$ value of the ammonium nitrate added during the spraying at Deepsyke and found it to be slightly enriched in ^{15}N, i.e., 1.6‰. Overall, we assume no differences across the sites in the $\delta^{15}N$ of added N as ammonium nitrate either in the soil or to tree canopies.

From each study, data points and corresponding error bars of $\delta^{13}C$ and $\delta^{15}N$ values were extracted using an image analysis programme (UTHSCSA Image Tool). We considered only the years when the experimental treatments were undertaken. For given $\delta^{13}C$ values, WUE_is were calculated, according to Eq. (6). Values are presented as differences between treatments and control, for both WUE_i and $\delta^{15}N$ values, in order to highlight the effect of the treatment and to account for differences in tree age, site and/or climate across studies.

Statistical analyses

Pretreatment (1995) and posttreatment (2004–2009) differences for $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ across plots were tested by ANOVA, considering the assigned treatments as fixed factors. For the pretreatment year (1995), we found no significant effect of 'block' as the random factor, so block was excluded from the subsequent analyses. The effect of different treatments was evaluated by repeated-measures ANOVA for the years 1996–2000, when replication of the experiment was balanced (four replicates for each treatment). Soil moisture was included as a covariate (Sheppard *et al.*, 2004) and with an interaction term with treatments. The year-by-year differences between the treatments and the wet control were investigated by ANOVA, using the least significant difference (LSD) test. Owing to the experimental design not being balanced during the recovery phase (2001–2003), differences between recovery ($n = 2$) and treatment ($n = 2$) for each treatment, were assessed using independent sample *t*-test.

A *t*-test was used for investigating the difference between needles collected in 1997 and the annual ring formed in the same year for $\delta^{13}C$ and $\delta^{15}N$ values, whereas differences among treatments for either $\delta^{13}C$ or $\delta^{15}N$ in needles and annual rings were tested by ANOVA.

All statistical analyses were carried out with SPSS 16.0 statistical package (SPSS, Chicago, IL, USA). Where not differently specified, the level of significance for statistical tests was $\alpha = 0.05$.

Results

Effect of treatments on changes in $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ in tree pools

Before the spray treatments began, $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ in 1995 showed similar values for all plots with different assigned treatments (Fig. 2, Tables 3–5).

After 1 year's treatment, $\delta^{13}C$ and $\delta^{15}N$ measured in wood could be compared with the corresponding values measured in needles formed in the same year (sampled in December 1997). When both values were plotted as differences from the wet control (Fig. 3) a significant increase in $\delta^{15}N$ in needles, but not in annual rings was observed, which seemed to be dose related (Fig. 3a). By contrast, the effect of treatment was not obvious for $\delta^{13}C$ measured in needles, while it was marginally significant ($P < 0.1$) in the case of the corresponding annual ring, with the 2NS treatments showing an increase in $\delta^{13}C$ within 1 year of treatment (Fig. 3b). It is noteworthy that the difference between the needle and annual ring for C and N isotopes has a different direction, significant only for 2NS: for $\delta^{15}N$, more positive values were observed in needles compared with the annual ring, whereas the opposite was true for $\delta^{13}C$.

Over the 5 years of the experiment, $\delta^{13}C$ and $\delta^{15}N$ were most affected by the addition of N, either alone or in combination with S. Although $\delta^{13}C$ showed a decreasing trend over time in all the treatments (Fig. 2, panels a–c), repeated-measures ANOVA from 1996 to 2000 also revealed a significant effect of treatment, with 2NS and N having higher $\delta^{13}C$ values than NS and wet control (Table 3). The tests of between-subject effects showed a nonsignificant effect of the covariate (i.e., the variability in soil moisture among plots) on changes in $\delta^{13}C$ ($F = 1.2$; $P = 0.3$).

The effect of simulated N and S deposition to the tree canopy for $\delta^{18}O$ was not as clear as for $\delta^{13}C$ (Fig. 2 panels d–f). Overall, there was a tendency for $\delta^{18}O$ to decrease for the first few years after the spraying started, with a trough in 1998, which was also the wettest year of the experimental period. Repeated-measures ANOVA did not show any significant treatment effect, even when variations in soil moisture were taken into account as a covariate (Table 4).

Overall, $\delta^{15}N$ values showed a slight tendency to increase over time (Fig. 2g–i), with a higher variability among years for the wet control compared with plots receiving the different treatments. Changes in $\delta^{15}N$ were not affected by treatment, when N was added in combination with S, with trees in NS and 2NS showing similar $\delta^{15}N$ values to the wet control. By contrast, N alone led to a more negative $\delta^{15}N$ values compared with the wet control and the NS treatment (Table 5). The covariate soil moisture was not significant for $\delta^{15}N$ ($F = 0.06$, $P = 0.8$).

The removal of S or N addition from the original treatment (Table 1) did not significantly affect stable isotope trends in the second phase of the experiment, with similar values measured for the recovery and the treatment plots (Tables 3–5). There was a tendency for $\delta^{13}C$ in the wet control to increase, whereas $\delta^{13}C$ tended to decrease in 2NS, NS and N (Fig. 2 a–c). Variation of $\delta^{18}O$ in treated plots followed the same time trend as in

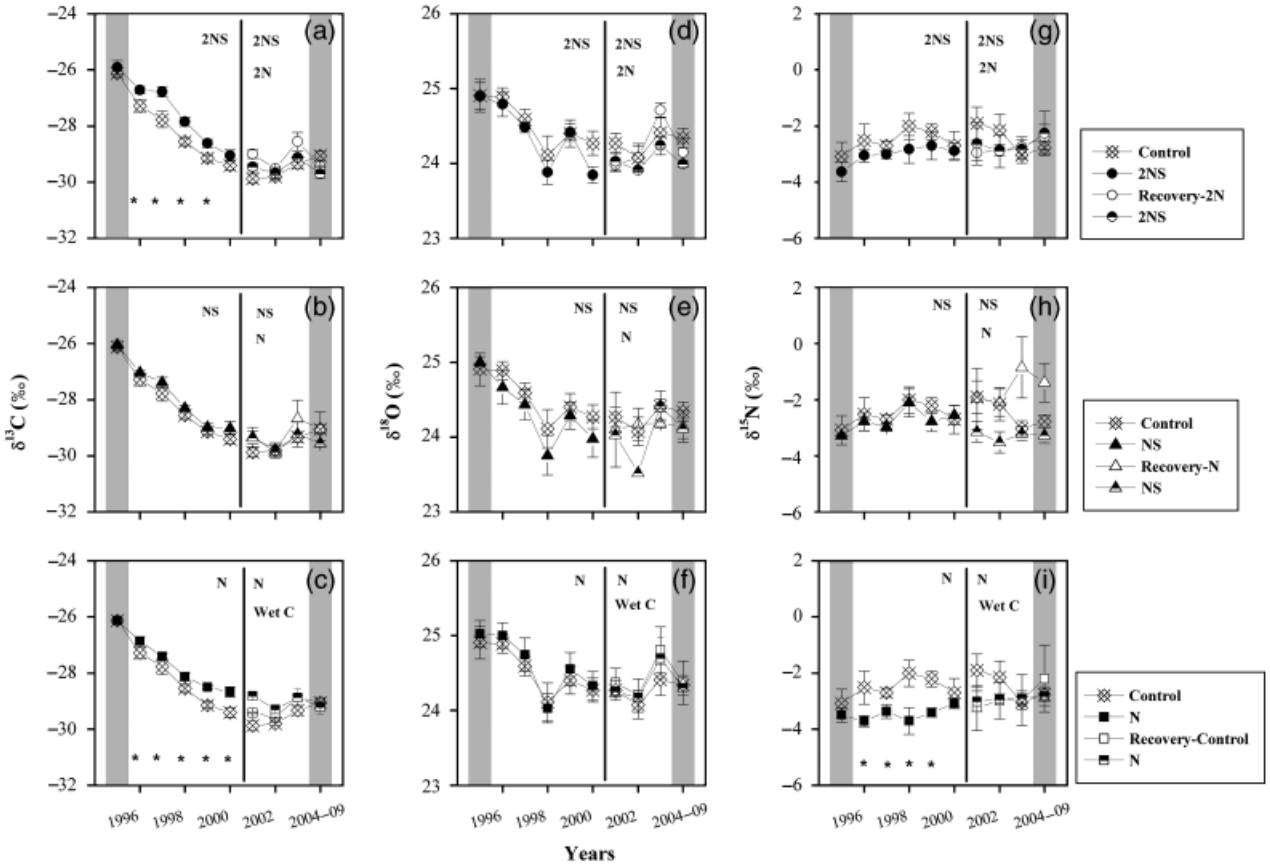

Fig. 2 Trend of δ^{13}C (a–c), δ^{18}O (panels d–f) and δ^{15}N (g–i) in tree rings from 1995 to 2009 for the different treatments (Wet control, NS, 2NS, N) applied at Deepsyke. For the years 1995–2000 each points is the mean ± SE of $n = 4$, whereas for years 2001–2003, means were calculated over $n = 2$ plots for the recovery and $n = 2$ plots for the original treatment. Please note that for δ^{18}O relative to the year 2002, in NS we show only a single value measured in one plot. Shaded areas mark the pretreatment and posttreatment periods and the vertical line, the start of the recovery period. A year-to-year significant (at least $P < 0.1$) effect of the treatment as assessed by least significant difference (LSD) test is indicated by the star. N, nitrogen; S, sulphur.

Table 3 Results of the statistical analyses of the differences between treatments in δ^{13}C for the period 1995, 1996–2000, 2001–2003 and 2004–2009

Treatments	1995 Pretreatment	1996–2000 Treatment I	2001–2003 Treatment II Recovery		2001–2003 Treatment II Treatment	2004–2009 Posttreatment
2NS	−25.91a (± 0.24)	−27.85a (± 0.14)	−29.43 (± 0.13)	ns	−29.02 (± 0.20)	−29.56a (± 0.11)
NS	−26.05a (± 0.15)	−28.15a (± 0.15)	−29.47 (± 0.19)	ns	−29.27 (± 0.29)	−29.29a (± 0.32)
N	−26.14a (± 0.09)	−27.74b (± 0.12)	−28.99 (± 0.12)	ns	−29.29 (± 0.14)	−29.12a (± 0.02)
Wet control	−26.14a (± 0.11)	−28.46b (± 0.12)				−29.05a (± 0.15)
Statistical tests	ANOVA $F = 0.479$ $P = 0.7$	Repeated-measures $F = 5.912$ $P < 0.05$	Independent t-test $t = -1.73$, $P = 0.11$ $t = -0.60$, $P = 0.56$ $t = 1.63$, $P = 0.13$			ANOVA $F = 1.6$ $P = 0.239$

Differences for the first and last period were tested by univariate ANOVA, with four replicates for each treatment. The effect of different treatments during the second period was explored by repeated-measures ANOVA, with four replicates for each treatment. Finally, differences in the recovery period were assessed by independent sample t-tests, with two replicates for each treatment. Different letters after the means indicate significant ($P < 0.05$) differences within each column.
ns, not significant.

Table 4 Results of the statistical analyses of the differences between treatments in $\delta^{18}O$ for the periods 1995, 1996–2000, 2001–2003 and 2004–2009

Treatments	1995 Pretreatment	1996–2000 Treatment I	2001–2003 Treatment II Recovery		2001–2003 Treatment II Treatment	2004–2009 Posttreatment
2NS	24.89a (± 0.18)	24.31a (± 0.14)	24.26 (± 0.15)	ns	24.06 (± 0.07)	24.07a (± 0.09)
NS	25.0a (± 0.07)	24.26a (± 0.14)	24.15 (± 0.14)	ns	24.08 (± 0.17)	24.12a (± 0.10)
N	25.02a (± 0.18)	24.38a (± 0.15)	24.46 (± 0.16)	ns	24.37 (± 0.13)	24.33a (± 0.12)
Wet control	24.91a (± 0.22)	24.54a (± 0.14)				24.22a (± 0.13)
Statistical tests	ANOVA $F = 0.145$ $P = 0.9$	Repeated-measures $F = 0.793$ $P = 0.5$	Independent t-test $t = 1.156$, $P = 0.8$ $t = 0.319$, $P = 0.3$ $t = 0.409$, $P = 0.7$			ANOVA $F = 1.587$ $P = 0.2$

Differences for the first and last period were analysed using univariate ANOVA, with four replicates for each treatment. The effect of different treatments during the second period was explored by repeated-measures ANOVA, with four replicates for each treatment. Finally, differences in the recovery phase were assessed by independent sample t-test, with two replicates for each treatment. For the treatment I (1996–2000), estimated marginal mean are provided (i.e., adjusted for the variability in the soil relative water content among plots). Different letters after the means indicate significant differences within each column.
ns, not significant.

the wet control, with the exception of 2003, when an increase of $\delta^{18}O$, with particular reference to N and 2NS, was observed.

After the experiment had ceased, in all the treatments $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ values approached those measured in the wet control (Tables 3–5), bearing in mind that stable isotopes were measured in the pooled annual ring from 2004 to 2009. With particular reference to $\delta^{15}N$, wood was ^{15}N-depleted compared with the soil samples, but ^{15}N-enriched compared with the litter sampled in June 2009 (Fig. 4).

Variations in WUE_i and its determinants following N and S spray treatments

For each treatment, variations of WUE_i were evaluated as differences from the wet control (Fig. 5 a–c), without distinguishing between recovery and original treatments in the last 3 years of the experiment. The three treatments (i.e., 2NS, NS and N) showed different temporal trends, with particular reference to the first phase of the experiment (i.e., 1996–2000). The 2NS treatment led to a rapid but short-lived increase in WUE_i, followed by a decline from 1998. Furthermore, for the posttreatment phase (2004–2009) this decline strengthened producing a larger reduction in WUE_i compared with the control (Fig. 5a). In the NS treatment, WUE_i showed a pattern similar to that observed for 2NS, but with a lower magnitude of variation and only a small nonsignificant posttreatment decrease (Fig. 5b). By contrast, for the N treatment, WUE_i kept increasing over the first phase of experiment and also for the beginning of the second phase. However, during the posttreatment years, WUE_i in the N treatment also decreased but only by a small amount, falling back to the value measured in the wet control (Fig. 5c).

The combination of $\delta^{13}C$ and $\delta^{18}O$ values, expressed in terms of their differences from the wet control, is shown in the main panel of Fig. 6. Values relative to the 8 years of the experiment (i.e., 1996–2003) were averaged, without distinction between recovery and treatment periods. This allows the different effects of N alone and N in combination with S to be assessed for the combined variations of $\delta^{13}C$ and $\delta^{18}O$. N addition led to an increase in $\delta^{13}C$, with a marginally significant effect on $\delta^{18}O$. By contrast, N combined with S (irrespective of the double or single dose) led to an increase in $\delta^{13}C$ combined with a decrease in $\delta^{18}O$. For the posttreatment phase (i.e., treatment vs. posttreatment phase), we observed a tendency to decrease for both isotopes, with a higher magnitude of change for $\delta^{13}C$ than $\delta^{18}O$. Different trends are discussed later in the paper in terms of variations in CO_2 assimilation and stomatal conductance, according to the conceptual model of Scheidegger *et al*. (2000) (Fig. 6, side panels).

Canopy vs. soil applications: a meta-analysis of data in the literature

We compared our results with those reported in previous studies, where N was added to the soil as either ammonium nitrate or urea, sometimes also in combination with S or other nutrients (e.g., K, P, Ca).

All values were considered as differences from the corresponding control, to standardize the differences in climate and soil conditions across sites. Overall, both

Table 5 Results of the statistical analyses of the differences between treatments in δ^{15}N for the periods 1995, 1996–2000, 2001–2003 and 2004–2009

Treatments	1995 Pretreatment	1996–2000 Treatment I	2001–2003 Treatment II			2004–2009 Posttreatment
			Recovery		Treatment	
2NS	−3.63a (± 0.31)	−2.89ab (± 0.15)	−2.85 (± 0.13)	ns	−2.78 (± 0.27)	−2.32a (± 0.37)
NS	−3.28a (± 0.36)	−2.64a (± 0.57)	−1.64 (± 0.49)	*	−3.29 (± 0.17)	−2.33a (± 0.62)
N	−3.49a (± 0.31)	−3.46b (± 0.12)	−3.06 (± 0.12)	ns	−2.94 (± 0.12)	−2.25a (± 0.54)
Wet control	−3.08a (± 0.359)	−2.43a (± 0.18)				−2.77a (± 0.23)
Statistical tests	ANOVA	Repeated-measures	Independent *t*-tests			ANOVA
	$F = 0.504$ $P = 0.69$	$F = 2.92$ $P = 0.07$	$t = -0.21$, $P = 0.83$			$F = 0.198$ $P = 0.89$
			$t = 3.22$, $P = 0.009$			
			$t = -0.30$, $P = 0.77$			

Differences for the first and last period were analysed using univariate ANOVA, with four replicates for each treatment. The effect of different treatments during the second period was explored by repeated-measures ANOVA, with four replicates for each treatment. Finally, differences in the recovery phase were assessed by independent sample *t*-test, with two replicates for each treatment. Different letters after the means indicate significant ($P < 0.05$) differences within each column.
ns, not significant.

above-canopy and below-canopy fertilization showed that addition of N leads to an increase in WUE_i, with a magnitude that was equal to or slightly greater for canopy compared with soil applications (Fig. 7). In two cases, no change (Balster *et al.*, 2009) or even a reduction (Heaton & Crossley, 1995) of WUE_i was observed with applied N. This latter study interestingly used the same NS combination as used here.

A different dynamic was observed for δ^{15}N, when the two different experimental approaches (canopy vs. soil N applications) were compared (Fig. 8). With increasing N dose applied to the soil, the δ^{15}N in tree rings became more ^{15}N-enriched compared with the control, whereas canopy applications seemed to lower the ^{15}N enrichment, although the low number of points prevents statistical analysis.

Interestingly, irrespective of the approach considered, the combination of N and S appears to restrict the difference between treatment and control for δ^{15}N. In fact, when N alone was added either to the soil (Nadelhoffer *et al.*, 2004) or to the canopy (i.e., Deepsyke forest), the difference in δ^{15}N from the control was always larger than in the case of combined N + S applications, with a higher difference in the case of soil fertilization. Changes in the δ^{15}N in tree rings in response to N application seemed to be species-specific (conifer vs. deciduous species) and compound-specific (ammonium nitrate vs. urea fertilizer). Conifers were possibly more sensitive than deciduous trees to N application. For the former group, the ^{15}N enrichment in tree rings compared with control was lower when N was added in combination with other nutrients (Elhani *et al.*, 2005).

Discussion

Changes in tree physiological traits following N and S mist above tree canopies at Deepsyke

One year after the experiment started (i.e., 1997) a clear effect of the treatment on the C isotope signal in the annual ring was already present, although no signal was detected in 1-year-old needles produced in the same year (Fig. 3b). Five years exposure to acid mist affected the isotopic signature in tree rings significantly, leading to higher δ^{13}C values (Table 3), thus a higher WUE_i, for N and 2NS treatments. This result is consistent with the increase in stem area increment reported by Sheppard *et al.* (2004) at the same site. Despite the similarity in all treatments with the positive response for tree growth rates, δ^{13}C enabled a treatment-specific change in terms of WUE_i to be identified. In fact, although both N and 2NS showed an increase of WUE_i, the different temporal trends (Fig. 5) demonstrate that

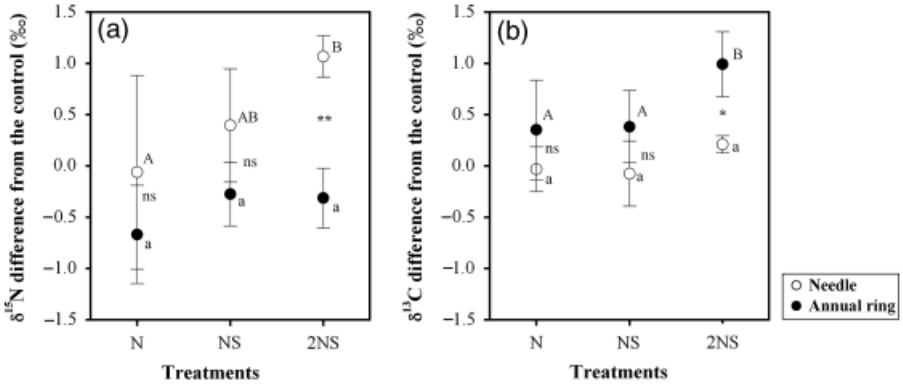

Fig. 3 Differences between treatments (2NS, NS and N) and control for δ^{15}N (a) and δ^{13}C (b) measured in needles (open points) and the corresponding annual ring (closed points) in 1997. For each isotope, stars [(*) for $P<0.1$ and (**) for $P<0.05$] indicate significant differences between annual rings and needles. Capital and small letters refers to ANOVA tests of differences across treatments for needles and tree rings, respectively. Each point represents the mean ± confidence interval ($n=4$). N, nitrogen; S, sulphur.

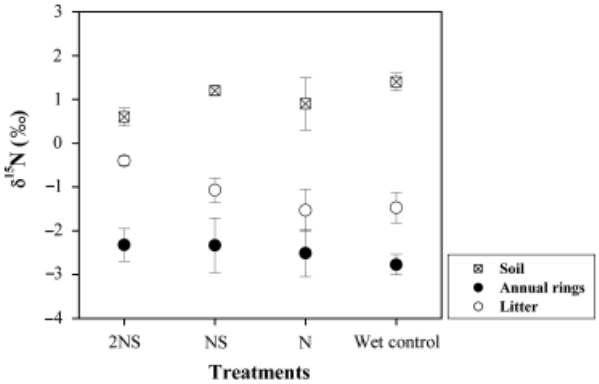

Fig. 4 δ^{15}N values measured in soil and litter sample collected in June 2009 and in the group of annual rings from 2004 to 2009. Each value is the mean ± SE for $n=4$ plots. N, nitrogen; S, sulphur.

the N application led to a long lasting positive effect of N availability on the ratio between assimilation (A) and stomatal conductance (g_s), whereas the 2NS application resulted in a short-lived pulse only.

The different patterns for WUE_i suggest different physiological mechanisms behind the responses observed for the three treatments. Combining δ^{13}C and δ^{18}O in a conceptual model (Fig. 6), according to Scheidegger *et al*. (2000), enabled us to better understand how N treatments with and without S affects N use in the tree. An important prerequisite of the conceptual model is similar δ^{18}O of source water and vapour water for the investigated trees. In our study, as the treatment plots were small and randomly distributed, we can assume no differences among treatments for the oxygen isotopic signature of soil water. Further, trees were growing in the same area with closed canopies giving rise to similar Rh and treated with rainwater collected on site, so we assume that possible differences in g_s, as inferred by δ^{18}O in tree rings, can be attributed to the treatments alone.

The increase in δ^{13}C observed in all treatments during the experiment (i.e., 1996–2003) indicates a reduction in the c_i/c_a ratio, explained by either an increase in A, at constant g_s or by a reduction of g_s at constant A. δ^{18}O, combined to δ^{13}C, helps to clarify whether changes in A, g_s or both accounted for the variations of WUE_i.

In the case of N addition, only small changes in δ^{18}O were observed (compared with the pretreatment time), therefore variation of WUE_i were mostly related to an increase of A, in response to a fertilizer effect of N (Fig. 6, side panels). In another experiment where spruce branches were sprayed with NH_4NO_3, N was shown to contribute significantly not only to enhance CO_2 assimilation but also to reduce the thermally dissipated light, suggesting an increase of photosynthetic efficiency (Tomaszewski & Sievering, 2007).

In the case of 2NS and NS treatments, the moderate reduction of δ^{18}O compared with the control suggests that together with A, g_s might have increased too. Acid mist has been shown to alter cuticular wax structure (Fowler *et al*., 1980; Shepherd & Griffiths, 2006 and references therein) and to affect stomatal opening, with a mechanism related to changes in apoplastic pH and in the electrochemical potential gradient across the guard cell plasmalemma (Eamus & Fowler, 1990).

The legacy of N and S treatments at Deepsyke forest as revealed by stable isotopes

Based on our measurements, the beneficial effect of N on A tended to disappear after the experiment stopped (Fig. 6, side panels), especially in the 2NS treatment. As suggested by the larger reduction of δ^{13}C compared with δ^{18}O, g_s did not change as much as A, which

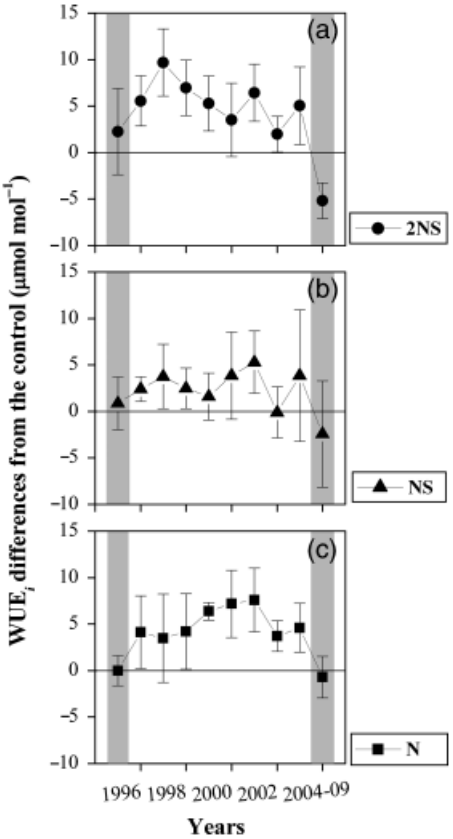

Fig. 5 Differences from the wet control (i.e., treatment-control) for intrinsic water-use efficiency (WUE_i) values measured in 2NS (a), NS (b) and N (c) treatments. Each point represents the mean of the values measured in $n = 4$ plots for each treatments; bars are confidence interval. Here, we averaged the values measured for each year from 1996 to 2003 in $n = 4$ plots within each treatment, without distinguishing between recovery and original treatment in the last 3 years of the experiment. Shaded areas indicate the pre- and posttreatment periods. N, nitrogen; S, sulphur.

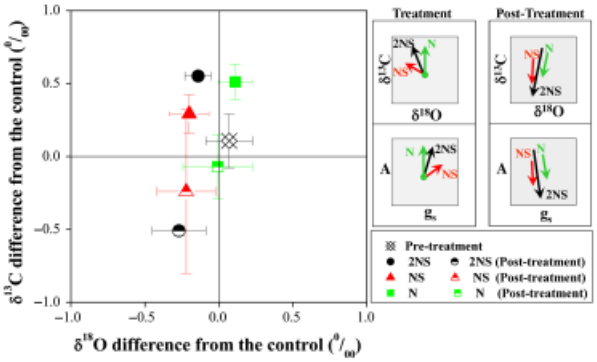

Fig. 6 (Main panel) Combination of $\delta^{13}C$ and $\delta^{18}O$ values for 2NS, NS and N treatment, evaluated as difference from the wet control (treatment-wet control). For the pretreatment year (i.e., 1995), we considered the mean of the differences from the wet control for $\delta^{13}C$ and $\delta^{18}O$ calculated over all the values measured in each treatments ($n = 12$). Bars represent the confidence intervals. Values relative to the 8 years of the experiment (i.e., 1996–2003) were averaged for each treatment, without separating recovery from the original treatment. Posttreatment refer to the grouped annual rings from 2004 to 2009 in each treatment ($n = 4$). (Side panel) Interpretation of the $\delta^{13}C$–$\delta^{18}O$ trends based on the conceptual model (Scheidegger *et al.*, 2000). According to the model, variations of c_i and therefore A are derived from $\delta^{13}C$, whereas changes in g_s are predicted from $\delta^{18}O$. N, nitrogen; S, sulphur.

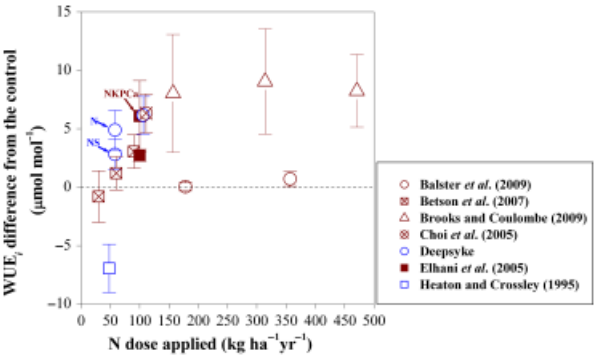

Fig. 7 Results of the meta-analysis: intrinsic water-use efficiency (WUE_i) vs. nitrogen (N) dose applied. Each point is the mean calculated from values measured during the fertilization experiments considered (see references in the legend and Table 1). Bars are confidence intervals. Differences between treatment and control for WUE_i are calculated from $\delta^{13}C$ values measured in either tree rings or needles. Soil and canopy N application are indicated with brown and blue symbols, respectively. Full symbols refer to deciduous species, whereas open symbols to conifers. We used crossed squares and circles when WUE_i was calculated from $\delta^{13}C$ in needles. S, sulphur; P, phosphorus; K, potassium; Ca, calcium.

decreased after the experiment stopped. Hence, the short-lived increase in WUE_i with the 2NS treatment was followed posttreatment by a significant decrease during 2004–2009 compared with the control, a tendency which was also apparent (but not significant) for the NS treatment, but not for the N treatment.

This means that S deposition had a longer lasting detrimental effect on tree physiological traits, even 6 years after the spray treatment stopped, suggesting a low resilience of the forest to recover from excess S_{dep} to either tree canopies and/or soil.

Nontreatment effects on stable C and O isotope trends for Sitka spruce at Deepsyke

Long dendro-isotope chronologies have often reported an increase of $\delta^{13}C$ in tree rings during the juvenile stage of tree growth (Duquesnay *et al.*, 1998; McCarroll & Loader, 2004), although this was not observed in our study. In fact, variations in $\delta^{13}C$ during the investigated

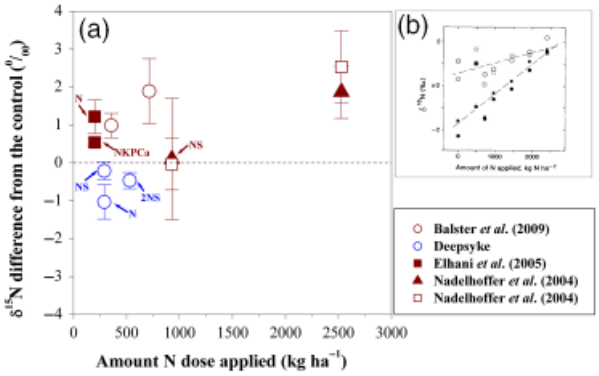

Fig. 8 Results of the meta-analysis (panel a): δ^{15}N in tree rings vs. amount of N applied (i.e., cumulated doses of N applied over the duration of the experiment). Each point is the mean calculated from values measured during the fertilization experiments considered (see references in the legend and Table 1). Bars are confidence intervals. Soil and canopy N addition are indicated with brown and blue symbols, respectively. Full symbols refer to deciduous species whereas open symbols to conifers. Each point is the mean calculated over the values measured during the fertilization experiments considered in the meta-analysis and published in the papers indicated in the legend. N, nitrogen; S, sulphur; K, potassium; P, phosphorus; Ca, calcium. The picture in the panel (b) is provided as an example of the ^{15}N-enrichment of δ^{15}N in a grass species (full symbols) and soil (open symbols) at increasing levels of N (i.e., urea) supplied to the soil, under experimental conditions (Johannisson & Högberg 1994).

period were quite consistent, with a reduction of about 4‰ over a period of 6 years (Fig. 2a–c). Since we considered a short chronology (i.e., 15 years) data were not corrected for the ^{13}C-depletion of atmospheric CO_2, and this may partially explain the decreasing trend for δ^{13}C in tree rings. Another explanation could be related to the age of the plantation. In fact, when the experiment started in 1996, trees were about 10-year-old, and canopy closure occurred during the experiment (Sheppard *et al*., 2004). This could have played a significant role, by increasing shading, which, in turn, reduced the δ^{13}C and increased the c_i/c_a ratio, due to a low CO_2 assimilation rate for a given g_s (Farquhar, 1980).

δ^{18}O in tree rings reflects both δ^{18}O of source water and the changes in δ^{18}O in leaf water during transpiration, modified by the Péclet effect (Craig & Gordon, 1965; Farquhar & Lloyd, 1993; Barbour *et al*., 2000a), in response to changes in environmental factors, e.g., precipitation and changes in summer relative humidity conditions (McCarroll & Loader, 2004).

Despite the fact that the additional spray water may have reduced the detection of the climatic signal, δ^{18}O proved to be a valuable archive for changes in evaporative demand from the atmosphere. In fact, the trough of δ^{18}O values in 1998, corresponding to the wettest year of the experiment, may have reflected 'over watering' conditions for trees, with particular reference to 2NS. Moreover, the response recorded in 2003 for both δ^{13}C and δ^{18}O might be related to drought conditions all over Europe, with particular reference to trees in the treatment plots where recovery phase started (i.e., the misting with S had ceased), suggesting that S might have affected tree responsiveness to seasonal changes in weather conditions.

Generalization of our findings: comparison of the Deepsyke experiment with other studies

Only a few studies met the most relevant conditions for inclusion in the meta-analysis: that the fertilizer response was assessed using δ^{13}C in either needles or tree rings and δ^{15}N in tree rings. It was beyond our aim to provide an estimation of the changes of absolute values of WUE_i and δ^{15}N for soil vs. canopy N application, rather we were interested in the extent of variations in relation to the two approaches, in order to account for differences in the underlying mechanisms.

Changes in WUE_i: same direction and magnitude? We are conscious that different variables might account for variations of WUE_i at each of the sites considered in the meta-analysis, such as age, tree species, and different climatic and environmental conditions. However, by considering the difference from the control, we were able to extract the influence of the treatment on WUE_i, through an effect on A, at a given g_s, given that the contribution of ambient N_{dep} is similar in all the sites considered.

The increase in N availability, through either soil or canopy application, enables trees to enhance the efficiency by which they assimilate CO_2, thus confirming the 'almost axiomatic and strongly positive relationship' (Warren & Adams, 2006) between A and N supply. Overall, there seems to be a steep increase of WUE_i up to 150 kg N ha^{-1} yr^{-1} (Fig. 7). At higher doses, the ratio between A and g_s tends to stabilize, though it must be noted that this conclusion is contradicted by the results of Balster *et al*. (2009), where urea was used as a fertilizer supply.

Although results for both above-canopy and below-canopy fertilization followed a similar trend for WUE_i, the magnitude tended to be higher in the case of canopy applications. In fact, for a similar doses of N applied (e.g., 48–50 kg N ha^{-1} yr^{-1}), the difference from the control for WUE_i was higher for trees receiving N as mist application over tree canopies (Fig. 8), compared with those receiving the same amount supplied to the soil (Betson *et al*., 2007). At higher dose (e.g., 96–100 kg N ha^{-1} yr^{-1}), the increase of WUE_i compared

with the control was in the same range reported by Elhani *et al*. (2005), where however, N was added in combination with other nutrients (e.g., K, Ca and P). Lower responses were observed by Elhani *et al*. (2005) when N was added alone (Fig. 8).

It must be acknowledged that duration of the fertilization experiment, differences in the N use efficiency and N–C allocation in young (i.e., the present study) and mature (Elhani *et al*., 2005; Betson *et al*., 2007) trees might also account for the differences in the WUE_i following N additions.

In the case of Balster *et al*. (2009) and Heaton & Crossley (1995), values deviated from others reported in Fig. 7. In the latter investigation, the spraying with acid mist resulted in a reduction of CO_2 assimilation (as assessed by the more negative $\delta^{13}C$ in the treated trees compared with the control), which was supported also by a decrease of radial growth increment during the experiment (Crossley *et al*., 1997). However, in the investigation of Heaton and Crossley, the Sitka spruce stand was potentially P limited, as a result of the acid treatment, which mobilized Al (Carreira *et al*., 1997). This result has important implication for forest management, indicating that in general, at low levels of N availability, an increase of N_{dep} can hardly produce a fertilization effect on CO_2 assimilation and growth if other conditions are not met (e.g., other nutrients or water availability).

Fingerprint of canopy and soil N applications as revealed by $\delta^{15}N$ in tree rings: what do they tell us about the N cycle in forests? Across the studies included in our meta-analysis, the fertilizers applied had a similar $\delta^{15}N$ values, thus we can assume that the differences between the treatment and the control for $\delta^{15}N$ measured in the tree rings reflect changes in soil and canopy processes, rather than differences in the isotope signature of the N applied.

Our meta-analysis showed that $\delta^{15}N$ in tree rings becomes enriched when the cumulative added N increases (Fig. 8a). Although the number of datasets used in our meta-analysis was limited, we observed a tendency for conifers to respond more strongly than deciduous trees to N supply (Nadelhoffer *et al*., 2004), suggesting a difference in the NH_4^+ vs. NO_3^- uptake (Gebauer *et al*., 2000), which is then reflected in their specific N isotope signature. The differences observed for the $\delta^{15}N$ values measured in trees subjected to ammonium nitrate vs. urea applications could reflect a different isotopic signature of these compounds as well as different N processing.

Soil N applications can speed up many biogeochemical processes, i.e., N microbial immobilization and/or losses, which in turn not only modify the immobilized N in a form more readily available to trees, but also its isotopic signature. In particular, losses of ^{15}N-depleted compounds, through denitrification, NO_3^- leaching or ammonia volatilization, which increase when the N dose exceeds the requirements of the trees, are reflected in plant material as an increase in $\delta^{15}N$ (Johannisson & Högberg, 1994) (Fig. 8b).

If N losses had occurred also at our experimental site in response to the spraying, they would have resulted in a significant enrichment of $\delta^{15}N$ in tree rings compared with the control. However, this was not the case. In fact, spraying of N over tree canopies tended to lower the $\delta^{15}N$ in tree rings compared with the wet control and the NS treatments (Fig. 2g–i, Fig. 8a).

A reduction in $\delta^{15}N$ values could suggest a contribution of N from micorryze (Högberg *et al*., 2010). In this case, discrimination against ^{15}N occurs during the N translocation from the fungi to the roots in a way that fungi are ^{15}N enriched compared with their host plants (Högberg, 1997). However, we exclude that this happened in our case, since Sheppard & Crossley (2000) reported a reduction of ectomycorrhizal fruiting bodies, with particular reference to N treatment, at Deepsyke.

We can exclude also a difference in the isotopic signature of the ammonium nitrate salt used during the experiment, which had a $\delta^{15}N$ value of 1.6‰. Likewise, the $\delta^{15}N$ value of the treatment was unlikely to have been diluted by the $\delta^{15}N$ of ambient N_{dep}, given that the rate of ambient N deposition ($8\,kg\,N\,ha^{-1}\,yr^{-1}$) was much smaller than the added rates (48 and $96\,kg\,N\,ha^{-1}\,yr^{-1}$). Also, based on $\delta^{15}N$ values of NH_4^+ (-3.8 ± 2.6‰) and NO_3^- (-3.8 ± 2.4‰) in rainfall, measured for six different sites across Scotland (T. H. E. Heaton, personal communication), only a small dilution effect would be expected. Hence, assuming no changes in the $\delta^{15}N$ of the atmospheric N source, the long-term trend measured for $\delta^{15}N$ in the different treatments reflected different dynamics of canopy N exchange rather than changes of N in the soil. This suggests that soil applications fail to capture all the dynamics and isotope fractionation that occur when deposition takes place on the canopy.

Before reaching the soil, the atmospheric N can be modified by the interaction with tree canopies, which can retain most of the N added by the spray treatments, leaving only a small amount to reach the soil. Up to 60% of the applied N at Deepsyke was retained in the high-N treatment (Cape *et al*., 2001), with different dynamics of canopy N exchange according to whether the N addition also contained S. In fact, the absolute amount of N retained as NH_4^+ and NO_3^- was similar in the 2NS treatment (i.e., 40% for both), whereas tree canopies in the N treatment retained more NH_4–N (i.e., 60%) than NO_3–N (i.e., 8%) (Cape *et al*., 2001). Further, throughfall

investigations showed also a net increase in the cations leaching losses from tree canopies, with particular reference to K^+ (Cape *et al.*, 2001; Chiwa *et al.*, 2004). The $\delta^{15}N$ measured in needles (for the year 1997) and tree rings at each treatment could therefore reflect the proportion of NH_4^+ and NO_3^- ions taken up and their relative isotope signature (Heaton *et al.*, 1997).

The substantial level of canopy N retention would have restricted the amount of N washed through to the soil, limiting any soil N accumulation or loss. This can explain why the $\delta^{15}N$ shift goes in the opposite direction to that found across studies when N fertilizer was added directly to the soil. No clear indication of excess N was apparent after 5 years, except in the 2NS treatment, where needle fall was significantly increased (reducing canopy N uptake) and N_2O emissions were recorded (Sheppard *et al.*, 2004).

Six years after the experiment stopped, $\delta^{15}N$ in the soil did not reveal any clear signal of the treatments (Fig. 4). This further suggests that only a fraction of the N reached the soil, which eventually was diluted in relation to the large soil N pool. Treatment effects, with particular reference to the N addition, were not detectable in tree rings once treatment ceased (Figs. 2g–i and 4). However, it must be recognized that pooling the annual rings from 2004 to 2009 precluded our ability to track the rate of loss of the treatment signal. Interestingly, a carry-over effect of the treatment-specific contribution to canopy N dynamics was still present in undecomposed litter sampled 6 years after the experiment stopped, i.e., more negative $\delta^{15}N$ values in the plot that received N treatment.

Conclusions

The combination of $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ in tree rings enhanced our understanding of how variations in atmospheric N and S deposition affect some key processes underpinning the C and N cycle in a Sitka spruce stand. Specifically we have shown that:

(i) Addition of N, independent of the presence of S, increased the ratio between assimilation (A) and stomatal conductance (g_s) in favour of A, thus supporting the fertilizer effect of N. However, the combination of N and S, as a double dose, counteracted the duration and the strength of the positive effect of N on WUE_i, likely related to a negative effect of S on stomatal control.

(ii) Based on our WUE_i values, trees largely returned to pretreatment values within 6 years (2004–2009) after the cessation of N_{dep}. However, this was not the case for S_{dep}, where WUE_i continued to fall. A carry-over effect of S excess could affect negatively the balance between A and g_s, by reducing A for trees subjected to 2NS. This suggests that the beneficial role of N_{dep} is counteracted by the negative effect of S, which may still be operating in forests that have received high loads of acid rain.

(iii) Treatment-specific variations in the $\delta^{15}N$ carried the effect of different canopy N dynamics, either in the short (in needles) and the long-term (tree rings), with an isotope signal imprinted also in the litter, even after treatment stopped.

(iv) In addition, our meta-analysis also demonstrates that soil and canopy N applications showed an increase of WUE_i in response to increased N_{dep}. However, for the same N dose applied, spraying over tree canopies led to a larger change in WUE_i compared with soil applications. By contrast, the two application methods produced different results with regard to the changes in the N dynamics. This suggests, on one hand, that experiments considering soil applications might underestimate the contribution of N_{dep} on the ecosystem N cycle, as they exclude possible N exchanges at the canopy level. On the other hand, they might overestimate the effect of N_{dep} to the soil, since canopy N retention tends to reduce the quantity and alter the quality of N reaching the soil, thus contributing to slowing down soil bioactivity and preventing N accumulation and losses.

Acknowledgements

R. G. was supported during field and part of the lab activities (May–August 2009) by the European Science Foundation (ESF), within the programme Nitrogen in Europe, grant n 2221. Since September 2009, R. G. has been supported by a Postdoctoral Newton International Fellowship (Grant no. NF082365) funded by the Royal Society, the British Academy and the Royal Academy of Engineering. Stable isotopes analyses were funded by the project 'Greenhouse gas management in European land use systems (GHG EUROPE) awarded to M. A. S., P. L. and L. J. S. for $\delta^{15}N$ and by Research funding within the Newton International fellowship for $\delta^{13}C$ and $\delta^{18}O$. M. M. acknowledges NERC for their support via the Grant no. NE/G00725X/1. We would like to thank C. McEvoy (Northern Research Station, Forest Research) and A. Crossley (Centre for Ecology and Hydrology) for their logistic support during the field work; M. Jarvis (University of Glasgow) for providing facilities for wood cores chemical pretreatment and C. Chilcott (University of Edinburgh) for measuring stable isotopes in needles. R. G. acknowledges R. Siegwolf (Paul Scherrer Institute) and T. Heaton (NERC Isotope Geosciences Laboratory) for stimulating discussions. We acknowledge the two anonymous reviewers and the Editor for their valuable comments on the manuscript.

References

Balster NJ, Marshall JD, Clayton M (2009) Coupling tree-ring $\delta^{13}C$ and $\delta^{15}N$ to test the effect of fertilization on mature Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) stands across the Interior northwest, USA. *Tree Physiology*, **29**, 1491–1501.

Barbour MM (2007) Stable oxygen isotope composition of plant tissue: a review. *Functional Plant Biology*, **34**, 83–94.

Barbour MM, Fischer RA, Sayre KD, Farquhar GD (2000b) Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and yield in irrigated, field-grown wheat. *Australian Journal of Plant Physiology*, **27**, 625–637.

Barbour MM, Schurr U, Henry BK, Wong SC, Farquhar GD (2000a) Variation in the oxygen isotope ratio of phloem sap sucrose from castor bean: evidence in support of the Péclet effect. *Plant Physiology*, **123**, 671–679. doi: 10.1104/pp.123.2.671.

Betson NR, Johannisson C, Löfvenius MO, Grip H, Granström A, Högberg P (2007) Variations in the δ^{13}C of foliage of *Pinus sylvestris* L. in relation to climate and additions of nitrogen: analysis of 32-year chronology. *Global Change and Biology*, **13**, 2317–2328.

Brooks JR, Coulombe R (2009) Physiological responses to fertilization recorded in tree rings: isotopic lessons from a long-term fertilization trial. *Ecological Applications*, **19**, 1044–1060.

Cape JN, Dunster A, Crossley A, Sheppard LJ, Harvey FJ (2001) Throughfall, chemistry in a Sitka spruce plantation in response to six different simulated polluted mist treatments. *Water, Air and Soil Pollution*, **130**, 619–624.

Carreira JA, Harrison AF, Sheppard LJ, Woods C (1997) Reduced P soil availability in a Stika spruce (*Picea sitchensis* (Bong.) Carr) plantation induced by applied acid-mist: significance in forest decline. *Forest Ecology and Management*, **92**, 153–166.

Cernusak LA, Tcherkez G, Keitel C *et al*. (2009) Why are non-photosynthetic tissues generally ^{13}C enriched compared to leaves in C3 plants? Review and synthesis of current hypotheses. *Functional Plant Biology*, **36**, 199–213.

Chandler JW, Dale JE (1993) Photosynthesis and nutrient supply in needles of Sitka spruce (*Picea sitchensis* (Bong.) Carr.). *New Phytologist*, **125**, 101–111.

Chiwa M, Crossley A, Sheppard LJ, Sakugawa H, Cape JN (2004) Throughfall chemistry and canopy interactions in a Sitka spruce plantation sprayed with six different simulated polluted mist treatments. *Environmental Pollution*, **127**, 57–64.

Choi J-W, Chang XS, Allen HL, Kelting DL, Ro H-M (2005) Irrigation and fertilization effects on foliar and soil carbon and nitrogen isotope ratio in a loblolly pine stand. *Forest Ecology and Management*, **213**, 90–101.

Comstock JP (2001) Steady-state isotopic fractionation in branched pathways using plant uptake of NO_3 as an example. *Planta*, **214**, 220–234.

Craig H, Gordon LI (1965) Deuterium and oxygen-18 variations in the ocean and the marine atmosphere. In: *Proceedings of Conference on Stable Isotopes on Oceanographic Studies and Paleotemperatures* (ed. Tongiorgi T), pp. 9–130. Lischi and Figli, Pisa, Italy.

Crossley A, Sheppard LJ, Cape NJ, Smith RI, Harvey FJ (1997) Stem growth reduction in mature Sitka spruce trees exposed to acid mist. *Environmental Pollution*, **96**, 185–193.

Dail DB, Hollinger DY, Davidson EA, Fernandez I, Sievering HC, Scott NA, Gaige E (2009) Distribution of nitrogen-1 tracers applied to the canopy of a mature spruce-hemlock stand, Howland, Maine, USA. *Oecologia*, **160**, 589–599.

Dezi S, Medlyn BE, Tonon G, Magnani F (2009) The effect of nitrogen deposition on forest carbon sequestration: a model-based analysis. *Global Change and Biology*, **16**, 1470–1486, doi: 10.1111/j.1365-2486.2009.02102.x.

Duquesnay A, Breda N, Stievenard M, Dupouey JL (1998) Changes of tree-ring δ^{13}C and water-use efficiency of beech (*Fagus sylvatica* L.) in north-eastern France during the past century. *Plant, Cell and Environment*, **21**, 565–572.

Eamus D, Fowler D (1990) Photosynthetic and stomatal conductance responses to acid mist of red spruce seedlings. *Plant, Cell and Environment*, **13**, 349–357.

Ehleringer JR, Hall AE, Farquhar GD (1993) Water use in relation to productivity. In: *Stable Isotopes and Plant Carbon–Water Relations* (eds Ehleringer JR, Hall AE, Farquhar GD), pp. 3–8. Academic Press, New York.

Elhani S, Guehl JM, Nys C, Picard JF, Dupouey JL (2005) Impact of fertilization on tree-ring δ^{15}N and δ^{13}C in beech stands: a retrospective analysis. *Tree Physiology*, **25**, 1437–1446.

Farquhar GD (1980) Carbon isotope discrimination by plants: effects of carbon dioxide concentrations and temperature via the ratio of intercellular and atmospheric CO_2 concentration. In: *Carbon Dioxide and Climate: Australian Research* (ed. Pearman GI), pp. 105–110. Australian Academy of Science, Canberra.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503–537.

Farquhar GD, Lloyd L (1993) Carbon and oxygen isotope effects in the exchange of carbon dioxide between plants and the atmosphere. In: *Stable Isotope and Plant Carbon–Water Relations* (eds Ehleringer JR, Hall AE, Farquhar GD), pp. 47–70. Academic Press, New York, USA.

Feng XH (1999) Trends in intrinsic water-use efficiency of natural trees for the past 100–200 years: a response to atmospheric CO_2 concentration. *Geochimica et Cosmochimica Acta*, **63**, 1891–1903.

Feng Z, Brumme R, Xu X-J, Lamersdorf N (2008) Tracing the fate of mineral N compounds under high ambient N deposition in a Norway spruce forest at Solling/Germany. *Forest Ecology and Management*, **255**, 2061–2073.

Fowler D, Cape JN, Nicholson IA, Kinnaird JW, Paterson IS (1980) The influence of a polluted atmosphere on cuticle degradation in scots pine. In: *Ecological Impact of Acid Precipitation* (eds Drabløs D, Tollan R), pp. 146. SNSF project, Oslo.

Fowler D, Smith R, Müller J, Cape JN, Sutton M, Erisman JW, Fagerli H (2007) Long-term trends in sulphur and nitrogen deposition in Europe and the cause of nonlinearities. *Water, Air and Soil Pollution*, **7**, 41–47.

Gaige E, Dail DB, Hollinger DY *et al*. (2007) Changes in canopy processes following whole-forest canopy nitrogen fertilization of a mature spruce-Hemlock forest. *Ecosystems*, **10**, 1133–1147.

Galloway JN, Cowling EB, Seitzinger SJ, Socolow R (2002) Reactive nitrogen: too much of a good thing? *Ambio*, **31**, 60–63.

Gebauer G, Zeller B, Schmidt G *et al*. 2000 The fate of ^{15}N-labelled nitrogen inputs to coniferous and Broadleaf forests. In: *Carbon and Nitrogen Cycling in European Forest Ecosystems. Ecological Studies* (ed. Schulze E-D), pp. 144–170. Springer-Verlag, Heidelberg.

Gessler A, Brandes E, Buchmann N, Helle G, Rennenberg H, Barnard RB (2009) Tracing carbon and oxygen isotope signals from newly assimilated sugars in the leaves to the tree-ring archive. *Plant, Cell and Environment*, **32**, 780–795.

Grams TEE, Kozovits AR, Häberle KH, Matyssek R, Dawson TE (2007) Combining δ^{13}C and δ^{18}O analyses to unravel competition CO_2 and O_3 effects on the physiological performance of different-aged trees. *Plant, Cell and Environment*, **30**, 1023–1034.

Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen cycle. *Nature*, **451**, 293–296.

Heaton THE, Spiro B, Madeline S, Robertson C (1997) Potential canopy influences on the isotopic composition of nitrogen and sulphur in atmospheric deposition. *Oecologia*, **109**, 600–607.

Heaton THE, Crossley A (1995) Carbon isotope variations in a plantation of Sitka spruce, and the effect of acid mist. *Oecologia*, **103**, 109–117.

Högberg P (1997) ^{15}N natural abundance in soil–plant systems. *New Phytologist*, **137**, 179–203.

Högberg P (2007) Nitrogen impacts on forest carbon. *Nature*, **447**, 781–782.

Högberg P, Fan H, Quist M, Binkley D, Tamm CO (2006) Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. *Global Change Biology*, **12**, 489–499.

Högberg P, Johannisson C, Yarwood S, Callesen I, Näsholm T, Myrold DD, Högberg MN (2010) Recovery of ectomycorrhiza after 'nitrogen saturation' of a conifer forest. *New Phytologist*, doi: 10.1111/j. 1469-8137.2010.03485.x.

ICP Forests-Executive Report UNECE/LRTRAP (2009) *The Condition of Forests in Europe*. ECEDG LIFE Unit, Hamburg and Brussels, Germany.

Johannisson C, Högberg P (1994) ^{15}N abundance of soils and plants along an experimentally induced forest nitrogen supply gradient. *Oecologia*, **97**, 322–325.

Létolle R (1980) Nitrogen-15 in the natural environment. In: *Handbook of Environmental Isotope Geochemistry*, **Vol. 1.4** (eds Fritz P, Fontes JC), pp. 407–433. Elsevier, Amsterdam.

Magill AH, Aber JD, William SC *et al*. (2004) Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA. *Forest Ecology and Management*, **196**, 7–28.

Magnani F, Mencuccini M, Borghetti M (2007) The human footprint in the carbon cycle of temperate and boreal forests. *Nature*, **447**, 848–850.

Magnani F, Mencuccini M, Borghetti M *et al*. (2008). Replying to: A. De Schrijver *et al*. Nature 451, doi: 10.1038/nature06578; W. de Vries et al. Nature 451, doi: 10.1038/nature06579 (2008). *Nature*. **451**, E3–E4

McCarroll D, Loader NJ (2004) Stable isotope dendroclimatology. *Quaternary Science Reviews*, **23**, 771–801.

Nadelhoffer KJ, Benjamin PC, Colman P, Currie WS, Magill A, Aber J (2004) Decadal-scale fates of ^{15}N tracers added to oak and pine stands under ambient and elevated N inputs at the Harvard Forest (USA). *Forest Ecology and Management*, **196**, 89–107.

Nadelhoffer KJ, Fry B (1994) Nitrogen isotope studies in forest ecosystems. In: *Stable Isotopes in Ecology and Environmental Science* (eds Lajtha K, Michener R), pp. 22–44. Blackwell, London.

Norby RJ (1998) Nitrogen deposition: a component of global change analyses. *New Phytologist*, **139**, 189–200.

Phoenix GK, Hicks WK, Cinderby S *et al*. (2006) Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. *Global Change Biology*, **12**, 470–476.

Rennenberg H, Gessler A (1999) Consequences of N deposition to forest ecosystems-recent results and future research needs. *Water, Air and Soil Pollution*, **116**, 47–64.

Ripullone F, Guerrieri MR, Saurer M, Siegwolf R, Jäggi M, Guarini R, Magnani F (2009) Testing a dual isotope model to track carbon and water gas exchanges in a Mediterranean forest. *iForest – Biogeoscience and Forestry*, **2**, 59–66, doi: 10.3832/ifor0491-002.

Robinson D, Handley LL, Scrimgeour CM (1998) A theory for $^{15}N/^{14}N$ fractionation in nitrate-grown vascular plants. *Planta*, **205**, 397–406.

Saurer M, Aellen K, Siegwolf R (1997) Correlating $\delta^{13}C$ and $\delta^{18}O$ in cellulose of trees. *Plant, Cell and Environment*, **20**, 1543–1550.

Saurer M, Siegwolf RTW, Schweingruber FH (2004) Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. *Global Change Biology*, **10**, 2109–2120.

Scheidegger Y, Saurer M, Bahn M, Siegwolf RTW (2000) Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia*, **125**, 350–357.

Shepherd T, Griffiths DW (2006) The effects of stress on plant cuticular waxes. *New Phytologist*, **171**, 469–499.

Sheppard LJ, Crossley A (2000) Responses of a Sitka spruce ecosystem after 4 years of simulated wet N deposition: effects of NH_4NO_3 supplied with and without acidity (H_2SO_4 pH 2.5). *Phyton*, **40**, 169–174.

Sheppard LJ, Crossley A, Harvey FJ, Skiba U, Coward P, Ingleby K (2004) Effects of five years of frequent N additions, with or without acidity, on the growth and below-ground dynamics of a young Sitka spruce stand growing on an acid peat: implications for sustainability. *Hydrology and Earth System Sciences*, **8**, 377–391.

Sheppard LJ, Crossley A, Cape JN, Harvey F, Parrington L, White C (1999) Early effects of acid mist on Sitka spruce planted on acid peat. *Phyton*, **39**, 1–25.

Sheppard LJ, Crossley A, Ingleby K, Woods C (2008) Implications of acidified S inputs on the fate and consequences of N deposition: results from a field manipulation of a Sitka spruce canopy in southern Scotland. *International Journal of Environmental Studies*, **65**, 409–430.

Sheppard PR, Thompson TL (2000) Effect of extraction pre-treatment on radial variation of nitrogen concentration in tree rings. *Journal of Environmental Quality*, **29**, 2037–2042.

Sievering H, Tomaszewski T, Torizzo J (2007) Canopy uptake of atmospheric N deposition at a conifer forest: part I – canopy N budget, photosynthetic efficiency and net ecosystem exchange. *Tellus B*, **59B**, 483–492.

Sparks JD (2009) Ecological ramifications of the direct foliar uptake of nitrogen. *Oecologia*, **159**, 1–13.

Sternberg LSL, DeNiro MJ, Savidge RA (1986) Oxygen isotope exchange between metabolites and water during biochemical reactions leading to cellulose synthesis. *Plant Physiology*, **82**, 423–427.

Sullivan PF, Welker JM (2007) Variation in leaf physiology of *Salix arctica* within and across ecosystems in the High Arctic: test of a dual Delta C-13 and Delta O-18 conceptual model. *Oecologia*, **151**, 372–386.

Sutton MA, Simpson D, Levy PE, Smith RI, Reis S, van Oijen M, De Vries W (2008) Uncertainties in the relationship between atmospheric nitrogen deposition and forest carbon sequestration. *Global Change and Biology*, **14**, 2057–2063.

Thomas RQ, Canham CD, Weathers KC, Goodale CL (2009) Increased tree carbon storage in response to nitrogen deposition in the US. *Nature Geoscience*, **3**, 13–17.

Tomaszewski T, Sievering H (2007) Canopy uptake of atmospheric N deposition at a conifer forest: part II – response of chlorophyll fluorescence and gas exchange parameters. *Tellus B*, **59B**, 493–501.

Tozer WC, Hackell D, Miers DB, Silvester WB (2005) Extreme isotopic depletion of nitrogen in New Zealand lithophytes and epiphytes; the result of diffusive uptake of atmospheric ammonia? *Oecologia*, **144**, 628–635.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, **7**, 737–750.

Warren CR, Adams MA (2006) Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis. *Plant, Cell and Environment*, **29**, 192–201.