

Large old trees influence patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in forests[†]

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Large old trees are the dominant primary producers of native pine forest, but their influence on spatial patterns of soil properties and potential feedback to tree regeneration in their neighbourhood is poorly understood. We measured stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in soil and litter taken from three zones of influence (inner, middle and outer zone) around the trunk of freestanding old Scots pine (*Pinus sylvestris* L.) trees, to determine the trees' influence on below-ground properties. We also measured $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in wood cores extracted from the old trees and from regenerating trees growing within their three zones of influence. We found a significant and positive gradient in soil $\delta^{15}\text{N}$ from the inner zone, nearest to the tree centre, to the outer zone beyond the tree crown. This was probably caused by the higher input of ^{15}N -depleted litter below the tree crown. In contrast, the soil $\delta^{13}\text{C}$ did not change along the gradient of tree influence. Distance-related trends, although weak, were visible in the wood $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of regenerating trees. Moreover, the wood $\delta^{15}\text{N}$ of small trees showed a weak negative relationship with soil N content in the relevant zone of influence. Our results indicate that large old trees control below-ground conditions in their immediate surroundings, and that stable isotopes might act as markers for the spatial and temporal extent of these below-ground effects. Copyright © 2008 John Wiley & Sons, Ltd.

Investigating stable isotopes has proven to be a promising way to study nutrient cycling in forest ecosystems.^{1,2} Various studies have focused on estimating the natural abundances of isotopes in different pools across different forest ecosystems,^{3–5} whereas others have followed nutrient deposition and uptake through the ecosystem by using stable isotopes as a tracer.^{6,7} In tree-rings, stable isotopes of carbon have been increasingly used to assess tree physiological responses to environmental changes, such as drought and light availability.^{8,9} Only a few studies, however, have used the stable isotopes of nitrogen in tree-rings to evaluate the dynamics in soil nitrogen,^{10–12} and more studies are needed to better understand the link between soil nutrients and $\delta^{15}\text{N}$ in trees.

In this study, we aimed to advance our knowledge on interactions between single trees and soil nutrient cycling, and on how these interactions influence spatial patterns of ^{15}N and ^{13}C in native pine forests. More specifically, we

investigated the influence of individual trees on soil N and C stable isotopes and potential feedback to N and C isotopic composition of tree-rings in regenerating trees growing in their neighbourhood. This was done by analysing C and N stable isotopes in the soil and in wood of regenerating trees along a small-scale gradient, defined by different zones of influence around a number of large old Scots pine (*Pinus sylvestris* L.) trees. Our hypothesis was that: (i) large old trees have marked effects on soil N and C content and isotopic composition close to their stems, below their crowns, and to a much lesser extent beyond their crown projections; and (ii) these below-ground soil patterns influence the isotopic composition of regenerating trees according to their spatial location relative to the large old tree.

EXPERIMENTAL

Study site

The study was carried out in Rothiemurchus Forest (57°9'N, 3°47'W) in the Cairngorms, Scotland, UK. The forest predominantly comprises Scots pine (*Pinus sylvestris* L.) trees and belongs to the eastern (Speyside), more continental group of ancient Caledonian pine forest in Scotland.¹³ These forests are characterised by strongly leached, acidic podzol soils with thick organic soil layers,¹⁴ and are assigned to the *Pinus sylvestris* – *Hylocomium splendens* forest community.¹⁵

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Sampling

Our sampling design included 16 large old and freestanding Scots pine trees, which were taken to represent 16 independent sampling plots. At each of the 16 sampling plots, we defined an inner (half of the crown projection of the centre tree), middle (from half to full crown projection) and outer (beyond crown projection) zone of influence (ZOI, cf. Fig. 1). Furthermore, altogether 48 small regenerating trees (1–3 m in height) were recorded around the 16 large old trees, if possible one each in the three ZOIs at each plot. In December 2005, a bulk sample of soil, which consisted of five soil cores of 2.5 cm in diameter and up to 12 cm depth, was taken from the surface organic horizon of each of the three ZOIs on each plot. In addition, a sample of Scots pine needle litter was collected randomly from the soil surface in the inner and middle ZOI of each plot. Wood cores were extracted from the 16 large old trees at breast height and from the 48 small trees at a height of approximately 50 cm. This was done using an increment corer, which was cleaned with ethanol before each extraction.

Analysis

Tree-ring width and age was measured using the Lintab3 measuring device (F. Rinn S.A., Heidelberg, Germany). Afterwards, the outer most ten rings were cut off the dried cores, ground with a liquid nitrogen mill and weighed into tin capsules to measure the stable isotopes of C and N. Since we analysed isotope contents to make comparisons between

single trees and in the sapwood only, we decided to use whole wood samples without any prior extractions. Sub-samples of 3–6 mg were processed for C and 10–20 mg for N. The measurements of wood C and N were processed separately due to the high C:N ratio in the wood of large and small trees (cf. Table 1). Total C and N were measured using a Carlo Erba NA1500 elemental analyser (Erba Science, Swindon, UK). The natural abundances of carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N) isotopes were determined with an interfaced continuous flow isotope ratio mass spectrometer (IRMS) (Europa Scientific Ltd., Crewe, UK) with measuring accuracies of 0.1‰ and 0.2‰, respectively. The sieved (mesh size = 0.5 mm) and oven-dried humus samples, which were cleared from coarse plant litter and roots >1 mm, were ground with a mortar and pestle; 8–16 mg were weighed into tin capsules and processed as described above.

Stable isotope ratios were expressed relative to the Vienna Pee Dee Belemnite standard for carbon ($\delta^{13}\text{C} = ((^{13}\text{C}/^{12}\text{C}_{\text{sample}})/(^{13}\text{C}/^{12}\text{C}_{\text{standard}}) - 1) \times 1000$; [‰]). The nitrogen isotope ratio was expressed relative to atmospheric air ($\delta^{15}\text{N} = ((^{15}\text{N}/^{14}\text{N}_{\text{sample}})/(^{15}\text{N}/^{14}\text{N}_{\text{standard}}) - 1) \times 1000$; [‰]). Differences in the average isotopic composition of soil, wood and litter were tested by an unpaired, two-sided t-test at the 95% confidence limit.

RESULTS

We detected a significant trend of increasing $\delta^{15}\text{N}$ values with distance from the stem of the centre tree (Fig. 2(a)): the soil $\delta^{15}\text{N}$ was about half as much enriched close to the stem (inner ZOI, median = 1.5‰) as it was beyond the influence of the crown (outer ZOI, median = 2.8‰). In contrast, we found no spatial gradient for soil $\delta^{13}\text{C}$ between the three ZOIs (Fig. 2(b)). Average values for $\delta^{13}\text{C}$ of surface soil integrated across all three ZOIs (−28.2‰) were significantly enriched compared with Scots pine needle litter (−29.1‰), but depleted compared with wood (−27.5‰), whereas average values for $\delta^{15}\text{N}$ were enriched in soil (2.1‰) but depleted in wood (−1.1‰) and even more so in needle litter (−1.6‰) (Table 1). While differences between soil and wood $\delta^{15}\text{N}$ were significant ($p < 0.001$), there was no significant difference between needle litter and wood $\delta^{15}\text{N}$. Wood of small regenerating trees was not significantly different from wood of large old trees with respect to C and N contents and isotopic composition (Table 1). The age of the old trees ranged between 120 and 250 years.

When examining stable isotopes in the wood of the small regenerating trees, which were grouped according to their location in the three ZOIs (Figs. 2(c) and 2(d)), we found no significant gradient, either for $\delta^{15}\text{N}$ or for $\delta^{13}\text{C}$; there was a slight tendency for wood to be isotopically lighter in the inner than in the middle and outer ZOI. Median values were greatest in the middle ZOI. Variability in the $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ of small regenerating trees was relatively high. The wood $\delta^{15}\text{N}$ of regenerating trees showed a weak negative relationship with N content of the soil in their relevant ZOI ($R^2 = 0.24$, $p < 0.001$, cf. Fig. 3).

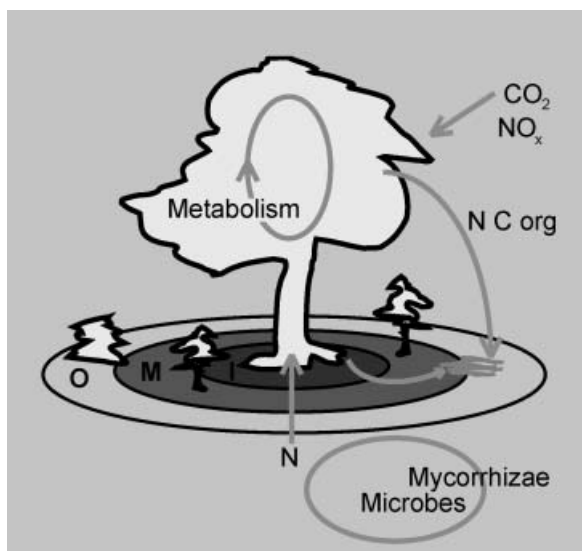


Figure 1. Sampling plot including inner, middle and outer zone of influence (ZOI) around large old trees and several regenerating trees. Large old trees measure approx. 15–20 m in height and represent crown projection areas of around 10 m in diameter. C and N are incorporated differently in wood by uptake via the air or the soil. Nutrients are translocated, stored and remobilised inside the tree. Needle and root litter, as well as root exudates, enter the soil. Mycorrhizae and microbes alter availability of C and N compounds in the soil. All the mentioned processes may discriminate against the heavy isotopes ^{13}C and ^{15}N .

Table 1. N and C contents and stable isotope ratios measured from the ten outer most tree-rings of large old and small regenerating Scots pine trees, in comparison with values for Scots pine needle litter and soil samples collected in their close neighbourhoods

	N [%] tw	C [%] tw	$\delta^{15}\text{N}$ [‰] vs air	$\delta^{13}\text{C}$ [‰] vs VPDB	C:N
Large trees (N = 16)	0.1 ± 0.0	49.3 ± 0.2	-1.1 ± 0.4	-27.5 ± 0.2	461 ± 19
Small trees (N = 48)	0.1 ± 0.0	49.1 ± 0.1	-1.5 ± 0.3	-27.1 ± 0.1	472 ± 9
Needle litter (N = 16)	0.8 ± 0.0	52.7 ± 0.2	-1.6 ± 0.2	-29.1 ± 0.1	67 ± 3
Soil (N = 48)	0.9 ± 0.1	31.6 ± 2.9	2.1 ± 0.2	-28.2 ± 0.1	35 ± 1

Means \pm SE, at 1 decimal point.

DISCUSSION

We found a clear increase in surface soil $\delta^{15}\text{N}$ from the inner to the outer zone of influence (ZOI) of large dominant pine trees, indicating a strong effect of individual trees on the stable isotope composition of forest soil. We cautiously propose that this effect of trees on soil $\delta^{15}\text{N}$ was due to greater inputs of pine litter within the inner and middle ZOI. This view is supported by the finding that, due to discrimination of ^{15}N during N uptake and incorporation (Fig. 1), pine needle litter on the plots was significantly depleted in ^{15}N by approximately 3.7‰ compared with soil. This suggests that litter input is a more important determinant of the low $\delta^{15}\text{N}$ values in the surface soil beneath pine trees than decomposition effects, which have been found to increase $\delta^{15}\text{N}$ values.¹⁶ For soil $\delta^{13}\text{C}$, no such gradient was observed, probably because litter $\delta^{13}\text{C}$ was more similar to soil

$\delta^{13}\text{C}$. Effects of needle litter input on surface soil $\delta^{13}\text{C}$ may therefore become observable only after trees have been present for longer than 250 years, and if ^{13}C -depleted organic matter has accumulated with time.¹⁷

While there was no clear spatial gradient in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in wood taken from regenerating trees growing within different spatial zones around large trees, we did detect a weak negative relationship between the wood $\delta^{15}\text{N}$ of regenerating trees and soil total N content. This finding supports the notion that $\delta^{15}\text{N}$ measurements in tree-rings have the potential to indicate soil N status, as previously suggested by a few studies: In a tracer experiment, ^{15}N -labelled nitrogen was applied to tree roots and produced a signal in the wood mainly 1 year after the application.¹⁰ In two other studies, a long-term decrease in natural $\delta^{15}\text{N}$ values in tree-rings of *Tsuga canadensis* in North America¹⁸ and *Quercus pubescens* in the western Mediterra-

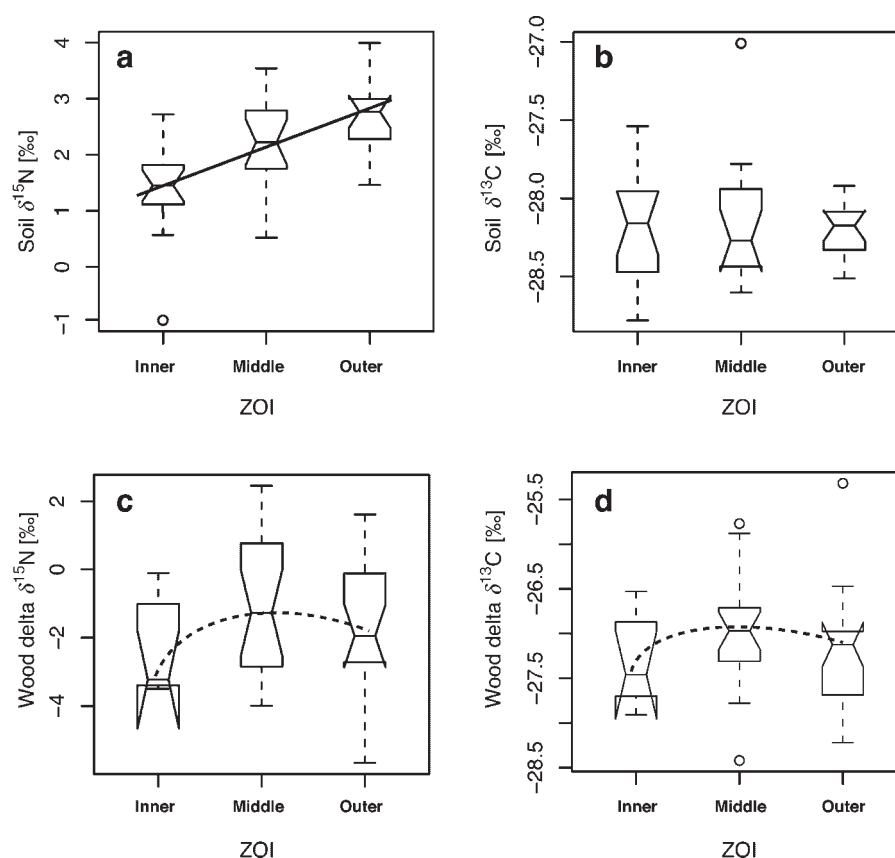


Figure 2. Notched boxplots of stable isotopes of N and C in soil (a, b) and in wood of regenerating trees (c, d) grouped according to zone of influence (ZOI) of the large old tree. If notches do not overlap, differences are significant ($p < 0.05$).

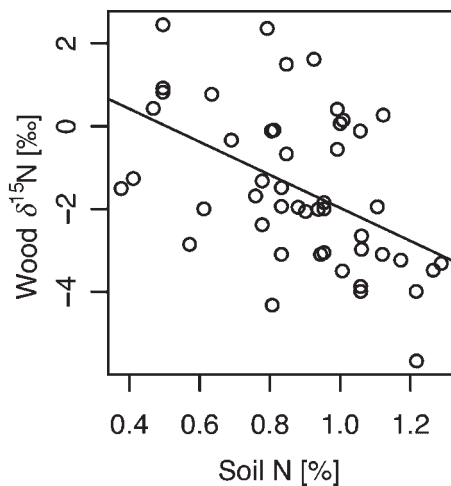


Figure 3. Relationship between wood $\delta^{15}\text{N}$ in regenerating trees and N content in the soil ($R^2 = 0.24$; $p < 0.001$).

nean¹⁹ was explained by potential effects of global change on soil N cycling. Linkages between soil total N content and $\delta^{15}\text{N}$ are more likely to occur in forest ecosystems where N is limiting, such as in many temperate boreal forests.²⁰ However, from this study we are not able to determine whether the reason for this relationship is that trees had to compete more intensely for ^{14}N -compounds under greater N limitation, consequently leading to higher $\delta^{15}\text{N}$ in wood. In general, assessing the contributions from different soil N sources based on plant $\delta^{15}\text{N}$ is complicated²¹ especially in tree species such as Scots pine which are associated with mycorrhizal fungi that uptake organic N from soil.²² It is possible that the $\delta^{15}\text{N}$ in wood of regenerating trees was depleted by the low soil $\delta^{15}\text{N}$ (inner ZOI) on the one hand, and by higher total N availability (outer ZOI) on the other (data not shown), leading to the observed pattern in wood $\delta^{15}\text{N}$ from the inner to the outer ZOI.

In contrast to $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ in wood is known to be mainly a function of above-ground processes at the leaf-atmosphere interface, i.e., of gaseous uptake of CO_2 during photosynthesis.^{8,9} As a consequence, in forests with sufficient water availability, such as the one studied here, one would expect $\delta^{13}\text{C}$ to be mainly a function of light availability, being lower below the crown due to the lower rate of photosynthesis. However, in this regard our results were ambiguous, in that we did not detect a significant increase in $\delta^{13}\text{C}$ in wood samples of regenerating trees growing on a light gradient from the inner to the outer ZOI. This lack of pattern might suggest that the gradient in light availability was not pronounced enough around our freestanding large old trees. Furthermore, small trees growing close to the soil surface may generally be depleted in ^{13}C , because they assimilate depleted CO_2 originating from soil respiration.²³

Our results confirm earlier studies that $\delta^{15}\text{N}$ is a useful parameter for studying N cycling in forests.²⁰ The weak

negative relationship which was found between $\delta^{15}\text{N}$ in the most recent ten tree-rings and total N content in the surface soil provides further indication of the potential of $\delta^{15}\text{N}$ in tree-rings for monitoring long-term N changes in forest ecosystems.^{10,11,18} However, our study also revealed that great care has to be taken when sampling forest soil or tree cores for stable isotopes of N due to high spatial heterogeneity in forests. Large old trees can be expected to play a significant role in altering surface soil isotopic composition in their surroundings in time and space. Even more complex patterns will evolve in mixed forests, where the litter quality between trees may differ substantially.

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