UvA-DARE (Digital Academic Repository)

Split-root labelling to investigate 15N rhizodeposition by Pinus sylvestris and Picea abies

Veerman, L.; Kalbitz, K.; Schoorl, J.C.; Tietema, A.

Link to publication

Taylor & Francis
Taylor & Francis Group

Split-root labelling to investigate ^{15}N rhizodeposition by *Pinus sylvestris* and *Picea abies**

Liz Veerman[a], Karsten Kalbitz[b], Jorien C. Schoorl[a] and Albert Tietema[a]

[a]Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam (UvA), Amsterdam, Netherlands; [b]Institute of Soil Science and Site Ecology, Technical University Dresden, Dresden, Germany

ABSTRACT

We investigated the transfer of ^{15}N into the soil via ^{15}N uptake and release by tree roots, which involves the principles of the split-root technique. One half of the root system received an injection of $(^{15}NH_4)_2SO_4$ and the other half equivalent amounts of $(NH_4)_2SO_4$ at ^{15}N natural abundance level. ^{15}N was transferred from one side of the root system (^{15}N side) to the other side (^{14}N side) and released into the soil. The method was conducted with Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* [L.] Karst). Two concentration levels of $(NH_4)_2SO_4$ were used, corresponding with annual N deposition in the Netherlands (30 kg N ha^{-1}) and a twelfth of that (2.5 kg N ha^{-1}). Samples were taken 3 and 6 weeks after labelling and divided into needles + stem, roots, rhizosphere and bulk soil. Already 3 weeks after labelling, Scots pine took up 23.7 % of the low and 9.1 % of the high amounts of ^{15}N, while Norway spruce took up 21.5 and 32.1 %, respectively. Both species transported proportions of ^{15}N to the rhizosphere (0.1–0.2 %) and bulk soil (0.3–0.9 %). The method is a useful tool to investigate the fate of root-derived N in soils, for example, for the formation of stable forms of soil organic matter.

1. Introduction

Trees can release organic nitrogen from their roots into the soil through rhizodeposition [1].The rhizodeposits can vary from low-molecular-weight soluble root exudates to high-molecular-weight insoluble substances [2]. The amount, type and function of these N-rich rhizodeposits are controlled by various biotic and abiotic factors such as moisture stress, vegetation type, soil type and microbial activity, influencing nutrient availability and organic matter dynamics in the soil [2,3]. As described by the multi-layered model of Kleber et al. [4], organic N compounds might be important for the stabilization of soil organic matter (SOM), due to the preferential adsorption of N-rich compounds to juvenile mineral surfaces.

Rhizodeposition of (in)organic N compounds can be investigated with several ^{15}N enrichment techniques, which are applied either above ground: leaf or stem labelling [5,6], or below ground: root labelling [7]. The latter approach follows direct uptake of ^{15}N via the roots and can be carried out by transplantation of previously labelled plants [8,9] or via split-root labelling [10,11]. Most of the ^{15}N enrichment studies focused on ^{15}N rhizodeposition in crop systems [3,12], while there is a knowledge gap on N rhizodeposition in forest ecosystems [13]. To be able to mimic the situation in a forest as much as possible, split-root labelling seems the most suitable method because this method follows the physiological pathway of N assimilation via roots and allows relatively homogeneous enrichment in all N pools, already in an early stage after labelling [3].

This split-root labelling technique enables the detection of root-derived N among the different pathways of N in forest soils. The potential of this technique will be investigated using two commonly occurring European coniferous species, Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* [L.] Karst). These species have different root structures depending on their function in a specific ecological niche [14]. However, when growing conditions are kept similar, flexibility in root development can produce similar root structures [15]. Experiments related to N uptake by coniferous seedlings showed a fast immobilization of especially ammonium (NH_4^+), within a few hours [16,17], though the time it takes for these conifers to release N back in the soil is unclear. Several studies [18–20] show that conifers prefer uptake of NH_4^+ over nitrate (NO_3^-), provided that the NH_4^+ nutrient levels are within an acceptable range. When the NH_4^+ levels are too high, it might cause seedling mortality due to toxicity [19,20]. Furthermore, high N addition might promote higher levels of mineral N rhizodeposition instead of organic N rhizodeposition [5].

The objective of this study was to test the suitability of the split-root method to measure ^{15}N rhizodeposition by coniferous tree seedlings and to investigate useful time frames and ^{15}N addition levels. This was investigated with Scots pine and Norway spruce seedlings at low and high levels of ^{15}N–$(NH_4)_2SO_4$ over a period of 3 and 6 weeks. We hypothesized that after 3 weeks, ^{15}N would be released by the roots in the soil compartment where it was not added. We expected to find higher ^{15}N rhizodeposition in Norway spruce due to its higher above- (needles + stem) and belowground (roots) tree biomass and its dominant lateral root structure in natural environments [13]. It was expected that ^{15}N rhizodeposition levels would be higher when higher concentrations of ^{15}N were added because there would be more ^{15}N available for the trees to take up. Furthermore, we expected ^{15}N levels to increase in the soil over time, due to microbial immobilization of organic and inorganic N rhizodeposition.

2. Methods

2.1. Experimental design

Twenty-two-year-old Scots pine (15 cm long) and 22-year-old Norway spruce (30 cm long) seedlings were planted individually in a split-root set-up (Figure 1). The plants were incubated in a greenhouse from November 2015 till January 2016. Prior to plantation, the roots were washed with water to remove the potting soil and separated in two (optically) equal parts. The plants were planted during autumn/winter time, when the sap flow was less active; to overcome desiccation of the roots. Greenhouse temperature was kept a 20 °C. The soil compartments were filled with approximately 2.4 kg sand derived from a haplic
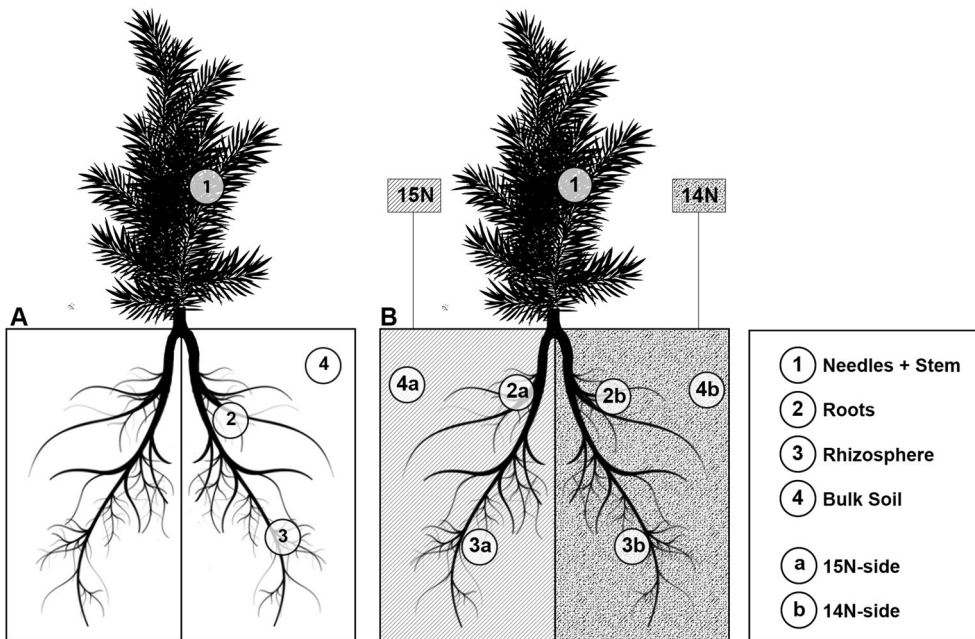
Figure 1. Diagram showing samplings prior to ^{15}N addition (A), and after addition of ^{15}N–$(NH_4)_2SO_4$ at one side (a) and $(NH_4)_2SO_4$ at ^{15}N natural abundance to the other side (b) of the root system (B).

podzol from an experimental site at Oldebroek, the Netherlands [21]. We used sand from the B-horizon of a depth of 5.5–27.0 cm (pH 4.3; C/N ratio 17.7), more details about the soil can be found in [21]. The sandy soil was sieved and homogenized prior to plantation. The seedlings were watered three times per week, to average soil moisture content (W_d) of 0.15 ± 0.04. $(NH_4)_2SO_4$ was injected in the soil at 8 fixed positions with 12-cm long needles. This was done to equally spread the amount of added $(NH_4)_2SO_4$ in each pot and to minimize the change of contamination at the ^{14}N side of the set-up. The needles were connected to a syringe pump, which pumped the $(NH_4)_2SO_4$ solution into the soil while the needles were pulled towards the soil surface at a constant speed. The soil containers were injected either with high (17.4 mg N kg^{-1}) or low (1.45 mg N kg^{-1}) concentrations of highly enriched $(^{15}NH_4)_2SO_4$ (98 at.%) at one (^{15}N) side of the split-root set-up and equivalent amounts of $(NH_4)_2SO_4$ (^{15}N natural abundance) at the other (^{14}N) side. The concentration corresponded with the annual N deposition in the Netherlands (30 kg N ha^{-1}) [22] and with one-twelfth of that (2.5 kg N ha^{-1}).

2.2. Harvest and laboratory analysis

Prior to N additions, reference samples were taken from above- (needles + stem) and belowground parts (roots, rhizosphere and bulk soil) (Figure 1(A)) in order to measure the natural abundance of ^{15}N. Then, 3 and 6 weeks after labelling, the abundance of ^{15}N was analysed in these N pools at both ^{15}N and ^{14}N sides (Figure 1(B)). At each time step, three seedlings of each species and treatment were harvested (12 trees per harvest). During harvest, trees were cut at ground level, and the above ground parts were separated into needles and stem. The roots were carefully removed from the bulk

soil, while the rhizosphere soil was still attached to the roots. The roots were washed with 45-ml water to separate the rhizosphere soil from the roots. Plant material was freeze-dried and soil samples were oven-dried at 40 °C. After drying, the soil samples were sieved (2 mm diameter) and ground with a ball mill. Organic samples were ground with a centrifuge mill, all to a homogenous powder. Approximately 10 g of the moist soil samples were dried at 105 °C to calculate total dry mass (g). ^{15}N was analysed in all samples (10 mg organic and 40 mg mineral) with a continuous flow elemental analyser (Vario ISOTOPE cube, Hanau, Germany) connected to an isotope ratio mass spectrometer (Vision Isoprime, Manchester, UK). Reference gas (high-purity N_2 gas) was calibrated to atmospheric N_2 standard (at-air) using certified reference materials (IAEA-N2, IAEA-NO3 and USGS-32; from the International Atomic Energy Agency, Vienna).

2.3. Calculations

2.3.1. Abundance and recovery of ^{15}N in plant–soil system

The abundance of ^{15}N in the N pools was either expressed as delta ^{15}N (δ^{15}N ‰) or as percentage ^{15}N (at.% ^{15}N) of total N. δ^{15}N (‰) was calculated as

$$\delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000\,\text{‰,} \tag{1}$$

in which R is the molar ratio of ^{15}N to ^{14}N, with atmospheric N_2 used as standard ($R_{\text{standard}} = 0.0036764$). The percentage ^{15}N (at.% ^{15}N) was calculated as

$$\text{at.\%}^{15}\text{N} = \frac{R_{\text{sample}}}{1 + R_{\text{sample}}} \times 100\%. \tag{2}$$

The ^{15}N mass balance approach of Wessel et al. [23] was used to calculate the recovery (%^{15}N$_{\text{rec},i}$) of the applied ^{15}N in each N pool (i) as

$$\%^{15}\text{N}_{\text{rec},i} = \frac{m_i(\text{at.\% }^{15}\text{N}_i - \text{at.\% }^{15}\text{N}_{i,\text{ref}})}{M_{\text{label}}} \times 100\%, \tag{3}$$

where %^{15}N$_{\text{rec},i}$ was the atom percentage of the added ^{15}N that was recovered in the N pool$_i$ and at.%^{15}N$_i$ as ^{15}N abundance in N pool i and at.%^{15}N$_{i,\text{ref}}$ as reference in N pool i, before ^{15}N was added. N pool sizes (m_i) were used as N mass (g N) in each compartment. To calculate N pool sizes (m_i), dry matter of each N pool was multiplied by the measured N percentage of that pool. The added ^{15}N (M_{label}) was the total mass (g ^{15}N) of the added tracer.

2.3.2. ^{15}N rhizodeposition

^{15}N rhizodeposition was estimated based on recovery (%^{15}N$_{\text{rec}}$) levels in the rhizosphere and bulk soil as calculated by Equation (3) and based on the Janzen and Bruinsma equation [24]:

$$\%\text{NdfR} = \frac{\text{at.\% }^{15}\text{Nsoil} - \text{at.\% }^{15}\text{Nsoil}_{\text{ref}}}{\text{at.\% }^{15}\text{Nroots} - \text{at.\% }^{15}\text{Nroots}_{\text{ref}}} \times 100\%, \tag{4}$$

where the ^{15}N rhizodeposition (%NdfR) is the ^{15}N atom percentage of the soil (at.%^{15}Nsoil–at.%^{15}Nsoil$_{\text{ref}}$) related to the atom percentage in the roots (at.%^{15}Nroots–at.%^{15}Nroots$_{\text{ref}}$).

Corrections for variation in the background signal of the non-labelled reference samples are given as ref. Enrichment (at.%^{15}N) in the soil was the sum of enrichment (at.%^{15}N) in the rhizosphere and the bulk soil, in proportion to the N pool sizes (g N).

2.4. Assumptions

Both methods that were used to estimate ^{15}N rhizodeposition assume (1) a homogenous ^{15}N distribution of the tracer within the N pools. Furthermore, ^{15}N rhizodeposition based on the Janzen and Bruinsma equation [24] assumes (2) comparable ^{15}N enrichment levels in both roots and rhizodeposits.

2.5. Data analysis

With the limited amount of observations per treatment and tree species ($n = 3$), normality of the data set could neither be accepted nor rejected. Assuming that the data set was normally distributed, we performed an independent (differences between species and treatments) and paired (change over time) two-tailed t-test with Welch correction for non-homogeneity of variance in R.

3. Results

3.1. Enrichment and recovery of ^{15}N in the plant–soil system

The total ^{15}N recovery per species, treatment and time step varied between 62 and 93 % of total ^{15}N added, of which the majority (40–70 %) remained available at the ^{15}N side (roots + soil) of the set-up (Figure 2). The proportions (%) that were taken up and released
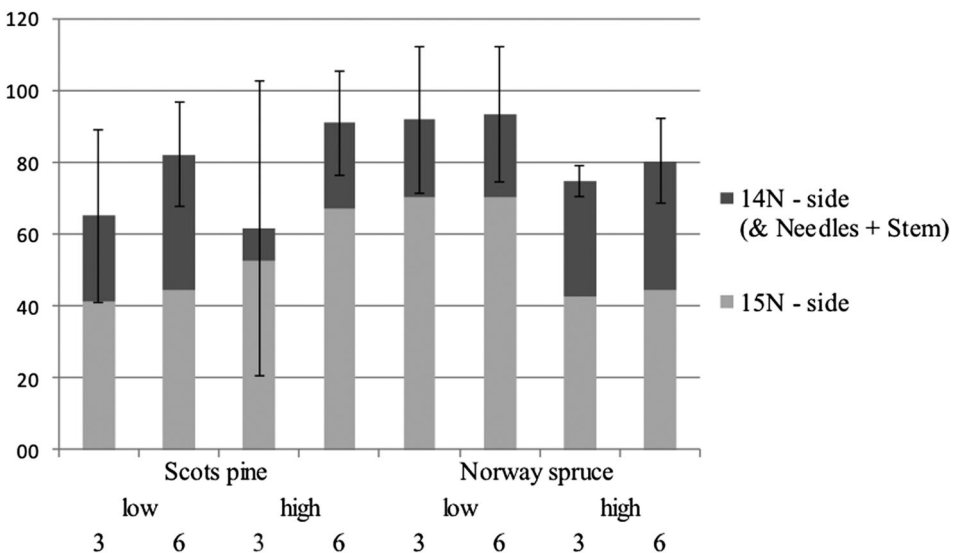
Figure 2. Average (± sd) recoveries (% of applied ^{15}N) for each species, treatment and time step. Divided into the ^{14}N side (& needles + stem) and the ^{15}N side soil compartments.
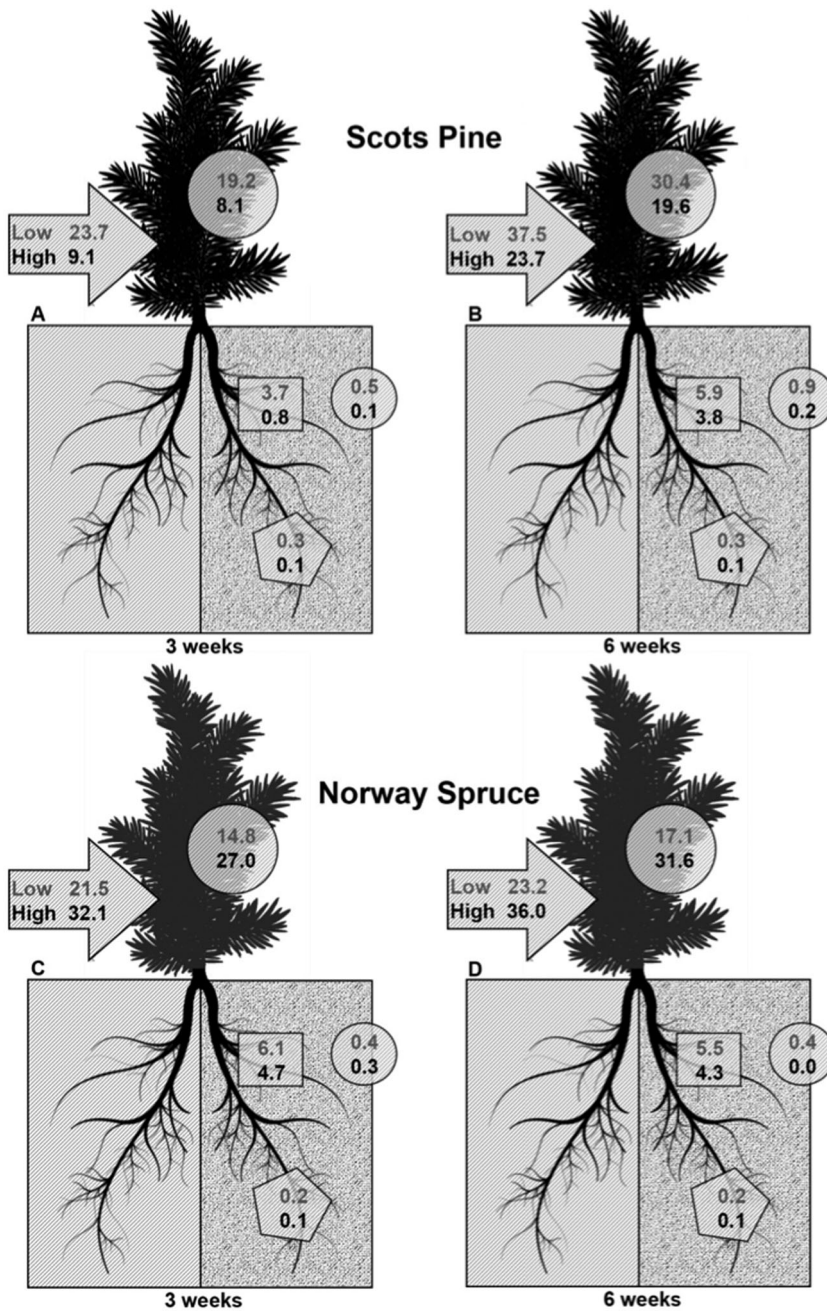
Figure 3. Average recoveries (% of applied ^{15}N) for Scots pine (A and B) and for Norway spruce (C and D) at three and 6 weeks after labelling. There are two ^{15}N addition levels, low (light grey) and high (dark grey). The arrow indicates total recovery ($\%^{15}N_{rec}$) in above- and belowground (^{14}N side) compartments. Belowground compartments are divided in roots (square), rhizosphere (pentagon) and bulk soil (circle). For further explanation of the soil compartments see Figure 1.

(at the ^{14}N side) in the various N pools are visualized in Figure 3. It shows a fast uptake and release of ^{15}N in the above- (needles + stem) and belowground (roots, rhizosphere and bulk soil) N pools, for both tree species and for low and high concentrations of ^{15}NH$_4^+$. Three weeks after labelling, Scots pine seedlings took up 23.7 % of the applied low and 9.1 % of the applied high ^{15}NH$_4^+$ concentrations (Figure 3(A)). Six weeks after labelling, these levels increased towards 37.5 and 23.7 %, respectively (Figure 3(B)). For Norway spruce this was 21.5 and 32.1 % after 3 weeks (Figure 3(C)), and 23.2 and 36.0 % after 6 weeks (Figure 3(D)), for the low and high treatment, respectively. The ^{15}N recovery values for the N pools are also shown in Table 1, combined with the associated ^{15}N abundance levels and pool sizes.

3.1.1. Treatment effects

Treatment effects were most evident when looking at the ^{15}N abundance levels (Table 1). Especially Norway spruce showed significant higher abundance levels in the needles + stem and roots within the high treatment compared with the low treatment. In Scots pine, significant higher abundance levels were only found in the needles + stem at time 6 within the high treatment ($P < .1$). Still the average abundance values of the high treatments were always higher compared with the low treatment, confirming our expectations that the trees took up more ^{15}N when there was more ^{15}NH$_4^+$ available. ^{15}N recovery levels in the (above- and belowground on the ^{14}N side) Scots pine N pools were generally higher within the low treatment compared to the high treatment, most likely because a relatively larger proportion of ^{15}N remained behind at the ^{15}N side of the set-up within the high treatment (Figure 2). In the bulk soil samples of Scot pine, this resulted in significant higher recovery levels ($P < .1$; $P < .05$) in the low treatment in comparison with the high treatment at both time steps. For Norway spruce, significant higher recovery levels ($P < .1$; $P < .05$) were found in the rhizosphere samples within the low treatment at both time steps. ^{15}N recovery in the needles + stem was significantly higher ($P < .01$) in the high treatment, 6 weeks after labelling.

3.1.2. Species effects

Species effects were most evident in terms of tree biomass. Tree biomass (needles + stem and roots) of Norway spruce was often significantly higher ($P < .05$; $P < .001$) compared to Scots pine. This resulted in generally (not significantly) lower ^{15}N abundance levels for Norway spruce compared to Scots pine. Despite the lower ^{15}N abundance levels, recovery levels of Norway spruce in the needles + stem and roots within the high treatment were often significantly ($P < .1$; $P < .05$) higher in Norway spruce compared to Scots pine.

3.1.3. Influence of time

To investigate seedling growth, tree biomass pool sizes (g) at 3 and 6 weeks after labelling were compared with the time before labelling (t_0). Compared to t_0, significant increases were found at t_6, for Norway spruce aboveground biomass (needles + stem) within the low treatment ($P < .1$) and for Norway spruce roots within the low ($P < .05$) and high ($P < .1$) treatment. To investigate changes in ^{15}N abundance over time, the abundance levels at 3 and 6 weeks after labelling were first compared with the natural abundance levels (t_0) to confirm that there was some actual ^{15}N uptake and release. As expected, ^{15}N abundance levels at t_3 and t_6 were in most cases significantly ($P < .1$; $P < .01$) higher

Table 1. Pool sizes (g), abundance ($\delta^{15}N$ ‰) and recovery ($\%^{15}N_{rec}$) levels at 3 and 6 weeks after labelling of above (needles + stem) and below ground (roots, rhizosphere and bulk soil) samples at the ^{14}N side of the split-root set-up.

Species	Treatment	Sample type	Pool size (g) 0	Pool size (g) 3	Pool size (g) 6	Abundance ($\delta^{15}N$ ‰) 0	Abundance ($\delta^{15}N$ ‰) 3	Abundance ($\delta^{15}N$ ‰) 6	Recovery ($\%^{15}N_{rec}$) 3	Recovery ($\%^{15}N_{rec}$) 6
Pine	Low	Needles + Stem	4.3±0.4	5.3±1.2	4.6±0.4	2.5±0.3	4240±1006 t0** sp*	7712±3991 t0*	19.15±8.57	30.42±18.33
		Roots	1.8±0.2	2.3±0.5 tr*	2.4±0.6	3.0±0.3	2943±591 t0**	5043±2538 t0*	3.69±2.16	5.89±1.87
		Rhizosphere	67.7±22.3	60.2±28.9	84.4±21.4	3.4±1.0	221±89 t0*	229±58 t0** sp*	0.25±0.24	0.27±0.14
		Bulk	2316.5±84.8	2159.3±132.4	2104.8	3.4±1.2	23±9 t0**	34±13 t0*	0.53±0.15 tr**	0.89±0.40 tr*
	High	Needles + Stem		4.4±0.5	5.2±1.1		25,305±13,715 t0** tr *	44,289±14,528 t0*	8.14±4.58	19.56±7.68 t*
		Roots		1.1±0.1	2.2±0.6		13,345±10,568	31,529±8183 t0**	0.80±0.54	3.83±1.03 t***
		Rhizosphere		33.0±9.8	77.3±34.0		1817±1065	1232±988 sp*	0.10±0.09	0.10±0.07
		Bulk		2103.2±89.3	2104.8		55±13 t0**	78±14 t0**	0.11±0.05	0.22±0.09 sp* t*
Spruce	Low	Needles + Stem	10.5±1.4	11.6±1.3 sp***	13.1±1.5 t0* sp***	2.6±0.1	2091±937 t0*	2461±903 t0*	14.79±7.71	17.13±3.48
		Roots	4.5±0.5	4.6±1.3	6.4±0.7 t0**sp***	2.3±0.2	2871±475 t0**	1884±951 t0*	6.05±0.91	5.45±3.48
		Rhizosphere	71.3±10.3	79.6±10.4	129.2±45.5	2.8±0.9	150±43 t0**	97±12 t0***	0.20±0.03 tr** t**	0.15±0.04 tr*
		Bulk	2472.7±43.6	1991.4±212.4	2104.8	3.0±0.8	19±9	16±10	0.37±0.12	0.42±0.34
	High	Needles + Stem		12.1±2.2 sp**	13.9±1.0 sp***		39,759±6009 t0*** tr***	40,753±3770 t0***tr***	27.02±0.78 sp**	31.59±3.81 sp* tr***
		Roots		5.5±0.3 sp***	5.9±0.1 t0* sp***		18,842±5108 t0** tr*	13,226±674 t0***sp* tr***	4.68±1.72 sp**	4.26±0.29
		Rhizosphere		142.1±16.6 sp*** tr*	110.5±41.5		460±217 t0*	391±182 t0*	0.07±0.04	0.05±0.01
		Bulk		2233.3±121.8	2104.8		159±116	19±6 t0**	0.31±0.23	0.04±0.02

Note: Given as mean ± standard deviation ($n = 3$). Bulk soil pool sizes (g) at week 6 are mean values of pool sizes at week 3, due to loss of the data. Significant differences between species (sp), treatments (tr) or time steps (t_0 or t) are given at levels: *** ($P < .01$), ** ($P < .05$) or * ($P < .1$).

Table 2. Rhizodeposition presented as the percentage of total soil ^{15}N, derived from roots (%NdfR), for each tree species and treatment, 3 and 6 weeks after labelling.

		%NdfR			
		3 weeks		6 weeks	
Pine	Low	1.01±0.2	sp** tr**	0.98±0.6	tr*
	High	0.75±0.6		0.29±0.2	
Spruce	Low	0.7±0.4	tr**	1.09±0.2	tr**
	High	0.46±0.6		0.3±0.1	

Note: Significant differences between species (sp) or treatments (tr) are given at levels: ** ($P < .05$) or * ($P < .1$).

in comparison with t_0, except for the high treatment of Scots pine at t_3. This was most likely affected by the high standard deviations. Actual changes in ^{15}N recovery from 3 to 6 weeks after labelling were small. However, significant increases in ^{15}N recovery were found within the high treatment of Scots pine in the needles + stem ($P < .1$), roots ($P < .001$) and bulk soil ($P < .1$). ^{15}N recovery in the rhizosphere of Norway spruce was significantly higher ($P < .05$) at t_3 compared to t_6.

3.2. ^{15}N rhizodeposition

We used two different approaches to estimate ^{15}N rhizodeposition, the mass balance approach and the Janzen and Bruinsma equation [24]. ^{15}N recovery levels in the rhizosphere varied between 0.05 and 0.27 % and in the bulk soil between 0.04 and 0.89 % (Figure 2 and Table 1). The relatively small pool sizes of the rhizosphere resulted in higher ^{15}N abundance levels in comparison with the bulk soil, but in lower recovery levels. ^{15}N recovery levels in both soil pools were often higher for Scots pine than for Norway spruce, but only significantly higher in the high treatment at t_6 ($P < .1$). For Scots pine, the ^{15}N recovery levels in the soil were generally higher in the low treatment compared to the high treatment, which were significantly higher in the bulk soil at both time steps ($P < .1$; $P < .05$). Scots pine ^{15}N recovery levels in the soil slightly increased over time, but significantly only in the high treatment at t_6 ($P < .1$). For Norway spruce, ^{15}N recovery levels in the soil (rhizosphere + bulk soil) were generally higher (not significantly) in the low treatment compared to the high treatment. ^{15}N recovery levels in the soil slightly decreased over time, except for the bulk soil pool within the low treatment at t_6. The soil (rhizosphere + bulk soil) to root ratio (%Ndfr) defined by Janzen and Bruinsma [24] to estimate ^{15}N rhizodeposition was often higher for Scots pine than for Norway spruce, only significantly higher ($P < .05$) within the low treatment at 3 weeks after labelling. For both species %NdfR values were significantly higher within the low treatment compared to the high treatment ($P < .1$; $P < .05$). Variations over time were not significant. Both approaches showed similar trends in terms of treatments, species and time effects.

4. Discussion

4.1. Suitability of the split-root method

The main purpose of this paper was to test the suitability of the split-root method to measure ^{15}N rhizodeposition. The technique proved to be a suitable method for that

purpose and could be applied to both treatments and tree species. Due to the pot growing conditions of the seedlings, both root systems consisted primarily of lateral roots, which made it easy to separate the roots into two parts without severe damage. We decided to use one pulse label instead of continuous flow or multiple pulses, because this approach provided information about the residence time of the applied ^{15}N in a specific N pool [3]. Thereby, not all injected ^{15}N was taken up by the roots at the same time. A part remained at the ^{15}N side of the soil (Figure 2) for later plant uptake or was immobilized by micro-organisms and not available anymore for plant uptake. The high variation between the replicates ($n = 3$) was most likely related to differences in tree biomass and possibly caused by an unequal separation of the roots in terms of size and mass. Unequal root separation would not only affect ^{15}N uptake by the roots, but due to the addition of natural abundance $(NH_4)_2SO_4$ at the non-labelled side, this would also cause unequal ^{14}N incorporation and dilution in the replicates [25]. Variation between the replicates might have been reduced when seeds were used to self-grow the seedlings and to separate the roots in an early stage.

4.2. Measuring ^{15}N rhizodeposition

^{15}N rhizodeposition was estimated based on two approaches: (1) ^{15}N recovery (%) in the rhizosphere and bulk soil, and (2) the percentage of enriched soil, relative to the roots (%NdfR). Using both approaches we observed slightly higher ^{15}N rhizodeposition levels for Scots pine compared to Norway spruce seedlings. Despite the lower above- (needles + stem) and belowground (roots) pool sizes of Scots pine, their ^{15}N rhizodeposition was higher. A possible explanation is that Scots pine seedlings reached their maximum [17] in terms of ^{15}N storage and translocation to shoot and roots, and consequently released more ^{15}N to the soil. Furthermore, the extra released ^{15}N by Scots pine seedlings might consist of higher proportions of inorganic N as shown by Janzen [5], which requires further identification of the N compounds. Comparing both approaches, we observed higher ^{15}N rhizodeposition within the low treatment in comparison with the high treatment. With increased addition levels, the percentage of enriched soil relative to the roots (%NdfR) or to the total mass balance (%$^{15}N_{rec}$) decreased.

For both methods, the first assumption (homogenous ^{15}N distribution) was largely encountered when most N pools (needles + stem, roots and rhizosphere) were completely milled and mixed prior to the ^{15}N analysis. The bulk soil samples were not entirely milled because of the large pool sizes (approximately 2 kg). For the Janzen and Bruinsma method [24], the second assumption (comparable ^{15}N abundance levels in both roots and rhizodeposits) was more difficult to meet because changes in root ^{15}N enrichment levels were not one-to-one related to changes in ^{15}N enrichment levels in the soil. ^{15}N levels in the roots could have been diluted due to shoot or root growth, causing overestimated results. Though, the remainder of ^{15}N in the soil (^{15}N side) might have contributed to a relatively constant uptake of ^{15}N by the roots. An important aspect related to the second assumption is time. The longer the duration of the experiment, the larger the potential for dissimilar ^{15}N abundance levels in both roots and rhizodeposits. In the current time frame (6 weeks), we saw that the roots did not take up all of the injected ^{15}N, possibly contributing to a relatively constant uptake of ^{15}N by the roots. With a longer duration of the experiment, it is expected to be more difficult to keep the tracer content of the

roots constant over the growing period of the trees. This will then disqualify the use of the Janzen and Bruinsma equation [24] for calculating rhizodeposition. In that case, the tracer mass balance equation would be the only suitable approach [26].

5. Conclusion

The split-root method proved to be suitable to measure ^{15}N rhizodeposition. The seedling roots could easily be separated in two parts for both tree species, and there was no ^{15}N contamination in the soil at the ^{14}N side of the split-root set-up. ^{15}N rhizodeposition was already visible at 3 weeks after labelling, for both tree species and for both low and high ^{15}N addition levels. In terms of ^{15}N rhizodeposition, some significant differences between the two treatments were observed, small trends between the two species, but no explicit trends over the time period of 6 weeks. These results offer potential applications of the method in studies related to ^{15}N rhizodeposition compound identification and its fate in the soil. Also, analysis of ^{15}N in various soil fractions can provide information about the stability of the ^{15}N rhizodeposits in the soil and ultimately about its contribution to soil organic compound stabilization.

References

[1] Weintraub MN, Scott-Denton LE, Schmidt SK, et al. The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem. Oecologia. 2007;154:327–338.
[2] Marschner P. Marschner's mineral nutrition of higher plants. 3rd ed. Amsterdam: Elsevier; 2012.
[3] Wichern F, Eberhardt E, Mayer J, et al. Nitrogen rhizodeposition in agricultural crops: methods, estimates and future prospects. Soil Biol Biochem. 2008;40:30–48.
[4] Kleber M, Sollins P, Sutton R. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry. 2007;85:9–24.
[5] Janzen HH. Deposition of nitrogen into the rhizosphere by wheat roots. Soil Biol Biochem. 1990;22:1155–1160.
[6] Yasmin K, Cadisch G, Baggs EM. Comparing ^{15}N-labelling techniques for enriching above- and below-ground components of the plant–soil system. Soil Biol Biochem. 2006;38:397–400.
[7] Merbach W, Schulze J, Richert M, et al. A comparison of different ^{15}N application techniques to study the N net rhizodeposition in the plant–soil system. J Plant Nutr Soil Sci. 2000;163:375–379.

[8] Hertenberger G, Wanek W. Evaluation of methods to measure differential ^{15}N labeling of soil and root N pools for studies of root exudation. Rapid Commun Mass Spectrom. 2004;18:2415–2425.

[9] Scandellari F, Ventura M, Gioacchini P, et al. Seasonal pattern of net nitrogen rhizodeposition from peach (*Prunus persica* (L.) Batsch) trees in soils with different textures. Agric Ecosyst Environ. 2010;136:162–168.

[10] Schmidtke K. How to calculate nitrogen rhizodeposition: a case study in estimating N rhizode-position in the pea (*Pisum sativum* L.) and grasspea (*Lathyrus sativus* L.) using a continuous ^{15}N labelling split-root technique. Soil Biol Biochem. 2005;37:1893–1897.

[11] Mahieu S, Fustec J, Faure ML, et al. Comparison of two ^{15}N labelling methods for assessing nitrogen rhizodeposition of pea. Plant Soil. 2007;295:193–205.

[12] Fustec J, Lesuffleur F, Mahieu S, et al. Nitrogen rhizodeposition of legumes. Sustain Agric. 2009;2:869–881.

[13] Nave LE, Gough CM, Maurer KD, et al. Disturbance and the resilience of coupled carbon and nitrogen cycling in a north temperate forest. J Geophys Res Biogeosci. 2011;116:G04016.

[14] Kalliokoski T. Root system traits of Norway spruce, Scots pine, and silver birch in mixed boreal forests: an analysis of root architecture, morphology, and anatomy (Dissertationes Forestales; 121) [academic dissertation]. Helsinki: University of Helsinki; 2011..

[15] Andersson F, editor. Ecosystems of the world. Vol. 6, coniferous forests. 1st ed. Amsterdam: Elsevier Science; 2005.

[16] Kamminga-Van Wijk C, Prins HBA. The kinetics of NH_4 and NO_3 − uptake by Douglas fir from single N-solutions and from solutions containing both NH_4 and NO_3. Plant Soil. 1993;151:91–96.

[17] Hangs RD, Knight JD, Van Rees KC. Nitrogen uptake characteristics for roots of conifer seedlings and common boreal forest competitor species. Can J For Res. 2003;33:156–163.

[18] Buchmann N, Schulze E-D, Gebauer G. ^{15}N-ammonium and ^{15}N-nitrate uptake of a 15-year-old *Picea abies* plantation. Oecologia. 1995;102:361–370.

[19] Ohlund J, Näsholm T. Growth of conifer seedlings on organic and inorganic nitrogen sources. Tree Physiol. 2001;21:1319–1326.

[20] Lupi C, Morin H, Deslauriers A, et al. Role of soil nitrogen for the conifers of the boreal forest : a critical review. Int J Plant Soil Sci. 2013;2:155–189.

[21] Kopittke GR, Tietema A, van Loon EE, et al. The age of managed heathland communities: impli-cations for carbon storage? Plant Soil. 2013;369:219–230.

[22] Waldner P, Marchetto A, Thimonier A, et al. Detection of temporal trends in atmospheric depo-sition of inorganic nitrogen and sulphate to forests in Europe. Atmos Environ. 2014;95:363–374.

[23] Wessel WW, Tietema A, Boxman AW. The fate of $^{15}NH_4^+$ labeled deposition in a Scots pine forest in the Netherlands under high and lowered NH_4^+ deposition, 8 years after application. Biogeochemistry. 2013;113:467–479.

[24] Janzen HH, Bruinsma Y. Methodology for the quantification of root and rhizosphere nitrogen dynamics by exposure of shoots to ^{15}N-labelled ammonia. Soil Biol Biochem. 1989;21:189–196.

[25] Leinweber P, Kruse J, Baum C, et al. Advances in understanding organic nitrogen chemistry in soils using state-of-the-art analytical techniques. Adv Agron. 2013;119:83–151.

[26] Rasmussen J. Why we need to restrict the use of 'rhizodeposition' and the Janzen and Bruinsma equation. Soil Biol Biochem. 2011;43:2213–2214.