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Original article

^{15}N partitioning and production of ^{15}N-labelled litter in beech trees following [^{15}N]urea spray

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Abstract – The leaves of 10-year-old beech trees grown in a plantation were sprayed once in late summer in 1993, 1994 and 1995 with [^{15}N]urea, to determine the ^{15}N utilization by beech (*Fagus sylvatica* L.) and to obtain homogeneous ^{15}N-labelled litter. One day after spraying, leaves had incorporated 42 % (1993) to 55 % (1995) of the applied [^{15}N]urea. The leaf amino acid content and N concentration increased shortly after application. During leaf senescence, approximately 88 % of the incorporated ^{15}N was translocated and mainly stored in the above-ground biomass. After spraying, N concentration and ^{15}N enrichment of leaves were measured until abscission. In spring, trees were sampled and ^{15}N allocation in above- and below-ground organs were determined to assess ^{15}N partitioning. Buds and bark showed the highest ^{15}N enrichment, but the largest amounts of foliarly applied ^{15}N were stored in bark and wood. ^{15}N enrichment of fallen leaves (i.e. litter) increased after each ^{15}N urea spray, from 2.11 % ^{15}N atom excess in 1993, to 2.97 % ^{15}N in 1994 and 3.14 % ^{15}N in 1995. Annual litter contained 4.7 %, 7.3 % and 7.8 % of the sprayed ^{15}N. Soluble and insoluble N fractions showed an identical ^{15}N atom excess indicating a homogeneous distribution of ^{15}N in the labelled leaves as well as litter. (© Inra/Elsevier, Paris.)

^{15}N / urea / beech litter / forest / nitrogen

Résumé – Répartition de ^{15}N dans le hêtre et production d'une litière marquée après pulvérisation de [^{15}N]urée. Afin de déterminer l'utilisation de l'azote par le hêtre (*Fagus sylvatica* L.) et pour obtenir une litière homogène marquée au ^{15}N, de l'urée enrichie en ^{15}N a été pulvérisée en fin d'été 1993, 1994 et 1995 sur le feuillage de jeunes hêtres. Après la pulvérisation, les concentrations en N et ^{15}N dans les feuilles et la litière ont été mesurées jusqu'à l'abscission. Un jour après la pulvérisation, les feuilles ont incorporé entre 42 % (1993) et 55 % (1995) de l'[^{15}N]urée. Une brève augmentation de la teneur en acides aminés et de la concentration en N foliaire a eu lieu peu après l'application. Au printemps 1994, un prélèvement sur cinq arbres a été effectué, pour déterminer la répartition de ^{15}N dans les organes aériens et souterrains des arbres. Pendant la sénescence des feuilles, 88 % de l'azote incorporé est transféré dont la plus grande part

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est stockée dans les organes aériens des arbres. Les bourgeons et l'écorce sont les tissus les plus enrichis en ^{15}N, mais l'écorce et le bois accumulent l'essentiel de l'azote ^{15}N. L'enrichissement en ^{15}N des feuilles sénescentes (litière) augmente après chaque pulvérisation d'[^{15}N]urée ; l'excès isotopique ^{15}N mesuré en 1993 est de 2,11 % et il atteint 3,14 % en 1995. La chute annuelle de litière représente 4,7, 7,3 et 7,8 % de ^{15}N pulvérisé. Les composés azotées solubles et insolubles présentent des enrichissements identiques en ^{15}N, indiquant une distribution homogène de ^{15}N dans les feuilles et également dans la litière. (© Inra/Elsevier, Paris.)

^{15}N / urée / litière / hêtre / forêt / azote

1. INTRODUCTION

Little is known on the dynamics of leaching, accumulation and release of N in decomposing forest litter in beech ecosystems. A major aim of the current investigations was to study the mechanisms involved in the uptake and release of N from litter. The determination of the sizes of the N pools involved and estimates of their turnover in litter and soil had been efficiently obtained by ^{15}N tracer experiments [1, 2]. Plant residues enriched in ^{15}N have been widely used as tracers in N-cycling experiments in agricultural systems [7, 11]. In forest ecosystems, ^{15}N-labelled corn straw was used as a source of N and C in studies on N mineralization [2]. However, replacing of straw from herbaceous species by labelled litter from the species under investigation (e.g. beech) would make it possible to quantify the decomposition and mineralization of litter with few modifications to the litter layer. ^{15}N-labelled needle litter has been used in several studies on N decomposition and mineralization in conifer ecosystems [1, 13]. This approach is, however, limited by the production of large amounts of ^{15}N-labelled litter for the species under investigation. In horticulture, massive labelling of trees has successfully been achieved by urea spraying [12, 14, 15]. Uptake of urea N by leaves is much faster than for NH_4^+-N and NO_3^--N [3], and this compound is rapidly converted into amino acids [10] and later to proteins.

In the present study, a field experiment was conducted to determine the ^{15}N utilization by beech (*Fagus sylvatica* L.) of ^{15}N urea sprayed on leaves and to obtain homogeneous ^{15}N-labelled beech litter. The major aims of this study were to ascertain i) that N originating from ^{15}N leaf labelling was properly distributed into beech organs, and ii) that fallen leaves (i.e. litter) exhibited homogeneous ^{15}N labelling.

2. MATERIALS AND METHODS

2.1. ^{15}N labelling

A field experiment was conducted on a calcareous brown earth soil (Calcisol) in a large natural regeneration area at Puvenelle near Pont-à-Mousson (France). The 10-year-old beech trees selected for the ^{15}N-labelling experiment, had a mean height of 1.25 m. A 25-m^2 plot containing 350 trees was carefully cleaned of understorey vegetation and litter from previous years. During late summer, tree shoots were sprayed in the evening with a 50-mM aqueous solution of [^{15}N]urea (99.5 % ^{15}N) (pH 6.2) using a hand-sprayer. The urea solution (3.0 g urea L^{-1}) was sprayed as a fine mist, which limited the formation of drops and consequently the contamination of the soil surface. Nevertheless, the uptake of ^{15}N by the roots could not be excluded. The following amounts of ^{15}N were applied per tree: 56.6 mg ^{15}N on 1 September 1993, 26.9 mg ^{15}N on 22 August 1994 and 58.2 mg ^{15}N on 30 August 1995. In 1994 and 1995, plots were covered with a plastic sheet during

spraying and for the following 36 h to avoid volatilization of ^{15}N, whereas in 1993 they were not covered. The highest application dose on 1 ha corresponded to 8.0 kg N.

2.2. Sampling

Fifteen leaves from the upper, inner and lower crowns were randomly taken from different trees at regular intervals (weekly and fortnightly in 1993; monthly in spring 1994 and 1995) and before and after the application of [^{15}N]urea. From mid September until November, senescent brown leaves (hereafter referred to as litter) were collected weekly just before abscission. In February 1994, five trees were harvested and separated into the following compartments: buds, bark (1993 = year of growth), wood (1993), bark (1991–1992), wood (1991–1992), bark and wood (branches), bark (1991 and earlier), wood (1991 and earlier), roots (< 1 mm), roots (> 1 mm), roots (> 3 mm). Soil samples (n = 5) were taken at a depth of 0–5 cm and 5–20 cm within the plot.

2.3. Analyses

Sampled leaves were rinsed twice with distilled water and weighed. One subsample was dried at 65 °C until constant mass. In 1993, the fresh leaves from the other subsample were stored at –20 °C and used later to determine the free amino acid composition after extraction in methanol/water (70/30, v/v) as described by Genetet et al. [4]. Fallen leaves (i.e. leaf litter) collected weekly in autumn were air-dried and stored for further use as substrate for N decomposition studies (Zeller et al., unpublished results). A composite litter sample from each harvest was dried at 65 °C.

All tree and soil samples were weighed and dried at 65 °C. After dry-weight determination, all samples (tree organs, litter, leaf, soil) were milled using a ball mill (Fritsch Pulverisette 6) to pass a 100-μm mesh. For N concentration and ^{15}N analyses of litter, tree and soil samples, about 6–10 mg of each sample were weighed in silver cups and carefully closed. Samples were then combusted, N reduced to N_2 and the $^{14}N/^{15}N$ ratio measured on a Finnegan MAT Delta S mass spectrometer at the Service Central d'Analyse (CNRS, Vernaison, France).

The incorporation of [^{15}N]urea into beech leaves was calculated on a tree basis as the difference between sprayed ^{15}N and the total amount of new ^{15}N in all leaves 1 day after spraying. ^{15}N excess = atom % ^{15}N sample – 0.3663 atom % ^{15}N

3. RESULTS

3.1. Amino acid composition of beech leaves

During late summer, the most abundant free amino acids in leaves (lower crown) of 10-year-old beech trees was asparagine (about 400 nmol g^{-1} fwt) followed by glutamate and glutamine (*figure 1*). After application of [^{15}N]urea, asparagine concentration in leaves dramatically decreased, whereas glutamine and glutamate concentrations drastically increased (*figure 1*). Glutamate concentration reached a peak (600 nmol g^{-1} fwt) 1 day after spraying and then rapidly decreased to its initial concentration. Glutamine concentration increased until day 3 after spraying to reach 400 nmol g^{-1} fwt and then levelled off. Increased glutamate and glutamine concentrations after urea application indicate a rapid assimilation of NH_4^+ produced by urea catabolism.

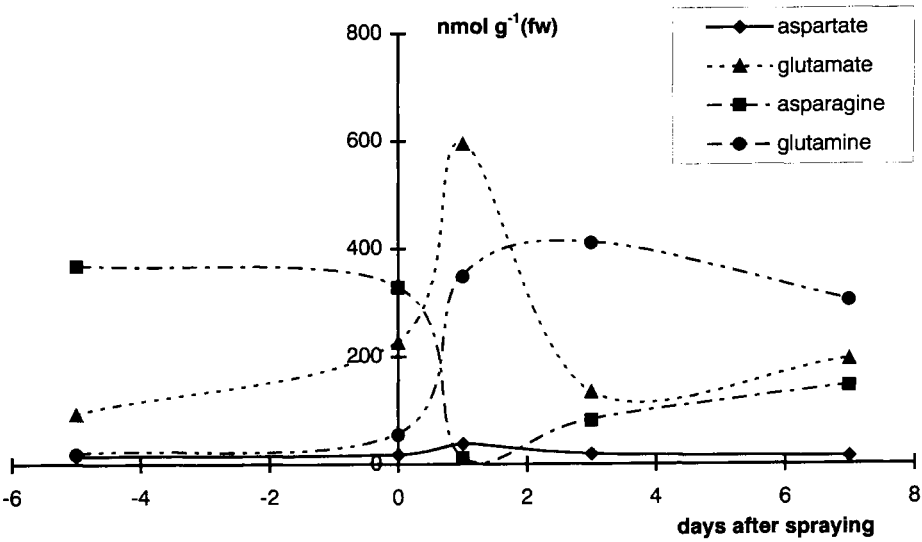

Figure 1. Concentration of major free amino acids in beech leaves before and after spraying with an aqueous solution containing 50 mM urea. The treatment was applied on 1 September 1993.

3.2. ^{15}N incorporation in leaves

^{15}N concentration in leaves sharply increased after [^{15}N]urea application and then rapidly decreased during the following weeks (*figure 2A*) suggesting that a significant part of the applied ^{15}N was rapidly translocated to other tree parts and/or lost by leaching. One day after spraying, leaves had incorporated 42 % (1993) to 55 % (1995) of the applied [^{15}N]urea, whereas 32.1 % of the applied ^{15}N reached the soil (1993). The difference (27.4 %) was most probably due to volatilization of NH_3 (*table I*). After the first [^{15}N]urea application in 1993, leaf ^{15}N concentration showed a high difference (*figure 2A*) resulting from a large variability in ^{15}N incorporation between upper- and lower-crown leaves (0.37 and 1.01 mg ^{15}N g^{-1} dwt, respectively). ^{15}N enrichment of leaves from the upper crown was approximately two times lower than in leaves of the inner and lower crowns (2.21 versus 6.12 excess % ^{15}N). Foliar uptake of [^{15}N]urea increased with leaf biomass as suggested by the increased urea incorporation from 1993 to 1995 (*table I*).

3.3. ^{15}N partitioning

Leaf senescence began in mid September at the upper crown and had spread to the whole tree crown approximately 3 weeks later. During leaf senescence, 88 % of the ^{15}N incorporated in leaves was allocated to perennial tissues of beech trees, whereas the remaining part was found in fallen leaves. ^{15}N distribution in the different perennial parts of trees harvested in February 1994 is presented in *figure 3*. ^{15}N from urea incorporated by leaves was allocated to the various organs of beech. The buds formed in 1993 showed the highest ^{15}N concentration of all plant parts (approximately 700 μg ^{15}N g^{-1} dwt). Bark and wood tissues of various

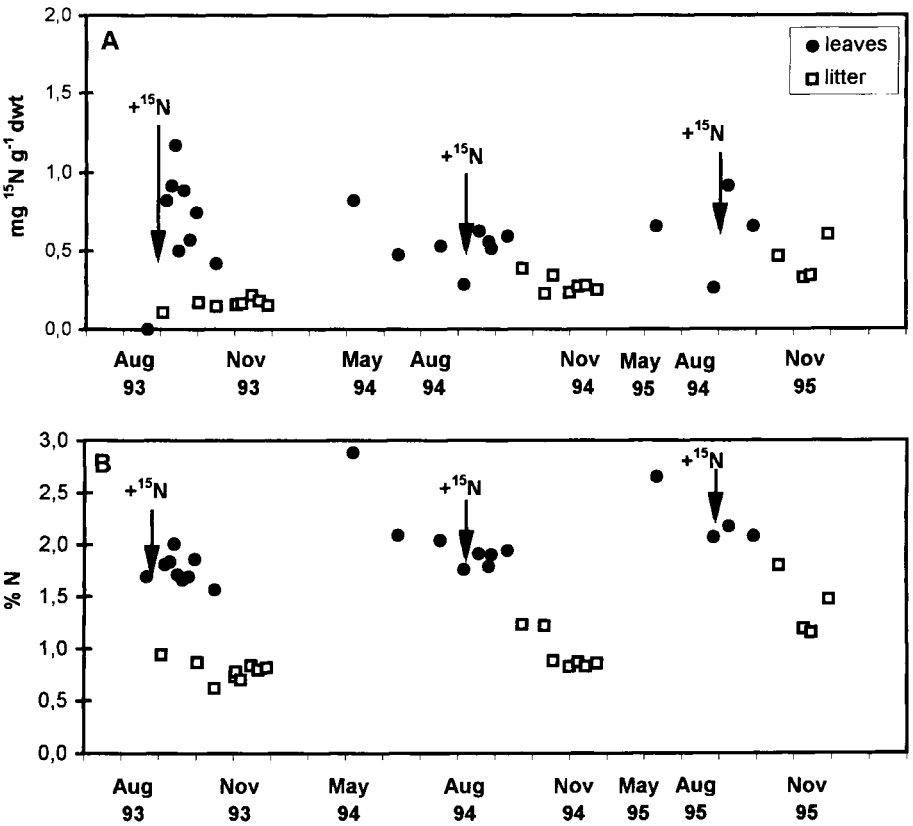

Figure 2. Concentration of ^{15}N, as μg g^{-1} dry weight, (**A**) and of total N (in %) (**B**) in leaves and leaf litter of 10-year-old beech trees sprayed with 50 mM [^{15}N]urea (99.5% ^{15}N). The amounts of ^{15}N applied per tree were as follows: 56.6 mg on 1 September 1993, 26.9 mg on 22 August 1994 and 58.2 mg on 29 August 1995. Total N and ^{15}N were measured in leaves at regular intervals between autumn 1993 and November 1995.

ages showed similar ^{15}N concentrations, whereas roots presented slightly lower ^{15}N concentrations. The highest amount of ^{15}N (25.2 %) was accumulated in wood (< 1991), the largest compartment of beech, followed by wood + bark of branches (16.6 %), bark (< 1991) (14.0 %) and coarse roots (16.3 %).

In May 1994, the ^{15}N stored in perennial tissues was remobilized and transported to the growing leaves, where the ^{15}N concentration reached the values observed after urea application (*figure 2A*). In August, the ^{15}N content of leaves drastically decreased indicating an active internal N cycling within the tree. The decrease in ^{15}N was stronger (–65 %) than the decrease in total leaf N (–40 %) suggesting that a large part of ^{15}N-labelled compounds corresponded to easily catabolized metabolites, such as amino acids.

Table I. Partitioning of incorporated ^{15}N in beech trees after spraying leaves with [^{15}N]urea for 3 subsequent years.

	1993 mg ^{15}N tree $^{-1}$	%	1994 mg ^{15}N tree $^{-1}$	%	1995 mg ^{15}N tree $^{-1}$	%
Sprayed N	56.6	100	26.9	100	58.2	100
N incorporated in leaf	23.5	41.5	12.8	47.6	32.4	55.6
N in litterfall	2.7	4.7	6.1	7.3	11.1	7.8
Stored N	20.8	36.8	27.5	32.9	48.8	34.4
Soil N		32.1		nd		nd

Results were expressed as mg ^{15}N per tree and % of sprayed ^{15}N found in the different compartments. The N incorporated in the leaves was measured 1 day after the spraying. The amounts of ^{15}N applied per tree were as follows: 56.6 mg on 1 September 1993, 26.9 mg on 22 August 1994 and 58.2 mg on 29 August 1995. nd = not determined

In this study, 5–8 % of the applied ^{15}N was found in fallen leaves, i.e. litterfall (*table I*). In these leaves, the ^{15}N enrichment of total N and insoluble N (i.e. protein and lignin N) was identical (2.06 and 2.11 excess % ^{15}N). Unlike the high variability of ^{15}N enrichment in the leaves, the ^{15}N enrichment of litterfall remained nearly constant in autumn (1993). In 1993, leaf litter showed a mean enrichment of 2.11% excess ^{15}N. In autumn 1994 and 1995, a higher enrichment (2.97 and 3.14% excess ^{15}N, respectively) was measured in this litter as a result of successive [^{15}N]urea applications. During the course of this labelling experiment, 4.9, 7.3 and 8.5 kg of ^{15}N-labelled beech litter were produced in 1993, 1994 and 1995, respectively.

4. DISCUSSION

Under field conditions, urea uptake by leaves of 10-year-old beech trees was efficient and a large and increasing proportion (42–55 %) of the applied [^{15}N]urea was incorporated into plant tissues. This increasing incorporation probably resulted from the increase in leaf biomass, as indicated by the amount of litter. Even higher rates of incorporation (60–80 %) have been reported for apple trees [5, 6]. Several reasons can be suggested to explain the lower urea incorporation in sun (upper)-crown leaves after the first spraying in 1993. Enhanced urea volatilization from upper leaves can be ruled out for the following years because the plot was covered with a plastic sheet. Higher application of urea on the lower crown leaves, was observed due to the fall of urea droplets from the upper crown to the lower crown. This is suggested by the soil contamination as measured in 1993. Lower urea incorporation in the upper crown was presumably due to a lower metabolic activity of these leaves senescing earlier than the leaves of lower crowns. The dramatic increase of free glutamate and glutamine in sprayed leaves (*figure 1*) suggests that the catabolism of incorporated urea is rapid and the released ammonium N is assimilated into glutamate and glutamine. The efficiency of the foliar uptake of sprayed [^{15}N]urea by beech trees grown in forest plantations depends on leaf density.

Remobilization of leaf N in senescent leaves allowed an efficient translocation of incorporated ^{15}N to perennial tissues of beech trees. About 88 % of incorporated

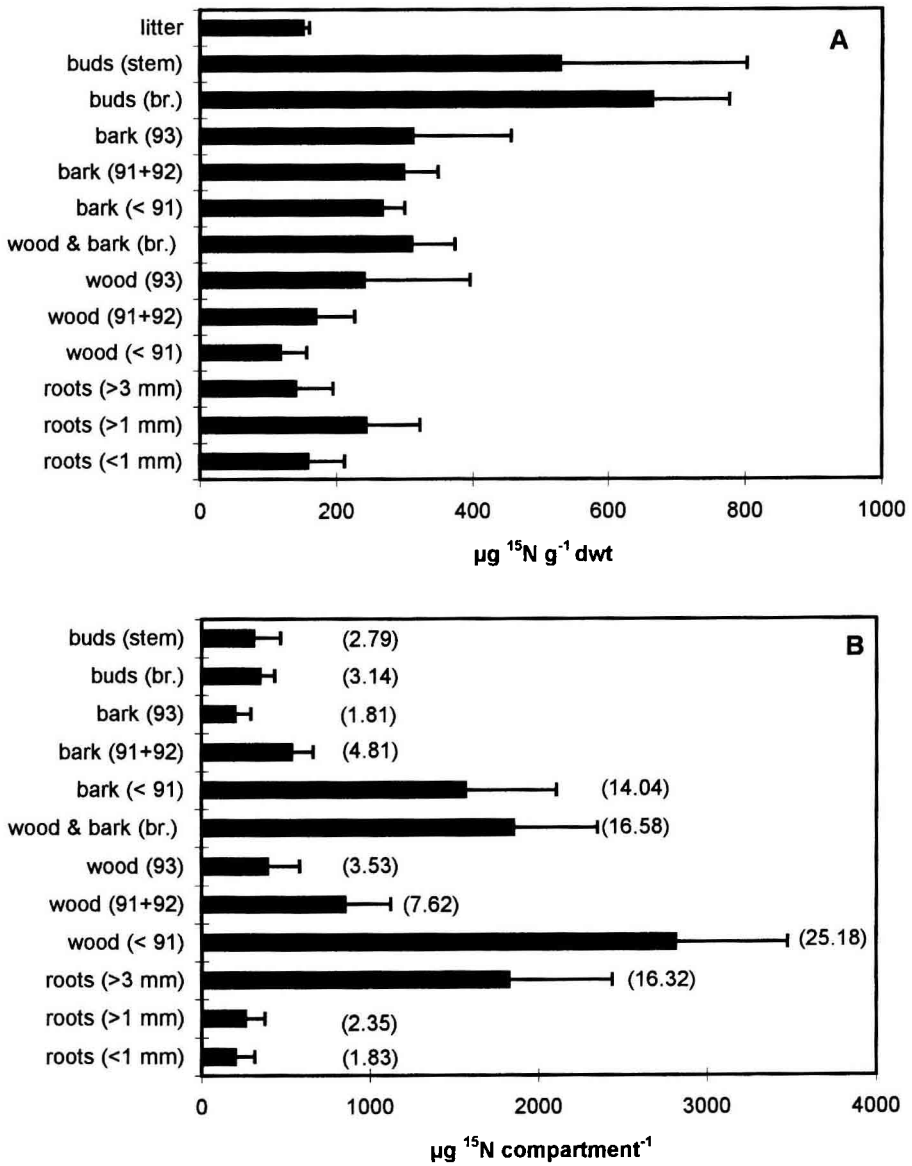

Figure 3. Concentration of ^{15}N as µg g^{-1} dry weight (**A**) and ^{15}N amounts as µg g^{-1} compartment (**B**) in perennial tissues of 10-year-old beech trees sprayed with 50 mM [^{15}N]urea (99.5 % ^{15}N; 56.6 mg ^{15}N per tree) in September 1993.
Labelled trees were harvested in February 1994 and ^{15}N analysis conducted on fallen leaves, buds, wood and bark tissues of different ages, and various types of roots. In (**B**), the numbers in brackets indicate the percentage of total incorporated ^{15}N in the various compartments. Values are the means of five replicates. br, branches; 91, 92 and 93, year of growth.

^{15}N was found in buds, bark, wood and roots. The buds of branches exhibited the highest ^{15}N excess, but most (>80 %) of the ^{15}N was found in bark and wood tissues. This partitioning is in agreement with that found in other deciduous trees [17]. In other tree species, retranslocated N is stored mostly in a specific family of glycoproteins, called vegetative storage proteins, in buds, bark, wood and roots [8, 9, 17]. Despite an efficient translocation of applied ^{15}N to perennial tissues, a significant proportion of ^{15}N was measured in fallen leaves and litter. As a result of [^{15}N] urea application during late summer, N translocation to other plant parts was limited to the remobilization processes taking place during leaf senescence with little dilution and transfer of ^{15}N by import/export mechanisms characterizing the developing leaves. After the first year of [^{15}N]urea application, 4.7 % of the ^{15}N sprayed was found in litter (*table I*), and this proportion increased to 7.3 % in 1994 and to 7.8 % in the last year (1995) of urea application. In 1995, the excess % ^{15}N of the harvested litter reached 3.14 and the incorporated ^{15}N was homogeneously distributed in the soluble and insoluble N fractions.

In summary, spraying [^{15}N]urea on leaves of beech trees grown in the forest during late summer readily generates large amounts of ^{15}N-labelled litter. Since incorporated ^{15}N is homogeneously distributed between the different leaf N fractions, the harvested litter produced could potentially be used to investigate uptake, leaching and mineralisation of beech litter in forest ecosystems.

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