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Soil Biology & Biochemistry 33 (2001) 439–448

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Rhizodeposition of nitrogen by red clover, white clover and ryegrass leys

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Received 4 April 2000; received in revised form 10 July 2000; accepted 29 July 2000

Abstract

Correct assessment of the rhizodeposition of N in grassland is essential for the evaluation of biological N_2-fixation of legumes, for the total N balance of agro-ecosystems, and for the pre-cropping value of grasslands. Using a leaf-feeding technique by which plants were ^{15}N labelled while growing in mezotrons in the field, the rhizodeposition of N by unfertilised red clover, white clover and perennial ryegrass growing in pure stands was shown to amount to 64, 71 and 9 g N m^{-2}, respectively, over two complete growing seasons. The corresponding values for red clover and white clover growing in mixtures with ryegrass were 89 and 32 g N m^{-2}, respectively. The rhizodeposited N compounds, including fine roots, constituted more than 80% of the total plant-derived N in the soil, and in all cases exceeded the amount of N present in stubble. In the mixtures of red clover–ryegrass and white clover–ryegrass and the pure stands of red clover, white clover and ryegrass, respectively, the rhizodeposition constituted a 1.05, 1.52, 1.26, 2.21 and 2.77 fold increase over the total N in the shoots harvested during the two production years. In pure stands and mixtures of clover, 84 and 92%, respectively, of this N derived from biological N_2 fixation. It is concluded that rhizodeposition provides a very substantial input of N to the legume-based grassland systems with great consequences for ecosystem N balance and turnover. Furthermore, the amount of atmospheric-derived N in the rhizodeposits may exceed that in the harvested shoots.

Keywords: Pasture; Red clover; Rhizodeposition; Roots; Ryegrass; White clover

1. Introduction

In legume-based leys knowledge about the below-ground N pools are important for correct estimates of the total biological N_2-fixation as well as for the potential N supply to the succeeding crops. The below-ground N pools can be divided into visible fibrous macro roots and in rhizodeposits that contain the rest. Rhizodeposition may contain many different organic compounds including water-soluble exudates, sloughed-off root tissue and dead roots (Rovira et al., 1979). The rhizodeposition of carbon can be very substantial, exceeding that present as fibrous roots at the end of the growing season by more than 20–50% (Whipps and Lynch, 1985; Cheng et al., 1993). Additionally substantial amounts of N can be deposited together with the carbon (Curl and Truelove, 1986; Janzen, 1990; Janzen and Bruinsma, 1993). The deposited compounds have major effects on the density and activity of microorganisms in the rhizosphere and, hence, on the turnover and plant availability of nutrients in the root zone. After mineralisation, the nutrients contained in the rhizodeposited compounds will be subject to plant or microbial uptake, adsorption on soil particles or lost from the plant–soil system. Consequently, rhizodeposition will always be measured as the net outcome of several interacting processes.

Deposition of plant-derived N, including rhizodeposits and litter fall, under field conditions is largely unknown for the common grassland species. Using a direct ^{15}N-labelling technique McNeill et al. (1997) conducted an experiment with pasture legumes growing in relatively small pots under controlled conditions. Under these conditions, rhizodeposition constituted 17–24% of the total below-ground clover-N. These values agree with the estimates obtained by the use of comparable ^{15}N-labelling techniques for pea in pot experiments (Sawatsky and Soper, 1991; Jensen, 1996), alfalfa in solution culture (Ta et al., 1979), and field bean and soybean in a pot study (Ruschel et al., 1979). Others found that rhizodeposition of N from alfalfa were insignificant under both field and controlled conditions (Lory et al., 1992). Importantly, all these studies are based on pure stands of the specific species. There is a particular need for measurements of rhizodeposition under field conditions by mixtures of common grassland species.

Isolation of mature root material from soil by wet sieving is commonly used in connection with the assessment of root

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0038-0717/01/$ - see front matter
PII: S0038-0717(00)00183-8

biomass (Böhm, 1979). However, this procedure only partly recovers fine roots as well as partly or fully decomposed root material (Sauerbeck and Johnen, 1976; Unkovich et al., 1994). Such partial recoveries obviously make predictions of N mineralisation difficult in a crop rotational context (Høgh-Jensen and Schjoerring, 1997; Høgh-Jensen 1999). A more direct determination of the below-ground plant-derived N pools and the subsequent turnover of plant-derived organic N during the growing period requires ^{15}N labelling of the plants (Hood et al., 1999). The labelling technique must uniformly incorporate ^{15}N in tissues and organs. Ledgard et al. (1985) and Ta et al. (1989) labelled legumes by immersing single trifoliate leaves into a $K^{15}NO_3$ solution. Recently, urea has been used to feed individual plants or leaves (Palta et al., 1991; Russell and Fillery, 1996a; McNeill et al., 1997). This technique enables the labelling of individual plants grown in close association with other species under field conditions.

Our purpose was to quantify the contribution of actively N_2-fixing red clover and white clover grown in either of the mixtures with perennial ryegrass or in pure stands to the below-ground N pools following a 2-year production period, and to quantify the proportion of these below-ground N pools that was derived from the fixation of atmospheric N. A direct technique in which ^{15}N-labelled nitrogen was fed to the leaves of either clover or grass plants growing in field-based mezotrons (PVC-cylinders, internal diameter 29.7 cm, depth 60 cm) was used to label the rhizodeposited compounds. At the end of the two production years, the mezotrons were excavated, followed by an analysis of the ^{15}N, total-N and total-C in macro roots, rhizosphere soil including fine roots, bulk soil, stubble and above-ground plant parts.

2. Materials and methods

The experimental area was located 18 km west of Copenhagen (55°40′N, 12°18′E; 28 m above MSL; mean annual precipitation 600 mm; growth period of 210 days) in a field that had been cropped mainly with cereals for the last 30 years without any organic residue or any phosphorus or potassium application. The experimental area was selected because of its history to obtain an agricultural soil depleted in fertility with a low soil microbial biomass pool and little amounts of particulate organic matter. Such circumstances are expected to represent conditions where rhizodeposition will be of greatest significance to agricultural systems.

Following its cropping history, the total-N content of the soil was on an average low, that is 0.133, 0.048, 0.059, 0.050 and 0.040% at the depths of 0–20, 20–40, 40–60, 60–80 and 80–100 cm, respectively. Furthermore, the C-to-N ratio was less than 9 in all depths. In the upper 40 cm soil, the $NaHCO_3$-soluble P content of the soil (Olsen et al., 1954; 0.5 g dry soil, 10 ml 0.5 M $NaHCO_3$ shaken for 24 h) determined spectrophotometrically was 10.8 mg P kg^{-1} dry soil, the NH_4-acetate extractable content (Page et al., 1982; 10 g dry soil, 100 ml 0.5 M NH_4-acetate + 3 mM LiCl shaken for 45 min, stand overnight) was 70 mg K, 38 mg Mg, 9 mg Na, and 1047 mg Ca kg^{-1} dry soil. The soil $pH_{(0.01\ M\ CaCl)}$ was 5.1.

The soil had 13.2, 14.9 and 22.6% clay, 17.5, 18.1 and 21.7% silt, 39.2, 36.2, and 34.8% fine sand and 30.1, 30.8 and 20.9% coarse sand at the depths of 10–25, 40–45 and 80–85 cm, respectively, as determined used the hydrometer method (Gee and Bauder, 1986).

2.1. Establishing the plant communities and experimental treatments

The experimental plant communities were established in spring 1997. Ley seed mixtures were undersown in an unfertilised spring barley crop in plots of 15 m^2 sizes, four replicates. The ley mixtures were established by seeding 10 kg seed ha^{-1} of white clover (*Trifolium repens* L. cv. Milkanova) or red clover (*Trifolium pratense* L. cv. Rajah) in mixture with 20 kg seed ha^{-1} of perennial ryegrass (*Lolium perenne* L. cvs. Tetramax and Borvi). Following a factorial experimental design, ryegrass in pure stand was established by seeding 25 kg seed ha^{-1} and white or red clover in pure stand by seeding 10 kg seeds ha^{-1}. The barley crop was harvested in August and the developing ley received no further treatment before the following spring except for defoliation in late autumn.

In the developing ley, during late autumn 1997, PVC cylinders with an internal diameter of 29.7 cm were inserted 60 cm into the ground leaving 5 cm above-ground. These cylinders are subsequently termed mezotrons. One mezotron were inserted per plot of pure stands and two in mixtures. Hence, four replicates were inserted in pure stands of ryegrass, pure stands of white clover and pure stands of red clover and eight replicates in mixtures of ryegrass-white clover and ryegrass-red clover. The mixtures were ^{15}N cross-labelled, i.e. either the clover component or the grass component was ^{15}N labelled.

The plant communities received no other treatment but the sward was cut four times during the first growing season and three times during the second growing season. At the end of the second growing season, after the final harvest, the mezotrons were excavated and sampled as described below.

2.2. Leaf labelling

One clover trifoliate leaf and two-four grass leaves, depending on leaf size, were each inserted into 2 ml micro-centrifuge vials containing 1.0 ml of a 0.5% (v/v) urea (99 at.%) (McNeill et al., 1997). The vial was then sealed using an inert plastic material (Terostat®, Henkel Surface Technologies, PA, USA) to avoid ^{15}N losses from the solution. Each time the leaves were selected from differing plants and enclosed in the vials between 2 and 7 days, depending on the climatic conditions. The grass was labelled three times between each harvest, except between

the third and fourth harvest, resulting in a total of nine vials between each harvest. This gave a total of 27 vials in each cylinder in both growing seasons. The clover was labelled 3–5 times between each harvest. This gave a total of 9–15 vials between each harvest, resulting in a total of 40 vials in the first and 33 vials in the second growing season. In the mixtures, a cross-labelling procedure was followed so that either the ryegrass or the clovers was labelled.

At the end of each labelling period, the vials were removed and the residual solution was analysed for N and ^{15}N content, as described below, in order to precisely determine plant uptake of ^{15}N. The uptake was calculated as the amount of ^{15}N offered to the plants subtracted the amount that was left in the vials at the end of each labelling period. The amounts of N added annually to the system this way was equivalent to 0.5–1.0 g N m^{-2}.

2.3. Biological N_2 fixation and dry matter production

Adjacent to the mezotrons, 2-m^2 plots with the same plant communities as those enclosed in the mezotron were labelled with ^{15}N fertiliser [$(NH_4)_2SO_4$; 99 at.%; equivalent to 0.22 g N m^{-2}] (described in detail by Høgh-Jensen and Schjoerring, 2000) to determine symbiotic N_2-fixation, using grass in pure stand as the reference. The plant populations in the ^{15}N labelled plots and in the PVC-mezotrons were managed similarly. Yields of dry matter and N accumulation was determined on the basis of these 2-m^2 plots.

2.4. Sampling

The last harvest took place late October in the second growing season leaving a stubble height of 5 cm. All PVC-mezotrons were excavated early November when the soil temperature had decrease below 5°C. The soil column within each mezotron was gently allowed to slide out. Then the stubble material was cut at the soil surface, separated into grass and clover, dried to constant weight at 80°C, weighed, pulverised to a fine powder and analysed. The time from excavation to isolation of the roots from the soil was less than three weeks, during which the intact mezotrons were kept at 5°C.

The intact soil columns were cut into sections of 0–10, 10–20, 20–40 and 40–60 cm depth. Each section was weighed and all visual roots were subsequently removed manually together with the adhering soil. This root material was dried at 80°C to constant weight. Subsequently, soil that had been adhering to the fresh roots was easily separated from the dry roots and termed the rhizosphere soil. The total mass of rhizosphere soil in white clover–ryegrass mixtures and ryegrass in pure stands amounted to approx. 1.5% and did not exceed 0.5% of total soil in the other plant communities. More soil remained on the macro roots in the upper 10 cm soil layer compared to roots in deeper soil layers. Relating the amount of dry soil in the mezotrons after root removal to the volume of 0–10, 10–20, 20–40 and 40–60 cm depth, respectively, gave average soil densities of 2.0, 2.3, 1.4 and 1.4 g cm^{-3}, respectively. This indicates small inaccuracies in determining section sizes during the slicing of soil columns.

The root material and the rhizosphere soil were finely pulverised and analysed for ^{15}N, total N and C content. A representative sample of the bulk soil was dried to determine the dry matter content of the bulk soil. This sample was sieved (2 mm) and analysed for ^{15}N, total-N and total-C. Soil adhering to the stubble and other plant debris on the soil surface were pulverised together with the organic matter. The dry matter contents were subsequently normalised to a C content of 43%. This correction affects only the reported dry matter yields, and not the estimates of N rhizodeposition.

The plant and soil materials were analysed for ^{15}N, total-N and total-C using the Dumas dry combustion method in a system consisting of an ANCA-SL Elemental Analyser coupled to a 20–20 Tracermass Mass Spectrometer (Europa Scientific Ltd. Creve, UK). Analytical SE for ^{15}N was 0.015%.

2.5. Proportion of absorbed ^{15}N in the living plant material, in rhizosphere and in bulk soil

^{15}N recovery in the living plant material was calculated as the sum of the ^{15}N content in shoots, stubble material at the final sampling time, and macro roots.

Assuming the deposited N to have the same ^{15}N enrichment as the sampled macro root N, the rhizodeposition relative to total-N in the bulk soil and in the rhizosphere soil was calculated as described by Janzen and Bruinsma (1989) using the following equation:

$$\%\text{Ndfr} = [\text{at.\% } ^{15}\text{N excess (soil)/at.\% } ^{15}\text{N excess (roots)}] \times 100 \quad (1)$$

where the at.%-excess of the roots was taken as the enrichment of the macro roots relative to 0.3663 which is the enrichment of atmospheric N_2. As the roots of the mixtures were not separated, the at.%-excess of the species in pure stands was used for calculations. The at.%-excess of the soil was taken as the enrichment of the soil relative to 0.3708 at.%, which was the average natural enrichment of ryegrass growing under unlabelled field conditions.

Potential losses of ^{15}N during the labelling period was estimated by calculating the total ^{15}N absorbed and subtracting the ^{15}N removed together with the ^{15}N present in the stubble and macro roots as well as rhizodeposition at the time of the final sampling.

2.6. Calculations

All ^{15}N enrichment values are given as excess relative to atmospheric N_2 (at.%-excess).

The proportion of absorbed ^{15}N that was removed in harvested shoots was calculated on an annual basis for each of the two production years separately.

3. Results

3.1. Seasonal dry matter production and N accumulation

The plant communities that included actively N_2-fixing legumes were growing well in both seasons, producing more that 1100 and 700 g dry matter per m^2 per year where red and white clover was included, respectively (data not shown). Ryegrass in pure stand, on the other hand, only produced 210–230 g dry matter per m^2 per year and contained 2.8 and 3.7 g N m^{-2} in the first and second growing season, respectively, confirming that the soil was indeed depleted in inorganic N.

3.2. Dry matter and nitrogen content in shoots, stubble, roots and rhizosphere at final harvest

The combined dry matter yield of stubble and roots varied from 1085 g m^{-2} for pure stands of red clover to 558 g m^{-2} for ryegrass (Table 1). The red clover–ryegrass and white clover–ryegrass mixtures contained 16 and 30% less dry matter in stubble and macro roots, respectively, than did the corresponding pure clover stands. The red clover–ryegrass mixture was totally dominated by the clover component, constituting 90 and 94% of the shoot and stubble biomass, respectively, while white clover only contributed 30 and 36% to the corresponding yields of the white clover–ryegrass mixture (data not shown).

The N content of the stubble plus macro roots in the pure stands of red clover, white clover and ryegrass was 30.7, 27.7 and 8.2 g N m^{-2}, respectively (Table 3). As was the case for the dry matter yields, the mixtures accumulated less N than the pure clover stands, 25 and 50%, respectively, for the red clover–ryegrass and white clover–ryegrass combinations (Table 2). The N content of the stubble exceeded that of the macro roots with more than 50% in all cases except the red clover–ryegrass mixture, where the difference was less pronounced (17%). These values demonstrate that stubble is a very important residue in the agroecosystems.

3.3. ^{15}N leaf labelling

Leaf feeding with ^{15}N-labelled urea succeeded in a high ^{15}N enrichment of all plants (Fig. 1). The obtained labelling efficiency varied with the climatic conditions and the dry warm weather promoting rapid absorption. The ^{15}N enrichment also varied with the actual N accumulation rate of the plant that was offered ^{15}N (Fig. 1). Consequently, high ^{15}N enrichments of ryegrass were obtained in the middle of each growing season when the growth rate was low.

Calculating the total balance for the ^{15}N administered over the two growing seasons demonstrated a high recovery ($\geq$83%) for the mixtures, whereas it only was 63–71% for species growing in pure stands (Table 3). The reason for the lower recoveries in pure stand plant communities is unknown. The ^{15}N balance further showed that between

Table 1
Dry matter yields (g m^{-2}) of shoots, stubble and macro-roots at the final harvest (means ± SE; $n = 4$ in pure stands and $n = 8$ in mixtures)

	Shoots above 5 cm	Stubble below 5 cm	Macro roots 0–10 cm	Macro roots 10–20 cm	Macro roots 20–40 cm	Macro roots 40–60 cm
RC–RG[a]	925 (60)	428 (23)	237 (20)	106 (13)	64 (7)	74 (1)
WC–RG[b]	196 (13)	317 (23)	174 (12)	47 (6)	49 (2)	44 (3)
RC (PS)[c]	650 (128)	612 (264)	240 (29)	88 (7)	73 (2)	72 (5)
WC (PS)[d]	167 (16)	520 (109)	211 (26)	59 (5)	59 (3)	58 (3)
RG (PS)[e]	89 (18)	266 (50)	160 (32)	47 (3)	42 (5)	43 (3)

[a] Red clover–ryegrass.
[b] White clover–ryegrass.
[c] Red clover in pure stand.
[d] White clover in pure stand.
[e] Ryegrass pure in stand.

Table 2
Nitrogen content (g m^{-2}) in shoots stubble and macro-roots at the final harvest (means ± SE; $n = 4$ in pure stands and $n = 8$ in mixtures)

	Shoots above 5 cm	Stubble below 5 cm	Macro roots 0–10 cm	Macro roots 10–20 cm	Macro roots 20–40 cm	Macro roots 40–60 cm
RC–RG[a]	20.7 (1.7)	12.3 (0.6)	5.7 (0.5)	2.3 (0.3)	1.3 (0.1)	1.3 (0.1)
WC–RG[b]	5.2 (0.4)	8.1 (0.6)	3.0 (0.2)	0.7 (0.1)	0.6 (0.1)	0.5 (0.1)
RC (PS)[c]	13.3 (0.8)	19.2 (2.8)	6.3 (0.7)	2.1 (0.1)	1.6 (0.0)	1.5 (0.1)
WC (PS)[d]	5.8 (0.5)	16.9 (3.1)	6.7 (1.3)	1.6 (0.2)	1.3 (0.1)	1.2 (0.1)
RG (PS)[e]	1.9 (0.3)	5.4 (1.0)	1.8 (0.1)	0.4 (0.1)	0.3 (0.1)	0.3 (0.0)

[a] Red clover–ryegrass.
[b] White clover–ryegrass.
[c] Red clover in pure stand.
[d] White clover in pure stand.
[e] Ryegrass pure in stand.

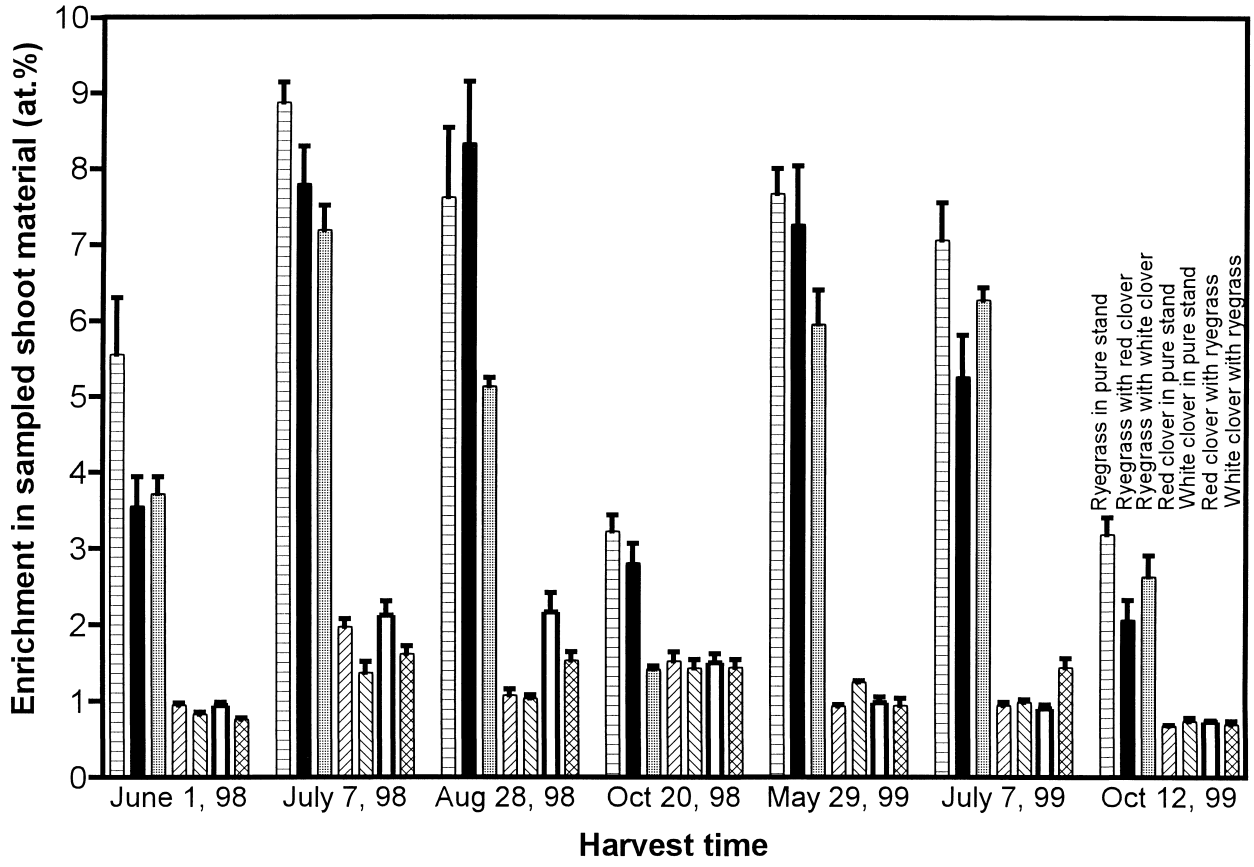

Fig. 1. ^{15}N-enrichment of shoot material during the two growing seasons of white clover, red clover and ryegrass growing in mixtures or pure stands over two

10 and 50% of the absorbed ^{15}N was recovered in the harvested shoot material (Fig. 2). The variation between years was small except for the ^{15}N-labelled red or white clover that had a greater proportion of the absorbed ^{15}N removed with harvested shoots in the first than in the second production year (Fig. 2). In all other cases, the differences between the plant communities were significantly greater than the differences between years.

The ^{15}N cross-labelling of both plant species in the mixtures clearly revealed that labelled white clover growing in a mixture with ryegrass retained the absolute greatest proportion of the absorbed ^{15}N. Importantly, the roots were relatively uniformly labelled except for ryegrass in pure stands, where the enrichment of the roots below 40 cm decreased rapidly. It must be noted that the measured enrichment of the roots of the mixtures was an average of the labelled and unlabelled species (Table 4) and the enrichment of roots from corresponding species in pure stands was used for calculating the rhizodeposition.

3.4. Rhizodeposition in rhizosphere soil and bulk soil

The proportion of the N in the soil that derived from rhizodeposition (%Ndfr) differed significantly between plant communities and between topsoil and deeper lying soil (Fig. 3). In pure stands of red and white clover and in

Table 3
Total balance of ^{15}N applied via leaf feeding to different grassland species growing in pure stands or mixtures over two growing seasons (means ± SE; $n = 4$)

	^{15}N absorbed (mg mezotron^{-1})	^{15}N recovered (mg mezotron^{-1})	^{15}N unaccounted (%)
^{15}N-RC w RG[a]	131 (11)	111 (13)	15 (6)
^{15}N-WC w RG	97 (10)	81 (5)	17 (6)
^{15}N-RG w RC	82 (3)	78 (3)	3 (5)
^{15}N-RG w WC	89 (3)	79 (4)	11 (4)
^{15}N-RC (PS)	135 (10)	85 (11)	37 (7)
^{15}N-WC (PS)	111 (7)	73 (5)	34 (7)
^{15}N-RG (PS)	87 (2)	62 (6)	29 (6)

[a] ^{15}N-labelled red clover with ryegrass; ^{15}N-labelled white clover with ryegrass; ^{15}N-labelled ryegrass with white clover; ^{15}N-labelled red clover in pure stand; ^{15}N-labelled white clover in pure stand; ^{15}N-labelled ryegrass in pure stand.

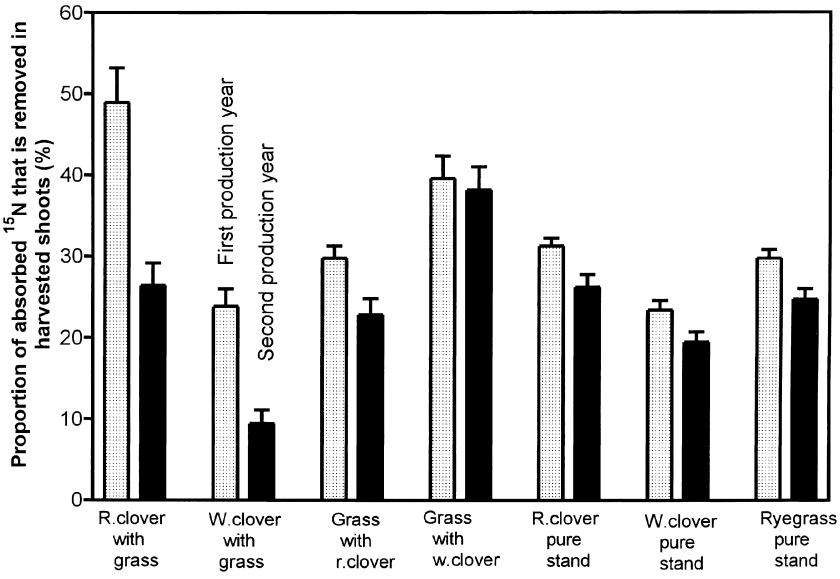

Fig. 2. Proportion of ^{15}N absorbed via leaf-feeding that subsequently was removed in harvested shoots of white clover, red clover and ryegrass growing in mixtures or pure stands over two complete growing seasons (means ± SE; $n = 4$).

Table 4
Average enrichment (at.%) of isolated macro roots at the final sampling. The ^{15}N enrichment of the roots of plants growing in mixtures is an average of the labelled and the unlabelled species as they cannot be separated (means; $n = 4$). Coefficients of variations were on average 9.6% and never exceeded 20%

	0–10 cm depth	10–20 cm depth	20–40 cm depth	40–60 cm depth
^{15}N-RC w RG[a]	0.6910	0.6201	0.5934	0.5944
^{15}N-WC w RG	0.9884	0.9590	0.9854	0.9396
^{15}N-RG w RC	0.6535	0.5629	0.5078	0.5195
^{15}N-RG w WC	1.0486	1.0184	1.0184	0.8663
^{15}N-RC (PS)	0.7181	0.6402	0.6036	0.6465
^{15}N-WC (PS)	0.9343	0.8712	0.8443	0.8157
^{15}N-RG (PS)	2.3740	2.2029	1.6760	1.6238

[a] ^{15}N-labelled red clover with ryegrass; ^{15}N-labelled white clover with ryegrass; ^{15}N-labelled ryegrass with white clover; ^{15}N-labelled red clover in pure stand; ^{15}N-labelled white clover in pure stand; ^{15}N-labelled ryegrass in pure stand.

Table 5
Deposition of N (g m^{-2}) in the rhizosphere and bulk soil of different grassland species growing in mixtures or pure stands (means ± SE; $n = 4$)

	0–10 cm depth	10–20 cm depth	20–40 cm depth	40–60 cm depth
^{15}N-RC w RG[a]	22.8 (9.2)	1.7 (0.2)	1.0 (0.8)	0.8 (0.1)
^{15}N-WC w RG	8.1 (3.9)	3.9 (1.6)	0.7 (0.2)	0.4 (0.1)
^{15}N-RG w RC	42.9 (10.4)	14.8 (2.1)	2.6 (1.6)	2.7 (2.0)
^{15}N-RG w WC	19.0 (3.7)	4.7 (1.7)	2.2 (0.4)	0.8 (0.3)
^{15}N-RC (PS)	52.1 (7.2)	10.2 (2.9)	0.8 (0.2)	1.1 (3.0)
^{15}N-WC (PS)	61.5 (14.9)	5.6 (0.7)	1.8 (0.4)	1.8 (0.6)
^{15}N-RG (PS)	6.7 (1.4)	1.8 (0.3)	0.6 (0.2)	0.3 (0.3)

[a] ^{15}N-labelled red clover with ryegrass; ^{15}N-labelled white clover with ryegrass; ^{15}N-labelled ryegrass with white clover; ^{15}N-labelled red clover in pure stand; ^{15}N-labelled white clover in pure stand; ^{15}N-labelled ryegrass in pure stand.

ryegrass growing in association with red clover more than 20% of the soil N in the top 10 cm soil layer derived from rhizodeposition. The corresponding value for the other plant communities was 3–7% (Fig. 3).

The quantity of N deposited over the 2 years, i.e. the product of %Ndfr and soil N content, amounted to 65–70 g m^{-2} in the pure stands of red and white clover. Assuming the N-rhizodeposits estimated from cross-labelling of either of the two species in the mixtures to be additive, the total amount of N deposited by red clover–ryegrass and white clover–ryegrass mixtures amounted to 89 and 32 g m^{-2}, respectively (Table 5). This deposition occurred predominantly in the top 10 cm soil layer.

The amount of the rhizodeposited N that remained in the

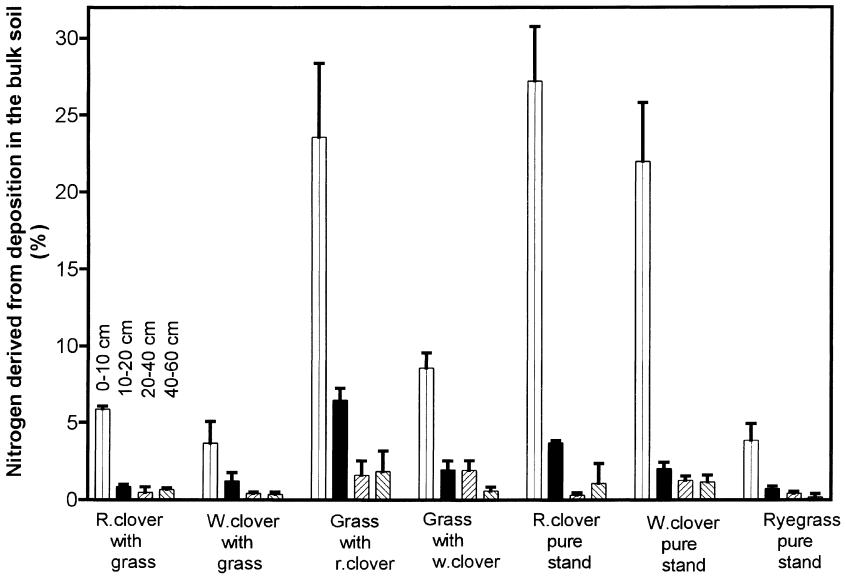

Fig. 3. Rhizodeposition of N in proportion of total soil N (bulk and rhizosphere) below white clover, red clover and ryegrass growing in mixtures or pure stands over two complete growing seasons (means ± SE; $n = 4$).

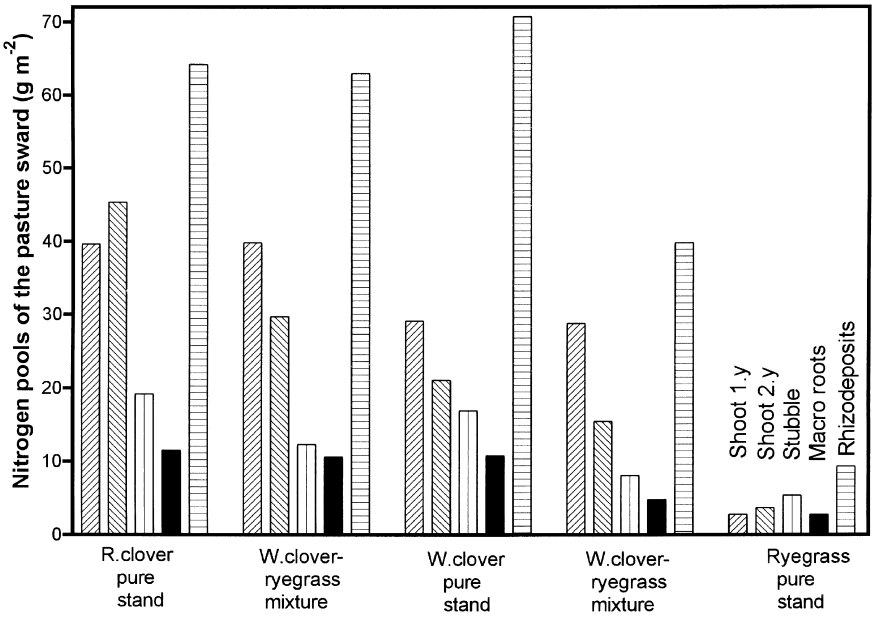

Fig. 4. Total nitrogen content of harvested shoots, stubble, macro roots and rhizodeposits of white clover, red clover and ryegrass growing in mixtures or pure stands over two complete growing seasons (means ± SE; $n = 4$ for pure stands, $n = 8$ for mixtures).

root-adhering soil only constituted a significant proportion of the total N deposition in the top 10-cm soil layer (data not shown). Here, the rhizosphere soil contained 12 and 15% of the N deposited by ryegrass in mixture with white clover and ryegrass in pure stands, respectively. In all other cases, the amount of rhizodeposited N remaining in the root-adhering soil did not exceed 3.5%.

3.5. The role of rhizodeposition in the N economy of the sward

Rhizodeposition constituted in all plant communities 80–87% of the total below-ground plant-derived N (Fig. 4). In the mixtures of red clover–ryegrass and white clover–ryegrass and the pure stands of red clover, white clover and ryegrass, respectively, the rhizodeposition constituted a 1.05, 1.52, 1.26, 2.21 and 2.77 fold increase over the total harvested shoot N during the two production years (Fig. 4).

4. Discussion

Using a ^{15}N leaf labelling approach directly on plants growing in the field the present work demonstrates that

rhizodeposition of N makes a very significant contribution to the N input in grasslands. In fact, rhizodeposition of N may even substantially exceed the amount of N removed by harvest of above-ground plant parts (Fig. 4). Expressed relative to the quantity of N recovered in macro roots, the rhizodeposition was in all cases more than 5-fold higher (Fig. 4). Not only rhizodeposition, but also stubble, using a cutting height of 5 cm, constituted a significant N pool, highlighting the importance of this fraction for the N dynamics in grasslands.

4.1. ^{15}N leaf labelling

The present use of the direct ^{15}N leaf labelling approach for assessing N rhizodeposition is based on two assumptions: (i) the ^{15}N enrichment of the N compounds deposited is assumed to be identical to that of the macro roots; and (ii) the ^{15}N enrichment of the soil N is accurately reflected in the ^{15}N enrichment of a non-leguminous crop.

Regarding the first assumption, the contribution of the ^{15}N to the total N content of particularly the legumes was minimal (Tables 2 and 3) and the labelling procedure resulted in a fairly uniform ^{15}N enrichment of shoots (Høgh-Jensen and Schjoerring, 2000) and macro roots in various depths (Table 4), indicating that the ^{15}N label was distributed relatively uniformly between all tissue and organs. Furthermore, the enrichment of the clover shoots was rather constant on a seasonal basis, while that of grass declined towards the end of each growing season (Fig. 1). Thus, %Ndfr may have been overestimated for grass, but not for legumes.

As regards the second assumption, the bioassay of ^{15}N enrichment of the soil-borne N is, even under relative undisturbed conditions, considered only to give an approximate value (see, e.g. Handley and Scrimgeour, 1997). However, even a 50% change in the estimate of the ^{15}N enrichment of the soil would only marginally change the estimates of N rhizodeposition. Furthermore, the fact that the estimated N rhizodeposition declined with depth in the soil profile and was very small in the deeper soil layers (Table 5) suggests that the procedure we used gave realistic results.

4.2. Input of residues

We removed visible root fragments by hand without any washing procedure. The recorded dry matter and N root yields of the ryegrass and clover species (Table 1) slightly exceeded yields of roots isolated from soil by wet-sieving under otherwise comparable experimental conditions (Haugaard-Nielsen et al., 1998; Høgh-Jensen et al., 1998). However, estimates of mature root biomass obtained by wet-sieving must be questioned due to the fragility of roots and due to the fact that fine roots, root hairs and partly or fully decomposed root materials are not retained in the wet-sieving procedure (Sauerbeck and Johnen, 1976; Unkovich et al., 1994). In addition to the lack of retention of such high quality root material, water-soluble compounds will also be lost from roots during wet-sieving, leading to lower concentrations of N in the recovered roots.

4.3. Rhizodeposition

Following deposition, some of the N will be stabilised in accordance with the theory of turnover of soil organic matter following a series of first-order reactions (Paul and Juma, 1981). This stabilised fraction is the one subsequently determined as deriving from rhizodeposition. The fraction that is not stabilised is subject to: (i) loss; (ii) re-absorption by the plant it originated from; or (iii) transfer to associated plant species (Høgh-Jensen and Schjoerring, 2000).

Even the N-deficient ryegrass plants growing in association with red clover or white clover contributed to a considerable N rhizodeposition (Figs. 3 and 4). Consequently, rhizodeposition can be viewed a net process where the C and the N input to the system may determine the amount of N that is immobilised in agreement with the rapid daily turnover of a relatively large pool of inorganic N below grassland (Ledgard et al., 1998).

Previous studies on N rhizodeposition have mainly been carried out using pure stands of plants grown for a relatively short period under controlled conditions in solution culture, pots or soil columns. Under such conditions, the rhizodeposition of N amounted to approx. 30% of the total below-ground plant-derived N in 3-month-old maize (Hétier et al., 1986) and, for mature plants, 46–48% in pea (Jensen, 1996; Sawatsky and Soper, 1991), 71% in barley (Sawatsky and Soper, 1991), 16–19% in wheat (Janzen, 1990; Janzen and Bruinsma, 1989; Janzen and Bruinsma, 1993) and 65% in lupin (Russell and Fillery, 1996b). Due to differences in rooting volume these values cannot be directly transferred to the field situation. Nevertheless, our estimates of field N rhizodeposition amounting to 80–87% of total below-ground plant-derived N (Fig. 4) agree reasonably well with the above-mentioned studies, also taking into account that a considerably longer period was covered. It agrees especially with the estimates of Russell and Fillery (1996b) who used soil columns. During two complete seasons of plants growing in a low fertility soil, the fine roots may turnover several times (Dubach and Russelle, 1994) and consequently contribute substantially to the deposition.

4.4. Turnover of plant-derived N in the soil

The ^{15}N cross-labelling approach revealed that ^{15}N-labelled white clover growing in mixture with ryegrass retained the absolute greatest proportion of the absorbed ^{15}N (Fig. 2). In addition, the amount of ^{15}N that was found in the stubble material at the end of the second growing season exceeded that removed in harvested shoots during one season (data not shown).

Studies of plant residue decomposition have often used macro roots as representing root material (e.g. Ladd and Amato, 1986). However, this approach will not give a

correct picture of the N turnover following ploughing-in of the grass sward due to differences in the quality of macro roots and that of rhizodeposited compounds (Jensen, 1996). Furthermore, the mass of macro roots isolated by wet-sieving or by hand picking underestimates the true input and will therefore not allow a correct prediction of crop rotational effects of grass swards (Høgh-Jensen, 1999). There is consequently a need for supplementing the empirical evaluations (Johnston et al., 1994; Haynes, 1999) with further studies of the quality of rhizodeposited N compounds and N-containing tissues.

Even N-deficient ryegrass growing in pure stand had a very substantial rhizodeposition of N expressed relatively to the N content of harvested shoots, stubble and macro roots (Fig. 4). This was the case despite a general high C-to-N ratio in grass tissue compared to clover and implies that the turnover of grass roots or excretion of N compounds from grass roots are higher than from red or white clover roots, in agreement with unpublished results by Andreas de Neergaard (pers. commun.) who, using $^{14}CO_2$ leaf labelling, found ryegrass to be the carbon donor when growing in mixtures with white clover. Ryegrass is well known for having a higher density and length of root hairs compared with clovers (e.g. Caradus, 1980). However, Dubach and Russelle (1994) found the turnover of fine roots and nodules in alfalfa to be high.

4.5. Conclusions

Our findings demonstrate the very important, but long unrecognised, role that rhizodeposition plays in the N economy of grassland. Thus, N rhizodeposition even exceeded the amount of N that was harvested in shoot material over two complete growing seasons for all communities. The N deposition also exceeded the amount of N contained in stubble and roots.

Expressed relative to the total amount of N in shoots, stubble and macro roots at the final harvest, the rhizodeposited compounds constituted 68, 70, 56, 91 and 64% of the N in the mixtures of red clover–grass and white clover–grass and the pure stands of red clover, white clover and ryegrass, respectively. The corresponding values for N rhizodeposition relative to total below-ground N were 80, 83, 85, 87 and 80%. Thus, the rhizodeposited compounds constituted by far the dominant pool of the plant-derived N in the soil.

The average contribution of fixed N_2 to total shoot N content as determined in plots adjacent to the mezotrons was for both red and white clover 92 and 95% in the first and second production year, respectively. In the pure stand of red clover it was 90% and in pure stands of white clover it was 84%. The major part of the deposited N would consequently derive from the atmosphere and as such exceed the amount of atmospheric-derived N in the harvested shoots. This very significant contribution must be taken into account when assessing the contribution of biological N_2 fixation to the N balance of terrestrial ecosystems.

Acknowledgements

Financial support from the Danish Agricultural Research Centre for Organic Farming is gratefully acknowledged. Further, the dedicated work of Mr Vagn Petersen in root isolation is much appreciated.

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