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REGULAR ARTICLE

Consequences of including adapted white clover in northern European grassland: transfer and deposition of nitrogen

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Abstract The interspecific transfer of nitrogen (N) between white clover (*Trifolium repens*) and smooth meadow grass (*Poa pratensis*) in legume-based grasslands was assessed under North European field conditions using ^{15}N individual plant leaf labelling. On average 50% of N in the grass was transferred from the white clover and about 6% of N in white clover was transferred from the grass. This corresponds to 2.5 and 0.3 g N m^{-2} being transferred over the growing season between the two species, respectively, and demonstrates that a significant part of the total N of the grass is coming through interspecific transfer. The majority of the ^{15}N transferred was within a period of 20 days at relatively low soil temperatures. This implies that there is a need for a new focus on direct transfer pathways or exudation and transfer of organic N sources. Rhizodeposition in the top 10 cm of the soil was found to be 2.98 g N m^{-2} on average over the growing season for the grass and white clover mixture. Inclusion of adapted white clover varieties in the low-input grassland systems of northern Europe will lead to a substantial contribution of N.

Keywords Bi-directional N transfer · Grassland · Legumes · N dynamics · N transfer · Rhizodeposition

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Introduction

Grassland is a central component of European agriculture and covers 37% of the total agricultural area (Olesen and Bindi 2002). White clover (*Trifolium repens* L.) is an important pasture legume in many temperate regions of the world and significantly affects the N status in agricultural systems through N_2 fixation. On average, 80% of the total N found in white clover grown in mixed grass swards is derived from the atmosphere, though this is influenced by such factors as the age of the sward, climate and cultivars (Carlsson and Huss-Danell 2003). This means that white clover is converting up to 545 kg of atmospheric N ha^{-1} $year^{-1}$ into potentially available N for other organisms. However, considerably lower values have been presented in sub-arctic regions. Fagberg and Sundqvist (1994) reported up to 99 kg ha^{-1} of fixed N in Northern Sweden (65° N) and the amount of fixed N has been calculated to range from 40 to 169 kg N ha^{-1} in Iceland (Helgadóttir and Kristjánsdóttir 1993). Using legumes

adapted to the northern regions of Europe may change the nutrient dynamics of the grass-dominated pastures in these regions. Little is, however, known about the potential impact of including such adapted varieties on the N status of the grassland.

In an N-limiting world, plants capable of N fixation have obtained well-earned attention. Legumes play an important role in enhancing the quality of the environment for other species. It has been generally assumed that N moves from N-rich legume plants to N-deficient grass plants but now it is acknowledged that N can also be transferred from non-legume plants to legumes (Høgh-Jensen and Schjoerring 2000). Nitrogen can be transferred both by direct and indirect pathways, below-ground and above-ground. Nitrogen can be transferred below-ground between species in a number of ways (Høgh-Jensen 2006). It can be released from living as well as decaying roots and nodules, and with the exudation of N compounds which are subsequently taken up by the receiver plant (Laidlaw et al. 1996; Paynel et al. 2001). Nitrogen can also be transferred through mycorrhizal fungi linking two plants (Bethlenfalvay et al. 1991; Johansen and Jensen 1996). Above-ground, N can be transferred via the atmosphere (Janzen and Gilbertson 1994), although volatilization of NH_3 from agricultural systems mainly is related to high-N input conditions (Herrmann et al. 2001), or via grazing herbivores (Høgh-Jensen and Schjoerring 2000).

Results of measured N transfer vary between studies and depend on the method used and whether the measurements are of direct or indirect transfer or both. Values ranging from 6 to 80% of total N in the grass have been published for N transfer from the legume to the associated grass (Broadbent et al. 1982; Brophy et al. 1987; Haystead and Marriott 1979). Up to 52% N transfer from white clover to its grass companion and about 12% transfer from grass to clover has been reported, which corresponds to approximately 6 and 0.36 g m^{-2}, respectively (Høgh-Jensen and Schjoerring 2000; Ledgard 1991).

It is difficult to investigate below-ground pathways, although over the years more precise methods have been developed (Høgh-Jensen 2006; Høgh-Jensen and Schjoerring 2000). Direct labelling of plants can be carried out by adding ^{15}N to enclosed canopy or by immersing plant parts in a solution containing ^{15}N. A direct ^{15}N leaf feeding technique enables labelling of individual plants in plant communities and, more importantly, it allows the detection of transfer from the grass plant to the clover plant and vice versa, i.e. bi-directional transfer. In addition, it enables the detection of transfer within shorter periods of time.

Rhizodeposition makes N compounds accessible for mineralization and therefore is one of the main factors that affect N availability in the soil (Heal et al. 1999). Rhizodeposition is a transfer of root-derived organic and inorganic compounds into the soil that occurs via exudation as well as decomposition of root litter (Hertenberger and Wanek 2004). Such rhizodeposition can vary between plant communities as the proportion of N in the soil derived from rhizodeposition varies between plant species (Høgh-Jensen and Schjoerring 2001). Breakdown of organic compounds in the rhizosphere has major positive effects on the density and activity of microorganisms, thus greatly increasing their productivity near the roots.

There is a lack of knowledge on the importance of transfer and deposition of N in low-input systems in the northernmost parts of Europe. Soil temperatures are low in these regions and low rates of N mineralization could therefore be expected (Stanford et al. 1973). High amounts of mineralized N are, though, important properties of Andosols (Nanzyo et al. 1993), a soil type that is dominant in Iceland. N transfer has not been measured in the field in regions where there is a short growing season characterised by low temperatures and long photoperiods. It is therefore of interest to study the effects of such environmental conditions on N dynamics, in particular whether N transfer occurs over a short period of time.

The objective of the present experiment was to (1) quantify the bi-directional N transfer between white clover and smooth meadow grass in Iceland over one growing season using the direct ^{15}N leaf feeding technique, (2) test whether leaves can represent the whole plant (roots, stolons, leaves) when N transfer is measured and (3) compare the rhizodeposition of N between white clover and smooth meadow grass over the experimental period.

Materials and methods

Experimental site

The experiment was carried out at Korpa Experimental Station in Iceland (64° 04′N, 21° 58′W, 30 m

above sea level) in summer 2003. The growing conditions are characterized by long photoperiods and relatively long and cool summers. The soil at the experimental site is a Gleyic Andosol (Arnalds 2004, 2005) with a mean bulk density of 0.52 and 0.57 g cm^{-3} for the top 10 and 10–30 cm depth, respectively, and a total N of 0.9% in 0–30 cm depth (C/N ratio of 12; Guicharnaud and Björnsson 2004).

Experimental design

The experiment was established in a grass field that had been sown with a mixture of white clover (*Trifolium repens* L. cv. Norstar from Norway) and smooth meadow grass (*Poa pratensis* L. cv. Fylking from Sweden) in the spring of 1999. The site had received the equivalent of 50 kg N ha^{-1}, 50 kg P ha^{-1} and 50 kg K ha^{-1} at sowing and 20 kg N ha^{-1}, 30 kg P ha^{-1} and 50 kg K ha^{-1} each spring for the three subsequent years. In early May 2003 the site was fertilized with the equivalent of 20 kg N ha^{-1}, 32 kg P ha^{-1} and 32 kg K ha^{-1} to prevent N deficiency in the plant community. Subsequently, 32 PVC cylinders with a diameter of 30 cm were inserted to a depth of 30 cm into the grass sward, thus making up the experimental plots. The experimental design was a randomised block with four replicates and a treatment consisting of four different times of first cut that cover the whole growing season (Fig. 1). For each cutting treatment, two cylinders were used to label either individual grass or white clover plants with ^{15}N.

The first treatment was cut on 30 June (H1), and other times of cutting were on 14 July (H2), 28 July (H3) and 12 August (H4). At each sampling occasion, eight separate cylinders were cut, except on 12 August when one cylinder had to be omitted because of damage. At the last sampling date, the regrowth (H5) in those cylinders that had been cut on the 30 June (H1) was also taken.

^{15}N leaf labelling

To detect bi-directional N transfer between white clover and smooth meadow grass either individual grass or clover plants within each cylinder were repeatedly labelled with a 0.5% (*w*/*w*) highly ^{15}N enriched (99 atom%) urea solution (McNeill et al. 1997). One newly expanded clover leaf or a few young grass leaves of unspecified leaf area were submerged for a few days at a time into 1 ml of urea solution in Eppendorf tubes. The tubes were closed with inert plastic material (Sticky Tac, Henkel Consumer Adhesives, Winsford, UK) in order to limit evaporation and to prevent rainwater entering into the

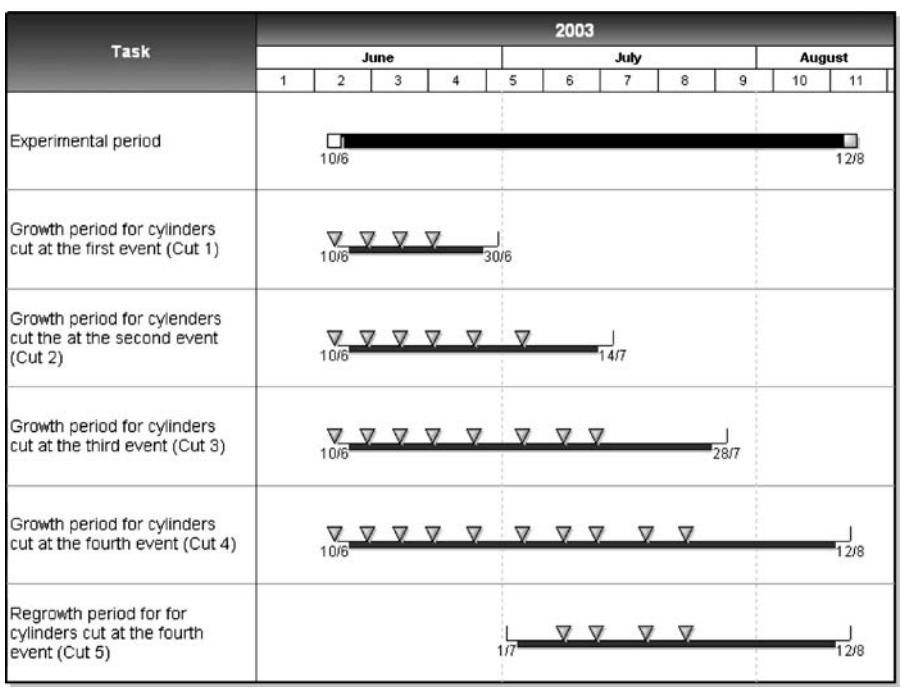

Fig. 1 Overview over labelling and cutting events for the different treatments (H1–H5) during the course of the experimental period (labelling events are depicted by the symbol *triangle*)

tubes. Care was taken to prevent contamination of ^{15}N to the plots during the labelling.

The labelling was started in all cylinders on 10 June. The cylinders were repeatedly labelled over the growing season prior to sampling but the number of labelling occasions varied between cuts (Fig. 1). Thus, the cylinders sampled on 30 June (H1), 14 July (H2), 28 July (H3) and 12 August (H4) were labelled repeatedly, four, six, eight and ten times, respectively, and ^{15}N was applied four times before the regrowth was cut on 12 August (H5). Leaves from different plants were used for each labelling occasion. The leaves were enclosed in the tubes between 6 and 9 days depending on weather conditions, influencing the evapotranspiration of the plants, the driving force in the uptake of the ^{15}N. At the end of each labelling period, the tubes were removed from the cylinders in order to give sufficient time for the ^{15}N to diffuse in the plant before each cut. The plots were cut 4, 6, 6 and 7 days after the labelling was completed for the four different sampling dates, respectively.

Sampling and analysis of above-ground and below-ground material

The cylinders were cut with hand shears to a stubble height of 5 cm and the herbage was separated into white clover, smooth meadow grass and unsown species. Each fraction was dried at 80°C for 60 h and dry matter determined. All fractions were ground using a ball mill (Retsch, Germany). After each cut, the cylinders, containing soil cores, were removed from the experimental site. The following day the soil cores were separated into two fractions; 0–10 and 10–30 cm. Cylinders originally cut on 30 June were not dug up until after the regrowth had been taken on 12 August.

Roots from the top 10 cm were carefully separated into clover and grass roots by hand and washed, and clover stolons and grass crowns were removed from three sub samples of turf taken by auger with a diameter of 10 cm. Utmost care was taken in separating the roots and roots of unsown species were discarded. All plant parts were washed and dried at 80°C for 60 h and ground by a ball mill.

All soil particles were carefully removed from roots by hand and mixed with the rest of the soil in the top 10 cm. The two soil fractions were weighted and homogenized and a subsample from each fraction was subsequently dried at 80°C for 60 h.

All three plant parts of clover and grass, leaves, stolons/crowns and roots, from all cuts as well as soil samples from all cylinders were analyzed for total-N and ^{15}N using a ANCA-SL Elemental Analyser coupled to a 20–20 Tracer Mass Spectrometer (Europa Scientific, Crewe, UK).

Identical samples were also collected and analyzed from an unlabelled area on 12 August to provide information on the natural ^{15}N concentration on the background or for each plant part and the soil.

Calculation of N transfer

The calculated N transfer between the species is presented as percentage of transferred N (%Ntrans) of the total N of the receiver plant. The %N transferred between white clover and grass and vice versa was calculated using a modification of an equation from Høgh-Jensen and Schjoerring (2000). Transfer from white clover labelled with ^{15}N to unlabelled grass was calculated as:

$$\%\mathrm{Ntrans} = \frac{N_{grass} * (G_1 - G_0)}{N_{clover} * (C_1 - C_0) + N_{grass} * (G_1 - G_0)} * 100 \quad (1)$$

where N_{grass} and N_{clover} denote N content of smooth meadow grass and white clover, respectively, G_1 is the atom% of grass and G_0 is the natural enrichment of grass growing under unlabelled field conditions next to the experimental plots, C_1 is the atom% of labelled clover and C_0 is the natural enrichment of clover growing under unlabelled field conditions. Calculations were carried out for each plant fraction, i.e. leaves, stolons/crowns and roots, separately as well as all plant parts together using the weighted atom% and thus transfer between different plants parts could be calculated. When calculating the N transfer from grass to clover Eq. 1 was modified accordingly. In these calculations it is assumed that ^{15}N enrichment of the donor plant stays constant over time and that the absorbed ^{15}N in all plant N pools subject to N transfer is equally distributed within the plant.

To determine the quantity of N transferred, the % Ntrans value obtained by Eq. 1 was subsequently multiplied by the total N content of leaves of the receiver plants within the cylinder.

Calculation of N rhizodeposition

The N rhizodeposition (%Ndfr) derived from the two species, white clover and grass, was calculated using an equation described in Schmidtke (2005) of excess atom% for each part:

$$\%\mathrm{Ndfr} = \left(\frac{\text{atom\% }^{15}\text{N soil} - \text{atom\% }^{15}\text{N background soil}}{\text{atom\% }^{15}\text{N roots} - \text{atom\% }^{15}\text{N background roots}}\right) * 100 \quad (2)$$

Atom% ^{15}N soil is measured ^{15}N abundance in soil. Natural abundance of soil samples measured outside the experimental plots were used as atom% ^{15}N background soil. Atom% ^{15}N roots is measured ^{15}N abundance in roots of labelled white clover or labelled smooth meadow grass. The natural abundance of unlabelled white clover and grass roots growing outside the experimental plots was used as atom% ^{15}N background roots. The rhizodeposition was calculated separately for cylinders with labelled white clover and for cylinders where grass was labelled, only taking into account roots from species that were labelled in each cylinder.

To determine the quantity of N in soil derived from rhizodeposition (Ndfr) the %Ndfr was multiplied by total N in top 10 cm of soil. In order to quantify the proportion of plant-derived N in the soil, Ndfr was divided by total N in white clover and grass, respectively (Schmidtke 2005).

Statistical analysis

The data were analyzed using standard ANOVA, *t*-test and correlation calculations in Genstat Version 7.1 (Lawes Agricultural Trust 2003). Missing value was estimated for the damaged plot for the cut on 12 August. Results for the transfer and rhizodeposition were analysed separately for each species component. For the leaf fractions, results from all five cuts (H1–H5) were included in the same ANOVA.

Results

Dry matter and N accumulation

The dry matter yield increased significantly from H1 to H3 with an overall mean of 484 g m^{-2} over the whole growing season (Table 1). The proportion of the clover in the sampled herbage did not change significantly over the growing season (Table 1), averaging 28% of the total dry matter.

The N content was calculated in the herbage as well as in the samples collected by the auger thus allowing determination of the total N of the plant (Table 1). For H1 values are only presented for N in the herbage as no soil cores were taken at this stage. The difference between the total N and N in the herbage is therefore the N content in stolons/crowns and roots of the plants. The N yield of the plant community generally increased from H1 to H3 reflecting increased dry matter yield.

Nitrogen concentration (%N) was higher for white clover than smooth meadow grass (Table 2) in all plant parts although both the amount of N in the herbage as well as total N was higher for the grass (Table 1). For both species %N in the leaves was highest at H1 and H5.

Ranking the %N for different plant parts at each cut separately (H2 to H5) showed that the grass leaves had higher %N than other grass organs at all cuts ($P<0.001$) and the %N was lower in roots than the crowns (H2, H4, H5, $P<0.001$; H3, $P=0.007$). For the white clover the %N was always highest in the leaves ($P<0.001$) but no significant difference was found between the stolons and the roots.

Table 1 Total dry matter yield and the proportion of clover in the herbage, total nitrogen in all plant parts (leaves, stolons/crowns, roots) and nitrogen accumulated in the herbage for white clover and smooth meadow grass at each cut (H1–H4) and in the regrowth of H1 (H5)

Cut	Herbage		Total N (g m^{-2})		N in herbage (g m^{-2})	
	Total (g m^{-2})	% Clover	Clover	Grass	Clover	Grass
H1	265	33	–	–	3.5	4.1
H2	370	24	5.6	7.8	3.2	5.1
H3	514	31	7.7	9.7	5.9	6.2
H4	543	30	8.7	8.4	6.0	6.4
s.e.d.	49.8***	5.17[a]	1.51[a]	0.77[a]	1.06*	0.60***
H5[b]	242	23	3.5	7.4	2.2	4.7

*$P<0.05$

***$P<0.001$

[a] Non-significant

[b] Regrowth of H1

Table 2 Percentage N (%N) of plant parts of smooth meadow grass (leaves, crows and roots) and white clover (leaves, stolons and roots) at each cut

Cut	Grass			White clover		
	Leaves	Crowns	Roots	Leaves	Stolons	Roots
H1	2.45	–	–	4.08	–	–
H2	1.99	1.47	0.98	3.71	2.66	2.71
H3	2.00	1.41	1.11	3.56	2.50	2.49
H4	1.96	1.38	1.02	3.65	2.68	2.64
H5[a]	2.72	1.80	1.10	3.77	2.50	2.71
Mean	2.23	1.52	1.06	3.76	2.58	2.64
s.e.d	0.112***	0.124**	0.618[b]	0.105***	0.122[b]	0.133[b]

**$P<0.01$

***$P<0.001$

[a] Regrowth of H1

[b] Non-significant

Labelling

The ^{15}N labelling of white clover and grass resulted in elevated ^{15}N atom% compared to natural abundance of samples taken outside the experimental plots, both in grass and white clover during the whole growing season (Table 3). This shows that the direct leaf feeding technique resulted in sufficient enrichment of ^{15}N in the donor plant for ^{15}N to be detected in the receiver plant. Further, the ^{15}N atom% exceeded the natural enrichment of the air for both white clover and smooth meadow grass grown under unlabelled field conditions at the experimental site.

The grass component generally had higher ^{15}N atom% than the clover component at all cuts (Table 3). For both species, the lowest atom% appeared in the roots and the highest in the stolons of the clover and crowns of the grass though this trend was not significant at all cuts. It is important to bear in mind that it was not possible to compare atom% of different plant parts in H1 as no soil cores were taken on that occasion.

The plants received ^{15}N either by direct labelling or by transfer from associated companion species. For white clover, the ^{15}N atom% was highest in the stolons and lowest in the leaves when the ^{15}N derived from transfer whereas it was highest in the leaves and lowest in the roots when the ^{15}N derived from direct leaf labelling. For the grass the atom% was lowest in the roots and highest in the crowns independent of the origin of the ^{15}N.

Relative and absolute transfer

Around half of the N in grass was derived from the associated white clover over the whole growing season, whereas only about 6% of the N in clover was derived from the grass companion (Table 4). The

Table 3 Atom% ^{15}N of grass and clover plant parts in mixtures where either grass or clover plants were labelled with ^{15}N

Cut	Grass only labelled						White clover only labelled					
	Grass			Clover			Grass			Clover		
	Leaves	Crowns	Roots	Leave	Stolons	Roots	Leave	Crowns	Roots	Leave	Stolons	Roots
H1	0.989	–	–	0.435	–	–	0.481	–	–	0.784	–	–
H2	1.138	1.225	0.726	0.422	0.412	0.397	0.541	0.544	0.452	0.685	0.578	0.453
H3	1.367	2.077	0.931	0.423	0.467	0.451	0.569	0.674	0.535	0.590	0.710	0.498
H4	1.675	3.344	1.460	0.442	0.505	0.527	0.562	0.622	0.533	0.641	0.675	0.495
H5[a]	1.163	1.436	0.801	0.474	0.535	0.501	0.584	0.657	0.501	0.783	0.760	0.585
Mean	1.266	2.021	0.980	0.439	0.480	0.469	0.547	0.624	0.505	0.697	0.681	0.508
s.e.d.	0.135**	0.603*	0.168*	0.014*	0.055[b]	0.053[b]	0.040[b]	0.088[b]	0.048[b]	0.133[b]	0.129[b]	0.057[b]

Natural enrichment of ^{15}N in white clover (taken out side the experimental plots); leaves=0.367, stolons=0.366, roots=0.367, and grass; leaves=0.369, crowns=0.367, roots=0.374

*$P<0.05$

**$P<0.01$

[a] Regrowth of H1

[b] Non-significant

calculated %Ntrans generally did not vary between cuts except for the following; transfer from clover leaves to grass leaves was significantly lower at H1 compared to later cuts (P=0.031), and transfer from grass leaves to clover leaves decreased between H1 and H2 (P=0.008).

The highest calculated %Ntrans (82.1%) was found for transfer from clover roots to grass roots but lowest transfer (23.2%) was found from clover stolons to grass crowns (Table 4). In contrast, higher %Ntrans values were found for transfer from grass crowns to clover stolons compared to transfer from leaves and roots. The values for %Ntrans based on calculations for the whole plant, on one hand, and the leaves, on the other, were however highly correlated both for white clover (r=0.924) and grass (r=0.966). The %Ntrans values from clover to grass were overestimated up to 8% (P=0.078) when leaf values were used compared to whole plant values and this overestimation increased as the growing season progressed. However, transfer from grass to clover was underestimated up to 20% by basing the calculations on leaf values instead of whole plant parts (P<0.001).

The observed N transfer is reflected in the N yield of the plant community. The amount of N transferred from clover to grass is considerably higher than the corresponding value for transfer from grass to clover (Table 4). The amount of N transferred from clover to grass increased from H1 to H3 but the same did not apply for transfer in the other direction.

Soil and rhizodeposition

Analyses of soil samples revealed no significant differences of the total N in the soil, between neither cuts nor different soil depths. The soil samples indicate a higher ^{15}N atom% in the soil than the natural enrichment of the atmosphere and the atom% was significantly higher in the top 10 cm compared to the lower soil layer (10–30 cm) in the experimental plots (P<0.001; Fig. 2). However, no significant difference in the natural abundance of ^{15}N was found at different depths for soil samples taken outside the experimental plots.

When the plant community and the top 10 cm of soil are considered as an isolated system and the contribution of each component of the system to total N and total ^{15}N, including all plant parts and the soil, is calculated it becomes evident that almost all (97%) of the N is found in the top 10 cm of soil (Table 5). However, the proportion of ^{15}N found in the soil is not as high as for the total N.

Rhizodeposition in the top 10 cm of soil was calculated according to equation (2). A significantly higher percentage of N in the soil was derived from

Table 4 The proportion of nitrogen (%Ntrans) derived from the associated species for H1 to H5, calculated on basis of the ^{15}N enrichment separately for each plant part (leaves, stolons/crowns, roots) and the whole plant, and the amount of transferred nitrogen derived from white clover and grass based on N in herbage

Cut	Transfer (%)								Amount transferred (g m^{-2})	
	From white clover parts to grass parts				From grass parts to white clover parts				From clover to grass	From grass to clover
	Leaves to leaves	Stolons to crowns	Roots to roots	All parts	Leaves to leaves	Crowns to stolons	Roots to roots	All parts		
H1	24.5	–	–	–	7.4	–	–	–	1.1	0.3
H2	43.7	29.5	76.7	43.0	3.5	8.8	2.5	4.4	2.0	0.1
H3	56.4	24.7	92.0	56.2	5.7	21.9	2.5	6.4	3.4	0.4
H4	46.6	13.4	79.2	43.0	5.6	21.2	5.4	7.4	3.0	0.4
H5[a]	60.2	25.1	80.4	54.2	6.0	28.9	2.2	7.5	2.8	0.1
Mean	46.3	23.2	82.1	49.1	5.7	20.2	3.2	6.4	2.5	0.3
s.e.d.	8.41*	6.10[b]	7.38[b]	9.14[b]	1.51[b]	9.92[b]	1.37[b]	2.27[b]	0.66*	0.15[b]

[a] Regrowth of H1

[b] Non-significant

*P<0.05

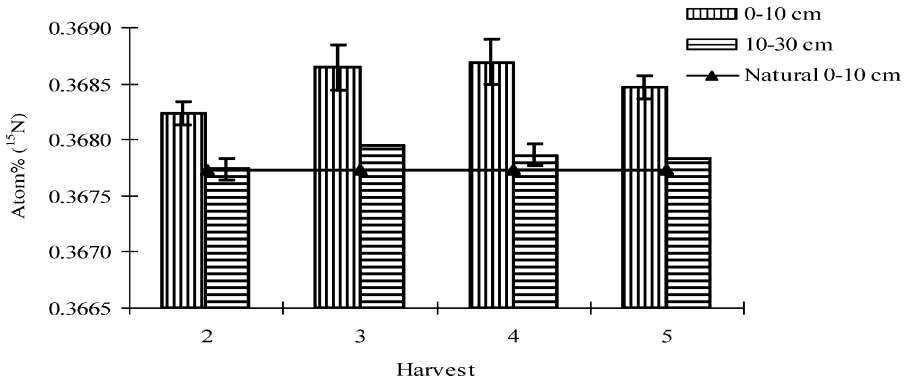

Fig. 2 ^{15}N atom% in the soil in two depths, 0–10 and 10–30 cm, in the experimental plots at different cuts, compared with the measured atom% of soil outside the experimental area taken in the same depth, 10–30 cm (designated 'natural'; values are means± standard error, n=8 except for H4 where n=7)

white clover deposition (%Ndfr) than its grass companion, or 0.46 and 0.18%, respectively, averaged over cuts (P=0.022). Similarly, the quantity of the N deposition (Ndfr) was significantly higher for white clover compared to the grass, or 2.16 and 0.82 g m^{-2}, respectively, averaged over cuts (P=0.029).

Further, the proportion of plant-derived N found in the top 10 cm of soil was significantly higher for white clover compared to grass, i.e. 47 and 10%, respectively, of the total N of the species (P=0.021).

Discussion

In the present work, transfer of N between white clover and smooth meadow grass as well as rhizodeposition was quantified over a full growing season in Iceland using direct ^{15}N leaf labelling technique under field conditions. Most grassland areas in northern Europe are characterised by a short growing season, low mineralization rates of the soil organic N and relatively low productivity. Thus, introducing adapted clover varieties into such areas may have important consequences for the whole N dynamics in the system (Nesheim and Oyen 1994).

Table 5 Proportions of nitrogen (N) and ^{15}N of the total N and total ^{15}N in the system found for different plant parts and top 10 cm of soil and the corresponding amounts (g m^{-2}; values are means for all cuts except H1)

Component	N		^{15}N	
	g m^{-2}	% of the system	g m^{-2}	% of the system
White clover				
Leaves	4.26	0.9	0.02	4.9
Stolons	1.63	0.4	0.01	2.1
Roots	0.37	0.0	0.00	0.4
Total	6.26	1.3	0.03	7.4
Grass				
Leaves	5.56	1.2	0.03	7.7
Crowns	0.61	0.1	0.01	2.3
Roots	2.16	0.5	0.01	3.4
Total	8.33	1.8	0.06	13.4
Soil	464.27	96.9	0.35	79.2
Total	478.86	100	0.44	100

Assumptions for the ^{15}N direct leaf labelling technique

The direct leaf feeding technique is straight forward, effective and more accurate than soil ^{15}N isotope dilution techniques. The use of 0.5% urea solution for labelling as used here has been shown to cause negligible damage to the labelled plant tissue (Khan et al. 2002). It has also been shown that the direct leaf feeding technique is valid for examining belowground N deposition (Hertenberger and Wanek 2004; Høgh-Jensen and Schjoerring 2001; McNeill et al. 1997).

The ^{15}N leaf labelling technique relies on the assumptions of (1) constant ^{15}N enrichment of the donor plant over time, and (2) equal distribution of the absorbed ^{15}N in all plant N pools subject to N transfer. The current study complied with the first assumption by applying frequent labelling during the experimental period that maintained a relatively high ^{15}N over the whole growing season (Table 3). Further, the study complied with the second assumption by having comparable enrichments of the N pools in the various organs, although the ^{15}N atom% was always lowest in the roots of both clover and grass (Table 3). This agrees with previous studies showing the highest atom% in the shoot material of labelled plants (Ledgard et al. 1985; McNeill et al. 1997) although

previous studies have mostly not reported enrichments of leaves, stolon and crown separately (Hertenberger and Wanek 2004; Ledgard et al. 1985; McNeill et al. 1997; Zebarth et al. 1991).

Senescence may influence the ^{15}N distribution in the plant organs. It has been reported that mature lupine roots can have somewhat lower concentration of ^{15}N than younger tissue and it is also known that plant parts are not all equally susceptible to the reduction of ^{15}N by age, with leaves and stems being less susceptible than roots (Russell and Fillery 1996). Further, nodules of legumes are not as highly enriched as roots. This can lead to a distortion in the calculation of below-ground N of legume systems as the assumption, that the atom% of recovered roots is representative of enrichment of all root derived N, may not hold (Khan et al. 2002). The distribution of ^{15}N is also influenced by the growth stage of the plant as N moves internally within the plant depending on the N need at a particular time (Table 2; Farrington et al. 1977). Høgh-Jensen and Schjoerring (2000) reported a decrease in atom% with increasing rooting depth. However, this should have negligible effect on the present calculations as the main root mass was in the top 10 cm.

The calculated N transfer based on leaves or the whole plants was comparable although there was a tendency to overestimate the N transfer from white clover to grass using only samples from leaves compared to all plant parts (Table 4). Similarly the data showed a small underestimation of N transfer from grass to clover using samples from leaves instead of samples from the whole plant.

N transfer

Bi-directional transfer of N between white clover and smooth meadow grass was confirmed in the present study (Table 4). Almost 50% of the N found in the grass derived from white clover and 6% of the N in the clover derived from the grass companion. This amounts to a mean transfer of 2.5 and 0.3 g N m^{-2} from clover and grass, respectively, over the growing season (Table 4). There was no significant difference in the amount transferred between different cuts, except that transfer from white clover leaves to grass leaves was significantly lower at H1 compared to later cuts.

There are only a few studies that have measured bi-directional transfer between legumes and grasses. The proportion of transferred N found in this study is comparable with previous results although the amount of N transferred is somewhat lower (Høgh-Jensen 2006; Høgh-Jensen and Schjoerring 2000; Ledgard 1991). Other studies have reported between 6 and 80% N transfer from legumes to grass under diverse conditions and using different methods (Broadbent et al. 1982; Brophy et al. 1987; Haystead and Marriott 1978) or failed to detect N transfer from legumes to their grass companions (Trannin et al. 2000; McNeill and Wood 1990).

Considering the northern growing conditions in Iceland, the above-mentioned difference in the amount of N transferred can be explained by lower growth rate and lower dry matter yields than in more southern regions. The average yield over the growing season in Iceland was 484 (Table 1) compared to 780 g DM m^{-2} on average in the experiment of Høgh-Jensen and Schjoerring (2000). Further, new nodules have to be formed every spring in temperate legumes (Bordeleau and Prevost 1994) possibly limiting the N transfer to the companion grass early in the growing season (Table 2).

It is surprising how quickly the ^{15}N tracer appeared in substantial amounts in the associated species (Table 4) as the soil temperature at 10 cm was only around 13°C during this period. Mineralization rates can be expected to be low at such soil temperatures and normal hierarchical mineralization concepts (Christensen 2001) therefore seem unable to explain such high rates of N transfer. Thus attention is drawn to direct transfer pathways and/or release and uptake of organic N compounds as possible transfer mechanisms. Transfer values based on indirect ^{15}N isotope dilution methodologies (Brophy et al. 1987; Høgh-Jensen and Schjoerring 1997; Ledgard et al. 1985) must thus be used with caution.

Soil deposition

The largest amount of N in the plant–soil system was found in the soil (Table 5). Decomposition is a very important process in the nutrient cycles of most terrestrial agroecosystems (Wachendorf et al. 1999). In grasslands, where the above-ground biomass is removed, the root turnover provides most of the dead organic matter in the soil (van der Krift et al. 2001). In the current study, clover had a higher ($P<0.05$)

proportion (%Ndfr) and amount of N (Ndfr) rhizodeposited than grass.

The Andosols in Iceland normally have high contents of organic carbon and N (Guicharnaud and Björnsson 2004; Satio 1990) but these soils do not supply the grass with more than 0.3–0.4 kg N ha^{-1} d^{-1} over the growing season (Björnsson 2004). However, rhizodeposition and mineralization are interacting soil pathways. In the current study rhizodeposition amounted to approx. 3.0 g N m^{-2} (Table 5; Fig. 2) demonstrating how the inclusion of appropriate legumes in these swards may lead to much more dynamic and short-term N fluxes (Tables 4 and 5; Høgh-Jensen 2006). The deposition amounted though to less than what was observed for pastures under more favourable growing conditions (Høgh-Jensen and Schjoerring 2001; McNeill et al. 1997).

Pathways for N transfer in light of the results

In the present study 1.1 g N m^{-2} were rapidly transferred from white clover to grass over the first period of 20 days in early summer. Mineralization can only explain a small part of this because of the limited time that was available for the ^{15}N to move from labelled clover leaves to the companion grass particularly under conditions with low soil temperatures. This contrasts at least partly with the perception that N transfer from clover to grass occurs mostly by decomposition of roots and nodules (Ledgard 1991). More rapid pathways for the N transfer must therefore be considered, such as direct uptake of organic N compounds (Chapin et al. 1993; Jones and Darrah 1993, 1994; Kielland 1994). Such organic N compounds may come from degradation of fresh organic materials or from exudations, in particular from clover (Paynel et al. 2001; Rasmussen et al. 2006). Transfer through mycorrhiza is another possibility which could explain the rapid transfer observed (Moyer-Henry et al. 2006; Zhu et al. 2000).

The applied labelling approach opens for the possibility that the newly labelled leaves, during periods of rain, could leach ^{15}N from the leaf surfaces to the soil and thus create artefacts. It cannot be excluded that such leaching has taken place and thus influenced the data for N transfer as well as deposition. However, such an effect is considered minor due to the fact that the top soil was only slightly more enriched with ^{15}N than the subsoil (Fig. 2). Further, such an effect should have been detectable in the grass component at H5 as grass is the strongest competitor for inorganic N (Høgh-Jensen and Schjoerring 1997) and therefore for any leached ^{15}N from the first labelling event (H1). This was, however, not observed in the current study (Table 5). Such potential leaching of ^{15}N needs nevertheless to be addressed in future studies.

In conclusion, the current study demonstrates a considerable bi-directional N transfer between legume and non-legume species showing that a significant part of the total N of the plant is coming through interspecific transfer. Basing calculations of N transfer on the N pools in different plant organs gave comparable results. The transfer took place within a period of 20 days indicating that transfer following mineralization may not be the major pathway. On average, 2.98 g N m^{-2} were deposited in the soil by white clover-based grassland. It may be expected that these results will encourage additional use of legumes in agriculture in the northern regions.

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