Plant and Soil **166:** 63–74, 1994.

Carbon distribution and nitrogen partitioning in a soil-plant system with barley (*Hordeum vulgare* L.), ryegrass (*Lolium perenne*) and rape (*Brassica napus* L.) grown in a $^{14}CO_2$-atmosphere

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Received 31 August 1993. Accepted in revised form 30 June 1994

Key words: ^{14}C, ^{15}N fertilizer, N transformations, N uptake, root-derived C, rhizosphere, soil biomass

Abstract

To examine the influence of plant-microorganism interactions on soil-N transformations (e.g. net mineralization, net immobilization) a pot experiment was conducted in a ^{14}C-labelled atmosphere by using different (two annuals, one perennial) plants species. It was assumed that variation in below-ground, microorganism-available C would influence N transformations in soil. Plant species were fertilized (low rate) with ^{15}N-labelled nitrogen and grown, during days 13 and 62 after germination, in a growth chamber with a ^{14}C-labelled atmosphere. Nitrification was inhibited by using nitrapyrin (N-Serve). During the chamber period, shoots were harvested, and associated roots and soil were collected on two sampling occasions, e.g. after 4 and 7 weeks in the growth chamber.

The distribution of net (%) assimilated ^{14}C was significantly affected by both plant and time factors, and there was a significant plant × time interaction. There were significant differences between plants in all plant-soil compartments examined as well as in the degree of the plant × time interaction.

Differences in the ^{14}C distribution between plants were due to both interspecific and developmental variation. In general, when comparing ^{15}N and ^{14}C quantities between species, many of the differences found between plants can be explained by the differences determined in the weight of shoot or root parts. Despite the fact that amounts of C released were greater in ryegrass than in the other plant-treatments no unequivocal evidence was found to show that the effects of plant-microorganism interactions on soil-N mineralization were greater under ryegrass. Possible mechanisms accounting for the partitioning of N found among plant biomass, soil biomass and soil residues are discussed.

Introduction

Plant-related factors that modify the flow of photosynthates to roots, and thus the input of C to soil, include species, age and stage of development (van Veen et al., 1989). Differences in the photosynthate distribution pattern were found between wheat and maize (van Veen et al., 1989), pea seedlings and tomato seedlings (dicotyledons; Whipps, 1987) and between pea seedlings and seedlings of wheat, barley or maize (monocotyledons; reviewed in Whipps, 1990). On the other hand, significant differences in the nature and quantity of root exudates have been found between monocotyledons and dicotyledons as well as between species within these groups (reviewed in Curl and Truelove, 1986; Hale et al., 1978; Hale and Moore, 1979).

Age and developmental stage are other plant factors influencing the distribution pattern of photosynthates during a given growing season. In cereals the translocation of C to the roots generally decreases as the growing season progresses (Jensen, 1993; Keith et al., 1986; Swinnen et al., 1994a, b). By contrast, in certain perennial grass species the translocation rate of C below ground appears to increase with time (Dormaar and Sauerbeck, 1983; Johansson 1991, 1993; Warembourg and Paul, 1977). The types of root-derived organic materials available to microorganisms

change with root age. For instance, the quantity of exudates usually decreases with plant age. Exudates also vary qualitatively during the growing season (Curl and Truelove, 1986; Hale et al., 1978; Hale and Moore, 1979). By contrast, the autolysis of cortical tissue (i.e. lysates) in wheat increases with plant age (Martin, 1977).

Plant-microorganism interactions in the rhizosphere have pronounced effects on N transformations in the soil. Because the rhizosphere is relatively rich in organic materials, the energy-consuming steps of the nitrogen cycle (N in reduced form) are likely to prevail, e.g. nitrogen fixation and/or ammonium mineralization-immobilization processes (Woldendorp, 1981).

To examine the influence of plant-microorganisms interactions on soil-N transformations (e.g. net mineralization, net immobilization) a pot experiment was conducted in a ^{14}C-labelled atmosphere by using different (two annuals, one perennial) plants species. The basic assumption in the pot experiment was that soil-N mineralization in the root zone is influenced by the amount of C available for microorganisms below-ground. It was expected that variation in below-ground, microorganism-available C would be achieved by using different plant species. Since it has been suggested that root-induced N mineralization (Clarholm, 1985) is most pronounced in non-fertilized soils and soils poor in mineral N (Clarholm, 1989), a low-N fertilizer rate was used.

Materials and methods

Soil

The soil (Örja) was a sandy clay loam with 1.15% C, 0.12% N, a pH of 6.7 and a microbial biomass C and N, measured in rewetted, air-dried soil by fumigation-incubation (FI) and fumigation-extraction (FE) techniques, of 161 μg C and 23 μg N g^{-1} soil, respectively (Zagal, 1993). The Örja soil was collected from a site used for a long-term soil fertility experiment in southern Sweden. The site and the experiment were described earlier by Ivarsson and Bjarnason (1988). The Örja soil is classified as a fine loamy, mixed, mesic Typic Eutrochrept according to Soil Taxonomy and as a Eutric Cambisol in the FAO system (Kirchmann and Eriksson, 1993).

Pot experiment

Spring barley (*Hordeum vulgare* L., var. Etu), ryegrass (*Lolium perenne*, var. Tove) and winter rape (*Brassica napus* L., var. Rustan) were cultivated in a $^{14}CO_2$-atmosphere. Shoots were harvested, and associated roots and soil were collected on two sampling occasions during the growth chamber period, e.g. after 4 and 7 weeks in the growth chamber. Three replicates (n=3) were used per plant species.

The pot technique employed in the experiment has been described earlier (Zagal et al., 1993; Zagal, 1994). Briefly, it consisted of a container in which a frame, containing a single layer of soil, was placed. The container was fitted with an airtight lid with two holes, through each of which a plant was allowed to grow. Sieved (2 mm), air-dried soil (348 g) soil was rewetted with the fertilizer solutions (N, P, K and micronutrients) and nitrapyrin (N-Serve, nitrification inhibitor) to reach 40% of the soil WHC. N, P and K fertilizer rates were approximately 25, 54 and 180 mg kg^{-1} wet soil, respectively, applied as ^{15}N-labelled $(^{15}NH_4)_2SO_4$, KH_2PO_4 and K_2SO_4. The former solution had a 13.17 atom% ^{15}N excess. Micronutrients were supplied in a solution (7 mL kg^{-1} wet soil; Zagal et al., 1993). Nitrapyrin was applied at a rate of 10 $\mu g\ g^{-1}$ soil after fertilization. The soil was packed in the frames to give a bulk density of 1.2 g $(cm^3)^{-1}$ and thereafter placed in the containers over a 2-cm-thick layer of washed sand. After fitting the containers with the lids, the soil was incubated for 13 days before sowing. To sow in the pots, the lids were lifted, and two pre-germinated (24–48 h) seeds of each plant species were planted per pot. Glass tubes were placed around each seedling in the soil surface and filled up halfway with sand. Glass tubes were used because once the plants have grown up through the tubes, their openings could be easily sealed. Thereafter, the pots were again fitted with their respective lids.

Thirteen days after germination (three leaves visible), the pots were sealed by applying silicon rubber around the stems and edges of the glass tubes. Thereafter, the plants were placed in a growth chamber with ^{14}C-labelled CO_2 in the atmosphere.

Plant growth conditions

The controlled growth conditions were as follows: light intensity, 218 $\mu E\ m^{-2}\ s^{-1}$; CO_2-concentration, 320–340 $\mu L\ L^{-1}$; specific activity in the atmosphere, 0.9 (0.7–1.0) kBq $mg^{-1}C$; air temperature, 21–22°C; rel-

ative air humidity, 70–80%. The daylength was kept constant at 16 h. Soil moisture was adjusted according to water losses. To determine the water loss, one pot of each plant species was weighed on a balance kept inside the chamber. Water was added to each pot through a PVC irrigation tube which was introduced inside the container through a hole at the bottom and then arranged so that it lay along the surface of the soil at the top of the pot. The part of the PVC tubing lying on the surface was perforated to allow water to infiltrate into the soil.

Evolved CO_2 in the root-soil compartment (rhizosphere respiration) was collected in 500 m*M* NaOH (CO_2 trap) by flushing each pot with CO_2-free air (flow rate: 24 mL min^{-1}). Air pumped into the pots was subjected to a vacuum, causing it to flow through the CO_2 traps.

Plant harvest and soil sampling

Six pots, two replicates per plant species, were sampled before starting the growth chamber period (13 days after germination). Shoots were harvested, and associated roots and soil were collected for plant dry weight and plant and soil C, N and ^{15}N analyses. Plant and soil samples from the chamber period were also subjected to ^{14}C analysis.

During the growth chamber period, rhizosphere respiration, measured as the production of CO_2-C and CO_2-^{14}C in the root-soil compartment, was determined twice a week.

Pots were collected twice during the chamber period. The first and second sampling dates were after 4 weeks of growth in the chamber (41 days after germination) and after 7 weeks of growth in the chamber (62 days after germination), respectively. Harvested shoots were dried at 50°C, weighed and finely ground prior to analysis. The frame containing the soil was dismantled, and the soil was gently sieved (2 mm). Collected roots were carefully washed with deionized water, dried and prepared as described above. Root-free soil samples (visible roots were picked up) were either used directly for biomass determinations, in which case soil moisture was adjusted to 20% (w/w) if necessary, or the samples were dried (50°C) and finely ground for ^{14}C, N and ^{15}N analyses.

Analyses

Methods for determining total C and N, as well as ^{14}C-labelled C and ^{15}N-labelled N, are described in detail elsewhere (Zagal et al., 1993; Zagal, 1994). A short description is given below. Total CO_2-C and ^{14}C-labelled CO_2-C from NaOH traps were determined by titration with HCl and liquid scintillation counting, respectively. C in soil and plant material was determined by dry combustion. To determine the ^{14}C contents of soil and plant material, samples were first combusted in a sample oxidizer. Microbial biomass C was determined by the FE method (Wu et al., 1990) with minor modifications (i.e. 20 h fumigation time; K_2SO_4 extractant, 250 m*M*). To estimate biomass C, extractable C (E_c) was multiplied by a factor of 2.42 (Zagal, 1993). The content of ^{14}C-labelled biomass was determined by measuring ^{14}C-activity (liquid scintillation counting). ^{14}C-activities of the different compartments in the soil-plant systems are presented as kBq pot^{-1}. Activities can be approximately converted to milligrammes of labelled carbon by dividing kBq by the specific activity of the labelled CO_2 (atmosphere: 0.9 kBq $mg^{-1}C$).

Organic N in the soil was estimated by regular Kjeldahl analysis (Bremner, 1965) after all mineral N had been removed. To remove inorganic N, samples were extracted twice with 250 m*M* K_2SO_4 and twice with 50 m*M* K_2SO_4. Total-N contents in soil and plants were also determined by regular Kjeldahl analysis. Contents of NH_4^+-N and NO_3^--N were measured in K_2SO_4 extracts by steam distillation. Microbial biomass N in soil was determined by the FE method (Brookes et al., 1984) with minor modifications introduced to estimate biomass C. Total N extracted with K_2SO_4 (E_N) was determined by the Kjeldahl technique (Bremner and Mulvaney, 1982) after treatments to exclude nitrates and evaporate water (Zagal, 1993). To convert E_N to biomass N estimates a factor of 2.22 was used (Jenkinson, 1988). To determine the ^{15}N content of mineral N and Kjeldahl digests, ammonia liberated by distillation was collected in H_2SO_4 and titrated with 25 m*M* NaOH (methyl red as indicator). Atom % ^{15}N excess was determined on a Micromass 602C mass spectrometer. Amounts of ^{15}N-labelled N (added ^{15}N ammonium-derived) in soil and plants were calculated on the basis of the atom % ^{15}N excess found in the ammonium pool 2 h after mixing the soil with the solutions (i.e. 12 % atom % ^{15}N excess). At the time of sowing in the pots the following amounts of ^{15}N-labelled N (mg pot^{-1}) were found: ammonium, 5.84±1.19; nitrate, 0.06±0.01; biomass-^{15}N, 0.74±0.39; soil residue, 2.67 (two replicates. Total organic ^{15}N in soil to which ^{15}N-biomass has been subtracted). Corresponding amounts of unlabelled N

were 6.45±1.31, 0.93±0.14, 4.77±0.78 and 334.6. Total biomass-C (mg C pot^{-1}) was 23.2±5.4.

Statistical analysis

^{14}C-labelled C results (expressed as % of net assimilated ^{14}C, Table 2) obtained during the growth chamber period were submitted to a two-way, multivariate analysis of variance (SAS Institute, 1991). The hypothesis of no overall factor effect was tested using four multivariate statistics: Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root.

^{15}N-labelled N results obtained during the chamber period were analyzed using a two-factor (plant species and time) analysis of variance. This was followed up by the calculation and testing of least-square means. The relationship between ^{15}N partitioning (Tables 4 and 5) and the distribution of assimilated ^{14}C (Table 2, kBq pot^{-1}) was analyzed using an analysis of covariance. Either the C distribution in the plant-soil system or the ^{14}C release below ground (i.e. rhizosphere respiration + soil biomass + soil residue) was introduced as covariate in the two-way analysis of variance (dependent variable: N-pool in the N partitioning). The GLM procedure in the SAS package (SAS Institute, 1989) was used for the analysis of variance and covariance.

Results

Plant development

On the first sampling occasion (chamber period, 41 days) the three plant species were in their vegetative phase. Barley was early in its inflorescence emergence stage (growth-stage decimal code of Zadocks et al., 1974); unvernalized ryegrass had developed at least one primary tiller, and unvernalized winter rape was at its leaf production stage, e.g. four true leaves exposed (growth-stage decimal code of Sylvester-Bradley and Makepeace, 1984). Ryegrass plants differed significantly from the other two species on this sampling occasion (Table 1): shoot dry weights were significantly lower ($p = 0.0027$) and root dry weights significantly higher ($p = 0.0001$) compared with the corresponding dry weights of barley and rape plants. The root dry weight of ryegrass plants was about three times higher than that of the other two plant species, and as a consequence, the shoot-to-root ratio of the ryegrass was much lower than that of the barley or rape.

On the second sampling occasion (62 days) barley was in its reproductive phase, whereas ryegrass and rape remained in their vegetative phase. Barley had reached the hard dough development stage; ryegrass plants consisted of four to five primary tillers, and rape had a seventh true leaf exposed. At this time, the species differed in root dry weights rather than shoot dry weights, and the differences were significant (ryegrass > rape > barley; $p = 0.0001$). The root dry weight in barley decreased with time. By contrast, in both ryegrass and rape, root dry weights increased with time by a factor of 2.5 and 1.8, respectively. Consequently, the highest shoot-to-root ratio was observed in barley plants and the lowest in ryegrass.

N contents (Table 1) of shoots and roots decreased with time in all three plant species.

Distribution of net assimilated ^{14}C

The ^{14}C distribution (% of net assimilated) was significantly affected by both plant and time factors as well as by the plant × time interaction (Tables 2 and 3). Both factors as well as the interaction between them had significant effects on each of the plant-soil compartments (Table 3), with two exceptions: time did not significantly affect soil biomass or soil residue.

On the first sampling date, the proportions of net assimilated ^{14}C measured in roots and as rhizosphere respiration (root and microorganism respiration) for barley were much lower than those measured for the other two plant species (Table 2). Rhizodeposition in soil (soil biomass + soil residue) was highest in ryegrass and lowest in barley plants on both sampling occasions. In contrast, on the two sampling dates, the proportion of net (%) assimilated ^{14}C measured in shoots was highest in barley and lowest in ryegrass. On the second sampling occasion, root and rhizosphere-respiration proportions of the net assimilated ^{14}C of roots were highest and lowest, respectively, in ryegrass plants (note, however, that in absolute amounts, kBq pot^{-1}, rhizosphere respiration was highest in ryegrass). On this sampling occasion rhizosphere respiration in rape was similar to that in barley. However, of the three species, barley plants showed the lowest root proportion of net assimilated ^{14}C.

On the first sampling date, the proportion of ^{14}C translocated below ground was much lower for barley (23.6%; Table 2) than for ryegrass (49.6%) and rape (31.0%). This proportion increased with time, so that by the second sampling occasion, values were 32.2%, 66.4% and 44.4% respectively. The greatest difference

Table 1. Dry weights (mg pot^{-1}) and N contents of shoots and roots and shoot-to-root ratios of barley, ryegrass and rape grown in a $^{14}CO_2$-atmosphere

	Growth chamber period								
	13 days[a]			41 days[a]			62 days[a]		
	Barley	Ryegrass	Rape	Barley	Ryegrass	Rape	Barley	Ryegrass	Rape
Shoot (dw)	305[b]	292[b]	288[b]	1093	882	1074	1113	1043	1220
				(53.9)	(5.6)	(69.8)	(134.3)	(60.3)	(60.8)
Root (dw)	122	28	55	156	517	175	103	1313	319
				(8.8)	(55.9)	(9.7)	(11.4)	(47.4)	(10.1)
Shoot-to-root ratio	2.48	10.4	5.87	7.02	1.72	6.14	10.8	0.79	3.82
				(0.29)	(0.19)	(0.42)	(0.55)	(0.04)	(0.14)
N content of shoot (% of dw)	4.57	5.01	4.34	1.42	1.51	1.10	1.26	0.83	0.73
				(0.10)	(0.16)	(0.07)	(0.55)	(0.05)	(0.01)
N content of root (% of dw)	2.20	2.42	2.74	1.24	0.97	2.20	1.27	0.53	1.55
				(0.15)	(0.87)	(0.04)	(0.55)	(0.02)	(0.11)

Standard deviation (SD) in parentheses (n=3).
[a]Days after germination. On the last sampling occasion barley was in its reproductive stage; ryegrass and winter rape were in their vegetative stage. See also text.
[b]Two replicates.

in below-ground ^{14}C between plants on this sampling occasion was for the C measured in roots. This proportion was several times greater for ryegrass plants than for barley and almost three times higher compared with rape. Differences in the proportion of ^{14}C released (rhizosphere respiration + soil biomass + soil residue) between plants were small but significant. On the first sampling occasion this proportion was significantly lower in barley (16.1±0.38%; Table 2) than in ryegrass (20.6±0.45%) and rape (20.2±0.99%) ($p < 0.001$). On the second sampling date this value had increased to 26±0.73% in ryegrass, which was significantly lower ($p < 0.01$) than in barley (29.0±1.62%) and rape (29.2±1.30%). Lower rhizosphere respiration in ryegrass plants was the main cause of the differences between plants in released ^{14}C measured on this sampling occasion.

During the chamber period, amounts of total biomass C (mg C pot^{-1}) increased in unvernalized ryegrass and rape plants between the two sampling occasions, whereas they decreased in barley. On the first sampling date (41 days) biomass C amounts were 44.3 (±2.0), 38.4 (±6.0) and 36.7 (±4.2) for barley, ryegrass and rape, respectively. Corresponding values on the second sampling occasion (62 days) were 38.4 (±0.0), 61.8 (±5.3) and 46.7 (±7.5).

Nitrogen partitioning

During the chamber period, amounts of soil mineral N were very low or undetectable. No labelled NH_4^+-N could be detected in the soil in any of the three plant species. Unlabelled NH_4^+-N varied, depending on the plant and sampling occasion, between 0.3 and 0.5 mg N pot^{-1}. Similarly, the amounts of unlabelled NO_3^--N varied between 0.2 and 0.5 mg N pot^{-1}.

The amounts of labelled and unlabelled N found in plants and associated soil are shown in Tables 4 and 5. Differences in N contents between plants were more marked for the root and shoot fractions than for the entire plant. Nevertheless, on both sampling occasions, the amounts of ^{15}N-labelled N (added ^{15}N ammonium-derived) were highest in ryegrass plants (shoots + roots) and significantly different ($p < 0.05$) from those in the other two species. Although the differences in the amounts of N measured in shoots and roots between each plant species appeared to be small, they were often significant (Table 4; $p = 0.05$). On both sampling dates the amounts of unlabelled and ^{15}N-labelled N found in the roots were highest in ryegrass plants and lowest in barley plants. The analysis of covariance performed to reduce the effects of differences in the ^{14}C distribution between plants (i.e.

Table 2. Distribution of assimilated ^{14}C in barley, ryegrass and rape grown in a $^{14}CO_2$-atmosphere

	Days after germination					
	41 days			62 days[a]		
	Barley	Ryegrass	Rape	Barley	Ryegrass	Rape
	Distribution (kBq pot^{-1})					
Shoot	755.4	687.7	799.1	730.3	747.4	848.7
	(52.3)	(14.3)	(61.5)	(103.1)	(47.2)	(55.0)
Root	73.9	398.3	125.3	41.4	896.3	232.3
	(4.9)	(49.7)	(5.4)	(1.3)	(45.6)	(6.8)
Rhizosphere	131.4	222.8	198.7	283.8	482.4	392.9
respiration	(15.1)	(12.1)	(6.3)	(37.8)	(46.3)	(14.8)
Soil biomass	11.3	16.8	7.7	6.8	42.0	11.0
	(2.2)	(4.4)	(1.0)	(0.2)	(3.9)	(2.5)
Soil residue	15.9	41.4	27.1	24.2	53.1	41.0
	(6.2)	(4.7)	(3.7)	(3.7)	(11.7)	(3.9)
	Distribution (% of net assimilated)					
Shoot	76.5	50.4	69.0	67.1	33.7	55.6
Root	7.5	29.1	10.8	3.9	40.4	15.2
Rhizosphere respiration	13.3	16.3	17.2	26.1	21.7	25.8
Soil biomass	1.2	1.2	0.7	0.6	1.9	0.7
Soil residue	1.6	3.0	2.3	2.2	2.4	2.7

Standard deviation (SD) in parentheses (n=3).

[a]Barley was in its reproductive stage; ryegrass and winter rape were in their vegetative stage. See also text.

Table 3. Summary of multivariate analysis of variance for distribution (%) of net assimilated ^{14}C in barley, ryegrass and rape plants and associated soil. The plants were cultivated in a $^{14}CO_2$-atmosphere

	Shoots	Roots	Rhizosphere respiration	Soil biomass	Soil residue	Total[a]
Source of variation						
Plant	***	***	**	***	*	***
Time	***	***	***	ns	ns	***
Plant × Time	**	***	***	**	*	***[b]

The difference between means is significant at: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant.

[a]Test criteria (four multivariate statistics) for the hypothesis of no overall factor effect. See also text.

[b]For all other multivariate statistics $p = 0.0001$, except for Pillai's Trace ($p = 0.0293$).

Table 4. Amounts of ^{15}N-labelled and unlabelled nitrogen in the shoots and roots of barley, ryegrass and rape grown in a $^{14}CO_2$-atmosphere. Results in mg ^{15}N of mg N pot^{-1}

	Growth chamber period									
	13 days[1]			41 days[1]			62 days[1]			SE from
	Barley	Ryegrass	Rape	Barley	Ryegrass	Rape	Barley	Ryegrass	Rape	ANOVA
Shoots										
Labelled	3.91[2]	4.61[2]	3.83[2]	4.71[a]	4.15[b]	3.75[c]	4.17[a]	2.73[b]	2.78[b]	0.20
Unlabelled	9.93	10.0	8.57	10.8[a]	9.13[b]	8.10[c]	9.76[a]	5.88[b]	6.14[c]	0.57
Roots										
Labelled	0.65	0.21	0.42	0.53[a]	1.45[b]	1.13[c]	0.34[a]	2.17[b]	1.44[c]	0.13
Unlabelled	1.88	0.48	0.97	1.39[a]	3.58[b]	2.73[c]	0.96[a]	4.75[b]	3.50[c]	0.25
Total plant										
Labelled	4.56	4.82	4.25	5.24[a]	5.60[b]	4.89[c]	4.51[a]	4.90[b]	4.22[c]	0.20
Unlabelled	11.8	10.5	9.54	12.2[a]	12.7[a]	10.8[b]	10.7[a]	10.6[a]	9.64[b]	0.54

In the chamber period (n=3). For each sampling occasion, values in a row with the same superscript letter are not significantly different ($p = 0.05$).
[1]Days after germination. On the last sampling occasion barley was in its reproductive stage; ryegrass and winter rape were in their vegetative stage. See also text.
[2]Two replicates.

Table 5. Amounts of ^{15}N-labelled and unlabelled nitrogen in soil of barley, ryegrass and rape grown in a $^{14}CO_2$-atmosphere. Results in mg ^{15}N or mg N pot^{-1}.

	Growth chamber period									
	13 days[1]			41 days[1]			62 days[1]			SE from
	Barley	Ryegrass	Rape	Barley	Ryegrass	Rape	Barley	Ryegrass	Rape	ANOVA
Soil biomass										
Labelled	1.29[2]	1.24[2]	2.08[2]	0.71[a]	0.61[b]	0.65[b]	0.47[a]	0.40[b]	0.45[a]	0.03
Unlabelled	6.43	5.49	9.82	5.78[a]	5.72[a]	5.34[a]	4.27[a]	4.45[a]	4.81[a]	0.76
Soil residue[3]										
Labelled	3.17	3.12	3.86	3.72[a]	4.05[b]	4.32[b]	4.37[a]	4.18[a]	4.31[a]	0.22
Unlabelled	340.9	336.0	321.6	345.9[a]	337.5[a]	344.3[a]	353.9[a]	333.2[b]	342.2[b]	7.52

In the chamber period (n=3). For each sampling occasion, values in a row with the same superscript letter are not significantly different ($p = 0.05$).
[1]Days after germination. On the last sampling occasion barley was in its reproductive stage; ryegrass and winter rape were in their vegetative stage. See also text.
[2]Two replicates.
[3]Total organic ^{15}N in soil to which ^{15}N-biomass has been subtracted.

different growth pattern) on the dependent variables (^{15}N-labelled N in shoots and roots) also resulted in significant differences between plants ($p = 0.0004$ for ^{15}N-labelled N in shoots and $p = 0.0028$ for ^{15}N-labelled N in roots).

In contrast to the situation with ^{15}N-labelled N in plants, labelled biomass ^{15}N in soil was lowest in ryegrass plants (Table 5) on both sampling occasions. Unlabelled biomass N did not differ between plant species. On the first sampling date, amounts of ^{15}N-labelled N in the soil residue were lowest in barley, and

the value differed significantly from those obtained with the other two plant species ($p < 0.05$). By the second sampling occasion, unlabelled N determined in the soil residue was higher in barley plants than in the other species.

Relating N partitioning to the C distribution

The link between the partitioning of labelled ^{15}N (N-pools) and assimilated ^{14}C was assessed using regression techniques (e.g. covariance analysis). Some of the relationships examined and described below between ^{15}N-labelled N and ^{14}C-labelled C in the plant-soil system were significant. This fact, however, does not necessarily imply that a causal relationship has been established (see Discussion).

For each plant species and harvest, the amounts of ^{15}N-labelled N measured in the shoots (dependent variable; Table 4) were related to the amounts of assimilated ^{14}C (kBq pot^{-1}; Table 2;) found in the shoots and the roots (independent variables). These C parameters were significant in the statistical model (shoots, $p = 0.0443$; roots, $p = 0.0445$). For assimilated ^{14}C in shoots the relationship was positive, whereas the opposite was true in the case of assimilated ^{14}C in roots. The other C-component parameters included in the model were not related significantly to amounts of ^{15}N-labelled N. Similarly, for each of the three species, amounts of ^{15}N in roots were positively related to amounts of assimilated ^{14}C in roots. In this case, ^{14}C-labelled C in the roots was the only component of the total C distribution in the model that was significantly related to the amount of ^{15}N-labelled N in roots ($p = 0.0414$). An additional relationship found in this study was that established between the amounts of ^{15}N-labelled N in roots and the release of ^{14}C from roots (rhizosphere respiration and ^{14}C remaining in soil). This relationship was positive. When introduced as a single independent variable in the model, ^{14}C release below ground was significantly related to ^{15}N-labelled N in roots ($p = 0.0273$). The relationship between the amounts of ^{15}N-labelled N in plants and the release of ^{14}C from roots was not significant ($p = 0.1459$).

Discussion

Plant growth

Shoot and root development were limited by the low-N conditions. Plant yields and N contents were low (Table 1). Thus, very little increase in shoot dry weight was observed between the first and second samplings in the chamber period. All plants had almost finished growing (shoots) by the time that the first harvest was made in the chamber period. However, the root dry weight of unvernalized grass and rape doubled between the first and second harvests in the chamber period. Apparently, plants grown under N-limiting conditions have a well developed root system, but a poorly developed shoot system (Troughton, 1957). For instance, short periods of very low N supply resulted in a marked increase in the ryegrass root growth (*Lolium multiflorum*) (Ennik and Hofman, 1983).

Carbon partitioning

Plant species and stage of development both contributed to the variation in the ^{14}C distribution between plants. The proportion of ^{14}C translocated below ground during the chamber period was greater in ryegrass than in rape and barley. The relatively higher investment below ground by perennial plants has been attributed to their need to withstand stressful conditions during winter, etc. (Whipps, 1990). On the other hand, Ennik and Hofman (1983) reported that short periods of very low N supply resulted in a marked increase in ryegrass root growth (*Lolium multiflorum*). Their findings suggest that the low-N conditions imposed here could have been a factor influencing the proportion of ^{14}C being translocated below ground in ryegrass (Table 2).

In this study, there were not enough sampling occasions to unequivocally show the effects of plant development stage on C partitioning. However, variations in the ^{14}C distribution between plant species due to developmental differences (i.e. vegetative vs reproductive stages) somewhat reflected the influence of the source-sink relationship regulating C partitioning. The existence and activity of sinks (i.e. properties, C demand) and factors affecting them have been postulated as determinants of C partitioning (Gifford and Evans, 1981; Wardlaw, 1990). Thus, in barley about 70% of the net ^{14}C assimilated was allocated to the shoots (Table 2) at the reproductive stage (e.g. second sampling), whereas second-sampling values for ryegrass and rape plants, which were in their vegetative stage, were 34% and 56% respectively.

The distribution of photosynthetically-fixed ^{14}C within barley plants in this study agrees well with that found in a previous experiment under similar conditions (Zagal, 1994). The ^{14}C distribution pattern found

here was also similar to that found for barley cultured in a growth chamber up to its reproductive stage (Liljeroth et al., 1990) and for wheat grown to maturity in the field (Keith et al., 1986; Martin and Merckx, 1992). Although the below-ground proportion of the ^{14}C translocated to the roots in this study differed from that obtained in field trials, there was good agreement between my results and those obtained with mature wheat, especially in the case where continuous labelling was used in the field (Martin and Merckx, 1992).

The proportion of net assimilated ^{14}C in ryegrass translocated below ground tended to increase as the growth period progressed. Similar patterns were found in other growth chamber experiments conducted with perennial grasses (Dormaar and Sauerbeck, 1983; Johansson, 1991, 1993) and in a field trial with native grassland using pulse-labelling technique (Warembourg and Paul, 1977). Nevertheless, pulse labelling of *Lolium perenne* plants has shown that the amounts of C translocated below ground were lower for 8 and 24-week-old plants than for 4-week-old ones (Meharg and Killham, 1990). In the present study, the proportion of assimilate partitioned to root tissues ended up being about 40%. In a glasshouse experiment employing pulse-labelling technique, Ryle (1970) found that up to 50% of the assimilate was allocated to the roots of young ryegrass (*L. perenne*) plants during a period of uninterrupted vegetative growth. This proportion declined to less than 10% later on as the crop increased in density and the root system became established. Although continuous labelling and pulse-labelling techniques are not absolutely comparable, it appears that in an established crop, only a small proportion of the assimilated is needed to maintain a comparatively large active root mass (Parsons, 1988). This can be explained by the fact that the active life of root tissue is greater than that of the shoot (Throughton, 1981).

The results regarding the ^{14}C distribution in rape are more difficult to compare with other studies. Of the many studies with rape in which pulse-labelling has been used, one of the main objectives has been to assess the degrees to which the various above-ground organs in the plant (i.e. leaves, stem, pods) contribute to seed formation and yield production (Chapman et al., 1984; Major et al., 1978; Rood et al., 1984). During the chamber period in this study, rape plants displayed uninterrupted vegetative growth; therefore, roots and their respiratory activity constituted an important sink in the partitioning of net assimilate.

Relating N and C partitioning to N flows in the root zone

Determinations of plant ^{15}N-uptake, made to measure net-mineralized amounts of ^{15}N in the soil during the chamber period, have been used earlier to evaluate root-induced N mineralization effects (Zagal, 1994). In this study, such an evaluation was complicated by genotypic differences in root mass between the three species and by the early uptake of plant N (by day 13 the plants had taken up about 86% of their ^{15}N-labelled N). Its use was, therefore, less relevant. For instance, during the chamber period ^{15}N-labelled N taken up by the plants was highest in ryegrass (Table 4), suggesting that greater amounts of ^{15}N (e.g. of relatively available nitrogen, mostly present in biomass) were mineralized in this treatment than in the barley or rape treatments. However, the higher uptake of ^{15}N observed was also in accordance with the very high degree of root proliferation found in these plants, which results in increased root exploration of the soil. Also, the positive relation between the release of ^{14}C from roots and amounts of ^{15}N found in them could be explained by genotypic differences in root mass between species. For instance, the root ^{15}N content and ^{14}C release from the roots are both linked to root dry mass (at both harvests, ^{15}N and ^{14}C in the roots were highest for ryegrass, which had the greatest root mass, and lowest for barley, which had the least root mass, Table 1). In general, when comparing ^{15}N and ^{14}C quantities between species, many of the differences found between plants can be explained by the differences determined in the weight of shoot or root parts (i.e. differences in plant growth and shoot-to-root ratios). To further examine plant effects on N mineralization in the soil, amounts of ^{15}N-labelled N as well as unlabelled N found in the plants were related to amounts of total and labelled N and C in the soil biomass and associated metabolites.

Amounts of ^{15}N-labelled N in soil residues at the first sampling (Table 5; 41 days) were higher in cultivated, unvernalized ryegrass and rape than in barley. Subsequently, there were no differences between treatments. However, on the second sampling occasion, amounts of unlabelled N found in soil residues were lower in ryegrass and rape than in barley. On the other hand, throughout the experimental period, the total amount of soil residue N (Table 5) remained constant under ryegrass whereas it tended to increase under barley and rape. Also, the amounts of labelled N and C (Tables 2 and 5) in soil residues increased. These results suggest that not only was soil-N mineralization

enhanced (e.g. ryegrass) but also that plant-derived material accumulated (e.g. in barley and rape). Mineralization of ^{15}N-labelled N at the time of the first harvest in the growth chamber was probably due to the turnover of N immobilized by the microbes early in the growth period.

The basic assumption here was that soil-N mineralization in the root zone is influenced by the amount of C available for microorganisms below-ground. Actually, amounts of C transferred below ground or released (rhizosphere respiration + soil biomass + soil residue) were in accordance with expectations: ryegrass > rape > barley. However, there was no positive correlation between the release of ^{14}C from roots and the amounts of ^{15}N-labelled N in the plants. ^{14}C release below ground was only related to ^{15}N-labelled N in the roots. Despite the significance of this correlation no causal relationship could be established. In this study the early uptake of plant N made it difficult to relate the subsequent measurements of plant C partitioning to plant absorbed N. For instance, amounts of ^{15}N in ryegrass and rape shoots decreased progressively from day 13 onwards, suggesting a large degree of internal recycling which could account for a substantial proportion of the root ^{15}N. The preferential allocation of ^{15}N to roots in ryegrass and rape plants could have been the result of higher priority being placed on root allocation under the low-N (N-limiting) growing conditions used in this study, when N absorption was reduced (the functional equilibrium hypothesis; Brower, 1983). Alternatively, it has been suggested that leaves are unable to incorporate all imported N under N-limiting conditions and that the excess N is, consequently, translocated back to the roots (Lambers, 1983).

As just mentioned, it was hard to relate plant C partitioning to plant absorbed N owing to the early uptake of plant N. Thus, even though amounts of C released were greater in ryegrass than in the other plant-treatments there was no unequivocal evidence that the effects of plant-microorganism interactions on soil-N mineralization were greater under ryegrass. The total amounts of labile N (i.e. plant N and microbe N) were only marginally greater in ryegrass than in barley and rape. On the other hand, the fact that amounts of plant N appeared to decrease between the first and second sampling occasions (41 and 62 days; Table 4) tends to reduce the importance of the coincident decrease in unlabelled N in soil residues under ryegrass (i.e. enhanced soil-N mineralization). In a model developed by Griffiths and Robinson (1992) it was assumed that root-induced N mineralization accounts for less than 6% of the total plant-N, and they even questioned whether this mechanism has any importance at all. Most probably, inorganic N was rapidly immobilized by plants and microorganisms early in the growth period. Earlier studies have shown that the energy supplied by growing roots makes microorganisms a significant N sink in the root zone (Breland and Bakken, 1991; Zagal et al., 1993). Remineralization of the microbial-N immobilized at the start of the experiment might have allowed the plants to absorb some of this N during the rest of the experiment, as indicated by the continuous decrease in amounts of microbial-N (Table 5), particularly ^{15}N.

Also, an increase over time was noted in the ryegrass-plant treatment, and to a lesser extent in rape, in total biomass C and labelled biomass C (see text and Table 2). However, this increase in biomass C was not followed by an increase in the immobilization of N. These findings were not in accordance with the mechanisms proposed in the root-induced N mineralization hypothesis (Clarholm, 1985). According to this hypothesis, bacteria utilizing root-derived C release soil organic N to support their own growth. Plants can benefit from this temporarily immobilized N when bacteria are grazed, especially, by protozoa. During the chamber period total amounts of N in the biomass decreased with time in all treatments. This resulted in higher C/N ratios for the soil biomass, i.e. from 6.1 to 12.7 (ryegrass) and 8.9 (rape) between the first and second harvest, respectively. For barley the increase in the C/N ratio of the microbes was lower (from 6.8 to 8.1) since some decrease in biomass C occurred between the first and second harvests in the chamber period. Whether or not these changes in C/N ratios of the microbial population reflected changes in the microbial composition or its activity (e.g. towards a fungi microflora inhabiting soil less under the influence of roots) is difficult to determine based on the present data. However, in a previous growth chamber experiment performed with barley under similar environmental conditions (Zagal et al., 1993), it was suggested that under low-N conditions, microorganisms utilized C from soil organic matter to a greater extent than they used C from root-released material.

Acknowledgements

I thank J Persson for support, Mrs I Juremalm and B Forsberg for valuable technical assistance, U Olsson for providing statistical advice, S Bjarnason and

G Johansson for critically reviewing the manuscript, and D Tilles for improving the language. This research was supported by grants from the Swedish Council for Forestry and Agricultural Research to the project 'Transformations of soil nitrogen — influence of the interactions between living plants and microorganisms'.

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Section editor: H Lambers