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Evidence for recycling of N from plants to soil during the growing season

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Abstract

In spite of the known below-ground biomass production of plant roots that concurrently introduce significant amounts of carbon and nitrogen into the soil, the effects of these inputs on N cycling in the soil–plant system are seldom considered. Here, we report on two field experiments carried out between 1995 and 1997 at the FAM Research Station Scheyern: (1) a N-turnover experiment to determine the N fluxes derived from ^{15}N-labeled clover residues incorporated into the plough layer of defined plots, and (2) a root production experiment to assess the above (shoot) and below ground (gross and net root) biomass production of winter wheat in different fields, but nearby the ^{15}N plots. An initial 50% decrease in soil organic ^{15}N at 0–20-cm soil depth was recorded between fall, 1996 (incorporation of clover straw) and spring, 1997 (138 days after incorporation), which was then followed by a period of stability in ^{15}N levels in the soil organic N until the harvest of winter wheat (286 days after incorporation). This stability may be explained in two ways: (a) actual stability of clover-derived ^{15}N remaining in the second phase, e.g., due to recalcitrant compounds or microbial immobilization; or (b) apparent stability, e.g., because the actual mineralization of clover-derived ^{15}N in the soil was compensated by secondary inputs of organic ^{15}N (recycling). Further results showed that the first explanation was unlikely, as (1) between 138 and 286 days after clover incorporation, the mean ^{15}N signature in soil mineral N was 2.1 at.%, indicating a persistent mineralization of clover residues; and (2) a decrease in soil microbial biomass ^{15}N occurred in the second phase, indicating a continued N turnover in the soil. The amount of clover-derived ^{15}N accumulated below the plough layer at 20–110-cm soil depth (11.5%) between early spring and the harvest of wheat also corroborated the return of mineralized ^{15}N into the soil being due to the root N inputs by winter wheat. Based on the depth distribution of winter wheat net root biomass (root production experiment) and on soil organic ^{15}N depth distribution (^{15}N-turnover experiment), the root N input into soil was estimated to be 282 kg ha^{-1}, equivalent to 54% of total net N assimilation of winter wheat. Thus, the results of this study give

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substantial evidence for a N loop between soil and growing plants, whereby a part of the net mineralized N taken up by plants is continuously returned into the soil by their roots. The implications of this N loop for the interpretation of ^{15}N experiments and for plant nutrition are discussed. © 2002 Published by Elsevier Science B.V.

Keywords: Nitrogen cycle; Nitrogen mineralization; Labeled N; Root biomass production; Soil microbial biomass; Clover

1. Introduction

Nitrogen is one of the most important nutrients limiting crop yield. Soil processes leading to net N mineralization and application of mineral N fertilizer are the main sources of plant available N in agricultural soils. Extensive research has been done to elucidate the role of the N cycle and related processes responsible for N supply to plants. However, fewer studies have addressed the backward flow of N from plants to the soil. Plant roots may release C and N into the soil as exudates, secretions, gases and lysates of dead root cells, often termed rhizodeposition (Lynch and Whipps, 1990). By means of isotope labeling techniques, the partitioning of plant biomass C and N between above (shoots) and below ground (roots and rhizodeposits) has been studied primarily in soil columns or pot experiments. According to studies reviewed by Lynch and Whipps (1990), between 30% and 60% of net fixed carbon is transferred to the roots in annual plants. The proportion of assimilated N recovered below ground at plant maturity ranges from 14% to 57% in legumes (Jensen, 1996; McNeill et al., 1996), and from 25% to 49% in winter wheat (Janzen, 1990) and barley (Jensen, 1996). The amount of N deposited by wheat roots into soil is enhanced in the case of a high plant N supply (Janzen and Bruinsma, 1993; Janzen, 1990), whereas the influence of plant water stress on root N deposition seems to be of lesser importance (Janzen and Bruinsma, 1993). It was shown that rhizodeposition represents a labile fraction of soil N contributing significantly to net N mineralization. Up to 79% and 50% of N rhizodeposition of pea and barley, respectively (at 7 weeks after planting), were mineralized during 15 weeks of subsequent soil incubation following removal of roots (Jensen, 1996). These amounts decreased to 30% and 23% at maturity of pea and barley, respectively.

Quantification of rhizodeposition has been done mostly in pot experiments, where rooting depth and volume were most likely to be restricted as compared to conditions in the field. Consequently, the assessment of rhizodeposition under field conditions remains an important and relevant question. Despite the fact that considerable N amounts enter the soil by plant roots, less attention has been paid to examining the consequences of N flows derived from roots in studies of N turnover in soil. In cases where ^{15}N techniques are utilized, confounding effects may result from unaccounted organic ^{15}N inputs derived from roots

In the present study, the turnover of ^{15}N-labeled clover residues incorporated into soil was followed for 286 days. An extensive data base of net and gross root biomass production was additionally used to provide a field-based estimate of rhizodeposition of winter wheat. Complementary results obtained in this experiment were interpreted with regard to the input of root-derived N.

2. Material and methods

2.1. Experimental site and experimental design

The present studies were carried out at the FAM Research Station Scheyern, located 40 km north of Munich in Bavaria, Germany (48°30.0′N, 11°20.7′E). The research station is situated 445 to 498 m above sea level. The mean annual precipitation is 803 mm and the mean annual temperature is 7.4 °C.

The experimental design consisted of field monitoring of net and gross root production of winter wheat (referred to as "root production experiment"), and a field experiment in which the size and turnover of different soil N pools was examined following the application of ^{15}N-labeled green manure (referred to as "N-turnover experiment").

The root production experiment was carried out between 1995 and 1997 on organic and integrated farming fields at the Research Station, having different soil types ranging from loamy (15–25% clay, 20–40% sand) to sandy soils (5–15% clay, 55–80% sand). On account of crop rotations, different fields had to be sampled each year to provide soils which were cropped to winter wheat.

The N-turnover experiment was conducted on field Ö5, also located at the Research Station. Its soil was classified as fine loamy, mesic, Dystric Eutrochrept with 40% sand, 42% silt and 18% clay in the plough layer (ca. 0–20 cm), and with a pH of 6.5. As plots were established at the beginning of the study, this soil contained 1.4% C and 0.14% N, and had a water content of 17%. The field was farmed organically since 1993 with a crop rotation of clover–grass–lucerne–ley, potatoes, winter wheat, winter rye and clover fallow. The experimental treatment consisted of incorporating dried, ^{15}N-labeled clover residues composed of stalks and leaves and small amounts of roots (mainly from *Trifolium resupinatum* and *T. alexandrinum*) into the soil. In corresponding controls, green manure was omitted. Manured and control variants were arranged in a randomized complete block design with four replicates.

The clover was grown in a greenhouse from May to August 1996, in hydroponic culture to which a modified standard nutrient solution for leguminous plants was applied that also contained NH_4NO_3. ^{15}N label was introduced by adding ^{15}N KNO_3 (99 at.%), instead of NH_4NO_3 to this nutrient solution three times during the growing period. The plant shoots were harvested after flowering (but before becoming senescent) and air-dried. Stubbles and adhering

roots were carefully washed with deionized water and also air-dried. The material was cut into pieces (0.5–1.5 cm in length) to avoid crushing and reduction of particle size. Some properties of this clover material are listed in Table 1.

On October 23, 1996, the vegetation cover of the clover fallow was removed and five rectangular areas (3.3 m by 1.2 m), representing the blocks, were dug out to a depth of 20 cm. The soil of each block was sieved through a 2-cm mesh and coarse roots and plant residues were separated out. Miniplots were established in open-ended PVC cylinders (30 cm in diameter and height), introduced approximately 2–3 cm into the subsoil and separated 20 cm from each other. A portion of the sieved soil (22 kg fresh weight) was mixed with 75 g dried ^{15}N-labeled clover material, placed in the PVC cylinders and compacted to a height of 22 cm (bulk density of 1.2 g cm^{-3}), corresponding to an application of 4080 kg ha^{-1} C, and 334 kg ha^{-1} N. Control miniplots were treated identically, except that clover material was omitted. Six manured and six control miniplots in each experimental block provided enough sampling area during the entire experiment (286 days). The area surrounding these miniplots (inner area) was filled with the remaining fresh soil and also compacted. Winter wheat ('Thasos') was sown in the plots (24 plants $plot^{-1}$) and surrounding area (200 kg seed ha^{-1}), and harvested on August 5, 1997; no further fertilization nor soil tillage was made. Weeds were pulled out of the soil by hand at irregular intervals and left in the respective plots.

2.2. Soil and plant sampling

In the N-turnover experiment, soil samples were usually taken within the miniplots with a short spade to depths of 0–10 and 10–20 cm, at 1- to 3-week intervals, until the harvest of winter wheat. At a few selected time points, the subsoil was also sampled to depths of 20–50, 50–80 and 80–110 cm, using soil corers 3 cm in diameter (for 20–50-cm depth) and 2 cm (for deeper sampling).

Table 1
Proportions of C, N and ^{15}N (% of total) and ^{15}N signature (at.% excess) in chemical fractions of clover material incorporated in the field

Fractions	Mineral N	Total N	Total ^{15}N	^{15}N signature	Total C	C/N
Water-soluble[a]	14.0	46.3	49.5	12.7	24.9	6.4
Acid-hydrolizable[b]	n.d.	87.9	85.2	11.5	48.0	6.6
Residual[c]	n.d.	12.1	14.8	14.5	52.0	52.5
Total	n.d.	100.0	100.0	12.2	100.0	12.3

n.d.: Not determined.
[a]Cold water extract (sample:water = 1:100 w/v) of milled clover material.
[b]Extracted after acid-hydrolysis (6 M HCl, 6 h, 100 °C).
[c]Difference between total and acid-hydrolyzable fraction.

The winter wheat plants growing in the sampled area (usually 1–2) were occasionally removed and air-dried to provide measurements of dry weight, N and ^{15}N contents. In this manner, three control and three manured miniplots within each block were consecutively sampled. Miniplots that were not sampled were kept undisturbed until sampling at harvest of winter wheat (viz. 286 days after incorporation of clover material). Harvesting occurred by first cutting the wheat plants within the miniplots in the inner area and also in two transects (50 × 250 cm), with a perpendicular orientation to the inner area. Soil samples in the miniplots were then collected as described before. Until November 18, 1996, soil samples were sieved (2 mm) on the day of sampling and stored overnight, prior to being processed for further analysis. After this date, sieving was replaced by crumbling the soil by hand. Extraction of fresh soil samples for determination of mineral and microbial biomass N was done within 24 h, following field sampling.

2.3. Meteorological data

Air, soil temperature (at 5-cm depth) and precipitation were recorded at the nearby meteorological station B1 at the Research Station Scheyern (cf. Fig. 3, Schröder et al., this volume).

2.4. Chemical and analytical methods

2.4.1. Soil and plant N

Total C, N and ^{15}N of finely ground soil and plant samples were determined on an elemental analyzer (NA 1500, Carlo Erba, Italy) coupled to an Isotope Ratio Mass Spectrometer (Delta E, Finnigan MAT, Germany). Samples whose ^{15}N contents were higher than approximately 1.5 at.% were measured on a similar system with an emission spectrometer (NOI-6PC, Fischer Analysen Instrumente, Leipzig, Germany).

2.4.2. Mineral N

Inorganic N (NH_4^+–N and NO_3^-–N) in soil extracts (100 g fresh soil with 200 ml 0.01 M CaCl) was determined by colorimetric reactions in a continuous flow autoanalyzer (Skalar 5100, Skalar Analytic, Erkelenz, Germany). Inorganic ^{15}N in these extracts was determined by the microdiffusion method of Jensen (1991), using the analysis equipment already described for soil and plant samples (Section 2.4.1).

2.4.3. Soil microbial biomass N

Soil microbial biomass N (N_{mic}) was determined using the fumigation–extraction method (FEM, Brookes et al., 1985). Aliquots of 40 g sieved, or 50 g unsieved moist soil were fumigated for 24 h with $CHCl_3$ at 25 °C and extracted with 160 ml 0.5 M K_2SO_4. Unfumigated control soil aliquots were extracted

identically. The amounts of ammonium and nitrate in the extracts were determined using the autoanalyzer system already described. Total N and ^{15}N in fumigated and unfumigated extracts were determined by persulphate oxidation with Oxisolv® (Merck, Mainz, Germany) in an autoclave at 145 °C for 30 min, followed by the same microdiffusion method as for inorganic ^{15}N. The efficiency of the persulphate oxidation method is similar to that of the Kjeldahl-method (Valderama, 1981), which was used by Brookes et al. (1985). The mean extract N recovery in the acid traps was determined on 30 samples by carrying out an additional analysis of total N on oxidized extracts by means of the total N line of the autoanalyzer (Skalar 5100). Based on these values ($n = 30$), a resultant linear regression ($r^2 = 0.941$) was used to correct the measured total N values for the lower observed recovery of 89%. In situ calibrations were performed on 10 sampling dates during the N-turnover experiment according to Bremer and van Kessel (1992), to determine a mean k_{EN} value of 0.25 used to convert the N Flush of FEM to soil microbial biomass N (N_{mic}).

2.4.4. Root-system measurements

The net development of the root system was measured by the auger sampling method (Böhm, 1979), taking soil cores of 8-cm diameter and 15-cm length each, which covered the soil profile of 0- to 90-cm depth. The roots in the soil cores were separated from adhering soil by washing over a 200-μm sieve. Root length was determined by the line intersection method according to Newman (1966). Root radius was measured directly. Both root length and radius were used to calculate root volume and fresh weight (assuming a specific root weight of 1 g cm^{-3}). Root dry mass was calculated from root fresh weight and the dry matter content of roots, which was assumed to be 10% (Gregory et al., 1978). Gross root growth in the upper 30 cm was measured by the ingrowth core method (Persson, 1983; Steingrobe et al., submitted a,b). Briefly, mesh bags were placed within PVC tubes (one per tube) that were inserted together into the soil in early spring. Every 2–3 weeks, some of the bags were filled with root-free soil from the experimental site and compacted to a soil density comparable to that of the bulk soil. The soil was then exposed to root ingrowth by removing the protecting PVC tube. After a period of usually 2–3 weeks, the mesh bags were removed, the roots were separated from the soil by washing over a sieve, and root length was determined as described previously. Each time mesh bags were removed, a new set of mesh bags was opened for root ingrowth. The gross root production was defined as the sum of root production inside the mesh bags which were consecutively sampled during the time between tillering to early dough stage of winter wheat. It was assumed that the period the bags were open for root ingrowth was always shorter than the life span of a root, so that no root mortality should occur inside the mesh bags (Steingrobe et al., submitted a,b). Ingrowth cores and soil core sampling were both determined with three to four replicates.

2.4.5. Calculations

Clover-derived N and ^{15}N in different soil pools was calculated according to Powlson and Barraclough (1993), assuming identical behavior of ^{14}N and ^{15}N. According to the "negative-discard" plot design (Müller and Sundman, 1988), the ^{15}N recovery in the N balance at harvest of winter wheat was defined in this study as the sum of clover-derived ^{15}N determined in the plot area (soil N at 0–110-cm depth and crop uptake), and that assimilated by winter wheat grown in the untreated surrounding area (transects, inner area, see Section 2.2).

Cumulative net N mineralization (NNM) of clover-derived organic N was calculated as the difference between clover-derived organic ^{15}N initially incorporated into the soil (SON_0) and that remaining in this pool after a time interval Δt, $SON(\Delta t)$ (Eq. (1)). This calculation is based on the assumption that only mineralized N is lost from organic N in the plough layer, e.g., by leaching, denitrification and plant uptake.

$$NNM(\Delta t) = SON_0 - SON(\Delta t) \quad \text{(N-balance approach)}. \tag{1}$$

According to the isotope dilution approach of Chaussod et al. (1988, Eq. (2)), the turnover rate of soil microbial biomass N (N_{mic}) was defined as the exponential decay rate (r_b) of ^{15}N in N_{mic} ($^{15}N_{mic}$). The exponential decay rate was obtained from the best fit of parameters in Eq. (2) to the measured $^{15}N_{mic}$ at different time points (t) by means of a nonlinear regression.

$$^{15}N_{mic}(t) = {}^{15}N_{mic}(t_0) \times e^{(t \times r_b)}. \tag{2}$$

2.4.6. Statistics

All variants were made at least in triplicate and data are presented as mean values of replicates with their standard error, unless otherwise stated. Statistical analyses were performed using the SPSS (SPSS, Unterschleissheim, Germany) software.

3. Results

3.1. Weather conditions

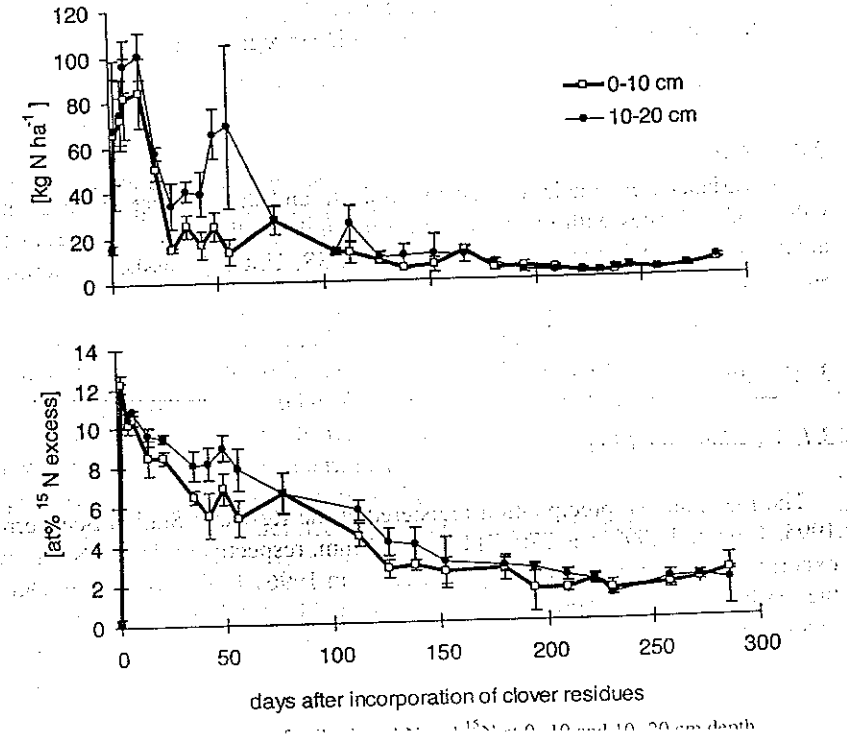
The mean annual precipitation registered at the Research Station Scheyern in 1995, 1996 and 1997 was 770, 711 and 684 mm, respectively. In the N-turnover experiment, the observed winter precipitation in 1996/1997 was 80 mm. During the early vegetation period between March 21, 1997 and June 17, 1997, precipitation was only 64 mm, in contrast to the subsequent period until the harvest of winter wheat (August 5, 1997) which was characterized by heavy rainfall events (141 mm). The soil remained frozen from December 22, 1996 to February 6, 1997.

Table 2
Recovery of clover-derived ^{15}N (% of total added ^{15}N) in the N-turnover experiment

Days after incorporation	Soil				Plant	Total recovery
	0–20 cm		20–110 cm			
	Mineral	Organic	Mineral	Organic		
7	25.9±5.2	71.4±10.1	n.d.	n.d.	–	97.5±11.3
287	0.5±0.1	41.8±2.7	0.2±0.0	11.5±4.1	33.9±1.8	87.0±5.2

3.2. N balance

The mean recovery of ^{15}N input 1 week after incorporation of ^{15}N-labeled clover straw was 97% and decreased to 87% at the harvest of winter wheat (Table 2). Crop uptake in plots and the surrounding area comprised 33.9% of added clover ^{15}N. The mean wheat crop N uptake in the manured and control plots was 240 and 188 kg N ha^{-1}, respectively.



3.3. Mineral N

Initial mineralization of clover residues proceeded rapidly as shown by the increasing ^{15}N signature and soil mineral N contents (Fig. 1). The subsequent decrease in mineral N during winter at the 0–10-cm soil depth and concomitant increase at 10–20-cm depth indicated NO_3 leaching into the subsoil. During growth of winter wheat in spring and summer, the level of mineral N in soil was minimal. The mean ^{15}N signature in soil mineral N decreased continuously from 12 at.% at two days after incorporation to 2 at.% at the harvest of the winter wheat.

3.4. Soil organic ^{15}N and net N mineralization

During the first 130 days after straw incorporation, a 50% decrease of initial soil organic ^{15}N levels was observed (Fig. 2). This stabilized after day 138, whereby ^{15}N contents remained constant until the harvest of winter wheat at day 286. The cumulative net N mineralization (NNM), which was inversely related and based on this data, also showed a biphasic course, with high mineralization rates initially and a subsequent stabilization. In early spring 1997, 42% of total applied ^{15}N remained in the topsoil (0–20 cm) almost exclusively as organic N, and nearly 10% was present in 20–50-cm depth, mainly as mineral N (Fig. 3). Between spring and the harvest of winter wheat, a significant increase of organic ^{15}N was found in the subsoil, located mainly at 20–50-cm depth.

3.5. Biomass ^{15}N

Within 14 days following incorporation of straw, maximum ^{15}N contents in soil microbial biomass were observed. The subsequent ^{15}N values used in Eq.

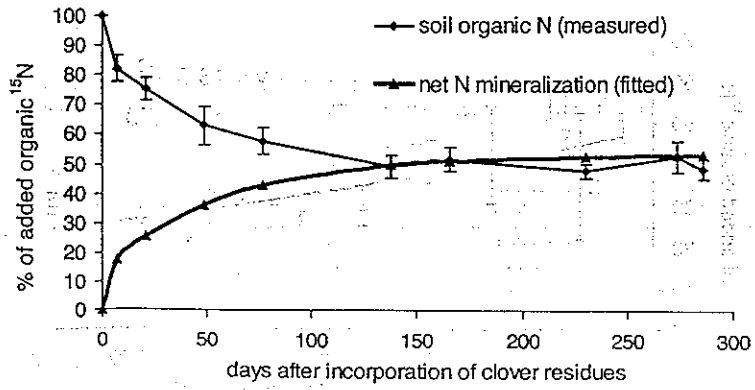


Fig. 2. Temporal course of clover-derived soil organic ^{15}N and net ^{15}N mineralization in 0–20 cm depth.

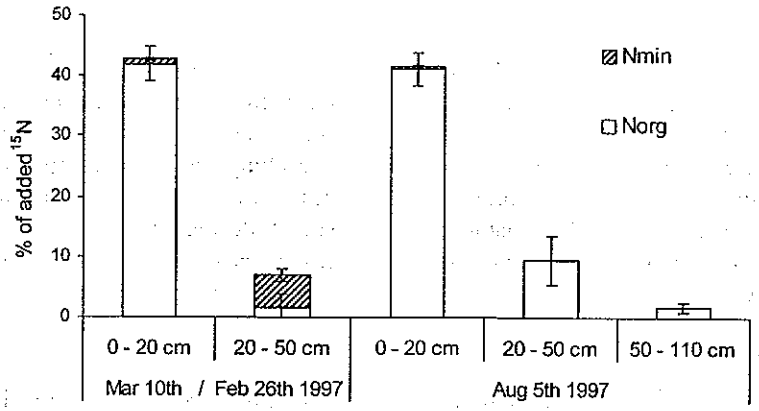


Fig. 3. Depth distribution of clover-derived ^{15}N in soil.

(2) gave a turnover rate of 0.0024 day^{-1} of soil microbial biomass N (Fig. 4), and the corresponding half-life was 289 days. Parameter variability during the first 4 weeks following straw incorporation was high due to high ^{15}N background in nonfumigated samples extracts, which were twofold to threefold higher than ^{15}N contents in the flush (fumigated minus nonfumigated).

The fitted cumulative values of clover-derived ^{15}N in net N mineralization ($N^{15}NM$) and decay of soil microbial biomass N ($\Delta^{15}N_{mic}$) differed markedly in their temporal course. Soil microbial ^{15}N turnover increased almost constantly until the harvest of winter wheat, whereas $N^{15}NM$ showed an initial rapid increase and reached an equilibrium in early spring, approximately 138 days after clover incorporation (Table 3). Therefore, the ratio $\Delta^{15}N_{mic}/N^{15}NM$ was

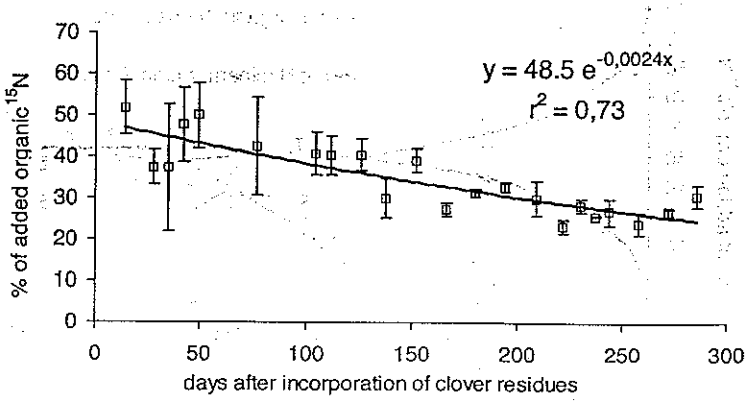


Fig. 4. Temporal course of clover-derived ^{15}N in soil microbial biomass.

Table 3
Cumulative net N mineralization ($N^{15}NM$) and soil microbial biomass N decay ($\Delta^{15}N_{mic}$) of clover-derived ^{15}N (% of added organic ^{15}N) in two time intervals after incorporation of clover residues

Days after incorporation	$N^{15}NM$[a]	$\Delta^{15}N_{mic}$[a]	Ratio $\Delta^{15}N_{mic}$ / $N^{15}NM$
7–138	31.8	13.1	0.41
138–286	2.2	10.6	4.82

[a]Values derived from the fitted temporal courses of soil organic ^{15}N (Fig. 2) and $\Delta^{15}N_{mic}$ (Fig. 4).

found to be higher in the second time interval (days 138–286) than in the first (days 7–138).

3.6. Root length and dry matter of winter wheat

The mean root dry mass of winter wheat at the flowering stage from 1995 to 1997 at different sites (sandy and loamy soils) and under different management (organic and integrated) was 247 g m^{-2} (0–90-cm soil depth) (Table 4). The coefficients of variation for the data of the different sampling populations were 20–40% (results not shown). A pronounced gradient in root length and root dry mass was found, with highest values in the upper 15 cm of the soil, decreasing towards deeper soil horizons. The average (1995 to 1997) cumulative gross root production in the upper 30 cm between tillering and early dough stage of wheat plants was 2.6 times the net root production, which showed lesser changes in this time interval (Table 5). From 1995 to 1997, the observed gross root dry

Table 4
Root distribution of winter wheat at flowering
Mean values from 27 winter wheat sites between 1995 and 1997 (every trial is the mean of three to four replications).

Soil depth (cm)	Root length		Root dry mass[a]	
	(km m^{-2})	(% of total)	(g m^{-2})	(% of total)
0–15	23.6±1.8	50	100±6.8	41
15–30	9.7±0.8	21	51±3.6	21
30–45	4.8±0.3	10	31±1.6	12
45–60	4.1±0.3	9	29±1.7	12
60–90	4.9±0.5	10	36±3.6	14

[a]Calculated assuming a root dry matter content of 10% from measured root lengths and radius (values represent means and standard errors).

Table 5
Dry matter production (g m^{-2}) of winter wheat root system between tillering to late milk/early dough and shoot at harvest on loamy soils (organic farming)
Average and cumulative values for the upper 30 cm of the soil are given for the net and the gross root system, respectively.

Year	Net root system	Gross root system	Shoot	Ratio gross/net	Ratio gross/shoot
1995	167±15	373±17	1354±206	2.2	0.28
1996	120±18	360±31	1318±137	3.0	0.27
1997	102±9	233±16	1001±55	2.3	0.23
1995–1997	129±25	322±38	1224±253	2.5	0.26

Values represent means and standard errors.

mass production at 0–30-cm soil depth at the organically farmed sites was 26% of the shoot dry mass at early dough stage of winter wheat.

3.7. Estimation of N input by winter wheat below-ground production

The estimation of below ground N production of winter wheat was based on both the organic ^{15}N found in the subsoil at harvest of winter wheat and the root dry matter depth distribution obtained in the root production experiment (Table 4). It was assumed that the entire clover-derived ^{15}N found in the subsoil at 20–50-cm depth (equivalent to 9.5% of added ^{15}N) was derived from winter wheat roots, and that ^{15}N deposition into soil by winter wheat roots was proportional to the root dry mass at each depth. The resulting N below-ground production of winter wheat in the N-turnover experiment was 161, 103 and 19 kg N ha^{-1} at 0–20-, 20–50- and 50–90-cm soil depth, respectively (Table 6), if

Table 6
N inputs by winter wheat roots into the soil in the field experiment deduced from the depth distribution of root dry mass and ^{15}N signature in organic N

Depth (cm)	Root dry mass[a] (% of total)	Soil organic ^{15}N		Soil organic N derived from roots (kg N ha^{-1})
		Total	Derived from roots	
		(% of added ^{15}N)		
0–20	48	41.1	15.0[b]	161
20–50	30	9.5	9.5[c]	103
50–90	22	1.8	1.8[c]	19

[a]Values from Table 4.
[b]Assuming that ^{15}N deposition by winter wheat roots was proportional to the root dry mass in each depth.
[c]Assuming that all ^{15}N in the subsoil was derived from winter wheat roots.

the mean ^{15}N signature of winter wheat roots was assumed to be identical to that measured in the shoots (3.71 ± 0.47 at.% ^{15}N).

4. Discussion

The net mineralization of clover-derived ^{15}N in soil proceeded in two phases, namely, high initial rates in the first phase up until 138 days after clover incorporation, and nearly zero values in the second phase up to the harvest of winter wheat. This N mineralization kinetics of residues with low C/N ratio in soil is similar to those commonly reported in the literature (Ladd et al., 1981; Jensen, 1992). There are two contrasting explanations for the constant level of soil organic ^{15}N in the second phase: (a) actual stability of soil organic ^{15}N due to, e.g., humification processes, or (b) apparent stability of soil organic ^{15}N resulting from actual ^{15}N mineralization with concomitant backflow of mineral ^{15}N into soil organic N pools (e.g., by microbial immobilization). Both mechanisms (a and b) are not exclusive and may act simultaneously, but in different compartments of soil organic N.

The hypothesis (a) (actual stability of clover-derived ^{15}N) was not consistent with several results of this study that indicated a continuing rather than stagnant ^{15}N turnover. Firstly, the soil microbial biomass (SMB), an important pool of soil organic N, showed a continued N turnover ($t_{1/2}$ = 289 days) throughout the entire experiment by means of a simplified isotopic dilution approach. This figure was much lower than that observed between the second and eighth month after incorporation of labeled *Medicago* residues at an arable site in South Australia (Ladd et al., 1981), with a half-life of 912 days. This discrepancy may reflect a reduced N turnover in SMB under the dry soil conditions in the Mediterranean climate of South Australia, compared to the humid climate at the Research Station Scheyern. It could be also argued that the comparatively high turnover of N_{mic} was due to several limitations inherent to the simplified isotopic dilution approach used here, as already discussed by Chaussod et al. (1988). Considering these limitations, nonetheless, a higher rather than a lower turnover rate would result.

Secondly, during the second mineralization phase, the decline in $^{15}N_{mic}$ was fivefold higher than the clover-derived ^{15}N net mineralization (balance approach). This was unexpected since the N_{mic}-turnover in soil should be closely linked to N mineralization. It is generally accepted that decay products of SMB (organic materials with low or high molecular weight, exoenzymes, dead cells) are rapidly mineralized to an extent of 37% within 28 days at 22 °C (Marumoto et al., 1982), and to an extent between 22% and 47% within 7 days at 28 °C (Nicolardot et al., 1986). The net decline in $^{15}N_{mic}$ should, therefore, appear at least partly as net ^{15}N mineralization, which was obviously not detected by the ^{15}N balance approach. Thus, this approach seemed to underestimate the actual

^{15}N net mineralization in topsoil (0–20 cm) and, consequently, to represent the apparent rather than actual stability of soil organic ^{15}N.

A persistent mineralization of clover-derived ^{15}N throughout the entire second phase (days 138 to 286) was also inferred by the mean ^{15}N signature in soil mineral N pool (2.1 at.% ^{15}N). The size of this pool during this phase was small and probably of the same magnitude as the daily turnover in soil mineral N, emphasizing that the signature in soil mineral N represented exclusively that being actually mineralized.

These findings support the previously mentioned hypothesis, of namely, the determination of clover-derived NNM based on soil organic ^{15}N decline (balance approach) being biased by the internal ^{15}N cycling in the soil–plant system, leading to apparent stability of soil organic ^{15}N.

This internal recycling of mineralized ^{15}N during the vegetation period may involve different processes, e.g., microbial immobilization [mineralization–immobilization turnover (MIT; Jansson and Persson, 1982)] or plant uptake and assimilation of mineral ^{15}N, followed by organic ^{15}N below ground inputs through roots and rhizodeposits.

Even though the direct measurement of plant root ^{15}N input into soil was not within the initial scope of our ^{15}N turnover experiment, we were able to assess it indirectly using the root dry matter input of winter wheat that was independently measured in adjacent fields at the Research Station Scheyern. The use of field instead of plot data for our purpose was justifiable on account of the similar soil and climate characteristics at these sites. Moreover, we used consistent relative proportions (depth distribution of net root production), instead of the absolute root production values, which were subject to certain annual and spatial variabilities. However, the mean root length and dry mass of winter wheat at flowering, as documented in this study, are comparable to results reported by Gregory et al. (1978) and Stoffel et al. (1995).

With this approach, the gross N input into the entire soil profile (0–90 cm) by winter wheat roots in the N-turnover experiment was estimated at 282 kg ha^{-1}, equivalent to 54% of total net N assimilation of winter wheat. Thus, the root input into the soil was of the same magnitude as the plant N net uptake at harvest.

The approach used to estimate root-derived N inputs was based on several assumptions carefully selected so as to closely reflect actual conditions. It was shown in the root production experiments that the depth distribution of net root production was relatively stable under the varying climatic and soil conditions at the Research Station Scheyern and, thus, applicable to the N-turnover experiment. Additionally, the root-derived N input was assumed to be proportional to the net input of root dry-mass at each soil depth. This assumption is likely to hold true if the root N contents at different depths were equal. The mean ^{15}N signature of the roots, also used in the calculation of root N inputs, was not measured, but assumed to be identical to that determined in winter wheat shoots.

This similarity of ^{15}N signature in roots and shoots has been shown in greenhouse experiments with ^{15}N-labeled wheat plants (Wagger et al., 1985).

It may be also argued that all of the organic ^{15}N found in the subsoil (20–90 cm) was derived from winter wheat root inputs, as inferred in our calculations. In spite of root inputs, three additional mechanisms could explain the transport of labeled N out of the treated topsoil and its accumulation as organic N in subsoil.

(1) Soluble organic N could have been washed out and immobilized in subsoil. This mechanism is improbable since concentrations of extractable organic N detected were higher during winter and early spring, where no significant accumulation of organic ^{15}N in subsoil was found. In contrast, lower concentrations (nearly 1 $\mu g\ N\ g^{-1}$) occurred during spring and summer, when the accumulation took place.

(2) It could be argued that endogeic earthworms fed on the ^{15}N-labeled clover residues in topsoil (Curry and Byrne, 1992) and deposited their feces or excretions in subsoil. However, this explanation is inconsistent with the stable ^{15}N content of soil organic matter observed between spring and wheat harvest, which should have been diminished by the activity of earthworms.

(3) The SMB may have immobilized a part of the labeled NO_3^- which had leached into the subsoil. This explanation is unsatisfactory as it implies the immobilization of approximately 100 kg N ha^{-1} during the relatively brief period between days 126 and 152 after clover incorporation, when most of the leached NO_3^- moved through the soil below 50 cm (data not shown). Assuming that SMB had a C/N ratio of 7 and a yield coefficient of 0.6, the corresponding microbial C-demand would amount to 1160 kg ha^{-1}, which is implausible for the C-deficient environment of the subsoil. During later drainage, nearly no net loss of ^{15}N in the topsoil was observed, thus indicating that this mechanism was also irrelevant during spring and summer.

The contribution of these mechanisms to explain the origin of organic ^{15}N found in the subsoil cannot be completely excluded, but is likely to be of minor importance compared to the prominent contribution of plant roots. However, considering a small contribution of these alternative mechanisms, the actual root N input into soil might be somewhat lower than the value we assessed. In contrast, it seems to be very probable that a portion of total root ^{15}N deposition into the miniplot subsoil was lost due to root growth in lateral direction, since the border of the miniplots (open-ended PVC cylinders) reached a depth of only 25 cm into the soil. However, the proportion of gross N uptake in winter wheat deposited into soil estimated in our field experiment (54%) is similar to that reported by Janzen (1990) from pot experiments with N-fertilized winter wheat (49%), by means of ^{15}N-pulse labeling of shoots with gaseous NH_3.

The ratio of gross-to-net root dry matter production (2.6) obtained in this study was comparable to other reports, ranging from 2.0 to 3.5 (Swinnen et al., 1995; Sauerbeck and Johnen, 1976), and indicates a large new root growth

which is in balance with a high root mortality. This high root turnover provides additional evidence for the importance of plant root N inputs into soil by means of an approximate estimate using measured root gross dry matter production and estimated N contents of root inputs ranging from 1% to 2.6% (Kleemola et al., 1998; Thomson et al., 1996; Zagal, 1994; Griffith and Robinson, 1992; Janzen, 1990). According to this estimate, the plant root N inputs would range from 49 to 128 kg N ha^{-1} (0–20-cm depth) and are lower than the value based on ^{15}N distribution in subsoil (161 kg N ha^{-1}). In spite of uncertainties on N contents of root inputs, our estimate does not include the contribution of root exudates and mucilage, thus leading to underestimation of total root N input into soil. Nevertheless, even these lower estimates emphasize the importance of root turnover in regard to the N cycling in the plant–soil system.

The N cycling by mineralization–immobilization turnover (MIT) seems not to be a reasonable mechanism to explain the discrepancy between concurrent turnover and apparent stability of soil organic ^{15}N. The rates of microbial ^{15}N decay, soil organic ^{15}N mineralization and microbial ^{15}N immobilization should be equal to achieve a stabilizing action of MIT. In contrast, our data show that the rate of microbial ^{15}N decay was higher than that of microbial ^{15}N immobilization.

Some limitations should be considered when interpreting the results presented here. The N-turnover experiment was performed in small plots and obtained results may have been biased by margin effects. This was indicated by the high wheat N yields observed: 188 and 240 kg ha^{-1} in the control and clover-treated plots, respectively. In addition, soil mixing and aeration during initial plot establishment may have artificially influenced the rooting and the mineralization kinetics in the plough layer, compared to tillage in agricultural practice. Our results provide valuable evidence for the N soil–plant loop; however, we have no direct measurements of root N deposition into the soil. However, these results represent to the best of our knowledge, the first field experiment report on N deposition of roots into soil and its consequence on the N cycle. In addition, the direct measurements of N deposition reported from pot experiments (Janzen, 1990; Jensen, 1996; McNeill et al., 1996) may also suffer from limitations due to artificial conditions, in respect to the rooting in the pots (e.g., restricted rooting space, soil compaction) or to the plants (e.g., light conditions, nutrition). Thus, ^{15}N-labeling techniques already applied in pot experiments must be adapted for field experiments, in order to prove the hypothesis and to exactly quantify N-root depositions.

In spite of the low net effect concerning the crop N nutrition, the N loop in the soil–plant system should be considered in respect to the interpretation of ^{15}N experiments and to ecological implications. As a consequence of N rhizodeposition, net ^{15}N losses of the plants could occur during the growing season, since the rhizodeposition may have higher ^{15}N signatures than the newly mineralized N which is subsequently assimilated by the plants. This mineral N will have a

lower ^{15}N signature, due to an increased contribution of unlabeled soil-derived N occurring in the later mineralization phase. This "pool substitution" mechanism, implicit to N cycling in the soil–plant system, may, therefore, contribute to the apparent "Added Nitrogen Interaction" (ANI, Jenkinson et al., 1985) in ^{15}N-labeling experiments, where lowered estimates of crop fertilizer use efficiencies are observed. The N loop may be also responsible for the lower net N mineralization observed in planted compared to unplanted soils (Jensen, 1992; Janzen and Radder, 1989), since a part of the mineralized ^{15}N-labeled soil organic N is returned into the soil via the root depositions.

The N loop may also have a stabilizing effect on N availability and N retention in the soil–plant system since N is kept mainly in organic forms in soil and plants and, thus, prevented from leaching, but still available to plants due to the high gross N fluxes.

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