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Nitrogen immobilization and mineralization during initial decomposition of ^{15}N-labelled pea and barley residues

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Abstract The immobilization and mineralization of N following plant residue incorporation were studied in a sandy loam soil using ^{15}N-labelled field pea (*Pisum sativum* L.) and spring barley (*Hordeum vulgare* L.) straw. Both crop residues caused a net immobilization of soil-derived inorganic N during the complete incubation period of 84 days. The maximum rate of N immobilization was found to 12 and 18 mg soil-derived N g^{-1} added C after incorporation of pea and barley residues, respectively. After 7 days of incubation, 21% of the pea and 17% of the barley residue N were assimilated by the soil microbial biomass. A comparison of the ^{15}N enrichments of the soil organic N and the newly formed biomass N pools indicated that either residue N may have been assimilated directly by the microbial biomass without entering the soil inorganic N pool or the biomass had a higher preference for mineralized ammonium than for soil-derived nitrate already present in the soil. In the barley residue treatment, the microbial biomass N was apparently stabilized to a higher degree than the biomass N in the pea residue treatment, which declined during the incubation period. This was probably due to N-deficiency delaying the decomposition of the barley residue. The net mineralization of residue-derived N was 2% in the barley and 22% in the pea residue treatment after 84 days of incubation. The results demonstrated that even if crop residues have a relative low C/N ratio (15), transient immobilization of soil N in the microbial biomass may contribute to improved conservation of soil N sources.

Key words Immobilization · Mineralization · ^{15}N-labelled crop residues · Residue quality · Soil microbial biomass

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Introduction

Incorporation of crop residues in soil causes a rapid increase in the soil microbial biomass on and around the residue particles and the soil microbial biomass will act both as a sink for nutrients and as a catalyst for decomposition. Microbial C and N metabolisms in soils are closely linked, since after adding a C substrate to soil the energy and growth substrates generated by heterotrophic metabolism are utilized to increase the microbial biomass and hence the N demand of decomposer populations (Ladd and Foster 1988). According to the hypothesis of mineralization-immobilization turnover (MIT) of N in the soil, the microbial biomass obtains the N needed for synthetic reactions associated with growth and metabolism from the soil inorganic N pool (Jansson and Persson 1982). It has been suggested that the soil microbial biomass is also capable of taking up and assimilating soluble, low-molecular-weight nitrogenous organic substances ("direct hypothesis", Molina et al. 1983). Both pathways (MIT and direct assimilation) may operate concurrently during initial residue decomposition, with low-molecular organc N forms dominating the N assimilation by the residue-specific population and the inorganic N immobilization operating at the level of the native soil population (Hadas et al. 1992).

The decomposition rate of fresh organic material added to soil is generally most rapid during the first weeks (Sørensen 1981). Many factors control the initial rate of plant residue decomposition, with soil moisture, temperature, size and composition of the soil microbial biomass and available nutrients, especially N, being of prime importance (Parr and Papendick 1978). The C/N ratio of incorporated organic materials gives a rough indication of whether net mineralization or immobilization of N will prevail during the initial stages of decomposition (Waksman and Tenney 1928; Jenkinson 1981). Generally, a C/N ratio of 25 will not cause any net mineralization or immobilization and with lower/higher ratios net N-mineralization/immobilization, respectively, take place (Paul and Clark 1989). However, net immobilization of N may take place during early decomposition with residue C/N as low

as 16 (Jensen 1994b). This is probably due to the C/N of readily decomposable residue-material being different from the overall C/N ratio of material. It has been suggested that the rate of initial residue decomposition and the amount of biomass produced depend on the size of the water-soluble and an intermediately available C pool in residues (Reinertsen et al. 1984).

The development of the fumigation-extraction technique (Brookes et al. 1985) enabled the determination of the soil microbial biomass C and N shortly after additions or organic material to the soil (Powlson 1994). Recent studies on the decomposition of ^{15}N-labelled crop residues indicate that significant amounts of residue-derived N may quickly become incorporated in the microbial biomass (Ocio et al. 1991). Even though the soil microbial biomass may be able to assimilate soluble organic residue-materials "directly", the availability or inorganic N significantly influences the amount of N immobilized and the rate of residue decomposition (Knapp et al. 1983; Mary et al. 1996; Recous et al. 1995).

My aim was to compare the effect of crop residues with contrasting chemical composition on the mineralization and immobilization of N during the initial 12 weeks of decomposition in a sandy loam soil. Mature ^{15}N-labelled barley and field pea residues were used in order to distinguish between indigenous soil N and residue-derived N.

Materials and methods

Soil

A sandy loam soil (Typic Hapludalf) was obtained from the 0- to 20-cm depth in the Risø experimental field. The soil particle size composition was 11.4% clay (<0.002 mm), 13.6% silt (0.002–0.02 mm), 48.6% fine sand (0.02–0.2 mm) and 26.4% coarse sand (0.2–2 mm). The soil contained 1.1% total C and 0.13% total N, and had a pH(H_2O) of 6.9. The soil was air dried and sieved (2 mm). The soil inorganic N-concentration (2 *M* KCl extract) was 9.7 μg N (NO_3+NH_4) g^{-1} soil at the start of the experiment.

Crop residues

Field pea (*P. sativum* L., cv. Bodil) and spring barley (*H. vulgare* L., cv. Golf) residues labelled with ^{15}N were produced in large pots containing quartz sand (Jensen 1996). Plants were harvested at maturity and the residues consistent of pea straw (70%) and empty pods (30%) and barley straw (70%) and awns (30%). Residues were ground to pass a 1-mm sieve. The chemical composition of the residues is shown in Table 1. Water-soluble substances were determined by extracting 100 mg ground residue with 10 ml cold water. Soluble C and N in extracts, total C and N and ^{15}N enrichment were determined using an on-line elemental analyzer-mass spectrometer (EA-MS) system (Jensen 1991). The lignin content of crop residues was determined using the acid-detergent fiber method (Van Soest 1963).

Experimental procedures

The experiment consisted of three treatments: soil without residues (control), soil with pea residues and soil with barley residues. Portions of 50 g soil (dry weight basis) were mixed with residues corresponding to 7.0 g kg^{-1} and filled into 250-ml polyethylene bottles.

Table 1 Chemical composition and amounts of crop residues added

Parameter	Pea residues	Barley residues
% N	2.51	0.98
% C	36.9	39.8
C/N	14.7	40.6
% soluble N	0.7	0.2
% soluble C	7.1	3.4
% lignin	8.1	6.7
Atom % ^{15}N excess	1.16	3.40
Residue added:		
μg N g^{-1} dry soil	160.2	63.4
mg C g^{-1} dry soil	2.4	2.6

The moisture content of the soil was adjusted to 55% WHC. Bottles were covered with aluminum foil with holes, placed in the dark and incubated at 20 °C.

After 0, 7, 14, 28, 56 and 84 days, three bottles of each treatment were analyzed for NO_3^--N, NH_4^+-N, soil microbial biomass N, total N and ^{15}N enrichments.

Analytical procedures

Inorganic N was determined by extracting 10 g fresh soil with 100 ml 2 M KCl, and NH_4^+-N and NO_3^--N in extracts were determined on a Technicon Autoanalyzer II (Jensen 1994a). Soil microbial biomass N was determined in 10 g fresh soil using the fumigation-extraction procedure (Brookes et al. 1985). The amounts of ammonium and organic N in K_2SO_4 extracts of fumigated and nonfumigated soil were determined using a semi-micro-Kjeldahl procedure, distillation and titration. A k_N of 0.45 was used for calculating the biomass N from the fumigations flush (Jenkinson 1988). Soil total N and ^{15}N enrichment were determined on subsamples of 60 mg air-dried (99.2% dry matter) soil using the EA-MS system (Jensen 1991). The ^{15}N enrichment of inorganic N in KCl and digested K_2SO_4 extracts was determined after concentrating the inorganic N by a diffusion procedure (Jensen 1991).

Calculations

The ^{15}N enrichments were corrected for the natural ^{15}N abundance of the soil (0.3703 atom% ^{15}N). Concentrations of residue-derived inorganic, microbial biomass and total N in soil were calculated under the assumption that the N in residue mixture components mineralized at the same rate. The net mineralization-immobilization of N due to residue incorporation was calculated by subtracting the inorganic N concentration in the control soil (no residues) from the inorganic N concentration in the residue-amended soil. The term soil-derived N will be used for soil inorganic N derived from indigenous soil organic N.

Analysis of variance was carried out using the ANOVA procedure in SAS (1990). $LSD_{0.05}$ was used for comparisons if the main effect of treatment was significant.

Results and discussion

Immobilization of N

The rapid proliferation of the microbial biomass on the ground substrate created a high demand for N. Both residue treatments caused a net immobilization of N during the initial 14 days of decomposition (Fig. 1 A). The concentration of soil-derived inorganic N in the residue-treat-

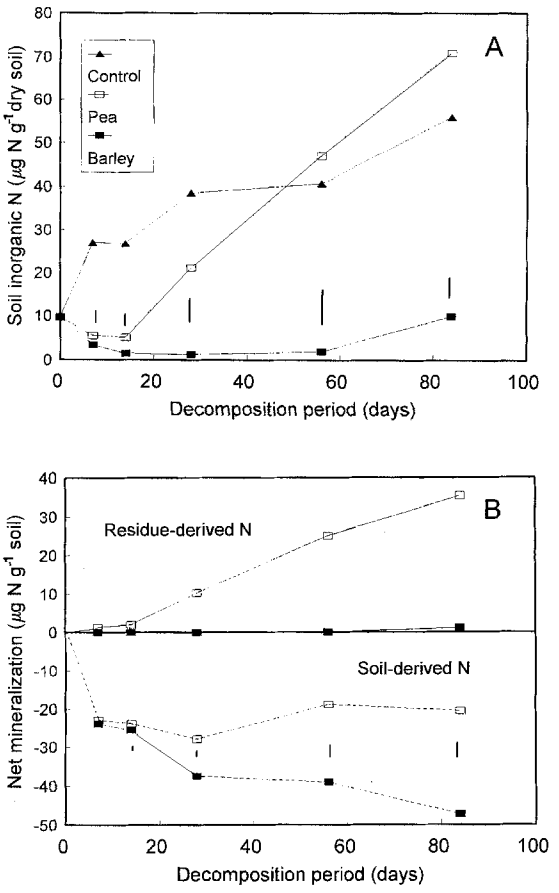

Fig. 1 **A** Soil inorganic N concentration as influenced by pea and barley residue incorporation. **B** Net mineralization of residue-derived N (*solid lines*) and net immobilization of soil-derived N (*broken line*). *Bars* represent $LSD_{0.05}$

ed soils was lower than in the control soil at all samplings (data not shown). Assuming that the mineralization and losses of indigenous soil N were similar in the control and residue-treated soils, the apparent net immobilization of soil-derived N was calculated by subtracting soil-derived N in the control treatment from soil-derived N in the residue treatment. Apparent net immobilization of soil-derived N was similar in the two residue treatments during the initial 14 days of decomposition (Fig. 1B). The immobilization of soil N increased in the barley residue treatment during the remaining period of incubation, reaching a maximum of 47 μg N g^{-1} soil or 18 mg N g^{-1} added C after 84 days. Maximum immobilization of soil N in the pea residue treatment was found to 28 μg N g^{-1} soil after 28 days of decomposition (Fig. 1B).

Reinertsen et al. (1994) reported that wheat straw having C/N ratios of 36–54 resulted in immobilization of 18–26 mg N g^{-1} added C in soil without addition of mineral N. In comparison, Bremer et al. (1991) found an immobilization of 13 mg N g^{-1} added C as wheat straw and 8 mg N g^{-1} added C in lentil (*Lens culinaris* Medik) straw with C/N ratios of 80 and 36, respectively. The initial immobilization of N per unit of residue carbon added is greater with increasing initial levels of organic N, reaching values as high as 39 mg N g^{-1} added C (Recous et al. 1995).

Even though the C/N ratio of the pea crop residue in the present study was significantly lower than the equivalence point of C/N suggested by Paul and Clark (1989), net immobilization occurred during initial decomposition. However, Marstorp and Kirchmann (1991) found that clover material with C/N ratios of 15–21 cause net immobilization of N during the initial weeks of decomposition. This was supported by McKenney et al. (1995), who found net immobilization of N during initial decomposition of clover material with C/N ratios of 15. Jensen (1994a) found net immobilization of soil N during early decomposition of pea residues with a C/N ratio of 16.

The overall net immobilization of N was much smaller due to the mineralization of pea residue N. After 56 days of incubation overall net mineralization was observed in the soil amended with pea residues. This indicates that the incorporation of pea crop residues may contribute to conservation of soil N in the autumn following cultivation of pea despite the narrow C/N ratio of residues. However, Jensen (1994b) found that the net immobilization of soil N was lower when pea residues were cut into 1-cm pieces than when ground to pass a 1-mm sieve. Thus, with conventional residue management in the field, net immobilization of N during the early decomposition of pea residues may not occur to the same degree as observed in the present laboratory incubation experiment.

Soil microbial biomass N

The soil microbial biomass N was not determined in the control soil at the start of the experiment. It is likely that the sieving and re-wetting of the soil may temporarily have increased the microbial biomass in the unamended control soil during the initial 7 days of incubation, but during the subsequent 3 weeks it decreased and was then stabilized at ca. 29 μg N g^{-1} soil (Fig. 2A).

The soil microbial biomass N increased by 92% after pea and by 30% after barley residue incorporation compared to the unamended control soil 7 days after residue incorporation and remained significantly higher than in the control during the complete incubation period (Fig. 2a). Maximum microbial biomass N was observed after 7 and 14 days in the pea and barley residue treatments, respectively. In the barley residue treatment the microbial biomass N was stabilized after 4 weeks of incubation, whereas there was a steady decline in the biomass N in the pea residue treatment during the complete incubation period, indicating the biomass formed during the first weeks of a pea residue decomposition turned over faster than the biomass formed during barley residue decomposition. However, the level of biomass N in the pea-residue-treated soil was still significantly higher than in the control after 84 days of incubation (Fig. 2A). The different dynamics of the microbial biomass N in the two residue treatments may be caused by differences in N availability. Due to a higher concentration of N, especially of soluble

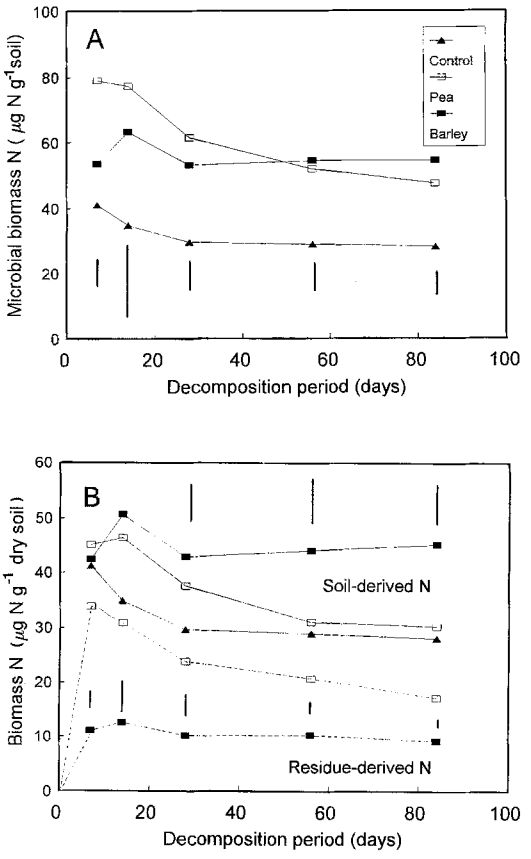

Fig. 2 **A** Total soil microbial biomass N as influenced by pea and barley residue incorporation. **B** Biomass N derived from indigenous soil N (*solid lines*) and residues (*broken lines*). **C** Percentage residue N recovered in the soil microbial biomass. *Bars* represent $LSD_{0.05}$

N (Table 1) in pea residues, the early decomposition proceeds fast. A major part of the substrate respires away quickly and a high proportion of the residue N is temporarily incorporated in the biomass. In the barley residue treatment the decomposition and incorporation of N in the microbial biomass is probably limited by available N and consequently residue C will be available over a longer period to support the growth of the microbial biomass. In an experiment with wheat straw decomposition, Ocio et al. (1991) found that addition of inorganic N increased the amount of N incorporated in the biomass compared to no inorganic N added, but the biomass N tended to decline faster in the treatment with N addition during the subsequent 15 days of incubation.

The greater immobilization of soil-derived N (Fig. 1B) was consistent with more soil-derived N being incorporated in the microbial biomass after barley than after pea residue incorporation (Fig. 2B). The decline of both residue- and soil-derived N in the microbial biomass was lower after barley than after pea residue incorporation (Fig. 2B).

After 7 days of incubation, the residue-derived N constituted 43% and 21% of the biomass N in the pea- and barley-amended soils, respectively (Fig. 2B). During the subsequent 77 days of decomposition the relative contribution of residue-derived N only decreased slowly to 36% and 17% of the biomass N in the pea and barley residue treatments, respectively. After 7 days the recovery of residue-derived N input in the biomass was 21% for the pea residue N and 17% for the barley residue N (Fig. 2C). Due to the slower turnover of the biomass in the barley residue N treatment, the percentage barley-residue N recovered in the biomass at the end of the incubation was significantly higher than of the pea residue-derived N (Fig. 2C). The above results for incorporation of N in the microbial biomass are in accordance with other reports on the assimilation of residue-derived N by the microbial biomass (Ocio et al. 1991; Thomsen 1993; Jensen 1994a). Maximum incorporation residue-derived N was found after 7 and 14 days for pea barley, respectively, and residue-derived N accounted for 90% of the increase in biomass N relative to the unamended control after 7 days. Ocio et al. (1991) similarly found maximum incorporation in the biomass after 5 days and Jensen (1994a) found maximum incorporation of pea residue N (14–22%) in the biomass after 10 days in a field experiment. The rapid early incorporation of residue-derived N may be related to the availability of N in residues. Approximately 28% of the pea and 21% of the barley N was soluble in water and this related well to the assimilation of 21% of the pea and 17% of the barley residue N after 7 days (Fig. 2C).

Experimentally, it is hard to prove the direct hypothesis for microbial assimilation of low-molecular soluble organic N, since it is difficult to trace inorganic ^{15}N-labelled N derived from ammonification in a soil, which has been amended with an organic substrate. Mary et al. (1996) suggested that the role of direct assimilation of low molecular organic N compounds from crop residue by the microbial biomass could be evaluated by comparing the mean ^{15}N abundance of the soil inorganic N and the newly formed biomass N pools. Unfortunately, the size of the biomass N pool was not determined before treating the soils with residues, but after 7 days the ^{15}N enrichment of the inorganic ^{15}N-pool was 0.293 and 0.109 atom% ^{15}N excess for pea and barley residues, respectively, whereas the ^{15}N enrichments of the total biomass N pools were 0.499 and 0.710 atom% ^{15}N excess, respectively. This suggests that residue-derived N was assimilated by the soil microbial biomass without entering the soil inorganic N pool. However, if the microbial biomass has a much higher preference for ammonium than for nitrate, it is likely that ammonium formed during mineralization of labelled residues is immobilized immediately before being nitrified in preference to a rather large pool of nitrate readily available (Jansson et al. 1955).

The percentage of indigenous soil N present in the soil microbial biomass was 2.4–3.8%, whereas the residue-derived N constituted 20–24% of the organic residue-derived N during the initial 14 days of decomposition, after which is gradually declined to 16–17% after 84 days (data not shown).

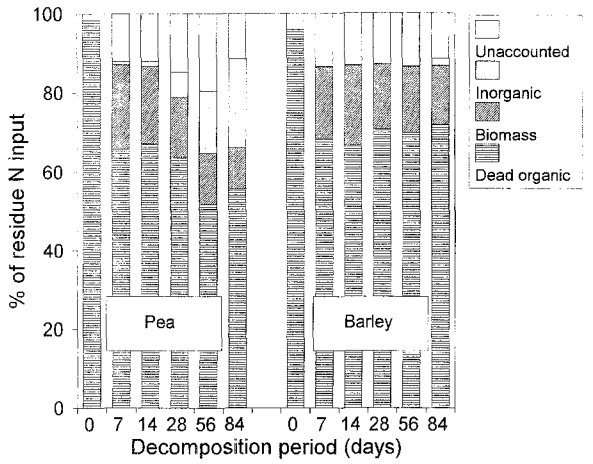

Fig. 3 Balances of ^{15}N-labelled pea and barley residue N during incubation

Mineralization of N

After the initial 14 days of overall net immobilization, pea residue N mineralized at a rate of 0.42 μg N g^{-1} day^{-1}, whereas the net mineralization of barley N was negligible until 84 days of decomposition (Fig. 1B). At 84 days 22% of the pea and 2% of the barley residue N was found in the soil inorganic N pool.

The mineralization of soil-derived N exceeds the immobilization of soil-derived N after 14 days in the pea-residue-amended soil, whereas no significant net mineralization of soil-derived N was observed in the barley residue treatment until day 84 (Fig. 1A). From day 7 to day 84 the decline in the residue- and soil-derived microbial biomass N due to remineralization was similar in size to the increase in residue- and soil-derived inorganic N in the barley-residue-treated soil (Figs. 1B, 2B). With pea residue the decline in biomass N only accounted for 60% of the increase in soil- and residue-derived inorganic N (Figs. 1B, 2B).

Balance of residue-derived N

At the start of the experiment 98.4±0.8% of the pea and 96%±8.9% (±SE) of the barley residue N was recovered (Fig. 3). During the subsequent 7 days of decomposition there was a loss of 10% of the pea residue N and 13% of the barley residue N, which could not be accounted for. This N may have been lost by denitrification. During the subsequent incubation period the amounts of labelled N unaccounted for did not increase (Fig. 3). This indicates that the net mineralization of residue N was probably underestimated.

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