Department of Organic Farming and Cropping

Faculty of Ecological Agricultural Sciences

University of Kassel

Root effects on the turnover
of grain legume residues in soil

Dissertation

submitted to the Faculty of Ecological Agricultural Sciences
(Fachbereich Ökologische Agrarwissenschaften) of the University of Kassel
to fulfil the requirements for the degree Doktor der Agrarwissenschaften
(Dr. agr.)

by

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Witzenhausen, February 2003

My parents

and

Runa

Preface

This thesis is submitted to the Faculty of Ecological Agricultural Sciences (Fachbereich 11) of the University of Kassel to fulfil the requirements for the degree Doktor der Agrarwissenschaften (Dr. agr.).

The dissertation is based on three papers published or submitted to internationally refereed journals which are included in chapter 4, 5 and 6.

Chapter 1 gives a general introduction and a short overview on relevant literature. Chapter 2 contains the objectives of the work and Chapter 3 gives an overview of the methodology used in the work.

Chapter 7 integrates in a synthesis the results and discussion of chapter 4, 5 and 6. Final conclusions and an outlook are given in chapter 8 and 9.

The following papers are enclosed:

Chapter 4

Mayer, J., Buegger, F., Jensen, E. S., Schloter, M. and Heß, J. (2003): Estimating N rhizodeposition of grain legumes using a ^{15}N *in situ* stem labelling method. Soil Biology and Biochemistry 35, 21 – 28.

Chapter 5

Mayer, J., Buegger, F., Jensen, E. S., Schloter, M. and Heß, J. (2003): Turnover of grain legume N rhizodeposits and effect of the rhizodeposition on the turnover of crop residues. Biology and Fertility of Soils, submitted.

Chapter 6

Mayer, J., Buegger, F., Jensen, E. S., Schloter, M. and Heß, J. (2003): Residual nitrogen contribution from grain legumes to succeeding wheat and rape and related microbial process. Plant and Soil, in press.

Content

List of plant names and abbreviations

Plant names

Faba bean	*Vicia faba* L.
Oats	*Avena sativa* L.
Pea	*Pisum sativum* L.
Rape	*Brassica napus* L.
Wheat	*Triticum aestivum* L.
White lupin	*Lupinus albus* L.

Abbreviations

ANI	Added Nitrogen Interaction
BGP-N	Below ground plant derived nitrogen
C	Carbon
C_{dec}	Carbon decomposed from residues
C_{input}	Added carbon as crop residues or rhizodeposits
C_{mic}	Microbial carbon
C_{micres}	Microbial residues carbon
C_{min}	Mineralised carbon
df	Derived from
dfr	Derived from residues
dfR	Derived from rhizodeposition
F	Flowering
fum soil	Fumigated soil
k_{EC} and k_{EN}	Extractable part of the total amount of carbon (k_{EC}) and nitrogen (k_{EN}) fixed in the microbial biomass
M	Maturity

Abbreviations continued from page before

MB	Microbial biomass
N	Nitrogen
N_{dec}	Nitrogen decomposed from residues
Ndfa	Nitrogen derived from air
NdfR	Nitrogen derived from rhizodeposition
Ndfr	Nitrogen derived from residues
NH_4^+	Ammonia
N_{input}	Added nitrogen as crop residues or rhizodeposits
N_{loss}	Losses of N derived from residues
N_{mic}	Microbial Nitrogen
N_{micres}	Microbial residues nitrogen
N_{min}	Mineral Nitrogen
NO_3^-	Nitrate
not fum soil	Unfumigated soil
SC	Subsequent crop
SOM	Soil organic matter
WHC	Water holding capacity

1. Introduction

Agricultural ecosystem serve to produce food of plant and animal origin. In contrast to natural ecosystems, agricultural ecosystems are formed by human beings by cultivation and selective alteration.

The aim of organic farming systems, in contrast to intensive conventional systems, is to integrate the self regulating mechanisms of natural ecosystems as basic element of the system. Thus, the aim is to achieve a high productivity from the system itself with largely closed substance and energy fluxes on farm level and an adaptation to the local site factors. The principle of the system 'Organic farming' can be described as 'principle of a selective organisation of a largely closed well-proportioned farm organism' (Köpke 1994). The term 'farm organism' corresponds to the term 'agricultural ecosystem' on farm level. The object of this principle is to maintain a sustainable productivity of the system without substantial dependence of external inputs into the system.

With respect to this concept the use of mineral N fertilizers is excluded. The husbandry is limited by a strict minimum area per livestock unit which is based on the productivity of the location. The allowed maximum nutrient import as fodder or organic manure is coupled with the nutrient amounts of the maximum stocking units per area (EEC 2092/91 1991).

The main primary nitrogen source in organic farming systems is provided by biological N_2-fixation by integrating legumes in the crop rotation. As a rule, a percentage of legumes of about one fourth to one third of the main crops is necessary to ensure the nitrogen supply of the crop rotation. Hence, nitrogen is the main limiting nutrient factor in organic crop rotations. The aim of agronomic management must be to save the legume derived nitrogen in the system by preventing nitrogen losses from the system and synchronising the nitrogen mineralisation from legume residues to provide a demand-oriented nitrogen supply for non-legumes. In particular in stockless legume based farming systems, farmyard manures are not available as a tool for a demand-oriented fertilization. Thus, in such systems the agronomic management of the legume derived residual nitrogen is the most important factor for an optimisation of N-fluxes in crop rotations.

In the last two decades, for temperate climates as in north-western Europe, the research on turnover of legume derived residual N focused mainly on

i)   the turnover of legume residue N in soil,

ii)   interactions between legume residue N turnover and soil N,

iii) the N transfer from legume residues to subsequent non-legumes,

iv)   tools for improving crop rotation management with respect to reducing N losses and improving demand-oriented N supply.

Turnover of legume residue N

The turnover of plant residues in soil over time is regulated by three interacting factors: (i) the physicochemical environment, (ii) the quality of the resource and (iii) the decomposer community (Heal et al. 1997). Most studies on legume residues focused on the relationship between quality and turnover. Dependent on its quality (chemical composition) the residue N mineralisation kinetics differs between various legume species.

In general, the N mineralisation follows a 1st order kinetics (Kirchmann and Bergqvist 1988; Klimanek 1988; Fox et al. 1990; Palm and Sanchez 1991; Marstorp and Kirchmann 1991; Azam et al. 1993; Franzluebbers et al. 1994; Jensen 1994b; Mary et al. 1996; Janssen 1996). However, the results show differing relationships between chemical parameters and the decomposability of plant residues. Franzluebbers et al. (1994) found for *Vigna unguiculata* the net-N mineralisation directly related to the total N content and the C/N ratio, respectively. Other authors found close correlations between lignin contents for *Trifolium repens* (Kirchmann and Bergqvist 1988), lignin + polyphenol contents (Fox et al. 1990) or only polyphenol contents in leaves of various legume trees. Mueller et al. (1988) found a close relationship between lignin, cellulose and hemi cellulose contents of *Trifolium pratense*, *Trifolium repens*, *Trifolium subterraneum*, *Vicia faba* and *Phleum pratense* and the net N mineralisation with strongly differing C/N ratios.

Interactions between residue N and soil N

Smith and Sharpley (1993) extended their investigations and included the effects of different soils on the turnover of plant residues. They found the soil type in comparison to the residue type had minor effects on the net N mineralisation of residues. Azam et al. (1993) investigated the interactions between soil N mineralisation of differing soils and legume residues which

are termed as 'Priming Effects' or 'Added Nitrogen Interaction' (ANI) (Jenkinson et al. 1985). An ANI occurs if the addition of plant residues causes an alteration of soil N mineralisation compared to a non treated soil. Azam et al. (1993) concluded from their results that both the soil type and the residue composition have an effect on the extent of ANI's during turnover of residues and cause as well positive as negative ANI's.

N transfer to subsequent non-legumes

The interactions between soil organic matter and plant residues and the related mobilisation-immobilisation-turnover may have an essential effect on the availability of N derived from residues (Ndfr) for subsequent crops. Several field and greenhouse studies showed that the direct contribution of legume derived N is relatively small for the first subsequent crop and almost negligible for the second and further subsequent crops. Thus Ladd et al. (1983) found after addition of ^{15}N labelled *Medicago littoralis* residues in a field experiment a N recovery between 20% and 28% in subsequent wheat (*Triticum aestivum*). The second subsequent crop, also wheat, recovered less than 5% of the added legume N. Harris and Hestermann (1990) found N transformations from *Medicago sativa* residues of 17% - 25% in subsequent maize (*Zea maize*) and only 1% in barley (*Hordeum vulgare*) as second subsequent crop on two differing locations. In the first year, on average 46% of the Lucerne N were found in the soil. The greatest amount, 96%, were incorporated in the soil organic matter (SOM) fraction and 18% of it immobilised in the microbial biomass.

Similar results were obtained for grain legumes: In field experiments with pea residues 7% (summer barley) – 15% (winter barley) were recovered in the first year, in the second year only 2% - 4% in summer and winter rape, respectively, and only 1% - 2% in summer and winter wheat in the third year after residue application (Jensen 1994a). Jensen (1994a) found a fast decomposition of pea residues during the first three months and a rapid decline after one year of decomposition. The decrease of residue N availability was explained by a stabilisation in slowly decomposable pools of SOM. Russell and Fillery (1996) found a small recovery of lupin below ground N in subsequent wheat of about 13% and concluded that the main value of lupin stubble N is to replenish the SOM pool.

Crop rotation management

The main focus in crop rotation management research was to reduce N losses by leaching and to develop strategies for a N saving crop rotation management. This research was strongly connected with the aim to increase non legume crop yields by optimising crop rotation positions of legumes and tillage systems. Heß (1989, 1993) and Fassbender (1999) developed strategies to reduce N losses after cropping of glover/grass leys and Justus (1996) for faba bean. The preceding crop values of clover/grass leys with differing cropping strategies (e. g. cutting or mulching, mixed cropping or open sowing) (Kaske et al. 1999; Schmidt et al. 1999; Schmidt and Leithold 2002) or grain legumes (Jensen and Haahr 1989; Eltun and Bjerke 2000; Wichmann et al. 2002) to subsequent crops were investigated. Cropping systems were improved by including clover undersowing to improve N nutrition of non-legumes (Henriksen et al. 2000; Tersbøl and Thorup-Kristensen 2000) or grass / crucifer undersowings in grain legumes or potatoes to reduce N losses (Justus 1996; Haas 2002). Also, classical tools such as the use of green manure catch crops have been improved and adapted to organic farming systems e.g. by integration of legumes in catch crop mixtures (Möller and Reents 1999). New management practices, e.g. strip cropping, wide rows, bi-cropping or direct sowing in plant cover have been examined (Schulz-Marquardt et al. 1994; Bourguignon and Gabucci 2000; Richter and Debruck 2001; Neumann et al. 2002).

These research activities have lead to a significant progress in N management of organic farming systems. But it also shows the limitations and difficulties related to the management of organic N sources in agriculture. The problem of most applied research is a lack of knowledge – the 'black box' – between a measure and its related effect. Major improvements of the system require a better understanding of the processes in the 'black box' as indicated for organic farming systems. From the basis of a fundamental understanding of the system the system can be adequately adapted and improved. Thus, the aim in organic farming research must be a better understanding of processes of the system.

The limitations of recent applied research and the necessity to understand processes can be exemplified with grain legumes.

Grain legumes

Grain legumes are an important legume group providing high quality fodder protein in a concentrated and easily digestible form in mixed farms. In stockless farms grain legumes are an interesting legume as cash crop for fodder and food production.

The integration of grain legumes in crop rotations have some beneficial effects to subsequent crops resulting in higher yields compared to non-legume pre-crops. These effects can be related to several factors as breaking pest and disease cycles, suppression of weeds, soil structural factors and as main factor an improved N supply derived from N inputs through N_2-fixation. However, after harvesting the grain the net N contribution from N_2-fixation is often only small, zero or negative. Thus, the beneficial N effect after grain legumes cannot solely be explained by the net N-Input due to N_2-fixation and was explained by various hypotheses: (i) The N effect is caused by 'N-sparing'. A reduced inorganic soil N demand of legumes compared to cereals leads to a better soil N availability for the subsequent crop. (ii) The grain legume residues have a higher decomposability compared to cereal straw (e.g. narrower C:N ratio) and will thus provide an easier available N source for subsequent crops (Peoples et al. 1995; Chalk 1998).

However, the N deposited by roots in the rhizosphere as low and high molecular weight substances has been only poorly quantified and the importance of this pool for the N-balance and its influence on nitrogen dynamics after harvesting grain legumes is only poorly understood. Jensen (1996) found for pea 7% of total plant N as rhizodeposits in soil at maturity and Sawatsky and Soper (1991) found rhizodeposition amounts between 8.7% and 12.0% of total N in laboratory experiments. Russsell and Fillery (1996), using an *in situ* stem feeding technique, found rhizodeposition rates for blue lupin of about 18%.

After harvest of the grain the rhizodeposition pool might be an important N pool for the nutrition of subsequent non-legumes. This is be supported by the findings of Jensen (1996) who found a high decomposability of the N rhizodeposits of pea. In an incubation experiment 30% of pea rhizodeposits were decomposed after 15 weeks at 20°C. Hence the N rhizodeposition may contribute significantly to the N nutrition of a subsequent non-legume. But the role of N rhizodeposition for the nutrition of subsequent crops was also poorly studied due to experimental difficulties and the effort related to ^{15}N labelling of rhizodeposits in field experiments.

Besides a quantitative effect of significant N input in the system due to N rhizodeposition, roots interact with the soil organic matter (SOM) and the soil microorganisms. These interac-

tions are mainly driven by root rhizodeposition (C and N compounds) (Kuzyakov 2002). Short term effects which major implications for the availability of plant nutrients as mobilisation of nutrients by lowering soil pH or complexation of ions, activation of the microbial biomass in the rhizosphere and related priming effects for SOM C and N were observed (Zagal 1994; Marschner 1995; Jones 1998; Kuzyakov 2002).

Rhizodeposition may also affect the SOM and the related turnover processes in the long term within weeks or months. Soluble root exudates can contribute to a greater aggregate stability (Traoré et al. 2000; Hütsch et al. 2002) or diminish the stability by disruption of soil aggregates in the rhizosphere (Reid and Goss 1981; Helal and Sauerbeck 1989). For positive or negative root effects on aggregate stability differences between plant species were observed (Haynes and Beare 1997).

These effects caused by root rhizodeposition may also affect the turnover kinetics of plant residues incorporated into the soil after plant harvest. Some authors reported direct effects of roots on the turnover of recently added plant residues and accelerating and obstructive effects have been found (Helal and Sauerbeck 1985). Probably there are differences between plant species (Van der Krift et al. 2001).

This might lead to differences in residue N recovery after incorporation of plant residues by contrasting succeeding plant species.

Hence the positive N effect in crop rotations after cropping of grain legumes might be a combination of examined factors such as 'N-sparing' or 'quality of residues' and factors such as N rhizodeposition, effects of root-soil interactions of the legumes on the subsequent turnover of its residues or root effects of the subsequent crop itself.

2.   Objectives

The general objective of this thesis was to investigate the root and rhizodeposition effects on the turnover of grain legume residues in soil and the related microbial process.

Specific aims were,

(i)   to examine the suitability of the *in situ* ^{15}N labelling cotton wick method in determining N rhizodeposition as well as the residue turnover for three grain legumes - faba bean, pea and white lupin,

(ii)   to quantify N rhizodeposition, N uptake and N distribution between the plant parts of the grain legumes and the N_2-fixation,

(iii)   to investigate the fate of the N derived from rhizodeposition in different soil pools,

(iv)   to determine the turnover of the N rhizodeposition of the grain legumes,

(v)   to determine the effects of rhizodeposition on the subsequent turnover of its crop residues,

(vi)   to determine the potentials of the residual N contribution of faba bean, pea and white lupin to the N supply of succeeding wheat and rape including the N-rhizodeposition of the legumes,

(vii)   to investigate possible interactions between the residue quality of the grain legume species, and

(viii)   to examine the effects of two contrasting subsequent crops – wheat and oilseed rape – on the residue N recovery and the soil microbial biomass.

The aims (i) – (iv) are dealt with in chapter 4, (iv) – (v) in chapter 5 and (vi) – (viii) in chapter 6. A synthesis of the results is discussed in chapter 7.

3. Methodology

The experimental concept was designed as an integrated experiment consisting of three parts (Fig. 3.1) in a combination of greenhouse pot and laboratory experiments. As basic experiment faba bean, pea, and white lupin were grown in pots and *in situ* ^{15}N labelled (experiment I). Based on this experiment, using the same soil and plant matter, the residual N transfer to subsequent non-legumes (experiment III) and the turnover of rhizodeposition N and effects of rhizodeposition on turnover of crop residues (experiment II) were investigated. The integrated concept enables a comparison of the results and their integrated evaluation.
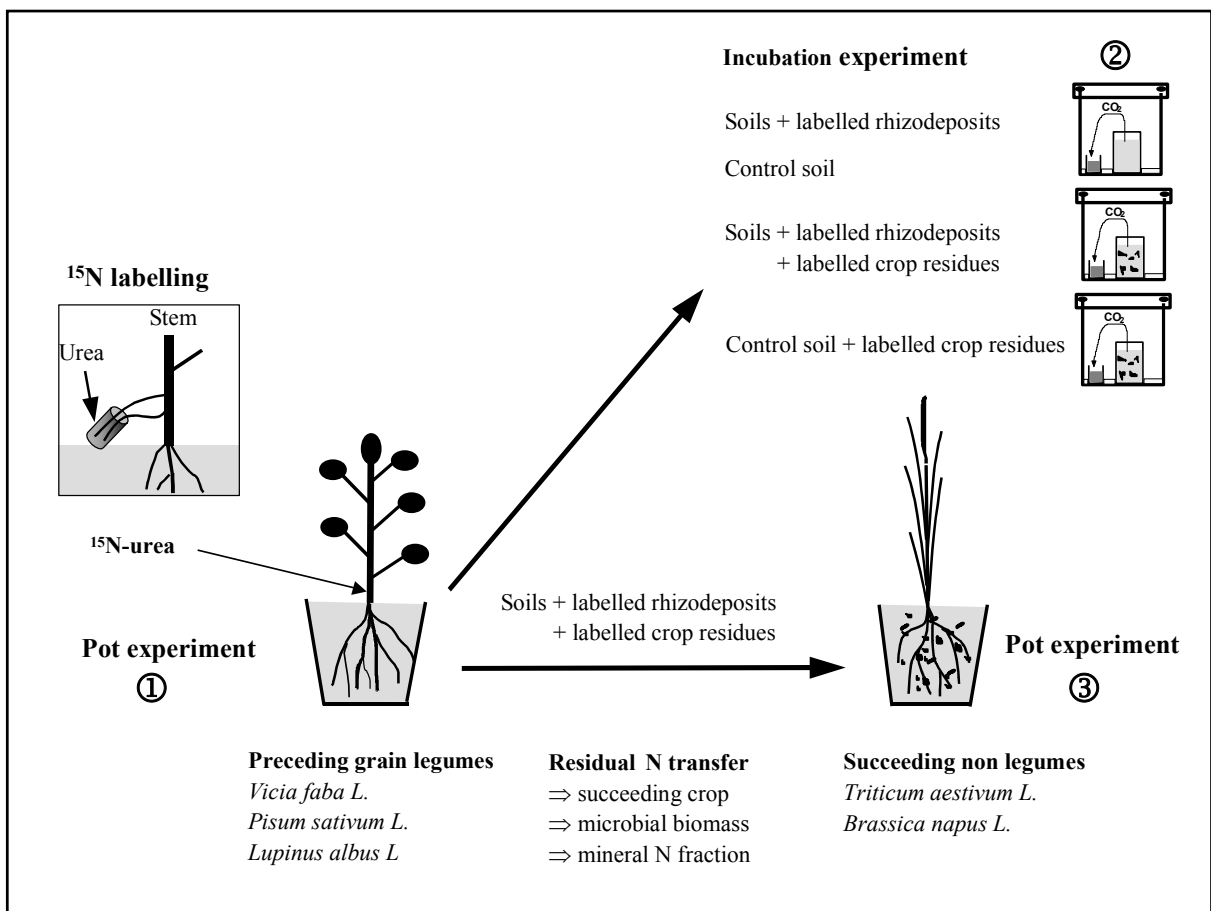

Fig. 3.1. Integrated experimental concept as combined greenhouse pot experiments (I+III) and laboratory incubation experiment (II). Experiment I: Determining N rhizodeposition; Experiment II: Effect of rhizodeposition on crop residue turnover; Experiment III: Transfer of crop residue and rhizodeposition N in subsequent non-legumes.

3.1. Soil

The criterion for the selection of the soil used in the experiment was to find a soil which has a distinct response and to added easily available substrates.

Based on the results of a screening of 12 soils in an incubation experiment examining the substrate use of added Glucose and KNO_3 (results not shown) the following selection criteria were used:

- middle to small N mineralisation potential (relative to investigated soils)
- small to middle N immobilisation capacity
- high N dynamic
- high microbial reactivity to applied substrates

Additional criteria have been

- pH (6.0 – 6.8) and soil texture suitable for the cropping of faba bean, pea and white lupin
- a soil which has been under organic management for more than 7 years (period of a typical crop rotation)

Detailed characteristics of the selected soil are given in chapter 4.2, 5.2 and 6.2.

3.2. Grain legumes

As grain legumes the three most important species for cropping under temperate climate conditions in North-western Europe were used. The selection of the varieties was based on the recommendations for use in organic farming in Germany.

The following grain legumes and varieties were used in the experiment:

Faba bean	*Vicia faba* L.	cv. Scirocco
Pea	*Pisum sativum* L.	cv. Duel
White Lupin	*Lupinus albus* L.	cv. Amiga

3.3 Experiments

Experiment I

The aim of the experiment was to determine the N rhizodeposition and fate of N rhizodeposition in soil of grain legumes using a ^{15}N stem labelling technique according to No. (i) – (iii), chapter 2.

The Experiment was established as pot experiment (12 replicates) in a plastic sheet covered vegetation hall. The legumes were *in situ* pulse labelled with a ^{15}N urea solution using a cotton wick technique which enables a ^{15}N label uptake through the xylem of the plants (Fig. 3.1).

At maturity the plants were harvested and a sub-sample of 20% of the soil was taken from each replicate. All visible roots and root fragments were removed from the soil by hand sampling and the remaining plant derived N in the soil was defined as N rhizodeposition.

The homogeneity of the ^{15}N distribution in differing plant parts (grain, stem, leaves, recovered roots) was determined. In the soil, the amount of not recovered micro roots and the fate of rhizodeposition N in the mineral N (N_{min}) pool and microbial N (N_{mic}) pool was determined.

The remaining undisturbed soil monoliths in the pots were stored at 4°C in the dark until initiation of experiment II. The remaining "root free" soil of the sub-samples was frozen at -22°C and stored until use for experiment III.

Details of the methodology and the determination of biological and chemical parameters are given in chapter 4.2.

Experiment II

The aim of the experiment was to determine the turnover of the N rhizodeposition of the grain legumes and to determine the effects of rhizodeposition on the subsequent turnover of its crop residues according to No. (iv) – (v), chapter 2.

The experiment was established as an incubation experiment. The soils with and without legume crop residue addition were incubated during 168 days at 15°C and 50% water holding capacity (WHC).

The experiment had two main treatments (Fig. 3.1):

Soils without grain legume crop residue addition

- *Soils + labelled rhizodeposits*

- Control soil

Soils with grain legume crop residue addition

- Soils + labelled rhizodeposits + labelled crop residues

- Control soil + labelled crop residues

During the incubation period the C and N mineralisation and the microbial C and N contents were determined. The proportion of N derived from crop residues and rhizodeposits in the respective pools were determined by ^{15}N analyses.

Details of the methodology and the determination of biological and chemical parameters are given in chapter 5.2.

Experiment III

The aim of the experiment was to determine the potentials of the residual N contribution (crop residues + rhizodeposits) of the grain legumes to the N supply of succeeding wheat and rape and to examine the effects of the two contrasting subsequent crops on the residue N recovery and the soil microbial biomass according to No. (vi) – (viii), chapter 2.

The experiment was established as a greenhouse pot experiment (5 replicates). The replicate undisturbed soil monoliths of experiment I were merged and mixed with the respective legume crop residues. As subsequent crops summer wheat (*Triticum aestivum* L.) and summer oilseed rape (*Brassica napus* L.) were sown on each of the legume treatments (Fig. 3.1). The wheat was harvested at flowering and at maturity and the oilseed rape at maturity only.

The residual N recovery in the subsequent crops, the microbial biomass N and the mineral N pool was determined by ^{15}N analyses.

Details of the methodology and the determination of biological and chemical parameters are given in chapter 6.2.

4.   Estimating N rhizodeposition of grain legumes using a ^{15}N *in situ* stem labelling method

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Abstract

Grain legumes in crop rotations cause significant increases in yield for succeeding non-legumes, which cannot be explained simply by the small effect that legumes have on the soil nitrogen balance, as found in the analysis of N in crop residues. Besides known positive non-N-effects, other effects, mainly rhizodeposition and its contribution to the N balance and nitrogen dynamics after harvesting the grain, are poorly understood. In this study, N rhizodeposition, defined as root-derived N in the soil after removal of visible roots, was measured in faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.) and white lupin (*Lupinus albus* L.). In a pot experiment the legumes were pulse labelled *in situ* with ^{15}N urea using a cotton wick method. About 84% of the applied ^{15}N was recovered for the three legume species at maturity. The ^{15}N was comparatively uniformly distributed among plant parts. The N rhizodeposition constituted 13% of total plant N for faba bean and pea and 16% for white lupin at maturity, about 80% of below ground plant N, respectively. Some 7% (lupin) - 31% (pea) of the total N rhizodeposits were recovered as micro roots by wet sieving (200 μm) the soil after all visible roots had been removed. Only 14% – 18% of the rhizodeposition N was found in the microbial biomass and a very small amount of 3% - 7% was found in the mineral N fraction. 48% (pea) – 72% (lupin) of N rhizodeposits could not be recovered in the mentioned pools and a major part of the unrecovered N was probably immobilised in microbial residues. The results of this study clearly indicate that N rhizodeposition from grain legumes represent a significant pool for N balance and N dynamics in crop rotations.

Keywords: Rhizodeposition, Nitrogen*, ^{15}N, Vicia faba, Pisum sativum, Lupinus albus*

4.1. Introduction

In crop rotations, grain legumes cause significant, positive yield effects on subsequent non-legumes when compared with rotations with non-legumes. In addition to its beneficial factors, such as improving soil structure, breaking pest and disease cycles and the phytotoxic and allelopathic effects of crop residues, nitrogen is a key factor in the positive response of cereals following legumes (Chalk 1998). Generally in sustainable and organic farming systems, biological N_2-fixation by legumes is the main source of nitrogen for the crop rotation. However, the calculated N contribution of grain legumes derived from biological N_2-fixation after harvest and removal of the grain is often small or negative (Peoples et al. 1995; Chalk 1998) and it alone cannot explain the yield effect observed in the crop rotations. A reduced inorganic N uptake of the legumes from soil in comparison with cereals termed "N-sparing" or a smaller N immobilisation of grain legume residues as compared with cereals may also contribute to the crop rotation effect (Chalk 1998). The N released from roots (N rhizodeposition) as low molecular weight substances, such as soluble root exudates, amino acids, hormones and enzymes, is poorly quantified, as are high molecular weight substances, such as mucilage, sloughed off cells and tissue, cell lysates, root debris and decomposed root materials (Lynch and Whipps 1990; Marschner 1995). The importance of this pool for the N-balance and its influence on nitrogen dynamics after harvesting grain legumes is only poorly understood.

Most of the experiments on N rhizodeposition in soil substrate have been carried out under controlled laboratory or greenhouse conditions. Different ^{15}N labelling techniques were used: $^{15}NH_3$ fumigation (Janzen and Bruinsma 1989), the split root technique (Jensen 1996b; Reining et al. 1995; Toussaint et al. 1995), ^{15}N leaf feeding (McNeill et al. 1997; McNeill et al. 1998) and ^{15}N pre-labelling (Rroco and Mengel 2000).

Only a few experiments have been carried out under field conditions. This may be due to the methodological problems and the amount of effort involved in ^{15}N *in situ* labelling. Høgh-Jensen and Schjoerring (2001) determined the N rhizodeposition of clover grass leys in the field over two years using ^{15}N urea leaf labelling.

Russell and Fillery (1996b) developed an *in situ* labelling method for lupin in which a ^{15}N urea solution is taken up by a cotton wick which is passed through the stem of the plants. This methodology is relatively simple and does not have the various disadvantages of some *in situ* labelling methods; e.g. leaf spraying / painting or solution uptake by cut leaves. The plants

tolerate a much higher urea concentration up to 4% by stem feeding compared to 0.5% tolerated by foliar feeding. The risk of run off with ^{15}N labelled solutions is small compared to spraying techniques as used by Zebarth et al. (1991). The results of Russel and Fillery (1996b) also show high recovery rates of applied ^{15}N and a comparatively uniform distribution of ^{15}N in various plant parts. A homogeneous ^{15}N distribution is necessary if the turnover of plant residues with different degrees of decomposability, for instance stems and leaves, is to be determined (Wagger et al. 1985; Hood et al. 1999).

The objectives of this study were (i) to examine the suitability of the *in situ* ^{15}N labelling cotton wick method in determining N rhizodeposition as well as the residue turnover of faba bean, pea and white lupin, (ii) to quantify N rhizodeposition, N uptake and N distribution between the plant parts of the grain legumes and (iii) to investigate the fate of the N derived from rhizodeposition in different soil pools.

4.2. Materials and methods

Soil

Soil samples from a site in north-west Germany were taken from the top 0 – 20 cm of an Eutric Cambisol. The field had been cultivated using organic farming management for 10 years. The soil was characterized as a sandy loam with 17.3% clay, 30.1% silt, 52.6% sand, pH (0.01 M CaCl$_2$) of 6.0 and 1.58% total C, 0.15% total N, 140 mg P kg^{-1} (Calcium-lactate extract), 208 mg K kg^{-1} (Calcium-lactate extract), and 100 mg Mg kg^{-1} (Calcium-lactate extract). The water holding capacity (WHC) (10 mm sieved soil) was 309 g H$_2$O kg^{-1} dry soil. After sampling, the soil was sieved to pass a 10 mm sieve and stored moist in a cool (6°C) and dark place until initiation of the experiment.

Plants and experimental design

In 1999 a pot experiment with 12 replicates designed in a Latin rectangle was established in a vegetation hall covered with greenhouse foil. The soil (11 kg dry weight pot^{-1}) was filled in 8.5 l PVC pots (height 31.5 cm, inner diameter 19 cm) and mechanically compressed to a density of 1.3 kg l^{-1}. Seeds of faba bean (*Vicia faba* L., cv. Scirocco), pea (*Pisum sativum* L., cv. Duel) and white lupin (*Lupinus albus* L., cv. Amiga) were inoculated with legume specific *Rhizobium* inoculants (Radicin, Radicin Institut, Iserlohn, Germany) according to the manufacturer and sown on the 4th of May (pea, white lupin: 5 plants per pot^{-1}; faba bean: 4 plants

per pot[-1]). The pots were watered daily with deionised water and kept at a WHC between 40% - 80% by weighing the pots two times a week.

The biological nitrogen fixation was estimated using an extended difference method, which includes N amounts of shed leaves, roots and the mineral N content of the soil at maturity in the calculation (Stülpnagel 1982). Four additional replicate pots were included in the experiment using oats (*Avena sativum* L., cv. Flämingslord) as a reference plant.

The mean temperatures during the growing period were 16.9°C for pea, 17.4°C for faba bean and 17.0°C for white lupin.

[15]N-labelling

To determine the N rhizodeposition and to separate legume derived N from soil N, the legumes were labelled *in situ* with a ^{15}N urea solution (99 atom% [15]N). A cotton wick was passed through a hole in the stem. The holes were drilled with a 0.5 mm drill 3 cm above the soil surface. To prevent solution losses, the wick was passed through a silicon tube and sealed with kneading mass against the stem. The wicks were put through two holes in the cover of a 2 ml vial containing the urea solution (Russell and Fillery, 1996a). The plant uptake of urea was driven by the transpiration stream.

The legumes were labelled 4 times in fortnightly intervals starting at 28 days after sowing (growth stage: 6 leaves unfolded). Due to fast maturation, the 4th labelling interval of the pea was reduced from 14 to 7 days. The amount of ^{15}N urea applied at each pulse was calculated to keep an average ^{15}N content of 2.5% [15]N excess of plant N during the growing period. Depending on the calculated demand, the amount and concentration of the urea solution varied between 0.24 – 0.69 ml and 1 – 3% urea (w/v), respectively. To ensure a complete uptake of the urea solution, 0.3 ml deionised water was added to the vials for cleaning the wick after the plants had emptied the vials. For the calculations, the N uptake curves of the three legumes were determined in a preliminary pot experiment.

Sampling and analytical methods

During plant growth, any shed leaves were collected with a mesh that was fixed as a funnel around the pots and sampled 3 – 4 times a week. At maturity (pea: 27th July, faba bean: 18th August and lupin: 6th September 1999), the above-ground plant parts were harvested and separated into grain, leaves and stem.

A sub-sample of 20% of the soil was taken from each pot throughout the entire profile. All visible roots and root fragments were collected by hand. The roots with adhering soil were shaken in a polyethylene-bottle with 50 ml deionised water for 5 min and washed with an additional 200 ml over a 2 mm sieve. The soil water suspension was filtered and the remaining soil was mixed with the "root free" bulk soil. The plant-derived N remaining in the soil is defined as the N rhizodeposition. Additionally, 100 g of the "root free" soil was wet sieved using a 200 μm sieve to determine the amount of remaining fine roots in the soil. The collected micro root material was burned to ash at 550°C to determine the ash-free dry matter and corrected with the ash content of the macro roots.

All plant and soil samples were dried at 60°C for 72 h and ground to a fine powder using a centrifuge mill and a ball mill and analysed for total N and ^{15}N. To determine the mineral N content, 60 g of fresh soil was extracted immediately after sampling with 250 ml 2 M KCL for 30 min. The NO_3^- and NH_4^+ contents were analysed using a continuous flow analyser (E-volution II, Alliance Instruments, France). The microbial biomass N was determined using the chloroform fumigation extraction method (Brookes et al. 1985). Living roots were separated from the soil by a pre-extraction step (Müller et al. 1992; Olfs and Scherer 1996). Two portions of 60 g fresh soil were extracted with 200 ml 0.05 M K_2SO_4 for 20 min, put on a 1 mm sieve and additionally washed with 150 ml 0.05 M K_2SO_4. The soil water suspension was collected in a beaker and left for 30 minutes to let the soil settle. The roots which were not kept back by the sieve were sucked with a tube connected with a vacuum pump. Subsequently the soil water suspension was filtered (Schleicher & Schüll 595 ½ filter paper). One sample (soil + filter) was immediately extracted with 200 ml 0.5 M K_2SO_4 for 30 min. The second sample was fumigated with 25 ml chloroform in a desiccator for 24 h at 25°C and later extracted with 0.5 M K_2SO_4. The total N content of the extracts was determined with a TOC / TN_b analyser (Dimatoc 100, Dimantec, Germany). Soil microbial biomass N was estimated from the relationship: biomass $N = E_N / k_{EN}$, where E_N is (total N extracted from fumigated soil) minus (total N extracted from unfumigated soil) and $k_{EN} = 0.54$ (Jörgensen and Müller 1996).

The ^{15}N content of the biomass and mineral-N extracts was determined using a modified diffusion technique by Goerges and Dittert (1998). Before diffusion, the N in the biomass extracts was oxidised to NO_3^- using $K_2S_2O_8$ and autoclaving (Cabrera and Beare 1993). For diffusion, the extracts were put into polyethylene bottles. 450 mg of Devarda's alloy (Merck) and 3 ml 6.25 M NaOH were added. Immediately afterwards, the opening was covered with a

double layer of PTFE tape (24 mm x 0.1 mm). A piece of glass fibre filter (diameter 5 mm) was put on the PTFE cover and 10 µl of 2.5 M KHSO$_4$ added to the filter. The filter was covered with an additional layer of PTFE tape, the bottle was closed with the screw cap immediately and shaken for 3 days on a horizontal shaker.

The plant and soil samples and the diffusion filters were analysed for N and ^{15}N content with an emission spectrometer (NOI-6PC, Fischer Analysen Instrumente, Germany; ^{15}N abundance > 1.5 atom% ^{15}N) and an isotope ratio mass spectrometer (delta E, Finnigan MAT, Germany; ^{15}N abundance < 1.5 atom% ^{15}N) both coupled with a Carlo Erba NA 1500 C / N analyser.

Calculations and statistics

The percentage of total N, mineral N and microbial N derived from rhizodeposition (NdfR) were calculated using the equation of Janzen and Bruinsma (1989):

$$\%\text{NdfR} = \frac{atom\%\ ^{15}N\ excess\ soil}{atom\%\ ^{15}N\ excess\ root} \times 100$$

The amount (mg) of total N derived from rhizodeposition was obtained by multiplying the N amount in this pool with the respective % NdfR value.

The ^{15}N excess enrichment in the microbial biomass (MB) was calculated as following:

$$atom\ \%\ ^{15}\text{N excess MB} = \frac{(N_{fum\ soil} \times \%^{15}\text{N excess}_{fum\ soil} - N_{unfum\ soil} \times \%^{15}\text{N excess}_{unfum\ soil})}{(N_{fum\ soil} - N_{unfum\ soil})} \times 100$$

fum soil = fumigated soil
unfum soil = unfumigated soil

The atom% ^{15}N excess values were obtained by correcting the ^{15}N enrichments with the background values of the soil and roots grown without labelling (faba bean: 0.3681, pea: 0.3676, white lupin: 0.3679 atom% ^{15}N).

The partitioning of nitrogen derived from rhizodeposition in the soil was calculated using the absolute ^{15}N label amounts in the soil, micro roots, microbial biomass and mineral N fraction. The ^{15}N amount of the total soil, microbial biomass and mineral N was calculated by multiplying its total N with the respective ^{15}N abundance. The ^{15}N amount in the micro roots was calculated by multiplying its dry weight with the ^{15}N abundance of the macro roots.

The following general assumptions were made in the calculations: (1) The N deposited has the same ^{15}N enrichment as the roots; (2) the ^{15}N content of the roots is constant over the growth period of the plants; (3) the micro roots recovered by wet sieving have the same ^{15}N abundance as the macro roots.

The GLM procedure of the SPSS 10.0 statistics package (SPSS GmbH, Germany) was used to carry out the statistical analysis.

Table 1. ^{15}N enrichment, recovery of ^{15}N label, distribution of recovered ^{15}N and total N of various plant parts at maturity after ^{15}N urea pulse-labelling

	atom % ^{15}Nexcess	recovery of ^{15}N label (%)	distribution of recovered ^{15}N (%)	distribution of total N (%)
Faba bean				
Grain	1.4 ±0.2[*]	51.3 ±3.1	60.6 ±3.2	64.0 ±3.4
Stem	2.0 ±0.2	10.1 ±1.1	11.9 ±1.3	9.2 ±1.4
Leaves	1.7 ±0.2	11.5 ±1.1	13.5 ±1.1	12.2 ±0.8
BGP-N	1.4 ±0.2	11.9 ±2.4	14.0 ±2.8	14.6 ±2.9
total		84.8 ±2.7	100	100
Pea				
Grain	2.4 ±0.3	62.8 ±9.1	75.3 ±6.1	71.6 ±8.4
Stem	2.6 ±0.3	7.0 ±1.7	8.5 ±2.2	7.5±2.0
Leaves	2.8 ±0.4	5.9 ±0.6	7.2 ±1.1	5.7 ±0.9
BGP-N	1.3 ±0.2	7.5 ±4.4	9.0 ±5.3	15.2±8.0
total		83.2 ±7.8	100	100
White lupin				
Grain	1.5 ±0.2	52.6 ±5.4	62.2 ±3.8	62.4 ±4.0
Stem	2.0 ±0.2	9.6 ±1.7	11.4 ±2.0	8.7 ±1.6
Leaves	1.6 ±0.2	10.3 ±1.3	12.2 ±1.6	11.7 ±1.7
BGP-N	1.2 ±0.2	12.0 ±2.6	14.2 ±3.0	17.2 ±3.3
total		84.5 ±5.6	100	100

[*] Values show means (± SD; n = 12).

BGP-N = below ground plant-N (roots + rhizodeposition)

4.3. Results

15N recovery and distribution

The three grain legumes showed a very similar recovery of the ^{15}N label, (about 84%) (Table 1). Faba bean and lupin had the highest ^{15}N enrichment in stems followed by the leaves, grain and below ground plant N (BGP-N = roots + rhizodeposition) (Table 1). Pea leaves, stem and grain had high and relatively homogeneous enrichments above 2.4 atom % [15]N excess, whereas the BGP-N enrichment was only half of these values.

N uptake and N rhizodeposition

Faba bean acquired the greatest amount of N followed by white lupin and pea (Table 2). The majority of the assimilated N was recovered in grains: Faba bean 62%; pea 72% and white lupin 61% (Fig. 1). The residue-N (stems + leaves + roots + rhizodeposition) in faba bean was only 38% of total plant-N, in pea 29% and in white lupin 39%. The quantity of the N derived from rhizodeposition (NdfR) was 0.67 g pot^{-1} for faba bean, 0.23 g for pea and 0.57 g for white lupin. Related to the total N, the percentage of NdfR was 13.4% for faba bean, 12.6% for

Table 2. Total plant-N, grain-N, residue-N (= stem + leaves + roots + rhizodeposition), below ground plant-N (BGP-N = roots + rhizodeposition), N derived from rhizodeposition (NdfR = rhizodeposition only, without roots recovered by hand collecting) and N derived from air (Ndfa) of faba bean, pea and white lupin at maturity

	Faba bean	Pea	White lupin
Total plant-N (g pot^{-1})	$5.0^a \pm 0.50^*$	$1.8^b \pm 0.29$	$3.6^c \pm 0.44$
Grain –N (g pot^{-1})	$3.1^a \pm 0.39$	$1.3^b \pm 0.28$	$2.2^c \pm 0.36$
Residual-N (g pot^{-1})	$1.9^a \pm 0.25$	$0.5^b \pm 0.06$	$1.4^c \pm 0.17$
BGP-N (g pot^{-1})	$0.86^a \pm 0.19$	$0.28^b \pm 0.05$	$0.67^c \pm 0.14$
NdfR (g pot^{-1})	$0.67^a \pm 0.13$	$0.23^b \pm 0.04$	$0.57^a \pm 0.13$
Ndfa (%)	$93^a \pm 0.7$	$80^b \pm 5.1$	$89^a \pm 2.0$

[*] Values with different letters show means (± SD; n = 12) with significant differences (LSD, P < 0.05)

pea and 15.7% for white lupin (Fig. 1). The proportion of symbiotically fixed N was high in the three species. The percentage of nitrogen derived from air (Ndfa) was 93% for faba bean, 89% for lupin and 80% for pea (Table 2).

Fig. 1. Relative distribution of nitrogen in grain, stems + leaves, roots and rhizodeposition
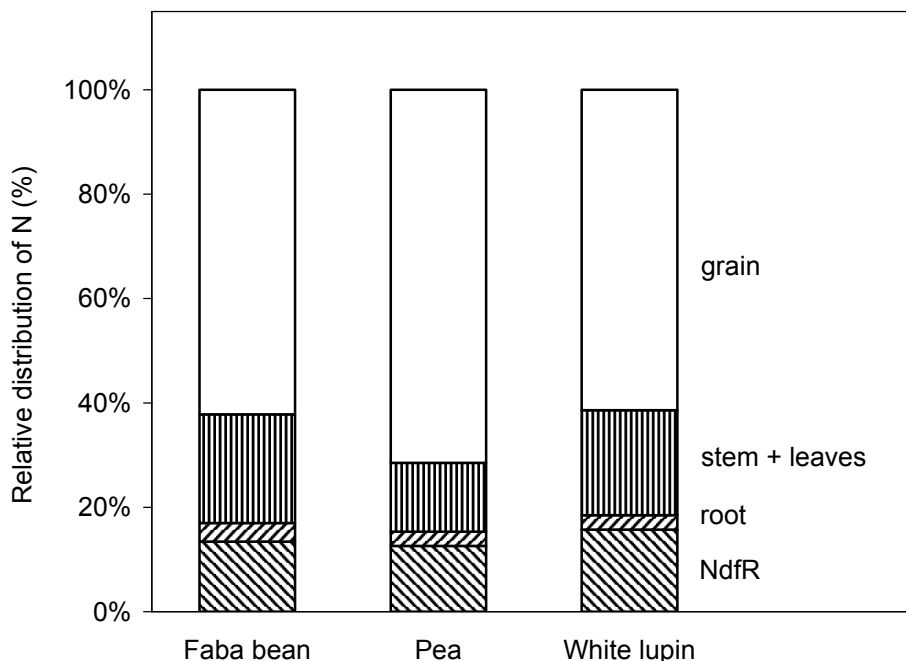
Partition of N rhizodeposition

The amount of micro root-derived ^{15}N recovered by wet sieving after removing all visible roots from the soil was 114 μg pot[-1] for faba bean, 70 μg for pea and 41 μg for white lupin, corresponding to 16%, 31% and 7% of total NdfR (Table 4). A relatively small amount of NdfR was found to be immobilised in the microbial biomass at maturity. In the faba bean soil, 22% of the microbial N was derived from rhizodeposition, in the pea soil only 8% and in the lupin soil 20% (Table 3). Thus, the amount of microbial ^{15}N derived from rhizodeposition was 110 μg pot[-1] for faba bean, 43 μg for pea and 89 μg for white lupin, corresponding to 14%, 18% and 14% of total NdfR, respectively (Table 4). The mineral N (N_{min}) content in the soil at maturity was very low (faba bean: 5 μg N_{min} g[-1] dry soil; pea: 2 μg; white lupin: 8 μg) but the percentage of mineral-NdfR constituted 43% for faba bean, 25% for pea and 41% for white lupin (Table 3). Consequently, the amount of mineral N derived from rhizodeposition was small: 42 μg pot[-1] for faba bean, 8 μg for pea and 46 μg for white lupin, corresponding to 5%, 3% and 7% total NdfR, respectively. Summing the three pools – micro roots, microbial biomass and mineral N – only 28% to 52% of the total NdfR could be recovered (Table 4).

Table 3. Microbial N and mineral N in the soil at maturity and percentage of N derived from rhizodeposition (NdfR) in these pools

	Faba bean	Pea	White lupin
Microbial N (μg g^{-1} dry soil)	32 $\pm$3.2^{a*}	37 $\pm$3.0^{b}	32 $\pm$1.3^{a}
Microbial NdfR (%)	22 $\pm$6^{a}	8 $\pm$2^{b}	20 $\pm$7^{a}
Mineral N (μg g^{-1} dry soil)	5 $\pm$3.0^{a}	2 $\pm$0.9^{b}	8 $\pm$3.9^{c}
Mineral NdfR (%)	43 $\pm$17^{a}	25 $\pm$6^{b}	41 $\pm$5^{a}

[*] Values with different letters show means ($\pm$ SD; n = 12) with significant differences (LSD, $P < 0.05$)

Table 4. Amount of ^{15}N label derived from rhizodeposition of faba bean, pea and white lupin in different soil pools at maturity

	Faba bean		Pea		White lupin	
	μg ^{15}N label pot^{-1}	recovery (%)	μg ^{15}N label pot^{-1}	recovery (%)	μg ^{15}N label pot^{-1}	recovery (%)
Total ^{15}NdfR	758 $\pm$139*	_100_	240 $\pm$55	_100_	629 $\pm$155	_100_
Micro root-^{15}NdfR	114 $\pm$40	_16^{a}_	70 $\pm$22	_31^{b}_	41 $\pm$15	_7^{c}_
Microbial-^{15}NdfR	110 $\pm$30	_14^{a}_	43 $\pm$17	_18^{b}_	89 $\pm$30	_14^{a}_
Mineral-^{15}NdfR	42 $\pm$31	_5^{ab}_	8 $\pm$4	_3^{a}_	46 $\pm$27	_7^{b}_
^{15}NdfR not recovered	493 $\pm$125	_65^{a}_	119 $\pm$44	_48^{b}_	454 $\pm$114	_72^{a}_

[*] Values show means ($\pm$ SD; n = 12). Figures in lines with different letters are significantly different (LSD, $P < 0.05$)

4.4. Discussion

^{15}N recovery and distribution

Russel and Fillery (1996b) using the cotton wick method for lupin (*Lupinus angustifolius* L.) found recovery rates between 81% and 102% in several pot experiments. In our study, the cotton wick method resulted in similar recoveries of about 84% of the ^{15}N label in the three grain legume species (Table 1). The ^{15}N not recovered in the soil plant system can be apparent

losses due to experimental errors in the mass balance between the calculated ^{15}N added through labelling and the various recovery and analysis of the different plant parts. Real losses caused by denitrification of plant N deposited into the soil were expected to be small because the water content in the pots was kept between 40 - 80% of WHC. NH_3 losses should also be less than 1% – 4% of total plant N (Schjoerring and Mattsson 2001). This is supported by the results of Rroco and Mengel (2000) who found ^{15}N losses between 3.6% and 4.7% in a pot experiment with wheat using a ^{15}N pre-labelling method. But losses could probably occur in the non-sterile wick system. After the first application, the ^{15}N urea solution was taken up within 1 – 3 days, but after the 2nd, 3rd and 4th applications, the uptake rates decreased dramatically and the solution needed more than 10 days for uptake. In this period, NH_3 losses caused by urea from the wick system are probable and lead to apparent low recovery rates. This hypothesis is supported by the results Russel and Fillery (1996b) obtained in a 14-day labelling of lupin. The recovery rate decreased from 91% in 12-week old plants to 78% in 20-week old plants.

To investigate the N turnover of plant residues with different microbial decomposability, such as stems, leaves, roots or constituent molecules within those parts (e.g. structural and non-structural components)(Vanlauwe et al. 1994), a ^{15}N distribution is required in the plants that is homogeneous (Wagger et al. 1985; Hood et al. 1999). The results of faba bean and white lupin showed a similar ^{15}N distribution pattern between the analysed plant parts compared to pea. The largest enrichment of the applied ^{15}N was recovered in the stem tissue and the lowest enrichment in the BGP-N fraction (Table 1). Russel and Fillery found an increasing ^{15}N enrichment in BGP-N with successive labelling. This finding was confirmed by the results of our study in which four labellings in fortnightly intervals were used. The comparatively uniform ^{15}N distribution may be supported by the concept of early labelling (6 leaves unfolded) and a pulse of ^{15}N label equivalent to the N uptake of the plant. However a very early labelling of young plant parts is impossible and this might contribute to an inhomogeneity between plant cell constituents. Compared with other methods which use shoot labelling techniques *in situ* (Zebarth et al. 1991; McNeill et al. 1998; Høgh-Jensen and Schjoerring 2001) or in the laboratory (Janzen and Bruinsma 1989), stem feeding through a cotton wick enables a more uniform ^{15}N enrichment and a high recovery in the plants.

N rhizodeposition and N uptake

Relative to total plant N, the NdfR amounted to 12.6% (pea) - 15.7% (lupin), but was not significantly different among the legume species (Tanle 2). This is in agreement with the results of Russel and Fillery (1996a) for blue lupin (*Lupinus angustifolius* L.) of 18.5% of total plant N using the same methodology. Jensen (1996b) found 7% NdfR at maturity relative to total plant N and Sawatsky and Soper (1991) found NdfR rates between 8.7% and 12.0% using the split root technique. The results are of the order of the N rhizodeposition found in other annual species: 10 – 23% were found in wheat at maturity (Janzen and Bruinsma 1989; Janzen 1990; Janzen and Bruinsma 1993; Rroco and Mengel 2000), 20% in barley at maturity (Jensen 1996b), 10% for subterranean clover and 20% for serradella (McNeill et al. 1997). However, over two growing seasons of clover grass leys, the rhizodeposition amounted to 36% - 48% relative to total plant N (Høgh-Jensen and Schjoerring 2001).

Typically for grain legumes about two thirds of the total N was grain N and will be harvested (Haynes et al. 1993; Schmidtke 1996). Thus a relatively small amount of the remaining residual N (stems, leaves, roots and rhizodeposits) contributed to the nutrition of a subsequent crop. Relative to the residual N, the contribution of the N rhizodeposition increased and constituted between 35% and 44%. N rhizodeposition constituted 79% - 85% of the belowground N at maturity (Fig. 1). But, relative to BGP-N, Jensen (1996b) and Sawatsky and Soper (1991) found that it constituted 47% NdfR. This may be due to the different experimental conditions in which the split root technique was used. With the same methodology and a similar experiment, Russel and Fillery (1996a) got a result of 65% for lupin.

However because of the specificity of the pot experiment with a relative small soil volume per plant the root density will be greater and the shoot-root ratio will be shifted in favour of shoot compared to field conditions. The percentage of N in the roots constituted between 2.7% (pea) and 3.6% (faba bean) related to total N (Fig. 1) and was only half of the values found under field conditions (Haynes et al. 1993). Thus under filed conditions the NdfR might be greater compared to the findings in our pot experiment.

Partition of N rhizodeposition

In this study, N rhizodeposition was defined as the total root derived N compounds left in the soil after removal of all visible roots and root fragments at maturity. The nature of these compounds released by the roots can be characterised as low molecular weight exudates (Brophy

and Heichel 1989; Wacquant et al. 1989), and as high molecular weight substances (Lynch and Whipps 1990; Marschner 1995). Additionally, about 20% - 50% of the recoverable root mass (by wet sieving) decomposes in the soil from flowering / pod filling until maturity (own results, data not shown) (Sawatsky and Soper 1991; Jensen 1996b) and are included in the rhizodeposition pool. A certain proportion of these N compounds mineralise and may be reabsorbed by the plant or lost through denitrification. It is difficult to estimate the reabsorption of N. In an experiment with an associated barley plant, it was found that the barley shoot contained 0.6 to 5.3 mg N plant^{-1} derived from pea, depending on the length of the growth period (Jensen 1996a).

A significant amount of "intact" micro roots was not removed from the soil by the hand sampling procedure and amounts differed among legume species. In the lupin soil, only 7% of total NdfR was recovered as micro roots, in faba bean 16% and in pea 31% (Table 4). The differences in the micro root content may be due to the different morphology of the roots. Fine pea roots in particular were more difficult to collect from the soil. The rapid maturation of pea (harvest 27th July) and the later maturation of faba bean and lupin (harvest on 18th Aug. and 6th Sept.) would have led to an increased turnover of root compounds in faba bean and lupin compared to pea. Due to its chemical characteristics, the rhizodeposits may be mineralised faster in the rhizosphere and reabsorbed by the plant or immobilised by the microbial biomass. The percentage of N_{min} derived from rhizodeposition – 25, 41 and 43% for pea, lupin and faba bean, respectively – indicates a mineralization of rhizodeposits (Table 3). Nevertheless, the N_{min} pool was extremely small at maturity (Table 3). Substantial N_{min} uptake by the dying plant roots in the late generative growing phase is unlikely (Haynes et al. 1993). Thus, the main part of N from rhizodeposits and decomposed roots may be immobilised by the microbial biomass or incorporated in the soil organic matter.

Experiments with wheat plants showed that most of the amino acids present in the rhizosphere were directly immobilised by the microbial population. Only a small amount of 6% of added amino acids was captured by the plants (Owen and Jones 2001). The increase in the net N rhizodeposition during plant development (Sawatsky and Soper 1991; Jensen 1996b; Rroco and Mengel 2000) combined with low mineral N contents will support the theory of an immobilisation of rhizodeposits in the generative growth phase. However, we found a relatively small percentage of NdfR in the microbial biomass of 8, 20 and 22% for pea, lupin and faba bean, respectively (Table 3). The absolute amount of NdfR immobilised as microbial N con-

stituted only 14% for faba bean and lupin, and 18% for pea (Table 4). The amount in the mineral N was also small, ranging from 3% to 7%. Summarising the three pools – micro roots, microbial and mineral N – substantial remains of 48%, 65% and 72% for pea, faba bean and lupin, respectively, were not recovered. Most of the N derived from decomposing roots in the generative growth phase of the plants may be from amino acids or polymeric amino acids (i.e. proteins and polypeptides). These components decompose very rapidly in the soil with half lives of 1 – 12 h. The direct sorption to the solid phase is by contrast negligibly low (Jones 1999). That indicates a fast turnover of the rhizodeposits through the microbial biomass and a subsequent immobilisation as microbial residues.

Conclusions

N rhizodeposition of grain legumes represents a significant pool in the N dynamics of crop rotations. The percentage of N rhizodeposition relative to the total plant N constituted between 12% and 16% and about 80% of the below ground plant N. Based on field data, NdfR contributes 16 - 68 kg N ha^{-1} to the soil for faba bean (Beck et al. 1991; Schmidtke 1996), 6 – 40 kg N ha^{-1} for pea (Jensen 1987; Beck et al. 1991; Schmidtke 1996) and 7 – 64 kg N ha^{-1} for lupin (Hartmann and Aldag 1989; Haynes et al. 1993). Taking this data into account, N rhizodeposition results in more positive N balances for grain legumes. Especially under field conditions where the N_2 fixation is lower than our findings in the pot experiment (Table 2), N rhizodeposition could be key to understanding the positive crop rotation effects of grain legumes.

As 48% - 72% of the rhizodeposition N was not recoverable in micro roots, microbial biomass and mineral-N, further investigations are needed to improve our understanding of the role of N rhizodeposition in crop rotations and the immobilization of N as microbial residues.

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5. Turnover of grain legume N rhizodeposits and effect of rhizodeposition on the turnover of crop residues

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Abstract

The turnover of N derived from rhizodeposition of faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.) and white lupin (*Lupinus albus* L.) and the effects of the rhizodeposition on the subsequent C and N turnover of its crop residues were investigated in an incubation experiment (168 days, 15°C, 50% WHC).

A sandy loam soil for the experiment was either stored at 6°C or planted with the respective grain legume in pots. Legumes were *in situ* ^{15}N stem labelled during growth and visible roots were removed at maturity. The remaining plant derived N in soil was defined as N rhizodeposition. In the experiment the turnover of C and N were compared in soils with and without previous growth of three legumes and with or without incorporation of crop residues.

After 168 days, 21% (lupin), 26% (faba bean) and 27% (pea) of rhizodeposition N was mineralised in the treatments without crop residues. Between 30% and 55% of mineralised rhizodeposition N was supplied by the microbial residue pool and a smaller amount of 15% to 17% was supplied by the microbial biomass.

The effect of rhizodeposition on the C and N turnover of crop residues was inconsistent. Rhizodeposition increased the crop residue C mineralisation only in the lupin treatment. A similar pattern was found for microbial C, whereas the microbial N was increased by rhizodeposition in all treatments. The recovery of residual ^{15}N in the microbial and mineral N pool was similar between the treatments containing only labelled crop residues and labelled crop residues + labelled rhizodeposits. This indicates a similar decomposability of both rhizodeposition N and crop residue N and may be attributable to an immobilisation of both N sources (rhizodeposits and crop residues) as microbial residues and a subsequent remineralisation mainly from this pool.

5.1. Introduction

Plant roots interact with the soil organic matter (SOM) and the microorganisms in the rhizosphere. These interactions are mainly driven by root rhizodeposition, which consists of low and high molecular weight C and N compounds released or sloughed off by the roots (Kuzyakov 2002). Most interactions have short term effects within hours or days and have major implications for the availability of plant nutrients. The mobilisation of nutrients by e.g. a lowering of pH in the rhizosphere or by complexation of ions by exuded organic acids are well-known mechanisms (Jones 1998). Freely available C and N rhizodeposits activate and increase the microbial biomass. This activation and the sufficient C supply can cause positive priming effects of SOM C and N mineralisation and contribute to N nutrition (Kuzyakov 2002; Zagal 1994). Dependent on the prevailing soil conditions and the plant species, negative priming effects have also been observed (Bottner et al. 1999; Kuzyakov 2002; Merckx et al. 1985; Wang and Bakken 1997).

Roots also influence the turnover of plant residues. Some studies have reported direct effects of roots on the turnover of recently added plant residues, while accelerating and obstructive effects have also been found (Dormaar 1990; Reid and Goss 1982; Wang and Bakken 1997). However, rhizodeposition may affect the SOM and the related turnover processes in the long term within weeks or months. Soluble root exudates can be directly absorbed by the clay fraction of soils (Kaiser and Zech 2000; Merckx et al. 1985) and contribute to a greater aggregate stability (Hütsch et al. 2002; Traoré et al. 2000). However, rhizodeposition can also result in a diminished stability and disruption of soil aggregates in the rhizosphere (Helal and Sauerbeck 1989; Reid and Goss 1981). Kuzyakov (2002) explained aggregate disruption as a mechanism behind positive rhizosphere priming effects. However, the positive or negative root effects on aggregate stability differ significantly between plant species (Haynes and Beare 1997). Additionally, apart from an alteration in size and activity, some authors have reported a plant species specific alteration of the microbial population in the rhizosphere compared to the bulk soil (Miethling et al. 2000; Smalla et al. 2001).

Most of C rhizodeposition is respired during plant growth (Hütsch et al. 2002). In contrast, the N rhizodeposition remains in the soil and contributes to the plant N supply. In studies with cereals, 10 – 23% (Jensen 1996; Rroco and Mengel 2000) of total plant N were found in rhizodeposits, 36 - 38% in clover-grass leys (Høgh-Jensen and Schjoerring 2001) and 7% - 19% in grain legumes (Jensen 1996; Russell and Fillery 1996) . Jensen (1996) and Janzen

(1990) found a mineralisation of rhizodeposits between of 23% and 38% in short term (15 and 11 weeks) incubation experiments. Thus the N rhizodeposition may contribute significantly to the subsequent N turnover after harvest of plants in an agricultural ecosystem.

In a complementary study, Mayer et al. (2003a) investigated the amount of N rhizodeposition of three grain legume species and found 12% - 16% of total plant N as rhizodeposition N in soil mainly immobilised in the microbial biomass and as microbial residues.

The aim of this study was to determine (i) the turnover of the N rhizodeposition of the grain legumes, *Vicia faba* L., *Pisum sativum* L. and *Lupinus albus* L.; and (ii) to determine the effects of rhizodeposition on the subsequent turnover of its crop residues.

5.2   Materials and Methods

Soil

Soil samples from a site in north-west Germany were taken from the top $0 - 20$ cm of a Eutric Cambisol. The field had been cultivated under organic farming management for 10 years. The soil horizon was characterised as a sandy loam with 17.3% clay, 30.1% silt, 52.6% sand, pH (0.01 M $CaCl_2$) of 6.0 and 1.58% total C, 0.15% total N, 140 mg P kg^{-1} (Calcium lactate), 208 mg K kg^{-1} (Calcium lactate), and 100 mg Mg kg^{-1} (Calcium lactate). The water holding capacity (WHC) (2 mm sieved soil) was 400 g H_2O kg^{-1} dry soil. After sampling, the soil was sieved to pass a 10 mm sieve and stored moist in a cool (6°C) and dark place until initiation of the experiment.

Legume residue and soil preparation

Faba bean (*Vicia faba* L., cv. Scirocco), pea (*Pisum sativum* L., cv. Duel) and white lupin (*Lupinus albus* L., cv. Amiga) plants were grown in 8.5 l pots (12 replicates; sowing: 4th May 1999) in a foil covered vegetation hall and *in situ* ^{15}N pulse labelled with a urea solution (99 atom % ^{15}N) using a cotton wick method (Russell and Fillery 1996).

At maturity (pea: 27th July, faba bean: 18th August, lupin: 6th September 1999) the above ground plant parts were harvested and separated into grain, stem and leaves. A sub-sample of 20% of the soil was taken from each pot throughout the entire profile. All visible roots and root fragments were removed by hand and the soil was sieved through a 2 mm sieve. The roots with adhering soil were shaken in a PE-bottle with 50 ml deionised water for 5 min and

washed with an additional 200 ml over a 2 mm sieve. Subsequently the soil water suspension was filtered and the remaining soil was mixed with the "root free" bulk soil. The plant-derived N remaining in the soil is defined as the N rhizodeposition. Details of the methodology are described by (Mayer et al. 2003a).

During the growing period of the grain legumes, a non cropped control soil was stored moist and cool (6°C) in a dark place. Because of the high NO_3^- + NH_4^+ nitrogen (N_{min}) content of the control soil (50 µg N_{min} g^{-1} soil) compared to the cropped soil at maturity (5 µg N_{min} g^{-1} soil), the N_{min} was leached with deionised water from the control soil by exchanging the complete water amount held in the profile. The sieved soil samples including the rhizodeposits and the control soil were separated into 3 replicates, frozen and stored at –22°C.

The plant parts were dried at 60°C for 72 h and ground to size < 2 mm with a centrifuge mill.

Experimental design

An incubation experiment was established to compare the C and N turnover of the soil containing legume rhizodeposits with the control soil without plant growth (Table, No. 1 – 4) and the rhizodeposition effects on the turnover of the crop residues (=stems+leaves+recovered roots) added compared to the control soil (Table, No. 5 – 10). The overall variations and the respective dry matter, C and N inputs added by the crop residues and the N inputs deposited by rhizodeposition are given in Table 1. Table 2 shows some chemical characteristics of the added crop residues.

The incubation experiment was designed in triplicate and the replicates were incubated separately and chronologically staggered. The soil with the respective residues was moistened to 50% of WHC and portions of 70g (= 58.3 g dry soil) were weighed in 100ml beakers and put in 900 ml preserving jars. To keep the soil moist, 20 ml of deionised water were put at the bottom of the jar. The soils were incubated at 15°C over a period of 168 days and sampled at day 0, 7, 14, 21, 28, 56, 84, 112, 140 and 168. The CO_2 respired from the soil was trapped in 15 ml 0.5 M NaOH put in the jars sampled at day 168. Five additional NaOH controls without soil were used per replicate. The NaOH was changed at weekly intervals from day 7 – 84 and at two-week intervals from day 84 – 168. In parallel, the jars without NaOH were aerated for at least 15 minutes.

Table 1. Experimental treatments and amounts of grain legume residue inputs to the soil

No.	Soil previously cropped with	Crop residues added	Abbreviation	Crop residues (stems, leaves, roots)			rhizo-deposition	total
				DM*	C	N	N	N
				mg g^{-1} soil		µg g^{-1} soil		
1	without	without	-GL/-Res	-	-	-	-	-
2	Faba bean	without	+FB/-FBres	-	-	-	56	56
3	Pea	without	+P/-Pres	-	-	-	17	17
4	Lupin	without	+Lu/-Lures	-	-	-	54	54
5	without	Faba bean residues	-GL/+FBres	8	3529	110	-	110
6	Faba bean	Faba bean residues	+FB/+FBres	8	3529	110	56	166
7	without	Pea residues	-GL/+Pres	8	3491	58	-	58
8	Pea	Pea residues	+P/+Pres	8	3491	58	17	76
9	without	Lupin residues	-GL/+Lures	8	3606	68	-	68
10	Lupin	Lupin residues	+Lu/+Lures	8	3606	68	54	122

* DM = dry matter; -GL = control soil without previous growth of grain legumes; +FB. +P, +Lu = soils previously cropped with faba bean, pea and lupin; -Res = treatment without added grain legume residues; + and – FBres, Pres and Lures = treatments with added (+) or without added (-) faba bean, pea and lupin residues.

Analytical methods

At each sampling date three replicate jars were removed from the incubation chamber. Mineral N was determined by extracting a first portion of 70 g moist soil with 240 ml 0.5 M K_2SO_4 for 30 minutes. The NO_3^- and NH_4^+ contents were analysed using a continuous flow analyser (Evolution II, Alliance Instruments, France).

The microbial biomass C and N was determined using the chloroform fumigation extraction method (Vance et al. 1987). A second portion of 70 g fresh soil was fumigated with $CHCl_3$ for 24 h at 25°C and afterwards extracted with 240 ml 0.5 M K_2SO_4 for 30 minutes. The or-

Table 2. Chemical composition of the crop residues (stems, leaves, roots) of the grain legumes

Parameter	Faba bean	Pea	Lupin
C (%)	44	44	45
N (%)	1.4	0.7	0.8
H_2O soluble C (%)	10.9	7.6	10.1
H_2O soluble N (%)	0.34	0.16	0.25
Cellulose (%)	39	49	42
Lignin (%)	15	10	14
C : N	32	60	53
H_2O soluble C : N	32	47	41
Lignin : N	11	14	16

ganic C and total N contents of the 0.5 M K_2SO_4 extracts were determined with a TOC/TN_b analyser (Dimatoc 100, Dimantec, Germany). The soil microbial biomass C and N was estimated from the relationship: biomass C or N = $E_{C \, or \, N}$ / $k_{EC \, or \, EN}$, where $E_{C \, or \, N}$ is [organic C or total N extracted from fumigated soil] minus [organic C or total N extracted from unfumigated soil] and k_{EC} = 0.45; k_{EN} = 0.54 (Jörgensen 1996; Jörgensen and Müller 1996).

The ^{15}N contents of the microbial biomass and N_{min} extracts were determined using a modified diffusion technique by Goerges and Dittert (1998). Before diffusion, the N in the biomass extracts were oxidised to NO_3^- using $K_2S_2O_8$ and autoclaving (Cabrera and Beare 1993). However, the ^{15}N enrichment of the mineral N could not be determined in all variations over the whole incubation period by the diffusion technique because of the very small N contents in the extracts.

A portion of 25 g soil was taken from the third jar, dried at 60°C for 72 h and ground to a fine powder using a ball mill.

The soil samples and the diffusion filters were analysed for N and ^{15}N content using an isotope ratio mass spectrometer (Finnigan MAT 251, Germany) coupled with a Carlo Erba NA 1500 C/N analyser. The total C, N and ^{15}N contents of the legume residues were analysed using a Carlo Erba NA 1500 C/N analyser coupled to an emission spectrometer (NOI-6PC, Fischer Analysen Instrumente, Germany).

The CO_2 amount trapped in the NaOH was precipitated by 0.5 M $BaCl_2$ and the remaining NaOH quantified by titration with 0.1 M HCl.

The cellulose and lignin contents were determined by the acid detergent fibre method (Van Soest 1963). The cold water soluble C and N contents of the crop residues (leaves + stems + roots) were determined according to Collins et al. (1990). A portion of 500 mg finely ground dry residue matter was extracted 7 times for 20 min with 50 ml deionised water at room temperature (ca. 20°C) on a horizontal shaker. After each extraction the suspension was centrifuged (3000 G) for 20 min and the clear supernatant was decanted. The 7 extracts were combined and analysed for total C and N using a TOC/TN_b analyser (Dimatoc 100, Dimantec, Germany).

Calculations and statistics

The recovery of the nitrogen derived from the legume residues (% of input) in the N_{min} and microbial biomass N (N_{mic}) pool and the recovery of the total ^{15}N label in the soil was calculated as follows:

$$\% \text{ recovery } ^{15}N_{\text{min or mic}} = \frac{^{15}N \text{ excess } N_{\text{min or mic}} \ (\mu g\,g^{-1} soil)}{^{15}N \text{ excess } Ndfr \ (\mu g\,g^{-1} soil)} \times 100 \qquad (1)$$

Ndfr = nitrogen derived from legume residues

$$\% \text{ recovery } ^{15}N \text{ label total soil } = \frac{^{15}N \text{ excess } \text{soil } t_n \ (\mu g\,g^{-1} soil)}{^{15}N \text{ excess } \text{soil } t_0 \ (\mu g\,g^{-1} soil)} \times 100 \qquad (2)$$

t_0 = start of the experiment (day 0)
t_n = samplings during experiment (n = 7 to 168)

The calculations were done under the assumption that the plant parts of the legumes (e.g. leaves, stems, roots) with differing ^{15}N enrichments mineralise equally and that the ^{15}N enrichment of the residue N mineralised was equal to the ^{15}N enrichment of the legume residue N.

The amount of ^{15}N label in the microbial biomass (N_{mic}) was calculated:

$$^{15}Nexcess\ label\ _{Nmic} = \frac{(N_{fum\ soil} \times \%^{15}Nexcess\ _{fum\ soil} - N_{not\ fum\ soil} \times \%^{15}Nexcess_{not\ fum\ soil})}{k_{EN}} \qquad (3)$$

fum soil $\qquad$ = fumigated soil

not fum soil $\quad$ = unfumigated soil

k_{EN} $\qquad\qquad$ = 0.54 (Jörgensen and Müller 1996)

The atom % ^{15}N excess values of the respective soil pools were obtained by correcting the ^{15}N enrichments with the respective background values of the control soil without residue addition. The ^{15}N values obtained by the diffusion procedure of the N_{min} and N_{mic} extracts were blank corrected (Sørensen and Jensen 1991).

The N derived from the residues in the N_{min} and N_{mic} pools was calculated as follows:

$$N_{min\ or\ mic}\ dfr = residue\ N\ input\ (\mu g\ g^{-1}\ soil) \times \%\ recovery\ N_{min\ or\ mic} \qquad (4)$$

Calculation of the newly formed microbial residue N derived from legume residues (N_{micres})

We used an approximate calculation to estimate the size of the newly formed microbial residue N derived from plant residues by combining the CO_2 respiration and microbial C data with the ^{15}N data. The calculations were done using several assumptions as follows:

A: Microbial residue N derived from crop residues (leaves+stems+recoverable roots) ($N_{micres\ dfr}$)

The decomposition of added carbon derived from crop residues ($C_{dec\ dfr}$) was calculated as follows:

$$C_{dec\ dfr} = C_{min\ dfr} + C_{mic\ dfr} + C_{micres\ dfr} \qquad (5)$$

$C_{min\ dfr}$: $\quad$ mineralised C derived from crop residues

$C_{mic\ dfr}$: $\quad$ microbial C derived from crop residues

$C_{micres\ dfr}$: microbial residue C derived from crop residues

$\left.\begin{array}{l} C_{min\ dfr} \\ \\ C_{mic\ dfr} \end{array}\right\}$ was calculated as: Soil with residues – control soil (Difference method) (6)
$\qquad\qquad\qquad$ (see Table 1, No. 5; 7; 9 and 1)

However, the size of $C_{micres\ dfr}$ is unknown. Hence the size of the $C_{dec\ dfr}$ can only be calculated as a 1st approximation:

$$C_{dec\ dfr\ (approximation\ 1)} = C_{min\ dfr} + C_{mic\ dfr} \tag{7}$$

The percentage of $C_{dec\ dfr}$ related to the initial residue C input is:

$$\%\ C_{dec\ dfr\ (1)} = \frac{C_{dec\ dfr\ (approximation\ 1)}}{C_{input\ dfr}} \times 100 \tag{8}$$

Assumption 1:

The N of the plant residues decomposes in the same way as the carbon ($N_{dec\ dfr} = C_{dec\ dfr}$). Thus the 1st approximation of the $N_{dec\ dfr}$ is:

$$N_{dec\ dfr\ (approximation\ 1)} = \%\ C_{dec\ dfr\ (1)} \times N_{input\ dfr} \tag{9}$$

According to equation (5) the size of the microbial residue N as a 1st approximation is:

$$N_{micres\ dfr\ (1)} = N_{dec\ dfr\ (1)} - N_{min\ dfr} - N_{mic\ dfr} - N_{loss\ dfr} \tag{10}$$

$N_{loss\ dfr}$: Losses of N derived from residues by denitrification, determined by the total recovery of ^{15}N in soil.

Assumption 2:

The newly formed microbial residues have the same C : N ratio as the microbial biomass ($C:N_{micres} = C:N_{mic}$).

Thus, as in the 1st approximation, the $C_{micres\ dfr}$ can be calculated as:

$$C_{micres\ dfr\ (1)} = N_{micres\ dfr\ (1)} \times C:N_{mic} \tag{11}$$

The 2nd approximation of the $C_{dec\ dfr}$ can then be calculated using equation (5):

$$C_{dec\ dfr\ (approximation\ 2)} = C_{min\ dfr} + C_{mic\ dfr} + C_{micres\ dfr\ (1)}$$

The 2nd approximation of $N_{dec\ dfr}$ is calculated by inserting $C_{dec\ dfr\ (2)}$ in equation (8), obtaining $\%\ C_{dec\ dfr\ (2)}$ and inserting it in equation (9):

$$N_{dec\ dfr\ (2)} = \%\ C_{dec\ dfr\ (2)} \times N_{input\ dfr}$$

The 2nd approximation of $N_{micres\ dfr}$ is obtained by inserting $N_{dec\ dfr\ (2)}$ in equation (10):

$$N_{micres\ dfr\ (2)} = N_{dec\ dfr\ (2)} - N_{min\ dfr} - N_{mic\ dfr} - N_{loss}; \quad \text{etc.}$$

B: Microbial residues derived from rhizodeposition ($N_{micres\ dfR}$)

In contrast to the various crop residues added to the control soil (Table 1, No. 5; 7; 9), the total C input derived from rhizodeposition (Table 1, No. 2 - 4) was not determined. However, the N rhizodeposition was defined as plant derived N in the soil after removal of the visible roots. After hand sampling of the roots, a certain amount of the micro-roots remained in the soil and was determined by wet sieving over a 200µm sieve (Mayer et al. 2003a). The C and N inputs by micro-roots were estimated using the C and N contents of the macro-roots for the calculation. Thus the nitrogen derived from rhizodeposition (NdfR) consists of the following pools:

$$NdfR = N_{df\ micro\text{-}roots} + N_{min\ dfR} + N_{mic\ dfR} + N_{micres\ dfR} \tag{12}$$

Mayer et al. (2003a) concluded from their data that the C and N inputs derived from rhizodeposition except the micro-roots were completely decomposed. The decomposition of the C derived from micro-roots ($C_{dec\ df\ micro\text{-}roots}$) was calculated as a 1st approximation using equation ($5 - 8$). The $N_{dec\ df\ micro\text{-}roots\ (approximation\ 1)}$ was calculated using equation (9).

Assumption 3:

The microbial residues derived from rhizodeposition ($N_{micres\ dfR}$) consist of two pools: The newly formed microbial residues derived from micro-roots and the previously formed microbial residues derived from rhizodeposition. The microbial residues derived from micro-roots and from rhizodeposits have the same proportion as the N which was decomposed from micro-roots and rhizodeposits, respectively.

The N decomposed from rhizodeposits without micro-roots ($N_{dec\ dfR}$) was calculated as:

$$N_{dec\ dfR} = NdfR - N_{dec\ df\ micro\text{-}roots} \tag{13}$$

The size of the microbial residue N derived from rhizodeposition ($N_{micres\ dfR}$) was calculated according to equation (10):

$$N_{micres\ dfR} = N_{dec\ df\ micro\text{-}roots} + N_{dec\ dfR} - N_{min\ dfR} - N_{mic\ dfR} - N_{loss} \tag{14}$$

Using this concept and the principal calculation procedure of equation ($5 - 11$) enables an approximate estimation of the microbial residue N derived from rhizodeposition.

C: Microbial residues derived from crop residues and rhizodeposition (N$_{micres\ dfr\ +\ R}$)

The formation of the microbial residues in the variations soil with rhizodeposition + crop residues (Table 1, No. 6; 8; 10) was calculated combining the calculations of A and B.

The size of the microbial residues derived from rhizodeposition (N$_{micres\ dfr\ +\ R}$) was calculated according to equation (10):

$$N_{micres\ dfr\ +\ R} = N_{dec\ dfr} + N_{dec\ df\ micro\text{-}roots} + N_{dec\ dfR} - N_{min\ dfR} - N_{mic\ dfR} - N_{loss} \qquad (15)$$

The N$_{micres\ dfr\ +\ R}$ was then calculated according to equation (5 − 11).

For all calculations the iteration procedure was carried out until the last calculated approximation deviated from the previous one by less than 0.001%. A maximum of 8 iteration steps was necessary.

The GLM procedure of the SPSS 10.0 statistics package (SPSS GmbH, Germany) was used to carry out the statistical analysis. The treatments without residues (Table 1, No. 1 − 4) and the treatments with crop residues (Table 1, No. 5 − 10) were analysed separately. Means were compared by the Tukey-test ($p < 0.05$) if the analysis of variance was significant.

5.3. Results

C and N mineralisation

The cumulative C mineralisation of the soils without addition of crop residues showed no distinct differences between the soils containing grain legume rhizodeposits and differed only slightly from the control (Fig. 1 A).

The increased C mineralisation after the addition of the crop residues (= stems + leaves + roots) showed differences between the legume species (Fig. 1 B). The greatest C amount was mineralised from the lupin residues, followed by faba bean and pea, respectively. Calculated by difference [(soil +residues) − (soil −residues)], the percentage of mineralised C constituted 45% for pea, 48% for faba bean and 51% for lupin of initial crop residue C input after 168 days of incubation (data not shown). Only in the lupin treatment was a distinct effect of rhizodeposition observed. The lupin soil with rhizodeposition showed a greater C mineralisation as observed in the control soil until day 84, with a decline in the following period.
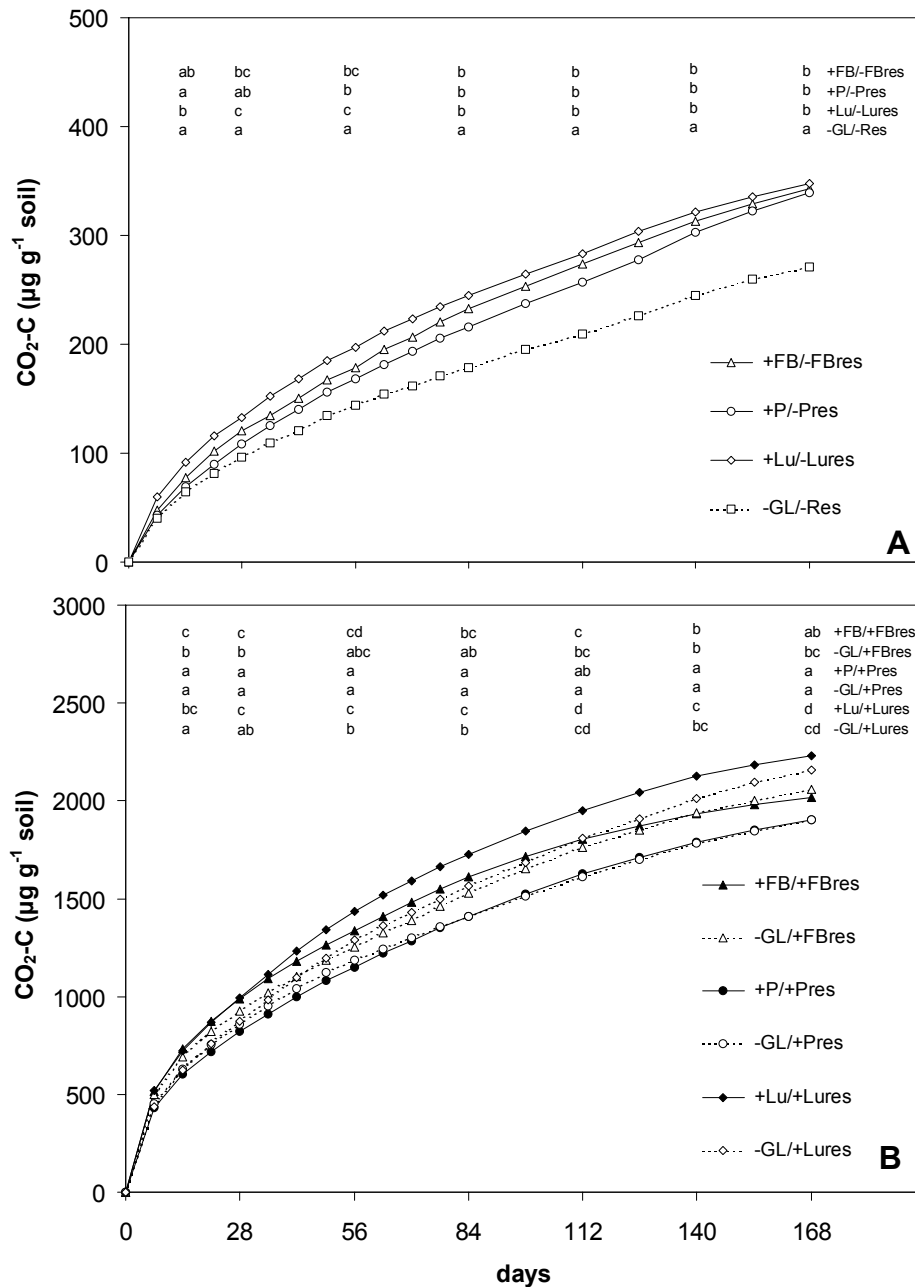
Fig. 1. Cumulative C mineralisation of soils without (A) and with (B) crop residue addition. Data show means at the respective sampling date (n=3). Means with different letters in columns differ significantly.

The N mineralisation in the soils without residues differed significantly between the soils with rhizodeposits and the control soil. The net N mineralisation constituted between 33 µg N_{min} g^{-1} soil (lupin) and 29 µg g^{-1} (pea) compared to 27 µg g^{-1} in the control soil after 168 days of incubation (Fig. 2 A).

The addition of the crop residues caused a complete N immobilisation in all variations during 84 days of incubation. The faba bean residues started mineralisation first, followed by lupin.

The pea residue N remained immobilised over the whole incubation period. However, the NdfR had no significant effect on the N mineralisation during 168 days of incubation (Fig. 2 B).
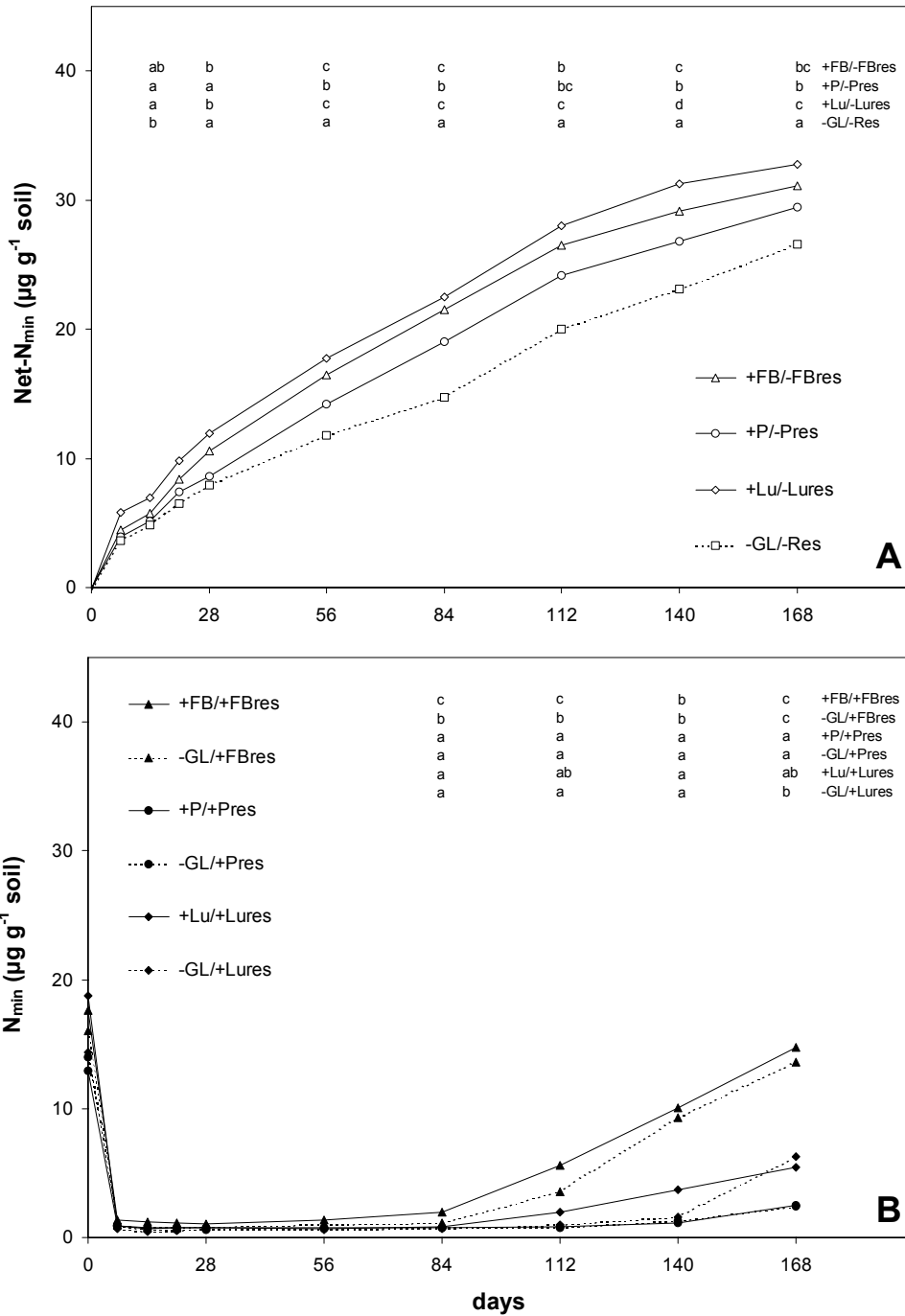

Fig. 2. Net N mineralisation of the soils without (A) and N mineralisation of soils with crop residue addition (B). Data show means at the respective sampling date (n=3). Means with different letters in columns differ significantly.

Microbial C and N

The microbial C content in the soils without residues showed similar patterns to the C mineralisation. Only small differences were found compared to the control soil and no distinct differences between the legume treatments (Fig. 3). No differences were found for microbial N (Fig.4).
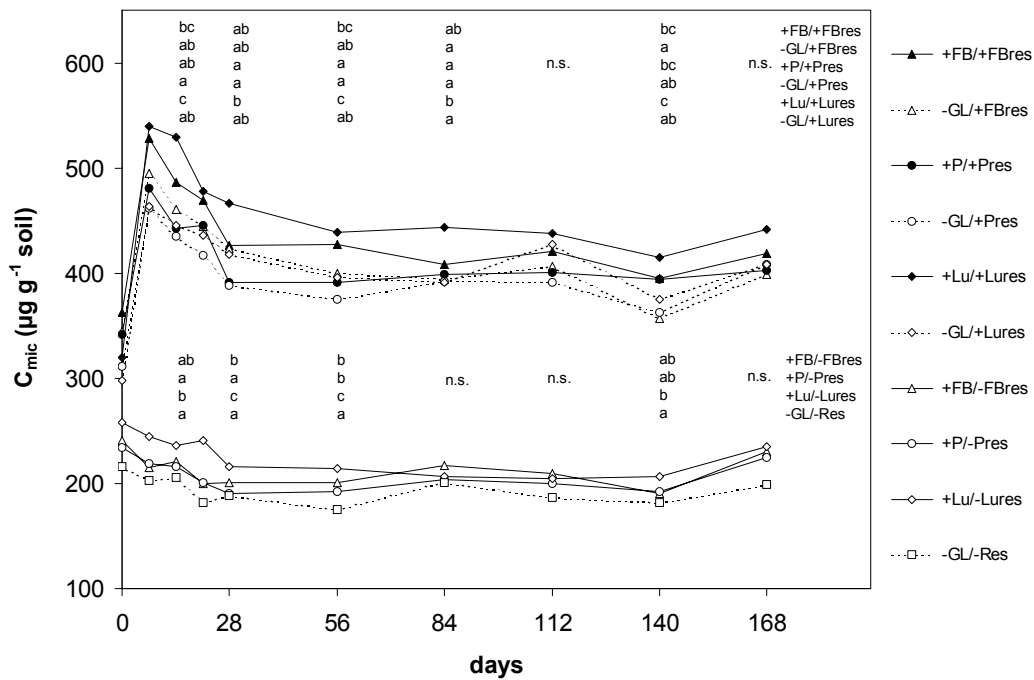

Fig.3. Microbial C contents in soils with and without residue addition. Data show means at the respective sampling date (n=3). Means with different letters in columns differ significantly.
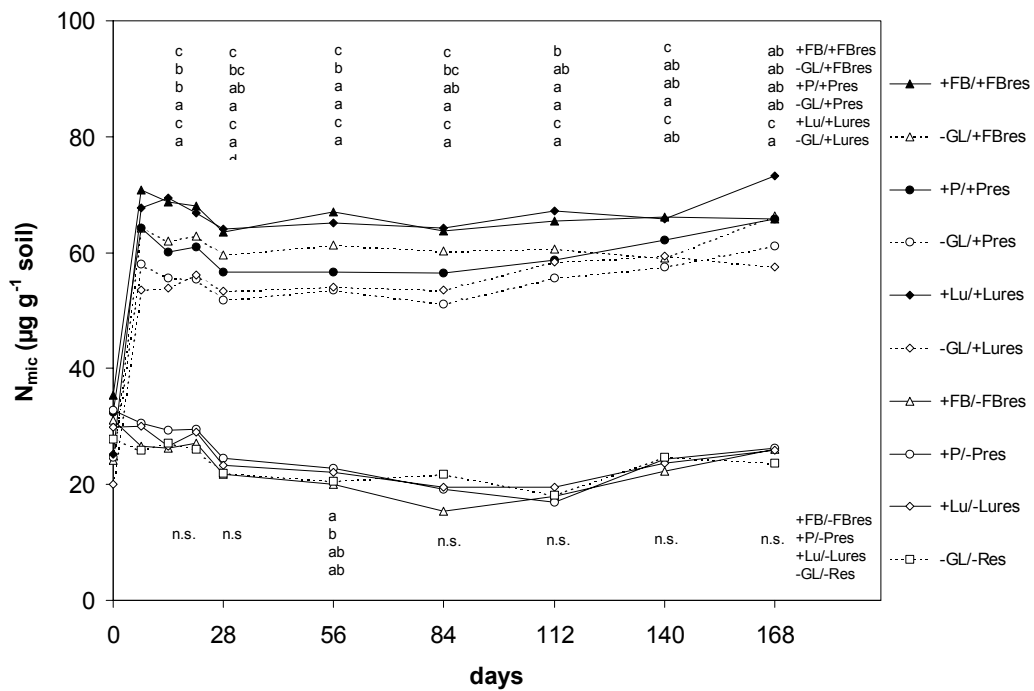

Fig. 4. Microbial N contents in soils with and without residue addition. Data show means at the respective sampling date (n=3). Means with different letters in columns differ significantly.

The addition of the crop residues caused an increase of microbial C and N and also a differentiation between the treatments. The microbial C content showed a similar pattern compared to the C mineralisation. However, significant differences could only be observed for soil without and with previous lupin growth. The other treatments showed no continuous differences during the incubation period. The rhizodeposition caused significant differences to the control in the lupin variation whereas the rhizodeposits of faba bean and pea had no significant effect on the microbial C content (Fig. 3).

However, in all treatments the presence of rhizodeposits caused an increase of microbial N compared to the control soil. The differing effect was strongest in the first three weeks and increased in the order pea, faba bean and lupin (Fig. 4).

Recovery of residual N

The total ^{15}N recovery in the soil was almost constant over the whole incubation period and the treatments. About 100% of initial ^{15}N were recovered after 28 and 84 days and decreased only slightly to about 98% after 168 days of incubation, indicating that losses from the system were very small (data not shown).

At the start of the experiment in the soils without residues about 10% of the NdfR was recovered in the mineral N pool and increased to 21% for lupin, 26% for faba bean and 27% for pea respectively after 168 days of incubation (Fig. 5).
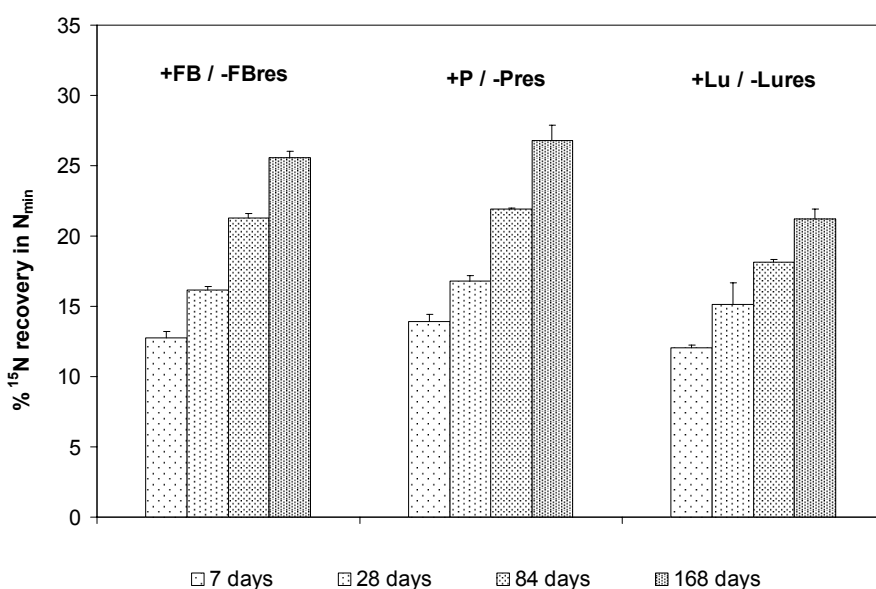

Fig. 5. Recovery of residue derived ^{15}N in the mineral N pool in soils without crop residue addition in the faba bean (+FB/-FBres), pea (+P/-Pres) and lupin (+Lu/Lures) treatment. Bars show means (± SD; n=3).

A similar percentage of NdfR was recovered in the microbial biomass and constituted 10% - 13% at the start of the experiment. It decreased until day 84 to 2% - 3% and increased afterwards to an extent of 6% (Fig. 6).

The net soil derived N_{min} differed only slightly between the variations and constituted between 23 µg N g^{-1} soil and 27 µg N g^{-1}.

In the treatments with crop residues 17% of the faba bean residue N, 18% of the lupin N and 27% of the pea N were recovered in the microbial biomass at day 7. The recovered percentage decreased only slightly and constituted 15% for faba bean, 18% for lupin and 23% for pea after 168 days of incubation. However, despite the differing residue N inputs in the treatments (Table 1), significant differences in the recovery were observed only between the legume species. The NdfR obviously had no differing effect on the recovery (Fig. 6).
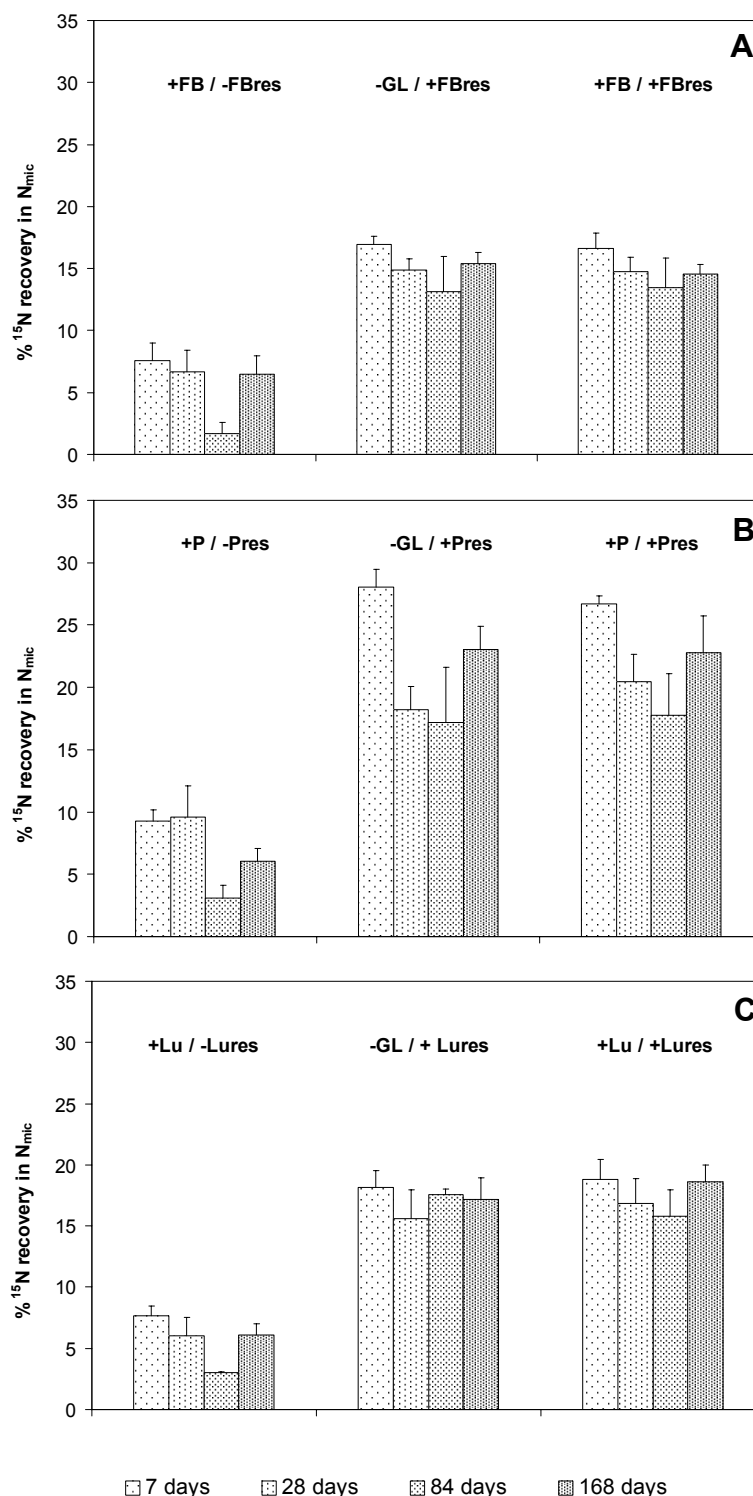

Fig. 6. Recovery of residue derived ^{15}N in the microbial biomass in soils without and with crop residue addition in the faba bean (A), pea (B) and lupin (C) treatment. Bars show means (± SD; n=3).

The soil derived microbial N was almost constant in the treatments with crop residues and constituted about 43 µg N g^{-1} at day 7 and 47µg N g^{-1} at day 168. The rhizodeposition had no differing effect. However, compared to the soils without crop residues, the residue addition caused an additional soil derived microbial N immobilisation of about 21 µg N g^{-1} (data not shown).

Formation of microbial residue N

For soils without incorporated residues containing only rhizodeposition it was assumed that the NdfR which was not recovered in the mineral N, the microbial N pool and still present as micro-roots in the soil was immobilised as microbial residues (Mayer et al. 2003a). Based on this assumption, differences in the formation of microbial residue N derived from legume residues (N_{micres}) were found between the treatments. The lowest N_{micres} amount was found in the pea soil (Fig. 7 A), which is consistent with the fact that this treatment had the smallest input of 17 µg NdfR g^{-1} soil (Table 1). It increased during the incubation from 9 µg N g^{-1} at day 7 to 13 µg N g^{-1} at day 168. A greater amount of N_{micres} was formed by faba bean and lupin NdfR and decreased slightly during the incubation period. The initial inputs of NdfR were similar between faba bean (56 mg N g^{-1}) and lupin (54 µg N g^{-1}; Table 1). However, in the lupin treatment a greater amount of N_{micres} of 41 µg N g^{-1} (day 7) to 39 µg N g^{-1} (day 168) was formed compared to faba bean with 36 µg N g^{-1} (day 7) to 33µg N g^{-1} (day 168) (Fig. 7 A).

According to the differing N inputs ranging from 58 µg N g^{-1} to 166 µg N g^{-1} in the treatments with crop residues (Table 1), the formation of N_{micres} differed both between the legume species and between the soils +rhizodeposition and –rhizodeposition. In all treatments the addition of the crop residues caused an increasing formation of N_{micres} until day 140 and stagnated in the last four weeks of incubation (Fig. 7 B).

However, considering the N_{micres} derived from crop residues, significant differences were found in the formation dynamics between the soils +rhizodeposition and -rhizodeposition. The soils +rhizodeposition increased the formation of N_{micres} derived from crop residues compared to the soils –residues in all treatments. This effect was strongest in the faba bean and lupin treatment and decreased from day 84 to the end of the incubation (Fig. 7 C). For faba bean the difference between +rhizodeposition and –rhizodeposition constituted 9 µg N g^{-1},

for lupin 8 μg N g^{-1} and for pea 2 μg N g^{-1} at day 28 and decreased to 3 μg N g^{-1} for faba bean, lupin and pea, respectively (Fig. 7 C).
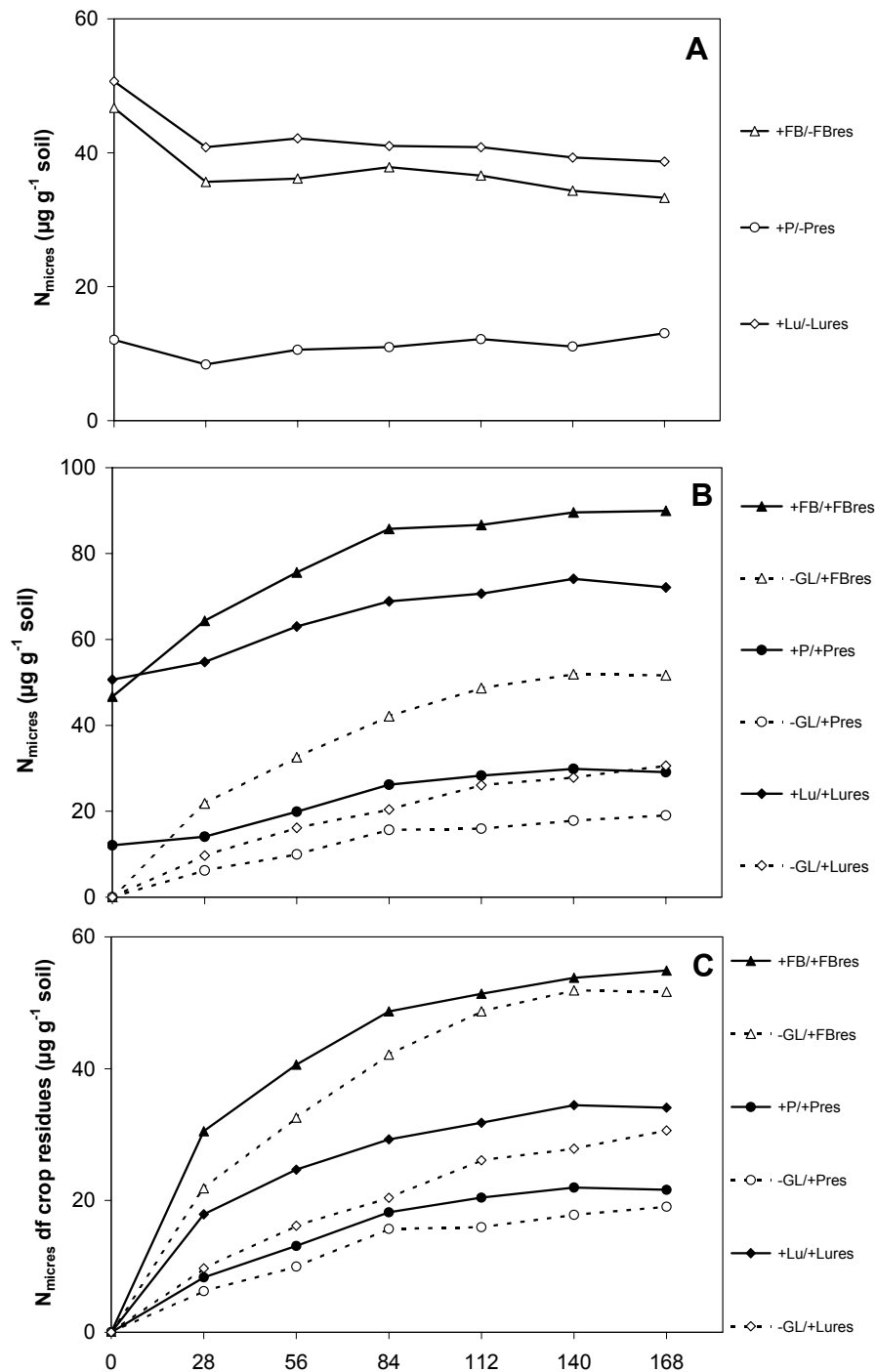

Fig. 7. Formation of microbial residues (N$_{micres}$) in soils without (A) and with (B) crop residue addition and microbial residues derived from crop residues (N$_{micres}$ df crop residues) in soils with crop residue addition (C).

Balance of residue N

In the treatments without crop residues the N_{micres} was the relatively greatest pool containing NdfR, followed by the mineral N pool and the remaining micro-roots in the soil. The microbial biomass recovered only a small amount of NdfR and did not alter markedly compared to the other pools. In the faba bean and the lupin treatment the N_{min} derived from rhizodeposition was mainly supplied by the N which was previously immobilised as microbial residues (55% and 52% for faba bean and lupin, respectively) or present as micro-roots (30% and 31% for faba bean and lupin, respectively). The NdfR immobilised in the microbial pool contributed only 15% and 17% for faba bean and lupin, respectively. In the pea treatment the N_{min} pool and the N_{micres} pool were supplied by the undecomposed micro-roots and to a smaller extent by the microbial N pool (Fig. 8, treatments +FB/-FBres; +P/-Pres; +Lu/-Lures).

The addition of crop residues caused strong N immobilisation. During the incubation the immobilisation of Ndfr in the microbial biomass constituted between 13% and 17% in the faba bean treatment, 17% and 27% in the pea treatment and 16% and 19% in the lupin treatment. The relative size of this pool kept almost constant during the whole incubation period and was not influenced by the further decomposition process. Hence the N mineralised from the crop residues was immobilised completely in microbial residues. The percentage of N_{micres} derived from residues increased and constituted at the end of the incubation 54% (+FB/+FBres) and 47% (-GL/+FBres) of total residue N for faba bean, 38% (+P/+Pres) and 33% (-GL/+Pres) for pea and 59% (+Lu/+Lures) and 45% (-GL/+Lures) for lupin, respectively (Fig. 8).

Fig. 8. Balance of residue N dynamics as percent of total residue N in soils containing only rhizodeposits (+FB/-FBres, +P/-Pres, +Lu/-Lures), control soils with crop residues (-GL/+FBres, -GL/+Pres, -GL/+Lures) and soils containing rhizodeposits and crop residues (+FB/+FBres, +P/+Pres, +Lu/+Lures) during the incubation period. Ndfr not dec = non-decomposed residue N; N_{micres} dfr = microbial residues derived from legume residues; $N_{mic\ dfr}$ = microbial N derived from residues; N_{min} dfr = Mineral N derived from residues.

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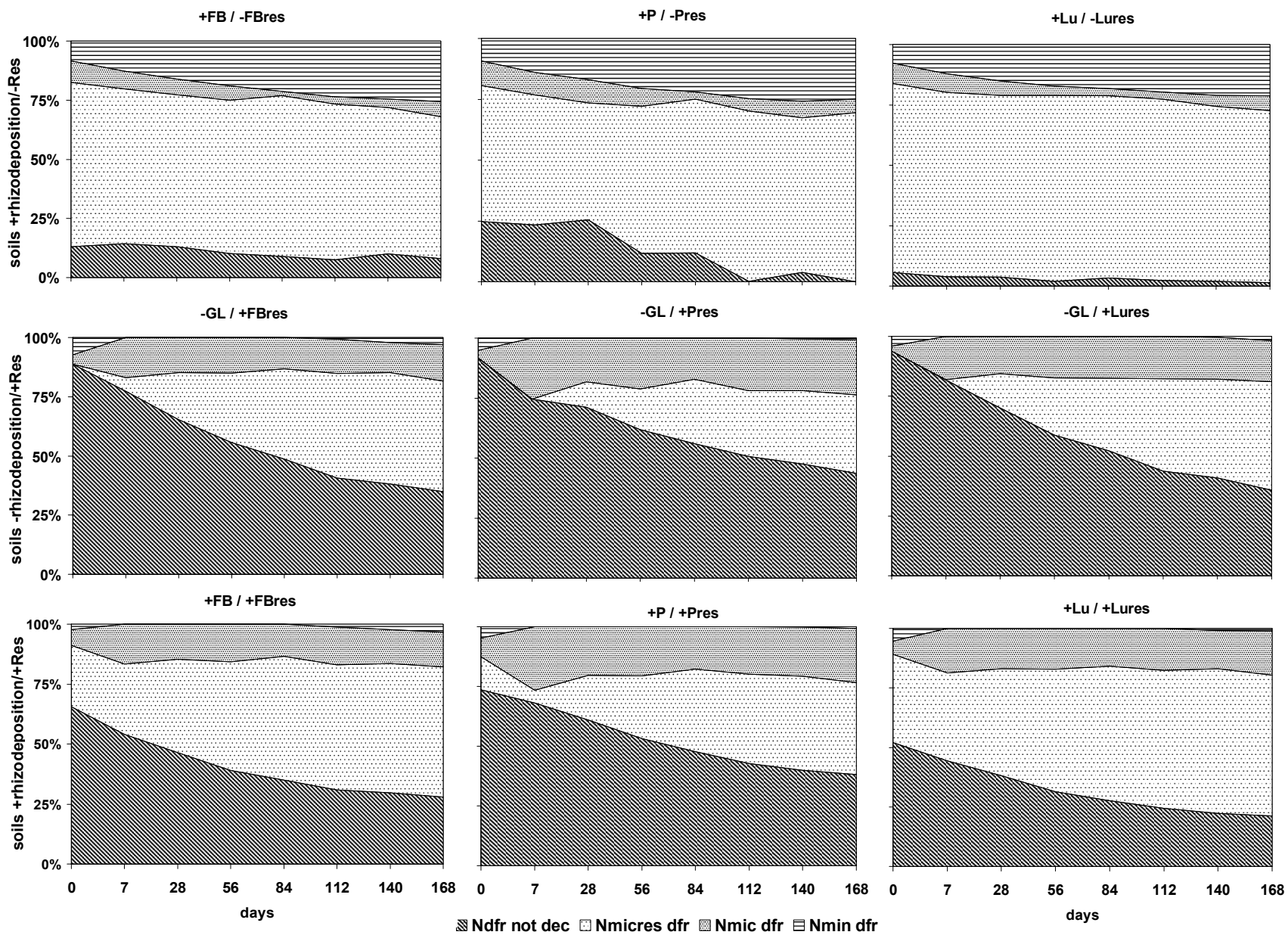
5.4. Discussion

Turnover of rhizodeposition C and N in soil

At maturity of annual crops only a small amount between 2% and 5% of C rhizodeposits remains in the soil (Hütsch et al. 2002; Kuzyakov and Domanski 2000) and it might be incorporated in the microbial biomass and the soil organic matter (Merckx et al. 1985; Molina et al. 2001) or bound in the clay fraction (Hütsch et al. 2002). Thus only a small amount of easily decomposable C will be available for decomposition. Our results show only a small increase in C turnover in the soils containing only rhizodeposits (treatments without crop residues) compared to the control (Fig. 1). This confirms a rapid turnover of C rhizodeposits during plant growth (Hütsch et al. 2002) and indicates a minor importance of C rhizodeposits in the turnover process in soil after plant maturity.

In contrast to C, the N derived from rhizodeposition (NdfR) remains in the soil. Most of these compounds consist of easily decomposable amino acids / amides (Gransee and Wittenmayer 2000) and will also rapidly be incorporated in the microbial biomass and the SOM pool (Owen and Jones 2001). Mayer et al. (2003a) concluded that most of the NdfR of the investigated grain legume species (faba bean, pea, white lupin) was immobilised as microbial residues and forms a pool of SOM with a high decomposability. Thus in our experiment 21% to 26% of NdfR was mineralised after 168 days of incubation and caused a greater net N mineralisation compared to the control without rhizodeposits (Fig. 2 A). Similar results were reported by Jensen (1996) who found 30% of pea and 23% of barley N rhizodeposits determined at maturity were mineralised after 15 weeks of incubation at 20°C. Janzen (1990) found 38% of wheat rhizodeposits mineralised after 11 weeks of incubation at 30°. The results could not be compared directly, but it also shows a relatively high decomposability of N rhizodeposits after harvest of the plants.

From the mineralised N, 12% to 32% was derived from rhizodeposition after 168 days of incubation. A smaller percentage of 4% - 13% was found in the microbial biomass, indicating that the N_{min} was supplied by microbial NdfR. However, the percentage of NdfR in the N_{min} pool decreased only slightly during the incubation period and was almost constant in the last third of the incubation in all treatments. This indicates a stabilization of rhizodeposits in an easily decomposable SOM pool and turnover dynamics similar to the native SOM in this pool.

The N balance shows that the microbial pool provides only a small amount for N_{min} (15 – 17%) and the main N_{min} dfR (52% - 55%) was supplied by the microbial residue pool. Additionally the micro-roots present in soil contributed a significant percentage to N_{min}, especially in the pea soil where a significant amount of NdfR was present as micro-roots. The results confirm our previously proposed hypothesis that most of the N rhizodeposits will be incorporated in the SOM pool as microbial residues (Mayer et al. 2003a).

Turnover of crop residues and rhizodeposition effects

The quality of plant residues, defined as its biochemical composition, determines the decomposition of plant residues in a given soil (Heal et al. 1997). On account of similar residue qualities of the grain legume residues (Table 2), the effects on C decomposition were small and were not significant during the incubation period (Fig. 1).

The principal aim of the study was to investigate the effect of previously cropped soil with grain legumes on the subsequent turnover of its residues. In the pea and faba bean treatments rhizodeposition had no effect on the C mineralisation of crop residues. In contrast, lupin rhizodeposition enhanced the C mineralisation of the residues (Fig. 1).

This effect could not be explained by the examined parameters in the soils without crop residues. No distinct differences were observed between the plant species either in microbial activity (C mineralisation) or in microbial parameters (Fig. 1; Fig. 3; Fig. 4). The differences to the control soil were also small. Thus rhizodeposition effects on the turnover of added residues cannot be related to a change in the size of the microbial population due to root rhizodeposition. However, in the soils without crop residues, C limited the activity of microorganisms as determined by CO_2 mineralisation, whereas the addition of available C (crop residues) showed a differentiation of the microbial activity in the case of lupin! This effect may be caused by two main factors: (i) a differing activation of the microbial population and (ii) a change in the composition of functional groups of the microbial population triggered by plant specific differences in the amount and composition of rhizodeposition. An activation of 'resting microbial' cells can be triggered by trace amounts of a range of trigger molecules with characteristics similar to rhizodeposits (De Nobili et al. 2001). The activation effect observed by De Nobili et al. (2001) led to an accelerated C turnover of recently added cellulose, which was maintained until the experiment was terminated after 24 days. In our experiment the absolute amount of rhizodeposition differed significantly between the species for N (and proba-

bly for C) and was lowest for pea, whereas faba bean and lupin had similar inputs (Table 1). Thus the quantity of rhizodeposits combined with an expected differing composition between the species (Gransee and Wittenmayer 2000) might cause a varying activation of the microbial population.

However; some studies have reported an alteration in the composition of the microbial population and its functional diversity, depending on the quality of substrate supply (Cheshire et al. 1999; Degens 1998; Smalla et al. 2002) and differences between crop species (Garland 1996; Miethling et al. 2000; Smalla et al. 2001). In a complementary study, using the same soil, grain legume species and varieties, the effect on the plant specific microbial diversification in the rhizosphere was investigated. Functional microbial diversity and activity was more similar in the rhizosphere of lupin and pea compared to faba bean (Aneja et al. 2003). These differences may lead to functional diversity and may be one reason for the rhizodeposition effect found in C mineralisation.

Griffiths et al. (1999) found that substrate availability strongly influenced microbial community structure, with fungi dominating over bacteria at high substrate loading as used in our experiment. In the case of lupin the diversification effect on the microbial population seems to affect the function of the microorganisms and can persist to significantly affect the turnover of plant residues. As Bending et al. (2002) observed, the addition of crop residues can also influence the microbial composition. Thus the effects obtained are a result of interactions between differing substrates (crop residues versus rhizodeposits) which seem to dominate the functions of the microbial community to a varying extent.

The patterns observed for microbial C were similar to C mineralisation and differed distinctly only for lupin, whereas microbial N tended to be increased by the presence of rhizodeposits in all treatments (Fig. 4). Obviously the influence of rhizodeposition results in a partition of C mineralisation and N immobilisation dynamics after addition of residues. This fact also indicates a functional diversification of the microbial community caused by rhizodeposition.

The recovery of residue N in the microbial biomass showed no differences between the treatments +rhizodeposition and –rhizodeposition during the whole incubation period (Fig. 6) and no differences in the N_{min} fraction at the end of the incubation (data not shown). This indicates apparently a similar availability of the N derived from either crop residues or rhizodeposits. Under N immobilising conditions the greatest part of crop residues will be immobilised as

microbial residues. In the treatments containing rhizodeposits the rhizodeposits were previously immobilised as microbial residues and will be supplemented by the microbial residues derived from crop residues. In the further decomposition and remineralisation process the microbial residues will be one of the main pools of C and N supply for the microorganisms (cryptic growth) and the N_{min} pool (Fig. 9). Thus the residue N recovered in the microbial biomass and N_{min} derives from a pool with the same decomposability and this might explain why the decomposability and recovery of N is apparently similar.

The results are also in good agreement with a complementary pot experiment of Mayer et al. (2003b) who found a similar availability of crop residue N and NdfR in subsequent wheat and rape derived from the same legumes and soil.

Considering the absolute amount of soil derived N in the microbial biomass, an effect was only observed by the addition of recoverable residues which increased the immobilisation of soil derived N. The presence of rhizodeposits had no effect on the absolute amount of soil derived N in the microbial pool after addition of crop residues (data not shown). The differences found in microbial N content and C mineralisation between treatments +rhizodeposition and –rhizodeposition can therefore be attributed to a differing utilisation of the legume residues by the microbial population triggered by rhizodeposition effects.

This hypothesis is supported by our observation of the formation of microbial residues derived from crop residues. The calculated amount was significantly increased by the presence of rhizodeposits in all treatments and approached that of the treatments without rhizodeposits during the incubation (Fig. 7). One possible explanation for this effect may be a pool substitution of previously immobilised NdfR in the labile pool of microbial biomass. However, the amount of NdfR in this pool was small and can explain less than half of the observed effect, assuming that the total of NdfR immobilised in the microbial pool will be substituted. Thus the effect of rhizodeposition seems to enhance the immobilisation of crop residue N either as microbial biomass or microbial residues.

However, the C:N ratio of the crop residues used in our experiment was comparatively high for grain legumes (Table 2) and caused a C excess over the whole incubation period. Thus our results must be considered under this aspect and may be altered with residues with a low C:N ratio.

Conclusions

This study confirms the important role of N derived from rhizodeposition for N dynamics after cropping of grain legumes. The N derived from rhizodeposition is mainly immobilised as microbial residues and forms a labile pool of SOM, which provides the main pool for remineralisation of rhizodeposition N.

The main rhizodeposition effect observed resulted in an acceleration of C-turnover for lupin residues and it encouraged the N immobilisation of added legume residues as microbial residues under C rich and N limiting conditions. The fixation of residual N in this pool explains on the one hand the relatively small direct N contribution to a succeeding non legume. On the other hand the size of this labile pool may be increased and provides an N source with a medium decomposability. In this context the relevance of the rhizodeposition N, which contributes about 35% - 45% of total residual N, becomes quite clear. Both rhizodeposition N and the N of the crop residues will be immobilised in the same pool with a similar decomposability and will thus contribute to a very similar extent to further mineralisation dynamics.

Abbreviations

C or N_{dec} = carbon or nitrogen decomposed from residues

C or N_{mic} = microbial carbon or nitrogen

C or N_{micres} = microbial residue carbon or nitrogen

C or N_{min} = mineralised carbon or nitrogen

C or N_{input} = added C or N as crop residues and/or rhizodeposits

dfr = derived from residues

dfR = derived from rhizodeposition

Ndfr = nitrogen derived from residues

NdfR = nitrogen derived from rhizodeposition

N_{loss} = losses of N derived from residues

SOM = soil organic matter

WHC = water holding capacity

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6. Residual nitrogen contribution from grain legumes to succeeding wheat and rape and related microbial process

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Abstract

The residual N contribution from faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.) and white lupin (*Lupinus albus* L.) to microbial biomass and subsequent wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) was studied in a greenhouse experiment. The grain legumes were ^{15}N labelled *in situ* with a stem feeding method before incorporated into the soil, which enables the determination of N rhizodeposition. Wheat and rape were subsequently grown on the soil containing the grain legume residues (incl. ^{15}N labelled rhizodeposits) and were harvested either twice at flowering and at maturity or once at maturity, respectively.

The average total N uptake of the subsequent crops was influenced by the legume used as precrop and was determined by the residue N input and the N_2-fixation capacity of the legume species. The succeeding crops recovered 8.6% to 12.1% of the residue N at maturity. Similar patterns were found for the microbial biomass, which recovered 8.2% to 10.6% of the residue N. Wheat and rape recovered about the same amount of residue N. The absolute contribution of soil derived N to the subsequent crops was similar in all treatments and averaged 149 mg N pot^{-1} at maturity. At flowering 17% to 23% of the residue derived N was recovered in the subsequent wheat and in the microbial biomass. 70% of the residue N was recovered in the microbial biomass in the flowering stage and decreased to about 50 % at maturity. In contrast, the recovery in wheat and rape constituted only 30% at flowering and increased to 50% at maturity in all treatments, indicating that the residual N uptake by the subsequent wheat was apparently supplied by mobilisation of residue N temporarily immobilised in the microbial biomass.

6.1. Introduction

In crop rotations, grain legumes contribute to a diversification of cropping systems and as N_2-fixing plant it can reduce the mineral N fertilizer demand. In the temperate climate of north-western Europe the implementation of a higher degree of cool season grain legumes as faba bean, pea or lupin, can improve the self-sufficiency in protein and reduce soybean imports. Generally in sustainable and organic farming systems, biological N_2-fixation by legumes is the main source of nitrogen for the crop rotation. Hence cropping of grain legumes is a prioritised area of research in the European Union.

Grain legumes cause significant, positive yield effects on subsequent non-legumes when compared with rotations with non-legumes (Chalk, 1998). In addition to its beneficial factors, such as improving soil structure, breaking pest and disease cycles and the phytotoxic and allelopathic effects of crop residues, nitrogen is a key factor in the positive response of cereals following legumes (Chalk, 1998).

However the improvement in N nutrition of non-fixing crops in grain legume-based cropping systems requires a more fundamental understanding of the microbial decomposition of grain legume residue processes and the interactions with soil organic matter. Grain legume species and varieties growing at the same location differ significantly in dry matter production, N accumulation, N_2-fixation, N-balance and residue quality (Beck et al., 1991; Evans et al., 2001; Haynes et al., 1993; Jensen, 1986; Schmidtke, 1996). These differences may be the main factors determining the residual N contribution to subsequent crops (Hood et al., 1999; Senaratne and Hardarson, 1988). In most experiments investigating the grain legume residue contribution to subsequent crops ^{15}N labelled above-ground residues or "recoverable" residues (= stems + leaves + roots) were incorporated in soil previously cropped with the respective non-labelled legume (Bremer and Van Kessel, 1992a; Hood, 2001; Jensen, 1994a; Stevenson and Van Kessel, 1997). However, this methodology does not take into account the below-ground nitrogen including the N in rhizodeposition. The N rhizodeposition constitutes a significant pool of the below-ground nitrogen in grain legumes ranging from 47% to 80%, and constitutes between 35% and 45% of total residue N (Jensen, 1996b; Mayer et al., 2003; Russell and Fillery, 1996a; Sawatsky and Soper, 1991). Some authors used the isotope dilution technique (Hood et al., 1999) or the A-value technique (Senaratne and Hardarson, 1988; Stevenson et al., 1998). In principle these techniques include the contribution of the N rhizodeposition pool, to determine the N contribution to subsequent crops. However, using

these techniques pool substitution effects occur by microbial immobilisation of added ^{15}N fertilisers and lead to an overestimation of the residue derived N contribution in subsequent crops (Hood et al., 2000; Hood et al., 1999). To overcome the problem of pool substitution Hood et al. (2000) and Hood (2001) proposed ^{15}N stabilisation in the soil by pre-labelling the soil. However, the pre-labelling method has not been used in studies to determine N contributions from rhizodeposits. Including the N rhizodeposition pool in residue N studies requires a direct *in situ* labelling methodology, which enables also the ^{15}N labelling of rhizodeposits e.g. by stem feeding (Mayer et al., 2003; Russell and Fillery, 1996b), leaf feeding (McNeill et al., 1998) or spraying (Zebarth et al., 1991) and ideally provides a homogeneous labelling of all crop residues (Hood et al., 1999). Stem feeding using cotton-wicks can be used for grain legumes. The method is relatively simple and provides a more homogeneous ^{15}N distribution in the plants compared to leaf feeding techniques (Mayer et al., 2003).

The turnover of plant residues and the N contribution to subsequent crops may not only be influenced by residue parameters such as the quantitative input and residue quality. During the decomposition process the growth of subsequent crops will compete with the microbial biomass for nitrogen and other nutrients (Kaye and Hart, 1997), leading to a differentiation in the decomposition process compared to a non cropped soil (Bottner et al., 1999). Plants also affect the residue turnover by excreting easily available organic C and N compounds which can interact directly with the microbial biomass and affect its size, activity, turnover rate or physiological status (Korsaeth et al., 2001). These interactions can have direct effects on the C and N mineralization of the native soil organic matter (SOM) and the decomposition of plant residues. Beside well-known adaptation mechanisms for nutrient acquisition such as root morphology, mycorrhiza or release of organic acids and phytosiderophores (Jones, 1998; Marschner, 1995), plants are able to influence the mobilisation-immobilisation turnover in soils (Zagal, 1994; Cheng and Coleman, 1990; Kuzyakov et al., 2000) and on top of that might be differences between plant species (Van der Krift et al., 2001). This might lead to differences in competitiveness for mineral N, N immobilisation-mineralization and residue N recovery after incorporation of plant residues by contrasting succeeding plant species.

Our objectives in this study were (i) to determine the potentials of the residual N contribution of faba bean, pea and white lupin to the N supply of succeeding wheat and rape including the N-rhizodeposition of the previous grown grain legumes, (ii) to investigate possible interactions between the quality of the grain legume species and (iii) the effects of two contrasting

subsequent crops – wheat and oilseed rape – on the residue N recovery and the soil microbial biomass.

6.2. Materials and methods

Soil

Soil samples were taken from the top 0 – 200 mm of an Eutric Cambisol in north-west Germany (52,31° nord; 8,13° east). The field had been cultivated under organic farming management for 10 years. After sampling, the soil was sieved to pass a 10 mm sieve and stored moist in a cool (6°C) and dark place until initiation of the experiment. The soil was characterised as a sandy loam with 17.3% clay, 30.1% silt, 52.6% sand, pH (0.01 M $CaCl_2$) of 6.0, 1.58% total C, 0.15% total N, 140 mg P kg^{-1} (Calcium-lactate), 208 mg K kg^{-1} (Calcium-lactate), and 100 mg Mg kg^{-1} (Calcium-lactate). The water holding capacity (WHC) (10 mm sieved soil) was 309 g H_2O kg^{-1} dry soil.

Preceding grain legumes

Faba bean (*Vicia faba* L., cv. Scirocco), pea (*Pisum sativum* L., cv. Duel) and white lupin (*Lupinus albus* L., cv. Amiga) were grown in 8.5 l pots (sowing: May 4th 1999) in a greenhouse experiment without additional fertilization; the plants were ^{15}N pulse labelled *in situ* using the cotton wick method (Russell and Fillery, 1996b). The biological nitrogen fixation was determined with additional pots using the extended difference method (Stülpnagel, 1982).

At maturity (pea: July 27th, faba bean: August 18th, lupin: September 6th 1999) the aboveground plant parts were harvested and separated into grain, stem and leaves. A sub-sample of 20% of the soil was taken to estimate the root mass, root N uptake and the amount of N rhizodeposition in the whole pot (Mayer et al., 2003). The remaining undisturbed soil monoliths were stored in the dark at 4°C.

Succeeding crops and experimental design

At January 3rd 2000 the replicate soil monoliths including undisturbed roots and rhizodeposition N were merged. The intact main roots of faba bean and lupin were cut into 5 mm pieces

Table 1. Harvest parameters, ^{15}N enrichment, residue input and residue quality of faba bean, pea and white lupin used in the experiment

	Faba bean	Pea	White Lupin
Harvest parameters			
Dry matter yield (g kg^{-1} soil)	14.2	6.6	11.9
Total N (incl. rhizodeposition) (mg kg^{-1} soil)	459	167	333
Grain N (mg kg^{-1} soil)	286	120	205
Ndfa (%)	93	80	89
N-balance (mg kg^{-1} soil)[*]	142	13	90
Residue input			
Leaves + stem + root N (mg kg^{-1} soil)	112	26	76
Rhizodeposition N[**]	61	21	52
Residue N total (mg kg^{-1} soil)	183	47	128
Leaves + stem + root C (g kg^{-1} soil)	3.4	1.3	3.6
^{15}N enrichment of residues			
Leaves (atom % ^{15}Nexcess)	1.66	2.84	1.56
Stem (atom % ^{15} excess)	1.95	2.75	1.95
Root and rhizodeposition[***] (atom % ^{15}Nexcess)	1.44	1.32	1.23
Weighted mean of residues (atom % ^{15}Nexcess)	1.69	2.19	1.56
Residue quality (Leaves + stem + root)			
Cellulose (%)	39	49	42
Lignin (%)	15	10	14
H$_2$O soluble C (%)	10.9	7.6	10.1
H$_2$O soluble N (%)	0.34	0.16	0.25
C:N	31	50	48
H$_2$O soluble C:N	32	47	41
Lignin :N	18	39	16

[*]   N-balance = total N * % Ndfa – grain N
[**]   Rhizodeposition N = Plant derived N remaining in the soil after removal of all visible roots and root fragments
[***] ^{15}N enrichment of rhizodeposition is assumed to be similar to the roots

and added to the soil. Subsequently the soil was mixed with the above-ground residues (stems and leaves) of each grain legume species (ground to a size of < 6 mm) according to the residual N uptake per kg soil. The residue C and N inputs and the residue quality are shown in Table 1. The ^{15}N natural abundance values were determined including controls of the experimental soil. Portions of 6.5 kg soil (dry weight basis) were filled into PVC pots (300 mm height, 150 mm inner diameter) and mechanically compressed to a density of 1.3 kg l^{-1} dry soil. Each pot was put in a bucket to collect a possible leachate after watering. The pots were placed in a greenhouse with 16 h additional lighting, watered daily with deionised water and kept at a WHC between 40% and 70% by weighing twice a week. The temperature regime was staggered in monthly blocks with increasing mean temperatures starting with 13°C in January up to 18°C in June. The mean temperature over the whole growing period was 16°C. The plants did not get any additional fertilization.

On January 19th summer wheat (*Triticum aestivum* L. cv. Kalistos; 14 plants pot^{-1}) and summer oilseed rape (*Brassica napus* L., cv. Licosmos; 5 plants pot^{-1}) were sown in pots incorporated with grain legume residues and without (control). The experiment was arranged as a randomised block design with five replicates. The wheat was harvested at flowering and at maturity and the rape at maturity only.

At harvest shoots were cut above the soil and the grain separated from the shoot. A subsample of about 15% of the soil was taken from each pot throughout the entire profile with an auger (diameter 34 mm). The mineral N (N_{min}) content and the microbial biomass were analysed directly after sampling. A portion of 200 g from the sub-sample was cleaned of all visible roots and root fragments and analysed for N and ^{15}N content.

Analytical methods

All plant and soil samples were dried at 60°C for 72 h and ground to a fine powder using a centrifuge mill and a ball mill.

To determine the mineral N content 60 g of the fresh soil was extracted immediately after sampling with 250 ml 2 M KCl for 30 minutes on a horizontal shaker (Schinner et al., 1996). The extracts were stored at –22°C until analysis. The NO_3^- and NH_4^+ contents were analysed using a continuous flow analyser (Evolution II, Alliance Instruments, France).

The microbial biomass C and N was determined using the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). Before extracting and fumigating the samples, the living roots were separated from the soil by a pre-extraction step (Müller et al., 1992; Olfs and Scherer, 1996). Two portions of 60 g fresh soil were extracted with 200 ml 0.05 M K_2SO_4 for 20 minutes, put on a 1 mm sieve and additionally washed with 150 ml 0.05 M K_2SO_4. The soil water suspension was collected in a beaker and left for 30 minutes to let the soil settle. The roots which were not kept back by the sieve were sucked with a tube connected with a vacuum pump. Subsequently the soil water suspension was filtered (Schleicher & Schüll 595 ½ filter paper). One sample (soil + filter) was immediately extracted with 200 ml 0.5 M K_2SO_4 for 30 minutes. The second sample was fumigated with 25 ml chloroform in a desiccator for 24 h at 25°C and later extracted with 0.5 M K_2SO_4. The organic C and total N contents of the extracts were determined with a TOC / TN_b analyser (Dimatoc 100, Dimantec, Germany). A k_{EC} of 0.45 and a k_{EN} of 0.54 were used for calculating the biomass C and N (Jörgensen, 1996; Jörgensen and Müller, 1996).

The ^{15}N content of the biomass extracts was determined using a modified diffusion technique (Goerges and Dittert, 1998). Before diffusion the N in the extracts was oxidised to NO_3^+ using $K_2S_2O_8$ and autoclaving (Cabrera and Beare, 1993).

The plant and soil samples and the diffusion filters were analysed for total N and ^{15}N content with an emission spectrometer (NOI-6PC, Fischer Analysen Instrumente, Germany; ^{15}N abundance > 1.5 atom% ^{15}N) and an isotope ratio mass spectrometer (delta E, Finnigan MAT, Germany; ^{15}N abundance < 1.5 atom% ^{15}N), both coupled with a Carlo Erba NA 1500 C / N analyser.

The cellulose and lignin contents were determined by the acid detergent fibre method (Van Soest, 1963). The cold water soluble C and N contents of the "recoverable" residues (leaves + stems + roots) were determined according to Collins et al. (1990). 500 mg finely ground residue matter was extracted 7 times for 20 minutes with 50 ml deionised water at room temperature (ca. 20°C) on a horizontal shaker. After each extraction the suspension was centrifuged (3000 G) for 20 minutes and the clear supernatant was decanted. The 7 extracts were combined and analysed for total C and N at a TOC / TN_b analyser (Dimatoc 100, Dimantec, Germany).

Calculations and statistics

The percentage of nitrogen derived from grain legume residues (Ndfr) in the succeeding crop (SC) and the microbial biomass (MB) were calculated as following:

$$\% \ Ndfr \ = \frac{atom \ \% \ ^{15}N \ excess \ SC \ or \ MB}{atom \ \% \ ^{15}N \ excess \ legume \ residues} \times 100 \tag{1}$$

The recovery of nitrogen derived from grain legume residues in the SC and MB (% of input) was calculated:

$$\% \ recovery_{grain \ legume \ residues} = \frac{\% \ Ndfr \times total \ N_{SC \ or \ MB}}{total \ N_{legume \ residues}} \times 100 \tag{2}$$

The ^{15}N excess enrichment in the microbial biomass was calculated:

atom % ^{15}N excess microbial biomass

$$= \frac{(N_{fum \ soil} \times \%^{15}N \ excess_{fum \ soil} - N_{non \ fum \ soil} \times \%^{15}N \ excess_{non \ fum \ soil})}{(N_{fum \ soil} - N_{non \ fum \ soil})} \times 100 \tag{3}$$

fum soil = fumigated soil

non fum soil = non fumigated soil

The atom % ^{15}N excess values were obtained by correcting the ^{15}N enrichments with the background values of the control soil without labelling (0.3689 atom % ^{15}N).

The calculations were done under the assumption that the N in different plant parts of the grain legumes (e.g. leaves, stem, roots) with different ^{15}N enrichments are mineralised at the same rate and that the ^{15}N enrichment of the residue N mineralised is equal to the ^{15}N enrichment of the grain legume residue N.

The GLM procedure of the SPSS 10.0 statistics package (SPSS GmbH, Germany) was used to carry out the statistical analysis. Means were compared by the Tukey-test ($p < 0,05$) if the analysis of variance showed significant main effects or interactions.

6.3. Results

Dry matter accumulation and N uptake by the plants

The residue incorporation caused significant differences in the dry matter accumulation of wheat and rape. Wheat accumulated from 15.3 g to 27.6 g dry matter pot^{-1}, rape from 16.8 g to 21.3 g pot^{-1} (Table 2).

Table 2. Dry matter yield, N uptake, ^{15}N enrichment, nitrogen derived from residues (Ndfr) and nitrogen derived from soil (Ndfs) in succeeding wheat and rape

Residues			Faba bean		Pea		White Lupin	
Succeeding crop			Wheat	Rape	Wheat	Rape	Wheat	Rape
Dry matter (g pot^{-1})	flowering	total	14.4^{a*}	-	10.2^{b}	-	9.5^{b}	-
	maturity	grain	9.0^{c}	4.9^{a}	5.8^{ab}	4.5^{a}	7.2^{bc}	4.6^{a}
		total	27.6^{c}	21.3^{b}	15.3^{a}	16.8^{a}	15.8^{a}	18.1^{ab}
Total N (mg pot^{-1})	flowering	total	132^{a}	-	88^{b}	-	116^{ab}	-
	maturity	grain	164^{a}	148^{a}	124^{a}	136^{a}	162^{a}	139^{a}
		total	244^{b}	238^{b}	176^{a}	202^{ab}	215^{ab}	228^{ab}
^{15}N enrichment (% ^{15}Nexcess)	flowering	total	0.77	-	0.47	-	0.52	-
	maturity	total	0.72	0.70	0.40	0.40	0.50	0.48
Ndfr (mg pot^{-1})	flowering	total	60^{a}	-	19^{b}	-	39^{c}	-
	maturity	total	103^{a}	99^{a}	32^{b}	36^{b}	69^{c}	70^{c}
Ndfs (mg pot^{-1})	flowering	total	72^{a}	-	69^{a}	-	77^{a}	-
	maturity	total	141^{a}	140^{a}	145^{a}	165^{a}	146^{a}	156^{a}

*Values in lines with different letters show means (n=5) with significant differences (Tukey, $p < 0.05$).

The N uptake for wheat at maturity ranged from 176 mg pot^{-1} in soil incorporated with pea residues to 244 mg N pot^{-1} in soil incorporated with faba bean residues (Table 2). For rape values for N uptake at the same plant development stage were from 202 mg pot^{-1} in the succession of pea to 238 mg pot^{-1} in the succession of faba bean. A plant-dependent N-uptake rate (wheat vs. rape) was not visible in these experiments. The percentage of the grain N related to total plant N was between 60% and 75% for both plants, regardless of the grain legume used (Table 2).

Microbial C and N

The mixed soil at the beginning of the experiment before incorporation of the above-ground residues containing roots and rhizodeposits had similar microbial C contents of 215 to 229 µg C g^{-1} soil and microbial N contents of about 35 µg N g^{-1} (Table 3).

Table 3. Mineral N and microbial C and N contents in soil containing "undisturbed" roots[*] and rhizodeposits of faba bean, pea and white lupin before above-ground residue application (stems and leaves) at start of the experiment (3rd January 2000)

Soil	Faba bean	Pea	White Lupin
Microbial C (µg g^{-1})	229	215	226
Microbial N (µg g^{-1})	35	36	35
Microbial C:N	6.6	6.0	6.5
Microbial N derived from rhizodeposition and "undisturbed" roots[*] (%)	30	8	23
Mineral N (µg g^{-1})	14	11	15

[*] "undisturbed" roots: Pots with intact soil profile were stored after harvest of grain legumes with the intact roots until start of the experiment. Samples were taken directly after merging the replicate pots. Except the main roots of faba bean and lupin, the roots were not chopped.

The above-ground residue addition caused an increase and a significant differentiation of the microbial C in the wheat pots at flowering from 333 µg C g^{-1} soil with faba bean residues to 268 µg C g^{-1} with pea residues (Table 4). This corresponds to an increase of 45%, 25% and 30% for faba bean, pea and lupin residues respectively. From flowering to maturity the absolute microbial C amount in wheat decreased by 26% for faba bean, 15% for pea and 17% for lupin residues and reached values of biomass C close to the levels before the above-ground residue addition. The pea residue soil differed significantly from faba bean and lupin. The soil grown with rape had slightly higher microbial C contents in the faba bean and lupin treatment at the maturity stage (Table 4).

Microbial N amounts from 61 to 45 µg N g^{-1} soil were found for wheat at flowering, corresponding to an increase from the start of the experiment of 74%, 25% and 29% for faba bean, pea and lupin respectively. The following decrease was higher compared to the C content and constituted 33%, 29% and 22% for the faba bean, pea and lupin soil respectively. This caused

a change to a wider C:N ratio of the microbial biomass (Table 4). In contrast to the microbial C content, significant differences were found for microbial N between the three residue types and between wheat and rape at maturity. The mean microbial biomass N ranged from 43 to 33 μg N g^{-1} for the residues and differed slightly from 36 to 38 μg N g^{-1} between the succeeding crops (Table 4).

As expected the mineral N was completely consumed by the plants or immobilised (Table 4).

Table 4. Microbial C and N, microbial ^{15}N enrichment, microbial N derived from residues (Ndfr) and from soil (Ndfs) and mineral N at harvest of wheat and rape

Residues		Faba bean		Pea		White Lupin	
Succeeding crop		Wheat	Rape	Wheat	Rape	Wheat	Rape
Microbial C (μg g^{-1} soil)	flowering	333^{a*}	-	268^b	-	294^c	-
	maturity	246^{bc}	262^{cd}	227^a	220^a	245^b	263^d
Microbial N (μg g^{-1} soil)	flowering	61^a	-	45^b	-	45^b	-
	maturity	41^a	45^a	32^c	33^{bc}	35^{bc}	37^b
Microbial C:N	flowering	5.5^a	-	6.0^{ab}	-	6.5^b	-
	maturity	6.0^{ab}	5.9^a	7.0^c	6.6^{bc}	7.1^c	7.1^c
Microbial ^{15}Nexcess (%)	flowering	0.61	-	0.34	-	0.46	-
	maturity	0.59	0.59	0.30	0.30	0.42	0.42
Microbial Ndfr (μg g^{-1} soil)	flowering	25^a	-	7^b	-	14^c	-
	maturity	15^a	16^a	5^b	5^b	10^c	10^c
Microbial Ndfs (μg g^{-1} soil)	flowering	36^a	-	38^a	-	31^b	-
	maturity	26^{ab}	29^b	28^{ab}	29^b	24^a	27^{ab}
Mineral N (μg g^{-1} soil)	flowering	1.0^a	-	0.7^b	-	1.6^c	-
	maturity	1.6^a	1.2^a	1.9^a	0.8^a	1.2^a	1.4^a

*Values in lines with different letters show means (n=5) with significant differences (Tukey, $p < 0.05$).

Residual N contributions to the succeeding crop and the microbial biomass

The nitrogen derived from the residues in the succeeding crop (Ndfr) constituted between 19 mg (faba bean) and 60 mg N pot^{-1} (pea), corresponding to 22% and 46% of total wheat N at flowering. At maturity the mean Ndfr in wheat and rape constituted 101 mg for faba bean, 34 mg for pea and 69 mg for lupin pot^{-1}, corresponding to 42%, 18% and 31% of total N, re-

spectively. The residue-derived N differed significantly between the residue types at flowering and at maturity but showed no difference between wheat and rape. The soil derived N was similar in all treatments and averaged 73 mg pot^{-1} at flowering and 149 mg pot^{-1} at maturity (Table 2).
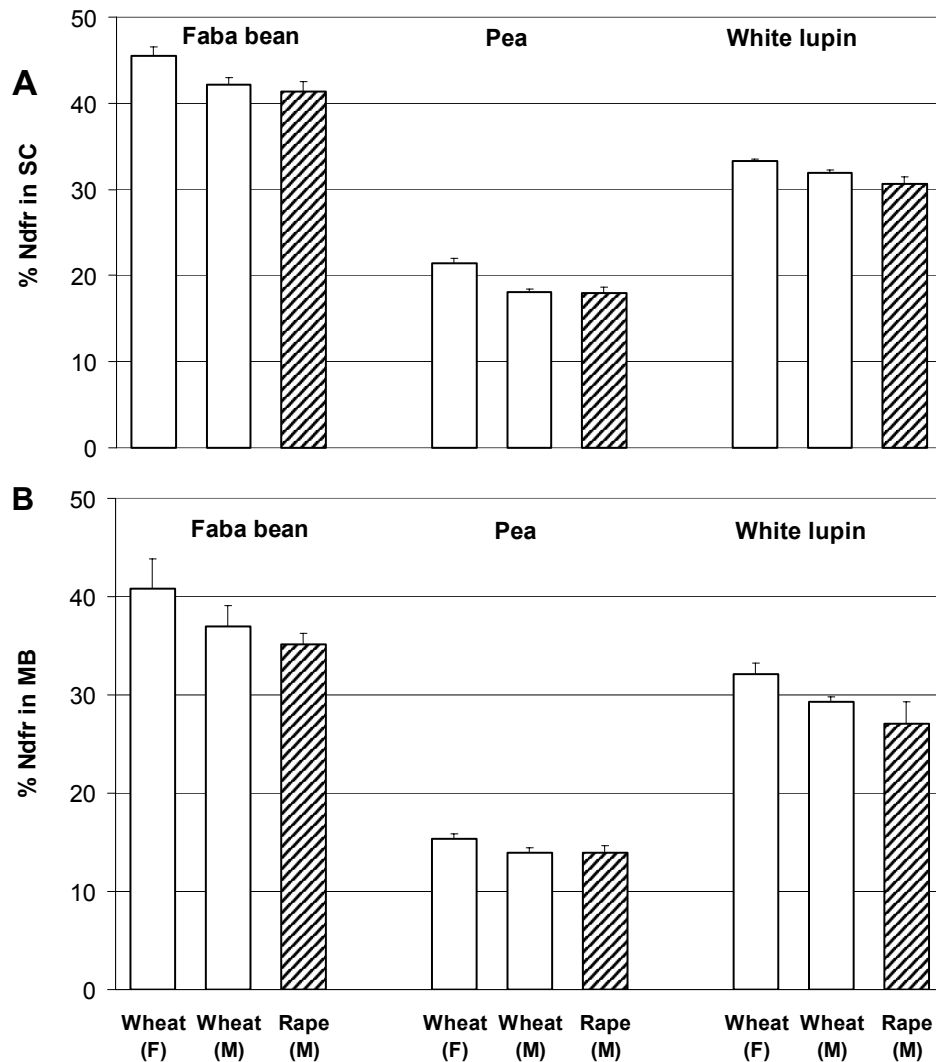

Figure 1. Percent nitrogen derived from residues (% Ndfr) from faba bean, pea and lupin measured in succeeding crops (SC) of wheat and rape (A) and microbial biomass (MB) (B) at flowering (F) and maturity (M). Error bars show the standard deviation.

Similar patterns were found for the Ndfr immobilised in the microbial biomass. At flowering the Ndfr constituted between 25 (faba bean) and 7 (pea) μg N g^{-1} soil corresponding to 41% and 15% of microbial N. At maturity the microbial Ndfr decreased and constituted as a mean of wheat and rape 16 μg N g^{-1}, 10 μg N g^{-1} and 5 μg N g^{-1} for faba bean, lupin and pea respectively (Table 4), corresponding to 36%, 28% and 14% of total microbial N (Figure 1). The

results clearly indicate that the absolute microbial residue derived N differed significantly between the residue types at flowering and at maturity. However, no differences between wheat and rape were found.

The soil derived microbial N (microbial Ndfs) differed significantly between the lupin soil with 31 µg N g^{-1} to 36 and 38 µg N g^{-1} for faba bean and pea at flowering. Rape, with a mean of 28 µg N g^{-1}, caused a significantly higher soil N immobilisation compared to wheat with 26 µg N g^{-1} (Table 4). The ^{15}N recovery of the residues in wheat was highest for the pea residues with 6.7% of input followed by faba bean with 5.7% and lupin with 4.8% at flowering (Figure 2). The differences were significant between pea and lupin. At maturity as mean val-
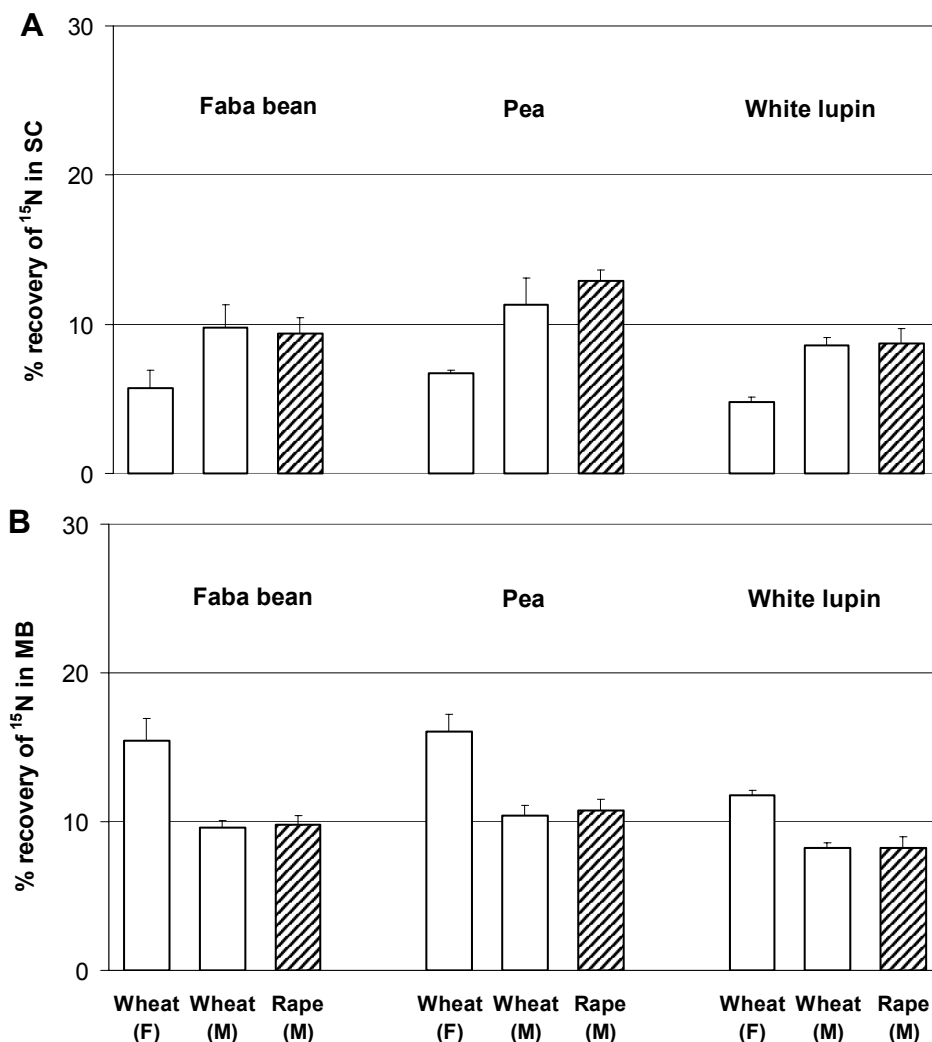

Figure 2. ^{15}N recovery (% of input) of faba bean, pea and lupin residues in succeeding crops (SC) of wheat and rape (A) and microbial biomass (MB) (B) at flowering (F) and maturity (M). Error bars show the standard deviation.

ues of wheat and rape 12.1%, 9.6% and 8.6% were recovered from pea, faba bean and lupin, respectively. Pea differed significantly between faba bean and lupin. No significant differences were obtained between wheat and rape (Figure 2).

The microbial biomass had recovered similar amounts of residue ^{15}N at maturity: from pea residues 10.6% slightly smaller compared to the plant recovery, from faba bean 9.7% and from lupin 8.2% similar to the plant recovery. As in the plants the recovery showed no differences between wheat and rape. At flowering the recovery of 16.0% to 11.8% was much greater compared to maturity (Figure 2).

At maturity the residue recovery in both pools - microbial biomass and succeeding crop - amounted to 22.7% for pea, 19.0% for faba bean and 16.9% for lupin. However, already at flowering this amount was recovered in wheat and the total pool size kept constant until maturity (Figure 3).
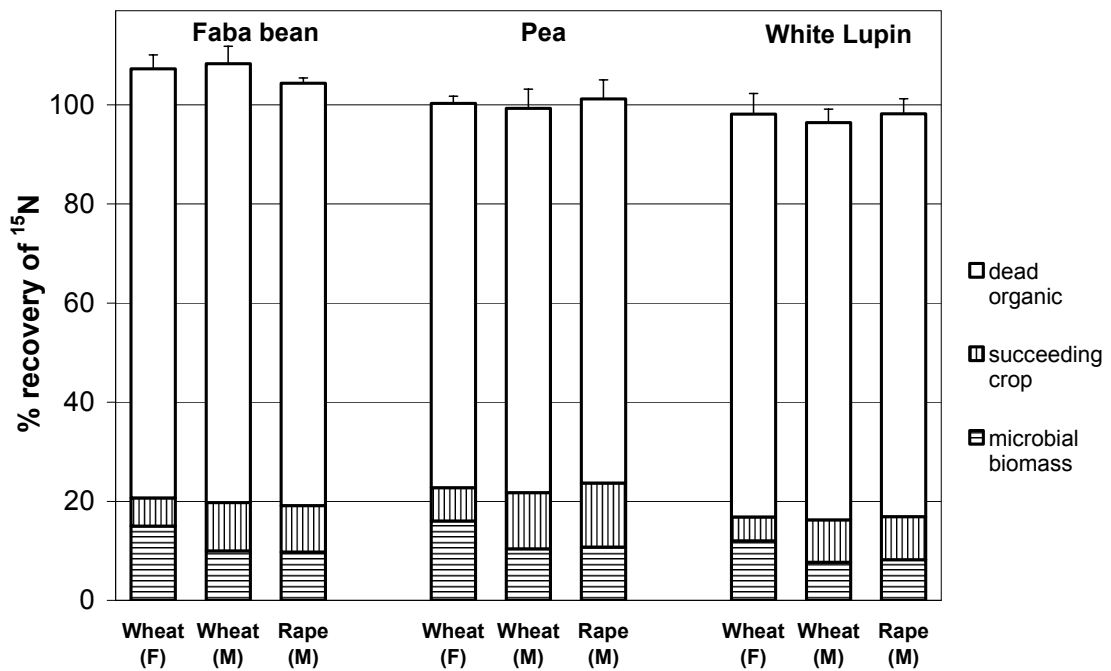

Figure 3. Total ^{15}N recovery at flowering (F) and maturity (M) of wheat and rape in the pools of soil-plant system - microbial biomass, succeeding crop and dead organic residue N - with faba bean, pea and white lupin residues. Dead organic: residue ^{15}N not decomposed or immobilised as dead microbial residues. Error bars show the standard deviation.

From the calculated added ^{15}N as residues about 106% were recovered in soil and plants for faba bean, about 100% for pea and 98% for lupin at flowering and maturity, respectively. No decline was observed from flowering to maturity, suggesting that no ^{15}N was lost from the system (Figure 3).

6.4. Discussion

The input in the system by the grain legume species varied significantly. The total residue N input of faba bean was almost four times greater than in pea and 1.4 times higher than in lupin. The C:N ratios varied between 31 and 50 for the "recoverable" residues (leaves + stems + roots) (Table 1). Due to these differences, major effects on the N dynamics in the soil-plant system were expected.

Dry matter accumulation and N uptake

The grain legume inputs caused differences in the wheat grain yield but no differences in the rape grain yield (Table 2). This response could not be observed for the N output, which suggests an uncoupling of the dry matter production and the N supply (Table 2). This may be explained by a non-synchronised N supply relative to the plant N demand mainly in the vegetative growing phase (Myers et al., 1997).

In agreement with Hood et al. (1999) reporting an N effect of different residues in subsequent maize, the N uptake in the subsequent crop in the present experiment increased linearly with the amount of the N input. Also the N balance was closely linked to the N uptake (Figure 4).

The differences in N uptake can be explained by the Ndfr, which differed significantly at flowering and maturity between the treatments. The nitrogen derived from soil (Ndfs) was constant between all treatments at flowering and at maturity (Table 2), indicating that pool substitution effects were not high in this experiment. The % Ndfr was greatest for faba bean, followed by lupin and pea (Figure 1). No residue effects on Ndfs were observed by Jensen (1996a) with two contrasting residues, pea and barley, cropped with ryegrass after two years and also no succeeding crop effect on Ndfs was observed by Jensen (1994a) in succeeding barley and rape after pea. However, Jensen (1996a) found a greater amount of soil derived N in ryegrass after pea residue incorporation compared to barley in the first two cuts (214 and 287 days after residue incorporation) and explained it by a stronger soil N immobilisation after barley residue incorporation. Bremer and van Kessel (1992a) found a similar immobili-

sation of soil N and fertiliser N after wheat and lentil straw incorporation, but an additional mobilisation after lentil green manure incorporation.
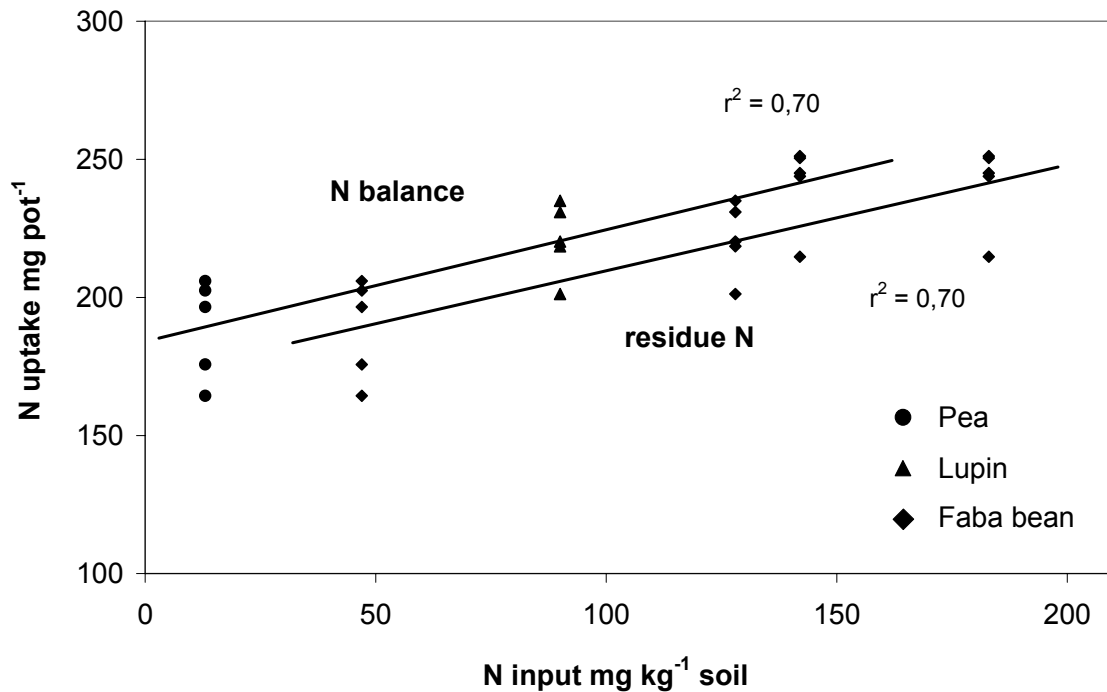

Figure 4. Relation between grain legume residue N input, N balance and N uptake of the succeeding crops.

Microbial C and N

As reported by several authors above-ground residue incorporation causes an increase in microbial C and N contents followed by a subsequent decrease (Bremer and Van Kessel, 1992b; Bending et al., 1998; Bending and Turner, 1999; Jensen, 1994b; Jensen, 1994c; Jensen et al., 1997). However, the disproportionate alteration of the microbial C and N contents led to a distinct decrease of the C:N ratio for faba bean residues at flowering but no change in the pea and lupin treatments. This might be explained by a better N supply to microorganisms under N limiting conditions by the relatively low C:N ratio of the faba bean residues compared to pea and lupin (Table 2; Table 4). The increasing C:N ratio combined with a decrease in microbial C and N from flowering to maturity in wheat can be explained by an improved competitiveness of the plants for N driven by an increasing root system and a root induced acceleration of the microbial turnover rate (Bottner et al., 1999). Under N limiting conditions (Table 4) and sufficient C supply by the roots combined with an increasing competitiveness of the plants for N, the microbial biomass will be severely N limited and this will reduce the

microbial growth (Korsaeth et al., 2001). However, following this concept, N will be set free from the microbial biomass as microbial residues. It is not clear how this N becomes plant available.

The significant differences in the microbial C:N ratio might be caused by the differences in residue N inputs and quality. However, the relation between the parameters is difficult to prove because data concerning the residue quality are only available for the "recoverable" residues and not for the rhizodeposits. At the start of the experiment the distribution in micro-bial C:N ratio varied from that observed at maturity (Table 3). This suggests that the changes in microbial C:N ratio are mainly caused by the "recoverable" residue inputs.

Wheat and rape showed no differences in microbial C:N ratio at maturity, suggesting that the alteration of the microbial turnover process was influenced by the same order. However, the rape caused a small but significantly greater microbial C and N immobilisation than observed in wheat (Table 3; Table 4). This might be caused by a greater C-input of rape rhizodeposits. However the rhizodeposition quality might also contribute to differences in microbial C and N immobilisation due to an alteration of the microbial population. Brassica species liberate isothiocyanates from the roots which can kill fungi, oomycetes and bacteria. Smith and Kirkegaard (2002) found that bacteria were generally more tolerant against isothiocyanates than the eukaryotic group, although both groups showed considerable variability in response.

Recovery of residue N

At maturity the residue N recovery (% of input) in the succeeding crops constituted 8.6, 9.6 and 12.1% for pea, faba and lupin, respectively (Figure 2). These amounts are in agreement with the findings of several authors in either field or pot experiments in the first succeeding crop. Between 15% and 7% were found for pea (Jensen, 1994a; Jensen, 1996a; Senaratne and Hardarson, 1988; Stevenson and Van Kessel, 1997), but 25% by Hood et al. (1999) for faba bean in a greenhouse experiment in which above-average values for all compared varying residue types were obtained. Russsell and Fillery (1996a) found 13% of below-ground N for lupin (*L. angustifolius*). Our results confirms that the direct contribution of legume residue derived N for subsequent crops is not high. The total N uptake in the differing succeeding crops had no significant effect on residue recovery, suggesting that the influence was equal, whereas Jensen (1994a) found a residue recovery of 15% in winter rape which was signifi-cantly greater than in winter barley with 13%.

In our experiment the N derived from rhizodeposition was ^{15}N-labelled and amounted to 35% to 44% of the total residue N (Table 1). Including this pool, we found similar residue recovery rates to other authors who did not include the N rhizodeposition, suggesting a similar contribution from both pools – N rhizodeposition and "recoverable" N. The relative contribution to the N uptake (% Ndfr) was expected to be greater because N rhizodeposition was included. The % Ndfr obtained in previous experiments ranged between 4.5% and 14% (Jensen, 1994a; Hood et al., 1999; Jensen, 1996a; Senaratne and Hardarson, 1988; Stevenson and Van Kessel, 1997) whereas we found between 18% and 42% at maturity (Figure 1). The extent of % Ndfr is mainly influenced by the availability of the unlabelled source – SOM or fertiliser N – and % Ndfr will be small if the unlabelled source is large (Hood et al., 1999). However, Russell and Fillery (1996a) using the same labelling methodology in a similar experiment found 40% of N derived from below-ground residues of lupin (including N rhizodeposition) in subsequent wheat in a non N fertilised variation. These results suggest that the % Ndfr found by many previous researchers tends to be underestimated and that the actual benefit of grain legumes to subsequent crops is much greater than previously assumed. However, with the design of our experiment this question could not be unambiguously clarified and requires further investigations. Using a cross labelling design with labelled rhizodeposits and unlabelled crop residues (leaves, stems and roots) and vice versa the contribution from the respective pool could be quantified (Hood et al., 1999).

The residue recovery in the microbial biomass was similar compared to that of the plants at maturity (Figure 2). Only the microbial recovery for pea residues was slightly smaller compared to that in the plants. This result apparently shows an equilibrium in competitiveness of plants and microorganisms. However, at flowering the residue recovery was 2 – 3 times greater in microbial biomass compared to the plants. Considering the recovery of both pools - microbial biomass and succeeding plant – the summed pool size kept constant from flowering to maturity. The increasing residue recovery in the plants was solely supplied by the recovered residues in the microorganisms (Figure 2; Figure 3). This confirms the above mentioned hypothesis of an increasing competitiveness of the plants for residual N during plant growth. However, an amount of about 80% of residual N was not recoverable for plants or microorganisms since flowering. It is not clear why this pool size does not change from flowering to maturity and what happened to the N. Was it completely immobilised as microbial residues and incorporated in the SOM or did it remain in the pool of undecomposed residues?

The patterns of the % Ndfr distribution were similar in the microbial biomass and in the plants between the residue types and differed significantly at flowering and maturity, respectively. The distinct differences can be attributed to the amount of the residue inputs. This suggests a similar acquisition capacity for either residue or soil N by both competitors.

However, the % Ndfr was smaller in the microbial biomass compared to subsequent crops in all treatments and differed at maturity between 4% and 28% from the succeeding crops (Figure 1). This can be explained by a differing size of the microbial pool which is dormant and present before incorporation of residues or has a slower turnover rate (Blagodatsky and Richter, 1998).

Comparisons of direct residue N recovery between treatments is the most appropriate way to compare treatments as different amounts of N were added as residues (Hood et al., 1999). We found significant but small differences in the residue N recovery in the plants as well as in the microbial biomass between the residue types. Combining both pools, the recovery ranged from 17% to 23% at maturity (Figure 3). These differences might be caused by a differing quality of the residues, but could not be clearly explained by the "recoverable" residue quality parameters (Table 1).

Because of the specificity of the pot experiment, such as a greater plant and root density, greater residual inputs to the soil, a strong N limitation and differing soil temperatures in the pots, no N-losses by leaching, etc., our results cannot be transferred directly to field conditions. However, the recovery rates obtained were similar to those in various field experiments (Jensen, 1994a; Jensen, 1996a; Russell and Fillery, 1999; Stevenson and Van Kessel, 1997), indicating that our experiment shows the potentials of the residue N recovery in a soil-plant system.

The direct N contribution from a preceding legume to a subsequent non-legume is small also taking into account N rhizodeposition, which represents a significant residual pool (Høgh-Jensen and Schjoerring, 2001; Jensen, 1996b; Mayer et al., 2003). The main part of the residue N derived from rhizodeposition and from the "recoverable residues" will be incorporated into a more or less active SOM pool and influence the N dynamic of the system in the long term (Jensen, 1994a; Russell and Fillery, 1999). To clear up these effects, the role of the N rhizodeposition and the possibilities of agronomic control may be of specific interest in legume-based cropping systems.

Conclusions

In a crop rotation system with temperate grain legumes the absolute N input determined by the respective N_2 fixation capacity of the grain legumes are the main factor for the N contribution in succeeding non-legumes. The recovery and the allocation of the N in the grain of the different residues were only slightly different and did not depend on the quantity of residue input. The effect of residue quality was small in this study, probably because of the relatively similar qualities of the three grain legume residues. However, the residue N effect could not clearly be explained by the quality of the "recoverable" residues and may be influenced by the rhizodeposition pool. The consideration of the N rhizodeposition leads to a greater proportion of N derived from residues in the subsequent crop compared to previous studies. However, a more detailed view on the role of this pool in the N benefit to subsequent crops requires further investigations. Wheat and rape had no differing effect on the residue decomposition and recovered similar amounts of residue and soil N.

The residual N uptake by wheat from flowering to maturity was supplied by the residue N incorporated in the microbial biomass at flowering. The total pool size – residue N in the microbial biomass and crops – did not change.

List of abbreviations

F	flowering
k_{EC} and k_{EN}:	extractable part of the total amount of C (k_{EC}) and N (k_{EN}) fixed in the microbial biomass
M	maturity
MB:	microbial biomass
Ndfr:	nitrogen derived from residues
SC:	succeeding crop

Acknowledgements

6.5. References

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7. Synthesis

15N labelling technique

The ^{15}N pulse labelling using a cotton wick enables progresses in research concerning N rhizodeposition of grain legumes and the related turnover processes in soil. The methodology is relatively simple and the effort is small compared to other methods. The ^{15}N tracer uptake pathway via xylem by a cotton wick can be separated reliably from the environment and thus the risk of a contamination of the soil by e.g. run off of tracer is relatively small. This enables the application under *in situ* conditions in field experiments and might be one reason for the high ^{15}N recovery found in the experiment (Chap. 4.3). The stem feeding pathway via xylem also tolerates high urea concentrations, mainly used as ^{15}N tracer. It encourages a comparatively homogeneous distribution of ^{15}N in differing plant parts as shown in chapter 4.3. Compared to other *in situ* labelling techniques such as leaf feeding or spraying, the cotton wick technique results in a more homogeneous ^{15}N distribution. Hence the improvement of the technique is the possibility to combine investigations on the turnover of N derived from crop residues and N rhizodeposits in soil under field conditions. However, full homogeneity of ^{15}N in differing plant parts could not be achieved and this will limit the use of the methodology for estimations of turnover of residue derived N and requires further improvements.

Root effects and related turnover of grain legume residues in soil

The processes investigated in the three experiments can be assigned to three phases which are typical for cropping systems in temperate climates (Fig. 7.1).

As annual crop with a low frost resistance the *phase I, growth of preceding grain legumes,* lasts from February – April until August – October. Subsequently, *phase II, turnover of grain legume residues in bare soil* after harvest of the grain follows. The length of this period depends on the harvest date of the grain legume species, the sowing date of the subsequent main crop, the type of the subsequent crop – winter or summer form and if a catch crop is used or not. In the best case the bare phase is very short, one or two weeks, in the worst case the phase can last more than half a year. The length of *phase III, growth of a subsequent crop,* depends also on the crop species and the form – winter or summer – and can last from August – November until July – October of the following year.

The results obtained in the experiments aimed to describe the principal processes in these phases and the interaction between the phases. The results of single pot and incubation experiments cannot directly be transferred to field conditions. But the examined processes may give an impression of what could happen under field conditions and help to develop new research strategies for field research.

The processes in the three phases related to root rhizodeposition, turnover of crop residues and role and interactions with microbial biomass can be described as follows (Fig. 7.1):
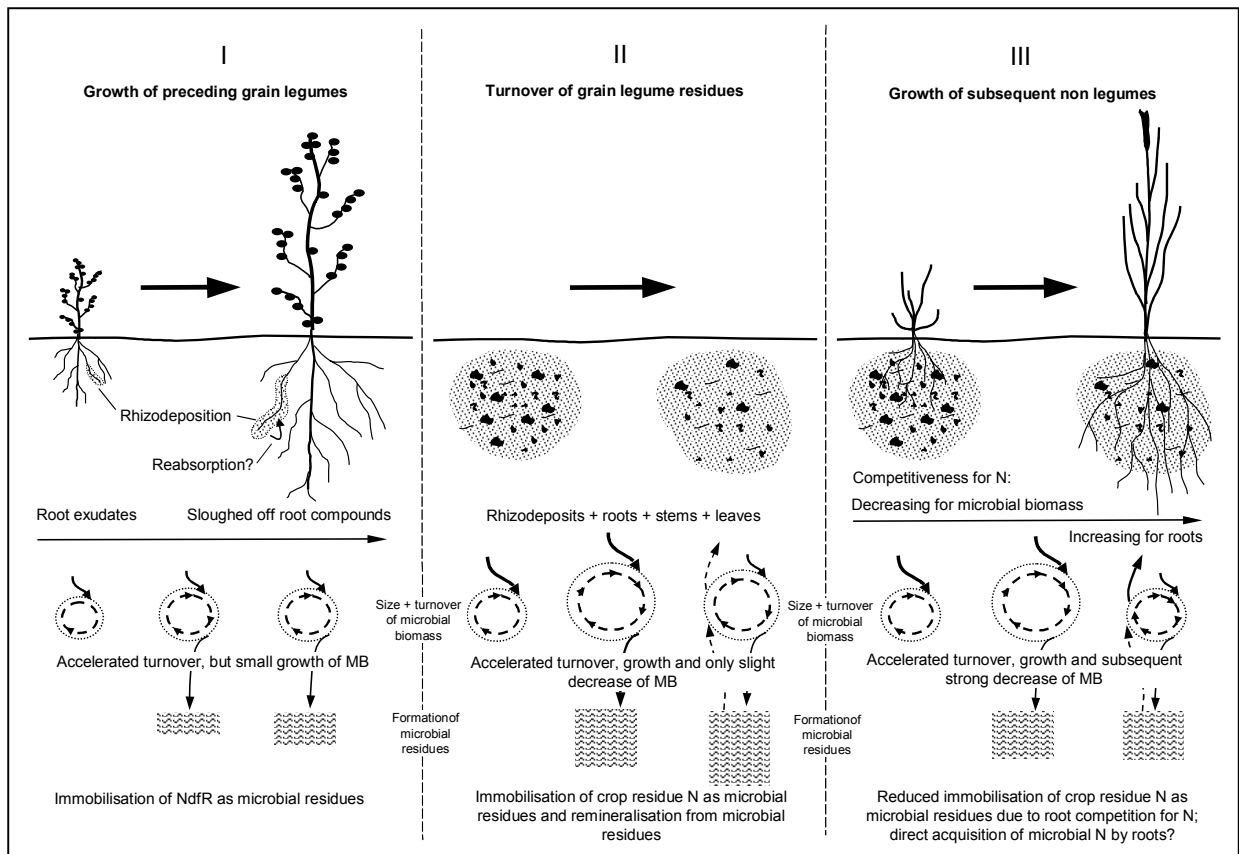
Fig 7.1: Rhizodeposition and residue decomposition of grain legumes and related microbial processes in soil during growth of grain legumes (I), turnover of residues in bare soil (II) and turnover of residues in cropped soil (III).

Phase I: Growth of preceding grain legumes

The N rhizodeposition of grain legumes amounts to about 12% - 16% of total plant N. After harvest, the percentage of rhizodeposition increase to 35% - 44% of residual N and constitutes about 80% of below ground N (Chap. 4.3). Hence it represents a significant pool for N dynamic in crop rotations. However, the role for the dynamics in soil depends mainly on the fate of rhizodeposition N and its decomposability. Rhizodeposits form an easily decomposable N

source deposited as root exudates, mucilage, sloughed off cells or tissue cell lysates and in the later growing phase of the plants it comprises also decomposing roots. The nature of the N compounds may be mainly amino acids, polymeric amino acids or amino sugars (Chap. 4.4). Only a small amount of these compounds, 14% - 18%, will be immobilised in the microbial biomass or is present in the N_{min} fraction (3% - 7%). Hence most of rhizodeposits, 48% - 72%, will be immobilised as microbial residues and form a labile pool of SOM.

As shown in chapter 5.3, the input through rhizodeposition did not increase the size of the microbial biomass N during cropping of grain legumes. The formation of microbial residues can therefore only be explained by a an increased turnover of the microbial biomass. This might be encouraged by a sufficient C supply due to root rhizodeposition which activates the microbial activity and accelerates its turnover (Fig. 7.1). The growth of microbial biomass, in this study, might be limited due to competition for limiting N in soil between the microbial community and the plant roots. It is an open question weather other factors such as Phosphorus are limiting the microbial growth.

However, the microbial biomass was only determined at maturity of the plants. It is also possible that the microbial biomass increased during plant growth in the rhizosphere and decreased in the late reproductive growing phase.

The processes and the ecological relevance related to N rhizodeposition and the turnover of rhizodeposits in soil are still only poorly understood. A certain proportion of rhizodeposits mineralise fast and my be reabsorbed by the plants or lost by denitrification. However, the reabsorption is difficult to estimate. Thus, the results show only the net rhizodeposition N. Gross rhizodeposition rates could be much higher and be of relevance for a better understanding of the ecological impact of N rhizodeposition.

Phase II: Turnover of grain legume residues

The rhizodeposition N mainly immobilised in the SOM has a relatively high decomposability. Differences in the N mineralisation of rhizodeposits were found and constituted 21% for lupin, 26% for faba bean and 27% for pea after 6 months of incubation. These difference may be due to differences in the quality of rhizodeposits between the species and the related immobilisation process. The role of the microbial biomass as a short term memory for nutrients with a high dynamic was small in the case of rhizodeposition N. Thus, the recovered amount in microbial biomass was small and almost constant during 6 months. The rhizodeposition N mineralised was mainly supplied by the pool previously immobilised as microbial residues.

This suggests that the function of the microbial biomass is more to drive the re-mineralisation from the microbial residue pool. Here might lie a possibility for a calculated manipulation of the microbial process by agronomic management and a better control of the N mineralisation.

The results indicate also a progressive stabilisation of rhizodeposition N in the SOM. Hence its decomposability will decrease with time and receive probably the same ratio as the native SOM (Chap. 5.3). This stabilisation process and the factors determining the course of the process is also poorly understood.

Under field conditions the decomposition of solely rhizodeposits is a theoretical consideration. At least roots and stubbles of plants and in most cases also above ground crop residues such as straw, leaves or pod walls are left in the field. If a greater amount of crop residues is incorporated into the soil, particularly with wide C:N ratios, the microbial response is a very fast and huge growth and incorporation of residual N (Chap. 5.3 and 6.3). However, similar to the turnover of only rhizodeposits, the growth is limited by external factors but lasts for a long period almost at the level achieved after some days. As shown in chapter 5.3, in the case of a sufficient C supply and relatively high C:N ratios of the legume residues, the decomposed crop residues were immobilised as microbial residues after some days of residue application. The following remineralisation, after two thirds of the incubation period, was in accordance with turnover of rhizodeposits mainly supplied by the crop residue N previously immobilised as microbial residues (Fig. 7.1).

However, the presence of rhizodeposits seems to have an effect on the turnover of the crop residues. But it was small and inconsistent between the investigated legume species. Only for lupin a distinct effect of rhizodeposition on the C turnover of legume crop residues was observed. In all treatments the presence of rhizodeposition caused an increased N immobilisation in the microbial biomass and in the first 3 months of the incubation an accelerated immobilisation of crop residue N as microbial residues. The recovery of the N of both residue sources – rhizodeposits or crop residues – was similar. This indicates a previous immobilisation of both sources, probably as microbial residues, and a later remineralisation from this source (Chap. 5.4).

An explanation of the factors responsible for the observed effects could not be given on account of the examined parameters. No differences were observed in the size or activity of the microbial biomass in soils without residue addition (Chap. 5.3). Also, the differing N inputs as rhizodeposits show no relationship to the observed effects. Thus, changes leading to a differing substrate use might be caused by changes within the microbial community. It was hy-

pothesised that probably two main factors are responsible: (i) a differing activation of the microbial population and (ii) a change in the composition of functional groups of the microbial population triggered by plant specific differences in the amount and composition of rhizodeposition (Chap. 5.4).

Phase III: Growth of subsequent crop

Phase III, turnover of legume residues (crop residues + rhizodeposits) in cropped soil and N recovery in a subsequent crop, was carried out with an assumed short term phase between crop residue incorporation and sowing of a subsequent non-legume of two weeks. Compared to phase II, the turnover process of legume residues was significantly influenced by the presence of growing crops. The plant roots compete with the microbial biomass for limited soil or residue N. In the case of high C:N ratios and a sufficient C supply, the competitiveness of the microbial biomass for N acquisition is greater than that of young plants with a relatively small root system. With an increasing root system and decreasing C supply derived from decomposing residues the competitiveness of the plants for N increases. Thus, in the early decomposition phase as shown at flowering in wheat (Chap. 6.3), the microbial biomass increases as shown in phase two and immobilises a certain amount of N. But, in contrast to the further process observed in phase II, the competition of plants for N leads to a N limitation for the microbial biomass. In consequence, the growth of the microbial biomass decreases probably due to an accelerated turnover of the microbial biomass driven by a sufficient C supply in the rhizosphere (Fig 7.1). As shown in chapter 6.3, the absolute pool size of residue N recovered in the microbial biomass and the subsequent crop did not alter between flowering and maturity but the percentage in the microbial biomass decreases and that in the subsequent crop increases. Thus, the released residue N from the microbial biomass was obviously directly captured by the plants but it is not clear how this N became plant available.

At maturity, the recovery of residual N in both subsequent crops, wheat and rape, showed a potential between 8% and 12%. The recovery in both pools, subsequent crop and microbial biomass, did not exceed 20% and an amount of about 80% remains unrecovered. It is not clear why this pool size does not change between flowering and maturity. As observed in phase II, the residues were probably immobilised as microbial residues, but in contrast, under the influence of living plants the immobilised N seems to be easier recoverable from the microbial biomass as from microbial residues. This might be a special adaptation of a co-evolutionary process between living plants and the turnover of residues.

However also in the subsequent crops the recovery from either rhizodeposits and or crop residues seem to be similar (Chap. 6.4) and this might be explained according to phase II: Crop residues and rhizodeposits were previously immobilised and the uptake was supplied by this pool with a similar decomposability.

Differences on residue decomposition and recovery due to root effects of contrasting subsequent wheat and rape could, contrary to the expectations, not be observed.

8. Conclusions

A prerequisite of studies related to root-soil interactions is the availability of a suitable methodology. The examined cotton wick technique for ^{15}N stem feeding of grain legumes provides a relatively simple methodology for *in situ* estimations of N rhizodeposition of grain legumes and can probably be used for other plants with a strong stem. The methodology enables a comparatively homogeneous ^{15}N labelling of different plant parts compared to other *in situ* methods. Therefore, it has advantages if also the turnover of residues will be studied. However, the ^{15}N labelling is not totally homogeneous and the methodology must be improved here.

It could be shown that roots have significant effects influencing the N-dynamics after cropping of grain legumes. The effects were related to the amount of N rhizodeposition of the grain legumes and its subsequent turnover, the interactions of grain legume rhizodeposition on the turnover of its crop residues and the effect of subsequent non-legumes on the turnover process of the residues. The progress obtained from the results can be summarised as follows:

- The N rhizodeposition of grain legumes represents a significant pool in crop rotations. The percentage of N rhizodeposition relative to total plant N constituted between 12% and 16%, relative to residual N 35% – 44% and about 80% of below ground plant N. Based on field data, N rhizodeposition contributes between 6 and 68 kg N ha-1 and results in more positive N balances for grain legumes. Especially under field conditions where the percentage of N derived from N_2-fixation is lower than the findings in the pot experiment, N rhizodeposition could be a key to understand the positive crop rotation effects.

- The effect of N rhizodeposition on the N dynamics in crop rotations depends mainly on its decomposability. During growth of the legumes, due to the nature of rhizodeposits as easily decomposable compounds, most rhizodeposition N have been immobilised in the microbial biomass, 14% - 18%, and as microbial residues, 48% - 72%. Thus, rhizodeposition N forms a labile pool of SOM with a relatively high decomposability. About one fourth of it mineralised during 6 months. The microbial residue pool provides the main pool for the remineralisation of rhizodeposition N in soils without growing plants. With progressive time the stabilisation of rhizodeposition N in the SOM increases.

- The rhizodeposition of grain legumes affected the subsequent turnover of its crop residues. But the observed effects are small and inconsistent. Only for lupin rhizodeposition

results in an accelerated C turnover of incorporated crop residues. In contrast, the presence of rhizodeposition causes a stronger N immobilisation in the first 3 months and an increased formation of microbial residues derived from crop residues in all treatments.

- Under conditions of sufficient C supply the recovery of either rhizodeposition N or crop residue N in the microbial biomass, the mineral N pool and in subsequent crops was similar. The similar recovery of two sources with an expected differing decomposability can be explained by a previous immobilisation in the same pool with the same decomposability: as microbial residues in the case of soils without living plants and in the microbial biomass in the case of growing plants.

- The potential of grain legume residue N for the N nutrition of a subsequent crop is small and constituted only 8% - 12% of residue N recovered in wheat and rape. However, due to including rhizodeposition the relative percentage of the residue derived N in subsequent wheat and rape was high and constituted between 18% and 46% of total N. This indicates that the benefit from grain legumes to subsequent crop is much greater than previously assumed.

- The residual N uptake by wheat from flowering to maturity was supplied by the residual N incorporated in the microbial biomass at flowering. The total pool size – residue N in the microbial biomass and crops – did not change. A rest of about 80% could not be recovered in the examined pools and may be immobilised as microbial residues or not be decomposed.

- The N uptake of the subsequent crops is directly correlated with the amount of residue N input of the legume species. The recovery of residue N differed slightly and due to small differences in the residue quality of the legumes.

- The two contrasting subsequent crops – wheat and rape – showed very similar patterns in N uptake, residue N recovery and microbial parameters for the three legume residues. Thus, no differing effect of the two species on the residue turnover or residue acquisition, respectively was found.

In general, it could be shown that the SOM as major N pool plays the dominant role as memory for residue derived N. The microbial biomass in contrast has a less important function as nutrient memory. The main role of the microbial biomass seems to be a short-term memory driving the turnover process. It works as a pump between the differing pools in the immobilisation-mineralisation process.

9.   Outlook

Most of work investigating the turnover processes and the related influencing factors has been done under controlled conditions in the laboratory or greenhouse. Our understanding of the processes related to root-soil interactions, turnover, immobilisation and remobilisation processes of C and N derived from crop residues and stabilisation and turnover of soil organic matter requires more basic knowledge under controlled but also under field conditions.

Nitrogen derived from rhizodeposition and related processes

The literature concerning N rhizodeposition comprises less than a dozen publications and only one was done under real field conditions. The methodology available for in situ ^{15}N labelling must be improved and adapted to the respective conditions, but in general a suitable methodology is available for investigation under field conditions. Hence the results obtained concerning N rhizodeposition of legumes and non-legumes should be confirmed in the field.

With respect to the management of organic or sustainable farming systems the amount of N rhizodeposition of legumes and non-legumes is a prerequisite for a new evaluation of N balances in crop rotations and the related effect on N dynamics.

Thus, future work should focus on

- the amount of N rhizodeposition of different legume and non-legume species,

- with respect to progresses in plant breeding, differences between varieties should taken into account,

- with respect to improvements in agronomic management the factors influencing the amount and quality of rhizodeposition should be investigated.

However, the questions "Why do plants release N as rhizodeposition?" and "What is the ecological function of N rhizodeposition?" are not answered. In a first view the N release by plant roots is an inefficient loss of the most limited resource in soils, and it seems to be an imperfection in the evolutionary development of plants. On the other hand, such a hypothesis is unlikely taking into account the long co-evolutionary process of plant development, formation of SOM and the living microbial community. Maybe N rhizodeposits play a role in nutrient mobilisation processes or alters selectively the microbial community structure in the rhizosphere. They could also contribute to the establishment of microorganisms in the rhizosphere in the early plant growth phase in annual crops. The understanding of these proc-

esses might give valuable information for plant breeding and related agronomic management. Investigations on quality of rhizodeposits, gross rhizodeposition rates, the fate of rhizodeposition and the diversification of rhizosphere microorganisms due to rhizodeposition could help understanding these processes.

Turnover of rhizodeposition N and related turnover of crop residues

As observed in this study, the turnover of the rhizodeposits differed between the grain legume species, but the differences were not large. However, the plant species were similar. Greater differences could be expected when comparing to e.g. non-legumes. How the quality of rhizodeposition affects the mineralisation-immobilisation-turnover has been only poorly investigated.

As observed in this study and several other studies, during decomposition of rhizodeposits and crop residues, N will be immobilised to a certain extent. With respect to possible management strategies and a synchronisation of N mineralisation and plant demand the understanding of these processes and the influencing factors should be improved. Important research areas are

- a more detailed understanding of the contribution of N-rhizodeposition in the turnover process of crop residues under field conditions,

- the reasons for and the ecological function of the small recovery of residue N in subsequent crops and related immobilisation processes,

- the effects of living plants on the turnover of residues and the N immobilisation-mobilisation-turnover in soils, especially the question if growing plants can reduce N immobilisation of fresh residues in SOM pools,

- a better characterisation of SOM pools in which "new" residue N will be immobilised, and

- possibilities for management of residue N immobilisation and re-mineralisation.

Perspectives for agronomic management

The knowledge can contribute to an optimisation of organic cropping systems e.g. breeding of "high N-input" legumes and "residue N" efficient non-legumes, improved management of crop residues, adaptation of tillage system, development of new cropping systems, etc. However, the basic knowledge for the development and establishment of new management practices is still small and requires more fundamental research.

10. Summary

The objective of the work has been to investigate the root and rhizodeposition effects on the turnover of grain legume residues in soil and the related microbial processes.

In an integrated experiment faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.) and white lupin (*Lupinus albus* L.) were investigated. The experiment consisted of three parts, two pot experiments and an incubation experiment, using the soil and plant matter from the pot experiments.

In experiment I, the N rhizodeposition, defined as root-derived N in the soil after removal of visible roots of the grain legumes and the fate of rhizodeposition N in the soil microbial biomass, the mineral N pool and remaining micro roots were measured. In a pot experiment the legumes were pulse labelled *in situ* with ^{15}N urea using a cotton wick method and harvested at maturity. The suitability of the labelling method was examined.

In experiment II, the turnover of rhizodeposition N of the grain legumes and the effects of the rhizodeposition on the subsequent C and N turnover of its residues were investigated in an incubation experiment. The turnover of the rhizodeposits in soil from experiment I was compared with the same soil as control stored cool during the growth of the legumes. The C and N turnover of the grain legume crop residues (stem, leaves, recovered roots) were compared in soils with and without (control) previous growth of the three legumes.

In experiment III, the residual N contribution from the grain legumes to microbial biomass and subsequent wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) was studied in a greenhouse pot experiment. Wheat and rape were subsequently grown on the soil with previously incorporated legume residues (incl. ^{15}N labelled rhizodeposits) from experiment I and were harvested at flowering and maturity.

The *in situ* cotton wick ^{15}N labelling technique resulted in high ^{15}N recovery rates of about 84% for the three grain legumes and showed a comparatively homogeneous ^{15}N distribution among plant parts at maturity. The method is relatively simple and can be used for the determination of N rhizodeposition and for turnover studies of grain legume residues in the field.

Roots had significant effects on the N dynamics after growth of grain legumes. The effects were related to the N rhizodeposition by the roots of the grain legumes and its subsequent turnover, the effects of grain legume rhizodeposition on the subsequent turnover of its crop residues (stem, leaves, recovered roots) and the effect of the subsequent non-legumes on the turnover process of the residues:

- The N rhizodeposition constituted between 13% of total plant N for faba bean and pea and 16% for white lupin at maturity. Relative to residual N it amounts to 35% – 44% and about 80% of below ground plant N. Hence, the N rhizodeposition of grain legumes represented a significant pool for N balance and could be a key to understand the positive crop rotation effects after growing grain legumes.

- Some 7% (lupin) – 31% (pea) of total N rhizodeposits were recovered as micro-roots by wet sieving (200 µm) after all visible roots were removed. Only 14% - 18% of rhizodeposition N was found in the microbial biomass and a very small amount of 3% - 7% in the mineral N fraction. In pea 48% and in lupin 72% of N rhizodeposits could not be recovered in the mentioned pools and a major part of the unrecovered N was probably immobilised in microbial residues.

- After 168 days of incubation 21% (lupin), 26% (faba bean) and 27% (pea) of rhizodeposition N was mineralised in treatments without crop residues. The mineralised N derived from mainly two pools: Among 30% and 55% of rhizodeposition N was supplied by the microbial residue pool and a smaller amount was supplied by the microbial biomass.

- The effects of rhizodeposition on the C and N turnover of the grain legume crop residues were inconsistent. Rhizodeposition increased the crop residue C mineralisation only in the lupin treatment, whereas the microbial N and the formation of microbial residues derived from crop residues was increased by rhizodeposition in all treatments.

- The potential of grain legume residue N for the N nutrition of subsequent crops was small. Only 8% - 12% of residue N was recovered in subsequent wheat and rape. Due to the inclusion of rhizodeposition the relative percentage of the residue derived N in subsequent wheat and rape was high and constituted between 18% and 46% of total N. This indicates that the N benefit from grain legumes to subsequent crop is much greater than previously assumed.

- The residual N uptake by wheat from flowering to maturity was supplied by the residual N incorporated in the microbial biomass at flowering. The total pool size – residue N in the microbial biomass + wheat did not change. A rest of about 80% could not be recovered in the examined pools and may be immobilised as microbial residues or not be decomposed.

- The two contrasting subsequent crops – wheat and rape – showed very similar patterns in N uptake, residue N recovery and microbial parameters for the three legume residues. A differing effect of the two species on the residue turnover or residue acquisition, respectively was not found.

11. Zusammenfassung

Das Ziel dieser Arbeit war, die Einflüsse von Wurzeln und Rhizodeposition auf den Umsatz von Körnerleguminosenresiduen und damit verknüpfte mikrobielle Prozesse zu untersuchen.

In einem integrierten Versuch wurden Ackerbohne (*Vicia faba* L.), Erbse (*Pisum sativum* L.) und Weiße Lupine (*Lupinus albus* L.) untersucht. Der Versuch bestand aus drei Teilen, zwei Gefäß-Experimenten und einem Inkubationsexperiment, in denen ausgehend von einem Gefäß-Experiment derselbe Boden und dasselbe Pflanzenmaterial verwendet wurden.

In Experiment I wurde die Stickstoff-Rhizodeposition der Körnerleguminosenarten, definiert als wurzelbürtiger N nach dem Entfernen aller sichtbaren Wurzeln im Boden, gemessen und der Verbleib des Rhizodepositions-N in den Pools mikrobielle Biomasse, mineralischer N und den im Boden verbliebenen Feinwurzeln untersucht. Dazu wurden die Leguminosen in einem Gefäßversuch unter Verwendung einer *in situ* ^{15}N-Docht-Methode mit einer ^{15}N-Harnstofflösung pulsmarkiert und zur Reife geerntet. Die Eignung der Markierungsmethode wurde überprüft.

In Experiment II wurde der Umsatz der N-Rhizodeposition der Körnerleguminosen und der Einfluss der Rhizodeposition auf den anschließenden C- und N-Umsatz der Körnerleguminosenresiduen untersucht. Der Umsatz der N-Rhizodeposition aus den Böden des Experimentes I wurde als Kontrolle mit demselben Boden verglichen, der während des Aufwuchses der Leguminosen kühl gelagert worden war. Der C- und N-Umsatz der Körnerleguminosenresiduen (Stängel, Blätter, erfassbare Wurzeln) wurde in den Böden aus Experiment I mit vorherigem Leguminosenbewuchs und im Kontrollboden ohne vorherigen Leguminosenbewuchs verglichen.

In Experiment III wurde der N- Transfer aus den Körnerleguminosenresiduen in die mikrobielle Biomasse und die Folgefrüchte Weizen (*Triticum aestivum* L.) und Raps (*Brassica napus* L.) in einem Gewächshaus-Gefässversuch untersucht. In die Böden aus Experiment I (mit markierten Wurzeln + Rhizodepositen) wurden die oberirdisch geernteten Residuen eingemischt, anschließend Weizen und Raps gesät und zur Blüte und Reife geerntet.

Die *in situ* ^{15}N Docht-Markierungs-Methode wies hohe ^{15}N Wiederfindungsraten von ungefähr 84% für alle drei Leguminosenarten auf und zeigte eine vergleichsweise homogene ^{15}N Verteilung zwischen verschiedenen Pflanzenteilen zur Reife. Die Methode ist relativ einfach und geeignet zur Bestimmung der N-Rhizodeposition und des Umsatzes von Körnerleguminosenresiduen in Feldversuchen.

Die Wurzeln zeigten deutliche Effekte auf die N-Dynamik nach dem Anbau von Körnerleguminosen. Die beobachteten Effekte konnten auf die N-Rhizodeposition und deren anschließenden Umsatz, Einflüsse der Rhizodeposition von Körnerleguminosen auf den anschließenden Umsatz ihrer Residuen (Stängel, Blätter, erfassbare Wurzeln) und die Wirkungen nachfolgender Nichtleguminosen auf den Umsatzprozess der Residuen zurückgeführt werden:

- Die N-Rhizodeposition betrug zur Reife der Pflanzen bezogen auf die Gesamt-N-Aufnahme 13% bei Ackerbohne und Erbse und 16% bei Weißer Lupine. Bezogen auf den Residual N nach Ernte der Körner erhöhte sich der relative Anteil auf 35% - 44% und betrug 80% des unterirdischen N (Wurzeln + Rhizodeposition). Die N-Rhizodeposition ist daher ein wesentlicher Pool für die N-Bilanz von Körnerleguminosen und trägt wesentlich zur Erklärung positiver Fruchtfolgeeffekte nach Körnerleguminosen bei.

- Ungefähr 7% (Lupine) – 21% (Erbse) des Rhizodepositions-N (Boden ohne sichtbare Wurzeln) wurden als Feinwurzel nach Nasssiebung (200 µm) wiedergefunden. Nur 14% - 18% des Rhizodepositions-N wurde in der mikrobiellen Biomasse und ein sehr kleiner Anteil von 3% - 7% in der mineralischen N Fraktion gefunden. Bei Erbse konnten 48% und bei Lupine 72% der N-Rhizodeposition in keinem der untersuchten Pools nachgewiesen werden. Dieser Teil dürfte als mikrobielle Residualmasse immobilisiert worden sein.

- Nach 168 Tagen Inkubation wurden 21% (Lupine), 26% (Ackerbohne) und 27% (Erbse) des Rhizodepositions-N in den Varianten ohne Residuenzugabe mineralisiert. Der mineralisierte N stammte im wesentlichen aus zwei Pools: Zwischen 30% und 55% wurde aus der mikrobiellen Residualmasse mineralisiert und eine kleinere Menge stammte aus der mikrobielle Biomasse.

- Der Einfluss der Rhizodeposition auf den Umsatz der Residuen war indifferent. Durch Rhizodeposition wurde die C Mineralisierung der Leguminosenresiduen nur in der Lupinenvariante erhöht, wobei der mikrobielle N und die Bildung von mikrobieller Residualmasse aus den Leguminosenresiduen in allen Varianten durch Rhizodepositionseinflüsse erhöht waren.

- Das Potential des residualen Körnerleguminosen-N für die N Ernährung von Folgefrüchten war gering. Nur 8% - 12% des residualen N wurden in den Folgenfrüchten Weizen und Raps wiedergefunden. Durch die Berücksichtigung des Rhizodepositions-N war der relative Anteil des Residual-N bezogen auf die Gesamt-N-Aufnahme der Folge-

frucht hoch und betrug zwischen 18% und 46%. Dies lässt auf einen höheren N-Beitrag der Körnerleguminosen schließen als bisher angenommen wurde.

- Die residuale N-Aufnahme von Weizen von der Blüte bis zur Reife wurde durch den Residual-N gespeist, der zur Blüte in der mikrobiellen Biomasse immobilisiert worden war. Die gesamte Poolgröße, Residual-N in der mikrobiellen Biomasse und in Weizen, verändertere sich von der Blüte bis zur Reife nicht. Jedoch konnte ein Rest von 80% des Residual-N in keinem der untersuchten Pools nachgewiesen werden und dürfte als mikrobielle Residualmasse immobilisiert worden sein oder ist noch nicht abgebaut worden.

- Die zwei unterschiedlichen Folgefrüchte – Weizen und Raps – zeigten sehr ähnliche Muster bei der N-Aufnahme, der Residual-N Wiederfindung und bei mikrobiellen Parametern für die Residuen der drei Körnerleguminosenarten. Ein differenzierender Effekt auf den Umsatz der Residuen bzw. auf das Residual-N-Aneignungsvermögen der Folgefrüchte konnte nicht beobachtet werden.

12. Acknowledgements

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