



# Carbon and nitrogen mineralization differ between incorporated shoots and roots of legume versus non-legume based cover crops

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## Abstract

**Aims** Cover crops (CC) have been widely used to improve soil quality and sustain agricultural productivity. However, the turnover of carbon (C) and nitrogen (N) from different species and parts of CCs incorporated into the soil under Northern European conditions remains unclear.

**Methods** In this study, we examined mineralization of C and N from isolated shoots and roots of two leguminous and two non-leguminous CCs labeled with  $^{15}\text{N}$  tracers in a 100-day incubation experiment at 10 °C.

**Results** After 100 days incubation, the average net N mineralization was 33% from the leguminous CCs and 20% from the non-legumes. Net N mineralization from shoots was 1–15% (% of the N input) higher than that from the corresponding roots, and the net C mineralization accounted for 53–62% and 18–39% of the total C applied with CC shoots and roots, respectively. The C/N ratio of plant residues was a good predictor for net N mineralization, while the lignin concentration was a good predictor for C mineralization from both roots and shoots. Roots of both legume and non-legume CCs caused net immobilization of (unlabeled) soil N, whereas no significant soil N immobilization occurred from shoots with low C/N ratios.

**Conclusions** Our results indicate that different CC characteristics (C/N ratio and lignin) control the turnover of residue N and C, respectively, in the soil. Our results further showed very limited interactions with soil N turnover (no priming effects) when materials with low C/N ratios were applied to soil.

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## Introduction

Nitrogen (N) cycling presents one of the greatest sustainability challenges for modern agriculture, which relies heavily on N fertilizer to support enhanced crop yields (Shibata et al. 2017). Globally, simplified cropping systems become increasingly dominant,

mainly by cereals with relatively short periods for N uptake (Ladha et al. 2016). This often results in low N use efficiency and high losses of N through gaseous emissions and leaching (Clark and Tilman 2017). Therefore, it is needed to retain and recycle excess N in cropping systems.

Growing cover crops (CC, also known as catch crops) is an important agronomic measure to capture excess N during the off-season. It is a common and sustainable agricultural practice to grow non-leguminous CC in cool seasons, e.g., from autumn to early spring, for preserving soil N for subsequent crops (Thorup-Kristensen et al. 2003; López-Bellido et al. 2004), and for decreasing N leaching losses in Western and Northern Europe (Askegaard and Eriksen 2008; Doltra and Olesen 2013). With leguminous CC or green manure crops, additional N can be added to the system via symbiotic N<sub>2</sub> fixation, which is of particular importance in organic arable farming. However, the decomposition of CCs may also contribute to N losses, such as through ammonia emissions from surface mulched residues (Whitehead and Lockyer 1989) and denitrification from soil incorporated materials (Li et al. 2015a, 2016). To fully utilize the N released from CCs in cropping strategies, it is thus essential to understand the N mineralization pattern of different CC residues.

An increasing focus on soil carbon (C) sequestration as a measure to mitigate climate change has drawn more interest in growing CCs (Lal 2004), including the aspect of C input to the soils for maintaining or increasing soil C concentrations (Olson 2013). Thus, understanding the C and N mineralization of CCs returned to soils is critical for the future management and sustainability of cropping systems.

Previous studies on C and N mineralization from CCs mainly focused on the aboveground parts (Andersen and Jensen 2001; Li et al. 2013). Roots of CCs are relatively less studied, although they may contain considerable amounts of N (Kumar and Goh 2002) and > 40% of total yearly plant C input (Chirinda et al. 2012). Also, root-derived C have been reported to contribute more to relatively stable soil C pools than the same amount of shoot-derived C (Kätterer et al. 2011; Ghafoor et al. 2017), although this has also been disputed (Hu et al. 2018a). As an ‘additional’ biomass production, CC shoots may be harvested for other purposes, e.g., winter forage or digestion for biogas, which makes the C and N contained in CC roots more important in the system, in particular in organically managed systems (Li et al.

2015a). Therefore, it is necessary to understand how the C and N mineralization differs between CC shoots and roots, in particular across leguminous and non-leguminous species commonly used in organic farming.

The decomposition of crop residues returned to the soil is controlled by several factors (Giacomini et al. 2007; Awad et al. 2012). Crop residue type has long been recognized as an important factor influencing the capacity for N retention and the potential for C incorporation into the soil (Gaillard et al. 2003; Mungai and Motavalli 2006). Furthermore, the biochemical composition of crop residues has a major effect on their decomposition and the release of N (Henriksen and Breland 1999). Several studies suggested that higher lignin/N ratios of plant residues result in slower degradation rates (Melillo et al. 1982; Yanni et al. 2011). In addition, the C/N ratio of plant residues has been used to predict the decomposition rate and N release patterns (Carpenter-Boggs et al. 2000). However, Jensen et al. (2005) did not find C/N ratios or lignin/N ratios of plant residue to be closely related to the N mineralization; total plant N concentration was a good predictor for the N mineralization potential, whereas holocellulose C concentration was the factor that explained most of the variability in C mineralization. Few studies have examined decomposition of isolated parts of different crop residues, e.g., roots and shoots (Shahbaz et al. 2017; Hu et al. 2018a).

Cover crops growing under Northern European conditions are at a relatively young physiological stage when terminated because of moderate temperatures and restricted light intensity during growth, and they are normally restricted in growth by limited N supply (Thomsen et al. 2010). This may influence their decomposition compared to other more mature plant residues. Previous studies have focused on C and N decomposition of fodder radish under Nordic conditions (Thomsen et al. 2016; Hu et al. 2018a), whereas the decomposition of other CC species remain largely unknown. However, such information is essential to assess the role of CCs in improving soil quality and crop N supply (Thomsen et al. 2016).

Based on the above considerations, we proposed the following hypotheses: (H1) Net N and C mineralization would be greater from shoots than the corresponding roots of CCs. (H2) Net N and C mineralization would be higher from leguminous than non-leguminous CCs. (H3) the C/N ratio of CC residues is a good predictor of net N and C mineralization. To test these hypotheses, we conducted a 100-day incubation to compare N and C mineralization of tops and roots of four leguminous and non-leguminous

CCs. The aim of this study was (1) to compare C and N mineralization between incorporated shoots and roots of contrasting CCs; and (2) to link several biochemical characteristics of CC residues and the C and N turnover in soil after incorporation.  $^{15}\text{N}$ -labeled materials were applied to quantify the N transformation processes involved in the decomposition of the materials and their interactions with the unlabeled soil N pool.

## Materials and methods

### Soil

Soil from the top 20-cm was sampled from an arable field at Research Centre Foulum, Aarhus University, Denmark (56°30'N, 9°34'E). The soil is classified as loamy sand with 95 g clay  $\text{kg}^{-1}$ , 115 g silt  $\text{kg}^{-1}$ , and 762 g sand  $\text{kg}^{-1}$ , 16.5 g C  $\text{kg}^{-1}$  and 1.4 g N  $\text{kg}^{-1}$ . The soil was air-dried and sieved (< 2 mm) to remove plant residues and stones, and then thoroughly mixed. Gravimetric soil water content was determined by oven drying at 105 °C for 24 h.

### Plant materials

Two leguminous and two non-leguminous CCs were sampled from a micro-plot experiment. The CC tops in the micro-plots were harvested by hand-cutting at the soil surface on 26 October 2012, before the first frost arrived. The materials were oven-dried at 60 °C (48 h) to determine dry matter content and then cut into small pieces (ca. 0.5 cm). Meanwhile, a subsample was ball-milled for  $^{15}\text{N}$  analysis. The four species were red clover (*Trifolium pratense* L., cv. Rajah), winter vetch (*Vicia villosa*, cv. Villana), perennial ryegrass (*Lolium perenne* L., cv. Foxtrot) and fodder radish (*Raphanus sativus* L., cv. Lunetta). Red clover and ryegrass were undersown in a preceding main crop of spring barley (*Hordeum vulgare* L.), while winter vetch and fodder radish were sown after barley harvest. Each micro-plot covered a surface area of 25 cm × 35 cm = 875 cm<sup>2</sup>. A  $^{15}\text{N}$ -enriched  $\text{KNO}_3$  (11.58 atom%  $^{15}\text{N}$  excess) solution was applied to all micro-plots on the soil surface along the crop rows. A total of 10 kg N  $\text{ha}^{-1}$  was applied, split into three doses (within 3 weeks after setting up of the micro-plots). Samples were collected from three replicated micro-plots per crop type (about 2 months after setting up of the micro-plots), pooled and then oven-dried at 60 °C for 48 h.

To extract the roots, the top 18 cm soil was excavated within the micro-plots in early November 2012. The excavated soil monoliths were kept at 2 °C until root washing for a maximum of 3 weeks from the time of excavation. The roots were washed on a sieve (0.425 mm) and then transferred to a tray with water, where organic debris was removed based on appearance and color as described by Chirinda et al. (2012). The recovered macro-roots were dried at 60 °C (48 h). The dried shoot and root residues were ground (< 1 mm) and stored for the incubation experiment with subsamples ball-milled for further analyses. The chemical composition of plant materials and  $^{15}\text{N}$  enrichment are presented in Table 1. In the following, we use ' $^{15}\text{N}$ ' as a short notion for the  $^{15}\text{N}$ -labeled N pools derived from the labeled plant material.

### Incubation experiments

The incubation experiment consisted of two parts for separate monitoring of the N and C mineralization dynamics after CC residue incorporation. The experiment was conducted using 100-cm<sup>3</sup> steel cylinders with an inner diameter of 6.04 cm. To monitor N mineralization, eight treatments with  $^{15}\text{N}$ -labeled CC materials (four shoots and four roots) and a control treatment without any amendment were included. Each treatment was replicated 15 times to allow three replicates to be destructively sampled for analysis of inorganic N concentration ( $\text{NO}_3^-$ -N and exchangeable  $\text{NH}_4^+$ -N) after 7, 14, 30, 60 and 100 d (see below). Cylinders were randomly arranged in five boxes insulated with foam rubber, which were then placed at 10 °C in a dark room, representing the typical soil temperature in spring under field conditions after CC incorporation in Denmark. To maintain moisture, deionized water was frequently added to the cylinders. To secure aeration and reduce moisture losses the cylinders were open-ended and the boxes were opened regularly. To monitor C mineralization, three additional replicates were prepared for the nine treatments (except for the control treatment, other treatments are shown in Table 1) during a continuous measurement campaign (for details see below). The total number of cylinders was 162.

Air-dried soil (140 g per cylinder) was weighed in four equal-sized portions and packed into cylinders using a piston manually resulting in a final bulk density of 1.38 g  $\text{cm}^{-3}$ . The soil was then moistened to 16% gravimetric soil water content with deionized water,

**Table 1** Biochemical characteristics of the cover crop roots (R) and shoots (S) used for the incubation experiment and amounts of C and N applied to soil

Treatments	Concentration in biomass											Applied to soil		
	C mg g <sup>-1</sup> dry matter	N mg g <sup>-1</sup> dry matter	Mineral N	C/N	NDF mg g <sup>-1</sup> dry matter	ADF mg g <sup>-1</sup> dry matter	Cellulose	Hemicellulose	Lignin	Total <sup>15</sup> N in plant atom % <sup>15</sup> N excess	Mineral <sup>15</sup> N in plant	<sup>15</sup> N in plant	C mg C g <sup>-1</sup> soil	N μg N g <sup>-1</sup> soil
S Red clover	414	34.4	0.26	12	307	204	36	168	103	0.39	0.46	0.46	2.37	197
S Winter vetch	418	46.7	0.56	9	299	212	26	186	87	0.43	0.57	0.57	2.39	267
S Ryegrass	418	20.9	0.26	20	439	228	16	212	211	1.27	0.9	0.9	2.39	119
S Fodder radish	390	22	0.17	18	171	161	5	156	10	1.03	0.99	0.99	2.23	126
R Red clover	414	30.9	1.41	13	483	286	84	202	197	0.31	0.31	0.31	2.37	177
R Winter vetch	390	27.6	1.74	14	599	374	153	221	225	0.29	0.43	0.43	2.23	158
R Ryegrass	416	17.7	0.93	23	723	367	108	259	356	0.49	0.32	0.32	2.37	101
R Fodder radish	411	20.6	0.72	20	577	365	137	228	212	0.36	0.41	0.41	2.35	118

Abbreviations: NDF Neutral detergent fiber, ADF Acid detergent fiber

corresponding to a matric potential of -100 hPa, which is near the optimal moisture content for soil microbial activity (Wu et al. 2010; Manzoni et al. 2012). To maintain soil moisture, deionized water was added once a week to keep a constant weight.

All cylinders with soil cores were pre-incubated at 10 °C for 3 weeks in a dark room. After pre-incubation, the plant materials were sandwiched in the middle of the original soil core to simulate the discrete distribution of residues after CC incorporation by plowing. To place the plant residues, the soil cores were carefully pushed half-way out from the cylinder and cut into equal halves. Homogenized plant material (800 mg oven-dried material per cylinder) was then placed on the surface of the remaining soil core in the cylinder, and the other half of the soil core was gently fitted back to the cylinder to form a residue layer in the middle of the soil core. After residue incorporation, the incubation continued at 10 °C for 100 days to monitor C and N mineralization. The application rates of C and N are presented in Table 1, and they correspond to the application of 2.8 Mg DM ha<sup>-1</sup>.

The 27 soil cylinders for monitoring C mineralization were weighed and put in respirometric jars with an approximate capacity of 0.5 L. The jars were randomized and transferred to a RESPICOND VI respirometer (A. Nordgren Innovations AB, Bygdev, Sweden) with a capacity of 48 respirometric units that were submerged into a highly insulated water bath. The water bath was used to ensure a constant temperature set at 10 °C. Each respirometric jar was equipped with a beaker containing 20 mL of 0.6 M KOH solution, which was used to trap the CO<sub>2</sub> emitted from the soil cylinders. The CO<sub>2</sub> capture was measured as a decrease in the conductance of the KOH solution, read automatically with platinum electrodes over a period of 100 days using logging intervals of one or two hours. The KOH solution was regularly replaced with fresh ones when accumulated CO<sub>2</sub> reached about 80 mg. The additional net CO<sub>2</sub>-C released from CC shoots and roots was calculated as the difference of CO<sub>2</sub>-C evolved from amended soil cylinders and the control without amendment.

#### Chemical analyses of plant and soil

The total C and N of plant materials were analyzed using an elemental analyzer (CE 1110, Thermo Electron, Milan, Italy). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin concentration of plant materials were analyzed using the method described by Van Soest

et al. (1991). Plant mineral N (0.5 g sample) was extracted with 100 mL 2 M KCl solution and shaken for 1 h. Mineral N ( $\text{NO}_3^-$ -N and exchangeable  $\text{NH}_4^+$ -N) concentrations were determined using a Technicon Autoanalyzer II (Bran+Luebbe GmbH, Norderstedt, Germany).

At each destructive sampling, soil cores were removed from the cylinders, put in transparent polyethylene bags and mixed thoroughly. Then 25 g moist soil were extracted for mineral N concentration in the same way as for the plant samples. Soil water content was measured by drying soils at 105 °C for 24 h.

The  $^{15}\text{N}$  enrichment of the mineral N ( $\text{NO}_3^-$ -N + exchangeable  $\text{NH}_4^+$ -N) in soil extracts was determined using a diffusion method, as described by Sørensen and Jensen (1991). The total N content and  $^{15}\text{N}$  enrichment of plant samples and soil mineral N samples were determined using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility, University of California, Davis, USA.

#### Data analysis

Net C mineralization from added plant materials was calculated by subtraction of the average  $\text{CO}_2$ -C evolution measured in the control soil from the amended treatment and expressed as the percentage of total C applied with plant materials. Net mineral N and  $^{15}\text{N}$  released from the applied plant materials were calculated in a similar way by subtraction of the average mineral N and  $^{15}\text{N}$  in the control soil sampled on 7, 14, 30, 60 and 100 d, respectively. The net N mineralization included both mineral N in the original CC residues and N mineralized from the material during incubation. For simplification, in this study, we did not consider the denitrification and  $\text{N}_2\text{O}$  losses as slow nitrification rate at low temperatures is generally considered to limit N losses by denitrification (Thomsen et al. 2016). Li et al. (2016) measured  $\text{N}_2\text{O}$ -N losses of 0.3–1.9% of N input at 20 °C in similar soil. Calculations of labeled N ( $^{15}\text{N}$ ) in a given pool were based on measurement of the  $^{15}\text{N}$  atom fraction in samples subtracted by the corresponding natural  $^{15}\text{N}$  fraction measured in a similar sample of control soil. The equations are as follows:

Net N mineralized (% of N input)

$$= (\text{mineral N in the amended soil} - \text{mineral N in the control soil}) / \text{total amount of N in applied CC} \times 100$$

A negative value indicates net N immobilization.

Ndfr (%) = the atom% excess of  $^{15}\text{N}$  in the soil mineral

$$\text{N} / \text{atom\% excess of } ^{15}\text{N} \text{ in the applied CC}$$

where Ndfr is the proportion of mineral N in the N pool derived from residues.

Mineral  $^{15}\text{N}$  in the amended soil

$$= \text{Mineral N in the amended soil} \times \text{Ndfr} (\%) / 100$$

$^{15}\text{N}$  mineralized (% of  $^{15}\text{N}$  input) = mineral  $^{15}\text{N}$  in the amended soil / total amount of  $^{15}\text{N}$  in applied CC  $\times$  100

Net mineralized of unlabeled soil N after residue addition (% of N input) = Net N mineralized –  $^{15}\text{N}$  mineralized

This difference is a measure of priming effects (positive or negative) of the plant residue additions on the release of mineral N from the soil N pool.

The cellulose concentration in the plant materials was calculated as the difference between the ADF and lignin concentrations, and the hemicellulose concentration was calculated as the difference between the NDF and ADF concentrations (Van Soest et al. 1991).

Statistical analyses were conducted using the SAS software package (Version 9.1). An analysis of variance (ANOVA in SAS) was performed and significant differences in the measured variables between treatments (residue types) were compared using the Least Significant Difference (LSD) test at  $\alpha = 0.05$ . Relationships between plant biochemical characteristics (i.e. C/N ratio, lignin, cellulose, and hemicellulose concentrations) and net C and N mineralization were tested using linear regression (GLM in SAS). The effects of different CC parts (shoot or root) on these relations were tested by including this variable as a fixed effect in the analysis affecting both the intercept and slope of the regressions. The assumption on variance homogeneity for both analyses of variance and linear regression was examined visually from plots of residuals versus predicted values.

## Results

### Biochemical properties of cover crops

Leguminous CCs (red clover and winter vetch) had higher N concentrations and lower C/N ratios than non-leguminous ones (ryegrass and fodder radish) (Table 1). The average of N concentrations of leguminous CCs was 72% higher compared with the non-leguminous ones, and the C/N ratios were 41% lower than non-leguminous CCs. Generally, for the same species, the N concentrations from shoots were 7–69% higher compared with the roots. In addition, the NDF, ADF and lignin concentrations were lower in shoots than roots. In particular, lignin varied from 5 to 36 mg g<sup>-1</sup> DM in shoots, and from 84 to 153 mg g<sup>-1</sup> DM in roots.

### Net N mineralization

In general, a relatively large proportion of plant N in legume shoots was mineralized in the first 30 days of the incubation, while for the legume roots N was predominantly released during the first 7 days of the incubation (Fig. 1; Supplementary Materials Table S1). In contrast to the net N mineralization in the legume treatments, N immobilization was observed in the non-legume treatments, during the first 30 and 60 days of the incubation for shoots and roots, respectively (Fig. 1). For the non-legumes, the shoot treatments had the largest proportions of N immobilized (equivalent to 10% and 14% of

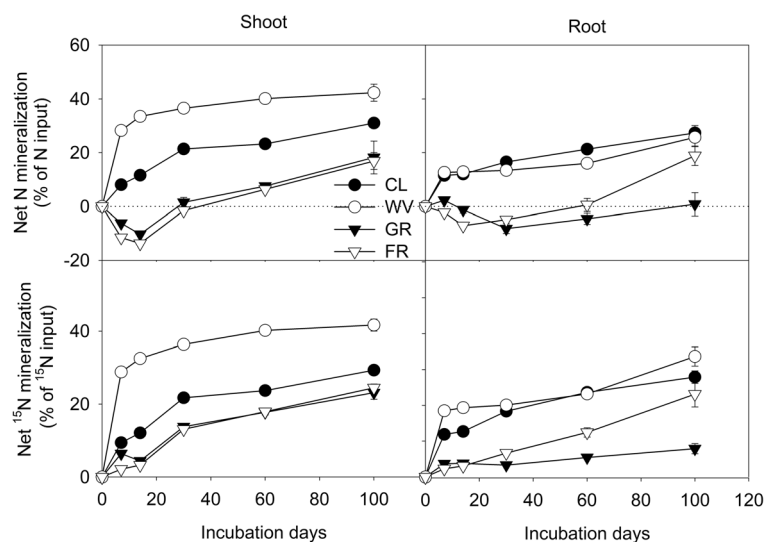
N input for ryegrass and fodder radish, respectively) at 14 d, followed by fodder radish root (7% of N input), while the largest N immobilization in the ryegrass root treatment (8% of N input) appeared at 30 d. Overall, at the end of the 100-day incubation, the net N mineralization was significantly higher from legumes (31% and 42% for shoots of red clover and winter vetch, and 27% and 26% for their roots, respectively) than from non-legumes (18% and 17% for shoots of ryegrass and fodder radish, and 1% and 19% for roots, respectively).

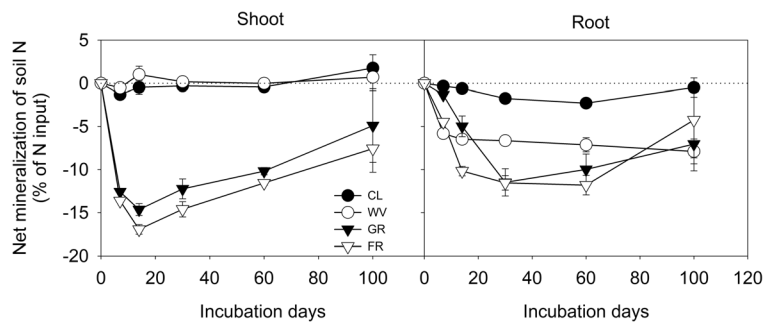
Net mineralization of the <sup>15</sup>N averaged 33% from the leguminous CCs and 20% from the non-legumes across the shoot and root treatments. Additionally, the net mineralization of <sup>15</sup>N from shoots of different CCs was 1–15%-points (% of N input) higher compared with the corresponding roots. The net mineralization rate of the applied <sup>15</sup>N in legumes was greatest during the first 30 d of incubation, after which the release of <sup>15</sup>N was slow (Fig. 1).

### Mineralization-immobilization of unlabeled soil N after residue addition

Net immobilization of soil N due to the addition of plant materials was observed during the whole incubation period (Fig. 2), except for the leguminous CC shoot treatments. In the root treatments, net immobilization of unlabeled soil N was lowest in the clover root treatment. Non-leguminous CCs resulted in higher soil N immobilization than legumes, with more pronounced differences observed during the initial incubation

**Fig. 1** Net mineralization of N and <sup>15</sup>N during 100 days incubation of <sup>15</sup>N-labeled roots and shoots of different cover crops (CC). Note: CL: red clover, WV: winter vetch, GR: ryegrass, FR: fodder radish. Error bars show the standard error of the mean





**Fig. 2** Net mineralization of soil N (unlabeled) due to the application of cover crop (CC) shoots and roots during 100 days incubation, calculated as the difference between net N mineralization from the materials and mineralization of labeled N derived

from the materials. Note: CL: red clover, WV: winter vetch, GR: ryegrass, FR: fodder radish. Error bars show the standard error of the mean

period, especially in the shoot treatments. Immobilization of soil N was highest at 14 days after the application of shoot residues, equivalent to 15–17% of the N applied, after which there was a steady remineralization of unlabeled soil N. After root application, soil N immobilization occurred more slowly and remineralization started later, but no remineralization was observed in the winter vetch root treatment.

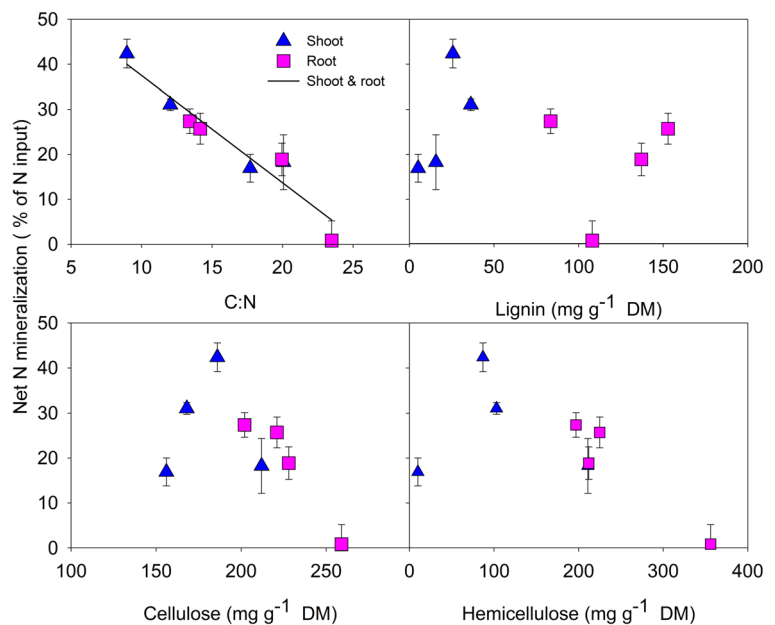
Net N mineralization correlated with plant biochemical characteristics

Carbon/N ratio was the plant characteristic that was most strongly correlated with net N mineralization from the plant materials ( $R^2 = 0.780$ ;  $P < 0.001$ ) after 100 days

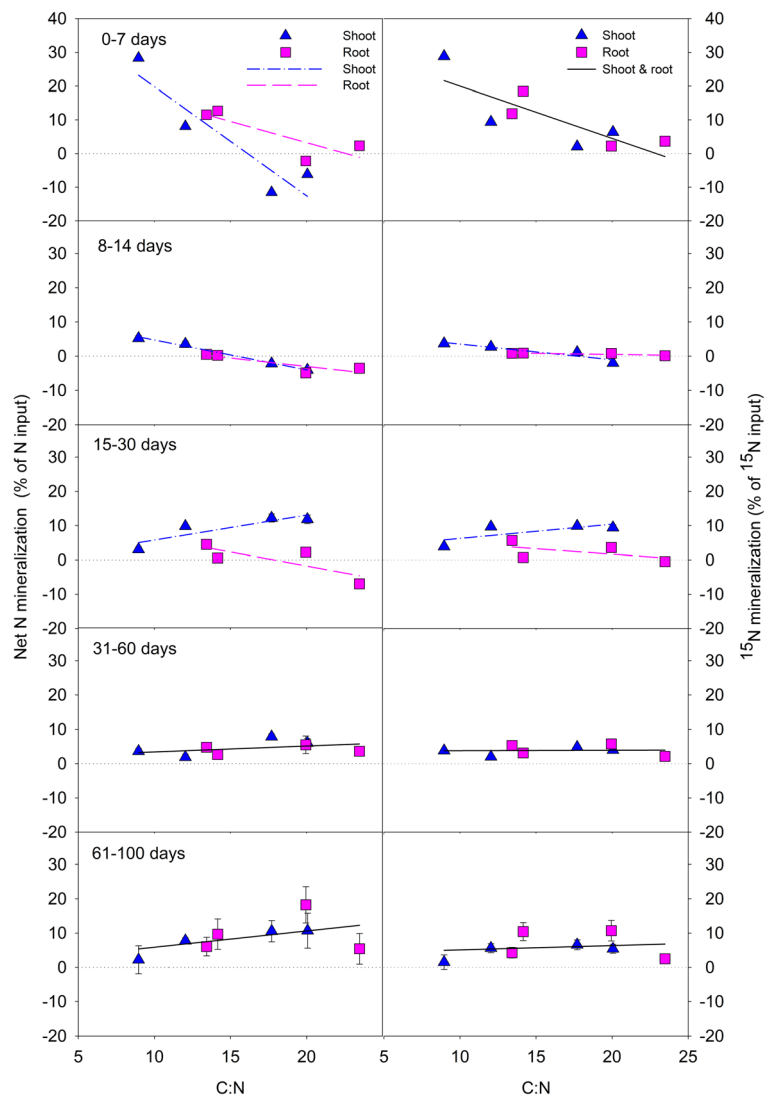
incubation (Fig. 3), which did not differ between the shoots and the roots. There was no significant relationship between net N mineralization and plant lignin, cellulose and hemicellulose concentration.

At different stages of the incubation, similar relationships were observed between the net N mineralization and C/N ratio to that between the mineralization of the labeled N and C/N ratio (Fig. 4). A significant negative correlation between the net N (and labeled N) mineralization and C/N ratio mainly appeared during 0–14 d of the incubation (Table 2). During 15–100 d, the N mineralization was little affected by the C/N ratio of plant materials. In addition, the relationship between the net N mineralization and C/N ratio

**Fig. 3** Net N mineralization after 100 days incubation in relation to C/N ratio, lignin, cellulose, and hemicellulose concentration in the incubated plant materials. Note: Regression lines for shoot and root were separated if the difference between them is significant. Significance levels: ns not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ . Error bars show the standard error of the mean. The net N mineralization is significantly related to C/N ratio ( $y = 61.4 - 2.39x$ ;  $R^2 = 0.780^{***}$ )



**Fig. 4** Net N (left) and  $^{15}\text{N}$  (right) mineralization from cover crop roots and shoots in relation to C/N ratio in the incubated plant materials. Results are shown for incubation intervals of 0–7, 8–14, 15–30, 31–60 and 61–100 days. Note: Regression lines for the shoot and root lines were separated if the difference between them is significant. The regression equations are shown in Table 2. Error bars show the standard error of the mean



differed somewhat between shoots and roots during the 0–30 days incubation period (Fig. 4). After the first 7 days of the incubation, the relationship generally showed a steeper slope for the shoots than the roots, resulting in an increasingly higher C/N ratio of roots compared with shoots needed to cause net N immobilization in the soil.

The relationship between net N mineralization and C/N ratio can be characterized by the slope of the linear relationship and the C/N ratio at which net N mineralization is zero (Figs. 3 and 4). These properties of the relationship changed during the incubation with different responses for shoots and roots, but similar responses for net N and  $^{15}\text{N}$

mineralization were observed (Fig. 5). Steeper slopes were obtained for shoots than for roots earlier in the incubation, which were reversed after about 90 and 60 days for net N and  $^{15}\text{N}$  mineralization, respectively. The C/N ratio at which net N mineralization was zero increased with increasing duration of incubation, especially for shoots. This means that as incubation proceeds, more materials will have a positive net N mineralization, whereas initially net N mineralization only occurs for shoots with C/N below 16 and roots with C/N below 20. For  $^{15}\text{N}$ , the corresponding values of C/N ratio at zero  $^{15}\text{N}$  mineralization were about 20 and 24 for shoots and roots, respectively.



**Table 2** Coefficient of determination ( $R^2$ ), intercept and slope of linear models relating net N and  $^{15}\text{N}$  mineralization to the C/N ratio of cover crop roots and shoots

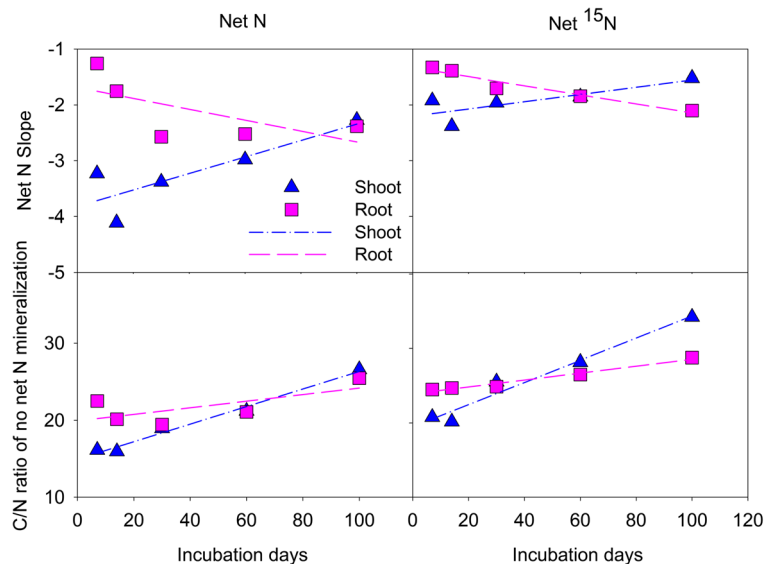
Time	Intercept	Slope	$R^2$
Net N			
0–7	37.7	-1.990	0.59 ***
8–14	10.8	-0.710	0.82 ***
15–30	10.1	-0.336	0.06 ns
31–60	1.7	0.175	0.11 ns
61–100	1.1	0.475	0.11 ns
Net $^{15}\text{N}$			
0–7	35.5	-1.551	0.66 ***
8–14	5.4	-0.269	0.50 **
15–30	7.7	-0.151	0.03 ns
31–60	3.6	0.013	0.00 ns
61–100	3.8	0.125	0.02 ns

Significance levels: ns not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

### Net C mineralization

The addition of CC shoots and roots caused a flush in net  $\text{CO}_2$  emission during the first 20 days of the incubation after which the C mineralization leveled off (Fig. 6; Supplementary Materials Table S1). At the end of the incubation experiment (100-d), net C mineralization corresponded to 53–62% of the C applied with CC shoots, which was higher than that from roots at 18–39% of the applied C. No significant difference was observed between legumes and non-legumes.

**Fig. 5** Regression parameters for net N and  $^{15}\text{N}$  mineralization estimated from the start of incubation against C/N ratio expressed as slope and C/N ratio of no net N and  $^{15}\text{N}$  mineralization and shown for different durations of incubation. Regression lines are shown separately for roots and shoots



### Net C mineralization negatively correlated with plant lignin concentration

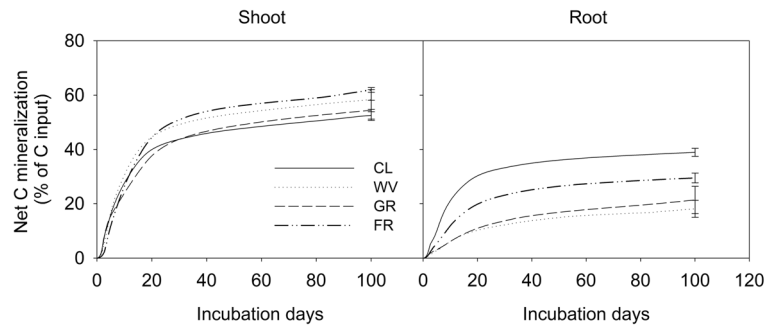
Net C mineralization was negatively correlated with lignin concentration of the plant materials during the incubation, which was the only significant correlation between C mineralization and the measured plant biochemical characteristics (Fig. 7). This significant negative correlation persisted during 0–60 d of the incubation (Table 3). The absolute value of the slope was greatest in the first 7 days and decreased as the incubation proceeded.

### Discussion

#### Carbon and N turnover in legume vs. non-legume and shoot vs. root treatments

In the present study, rapid N turnover was observed within the first 30 days of the incubation of CC shoots, while only during the first 7 days for the roots with a slower rate (Fig. 1). Similarly, Jensen et al. (2005) found that the initial phase with rapid mineralization or immobilization lasted for a maximum of 4 weeks at 15 °C, using 76 different crop residues (covering 37 species and several plant parts). Our leguminous CC treatments (red clover and winter vetch) showed relatively large net N mineralization while the non-leguminous treatments showed net N

**Fig. 6** Net CO<sub>2</sub>-C release during 100 days incubation after application of <sup>15</sup>N-labeled roots and shoots of different cover crops (CC). Note: CL: red clover, WV: winter vetch, GR: ryegrass, FR: fodder radish. Error bars show the standard error of the mean



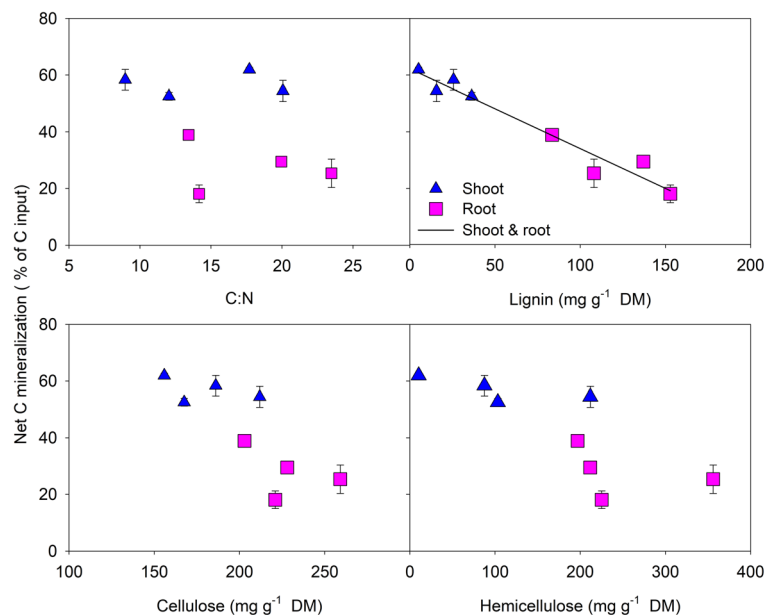
immobilization in 0–30 d of the incubation (Fig. 1). Studies have shown that the N concentration or the C/N ratio can be good predictors of net N mineralization from plant residues (e.g. Trinsoutrot et al. 2000). The present study showed that this also applies for cover crops and that the relationship is similar for CC roots and shoots.

Both C and N mineralization rates were higher in shoots than roots (Figs. 1 and 6), which aligns well with several previous studies on other crop residues (Yannie et al. 2011; Hu et al. 2018a). Using a 180-day incubation of fodder radish residues at 20 °C, Hu et al. (2018a) found a net N-mineralization of 42% of N added in shoots, while only 28% for N in roots. The higher N mineralization in the study by Hu et al. (2018a) compared with our results may be due to the lower incubation temperature and shorter incubation time in our study.

The lower net C mineralization rate from root compared with shoot residues similarly agrees with previous

studies (Rasse et al. 2005; Austin et al. 2017). Root-derived C has been reported to contribute more to stable soil C pools than the same amount of aboveground crop residue-derived C (Kätterer et al. 2011), although these findings have also been contested in both field and laboratory experiments (Hu et al. 2018a, b). Hu et al. (2018a) thus found that shoot C mineralized faster than root C in the initial phase of decomposition, but after 180 days incubation, the release of C did not differ between shoots and roots. We incubated residues at a lower temperature and for a shorter time than Hu et al. (2018a), so our results for C mineralization may not reflect the long-term effects. Therefore, we recommend considering the mineralization kinetic parameters of different isolated parts of plant residues, e.g. roots and shoots, instead of a mixture of the whole plant in studies of soil fertility and nutrient balance, especially if the shoots are harvested and removed from the field.

**Fig. 7** Net cumulative CO<sub>2</sub>-C release after 100 days decomposition in the soil in relation to C/N ratio, lignin, cellulose, and hemicellulose concentration in the applied plant materials. Note: Regression lines for the shoot and root lines were separated if the difference between them is significant. Significance levels: ns not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ . Error bars show the standard error of the mean. The net CO<sub>2</sub>-C release is significantly related to lignin ( $y = 62.3 - 0.281x$ ;  $R^2 = 0.948$ \*\*\*)



**Table 3** Coefficient of determination ( $R^2$ ), intercept and slope of linear models relating net  $\text{CO}_2\text{-C}$  release during different periods after application to soil to lignin, cellulose and hemicellulose concentrations in cover crop roots and shoots

Time	Intercept	Slope			$R^2$
		Lignin	Cellulose	Hemicellulose	
0–7	36.6	−0.089***	−0.081 <sup>ns</sup>	0.003 <sup>ns</sup>	0.84
8–14	8.4	−0.052***	0.061 <sup>ns</sup>	−0.041*	0.90
15–30	0.9	−0.054***	0.091 <sup>ns</sup>	−0.035*	0.83
31–60	−0.3	−0.021***	0.040 <sup>ns</sup>	−0.009 <sup>ns</sup>	0.54
61–100	−1.0	−0.028 <sup>ns</sup>	0.030 <sup>ns</sup>	0.004 <sup>ns</sup>	0.17

Significance levels: ns not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

The type of CC incorporated in the soil strongly affected the dynamics of soil mineral N. Net immobilization of soil N was observed for all materials, except leguminous CC shoots. Immobilization of soil N was greatest in the first 14 d after the application of shoots of non-legume CCs, at 15–17% of the N applied, after which there was steady remineralization of unlabeled soil N (Fig. 2). This greater initial immobilization of N in shoots versus roots is caused by the greater C mineralization in shoots, which increases the demand for N of the microbial decomposers. An initial soil N immobilization phase is frequently observed in mineralization of organic residues (Flavel and Murphy 2006; Mohanty et al. 2011), which largely depends on the C/N ratios of the amended plant residues, the amount of crop residues and the physical or chemical characteristics controlling microbial activity. In contrast, Dalias et al. (2002) did not find a clear initial decreasing trend of native soil mineral N after  $^{15}\text{N}$ -labeled wheat straw (C/N ratio 34:1) was incorporated into the soil in laboratory incubations. Such differences may be due to the C amount added; in our experiment, the addition of CCs corresponded to 14.5% of the soil C content. In the study of Dalias et al. (2002), the amount of added straw C corresponded to 4% of the C content of each experimental soil, which was probably too low to cause measurable immobilization of native soil N.

#### Biochemical characteristics of residues impact C and N mineralization

Our results show that among all the tested biochemical properties of plant materials, C/N ratio had the strongest

correlation with the net N mineralization (Fig. 3). The significant negative correlation between net N mineralization and C/N ratios in plant materials is in line with previous observations (Taylor et al. 1989; Seneviratne 2000; Trinsoutrot et al. 2000; Thomsen et al. 2008). In contrast, other studies argued that the lignin and N content of plant materials were good predictors of N mineralization (Yanni et al. 2011; Vahdat et al. 2011). Jensen et al. (2005) found that the initial net N mineralization rate was most closely correlated to water-soluble N content of the plant materials, and the C/N ratios and lignin/N ratios are not always well correlated with the N mineralization. In addition, Giller and Cadisch (1997) found that short-term and long-term net N mineralization were correlated with different biochemical properties of plant residues. Therefore, no single index can characterize the quality of plant residues. The diverging results between our study and these aforementioned studies could result from our study focusing on CCs, and these were by nature at a young stage having a relatively narrow range of C/N ratios (Table 1). Therefore, our results are most relevant for CCs under similar growing conditions as in Northern Europe.

We found that lignin concentrations showed the strongest correlation with the net C mineralization among all the tested biochemical properties of plant materials (Fig. 7). Our results were comparable with previous studies showing that lignin/N ratio, lignin content and N content of plant materials were good predictors of residue decomposition (Johnson et al. 2007; Yanni et al. 2011). In contrast, some studies showed that C/N ratios of plant materials could also be a good indicator for predicting C mineralization (Taylor et al. 1989; Seneviratne 2000; Thomsen et al. 2008). Jensen et al. (2005) found that holocellulose C content was the single factor, which can best explain the variability of C mineralization. They did not grind the plant materials, which could reduce the contact between plant materials and soil compared to adding finely grounded material as in our study. Grinding plant materials may increase the accessibility of residue C and N to decomposer organisms (Jensen 1994). It may also result in physical protection of a larger proportion of residue-derived C in microbes C (Sørensen et al. 1996). In the present study, the ground plant material was placed in a discrete layer in soil and the effect of grinding was therefore expected to be lower. Our study indicates that the lower mineralization of root C was mainly related to a higher lignin concentration in roots. This was clearly

reflected in the observed relationship between lignin content and net C mineralization in soil.

Net N mineralization was negatively correlated with C/N ratios of plant materials during the first 14 d of incubation, after which the significant relationship diminished (Table 2, Fig. 4). The result could be ascribed to that availability of C (and N) from remaining, non-decomposed residues declined as the increasing proportion of recalcitrant residues. Simultaneously, the decomposer community and microbial products change, which may influence both N demand and availability. The C mineralization rates were highest during the first few weeks (Fig. 6), and the net effect of this microbial C use was net N mineralization or immobilization, depending on C/N ratios in the added material (Trinsoutrot et al. 2000; Gentile et al. 2009; Li et al. 2016). Subsequently, the immobilized N may be re-mineralized. The insignificant correlation between the net N mineralization and the C/N ratio of crop residues during the 31–100 d after application may be attributed to re-mineralization of the immobilized N and differences in the C availability among plant materials. It shows that after the initial decomposition period other factors than initial C/N ratio determines the net N mineralization rate.

#### Laboratory vs. field measurements

The plant materials used in this study were derived from a field experiment reported by Li et al. (2015a, b). The N fertilizer replacement value of CC shoots was estimated in the following barley crop. The N fertilizer replacement value was expected to be proportional to the net release of mineral N from residues. The net mineralization of

legume shoot N measured in the incubation here was comparable with the N fertilizer replacement value estimated in the field: 31% vs. 31% for red clover and 42% vs. 39% for winter vetch (Table 4). For the non-legumes, the correspondence was less good: 18% vs. 9% for ryegrass and 17% vs. 2% for fodder radish, showing that more N was released in our incubation than found to be retrieved by the field crop. In our incubation study, a significant proportion of the net N mineralization occurred from the non-legumes in the last period of the incubation (Fig. 1), which may very well have been too late under field conditions for the barley crop to benefit. Such a delay in N release after incorporation of a grass-based CC was also reported by Doltra and Olesen (2013), and this is one of the main challenges for efficient use of N from CCs under North European climatic conditions.

In the field study, Li et al. (2015b) also calculated the apparent N recovery from CC root residues in the following barley crop, which however could not be directly related to the present incubation, since the amount of N in root residues in the field study was unknown. They observed a significant N release from red clover root residues (21 kg N ha<sup>-1</sup> extra N uptake in barley), and we measured net mineralization of 27% after the 100-d incubation. For winter vetch and fodder radish roots, however, they found nearly no extra N uptake in barley, whereas we measured net N mineralization of 26% and 19%, respectively, and for ryegrass roots, they observed a negative effect while the net mineralization in our incubation was close to zero from ryegrass roots. This indicates that the extracted roots used in the present incubation did not have the same composition as the intact roots

**Table 4** Comparison of the net N and <sup>15</sup>N mineralization of residues in the 100-day incubation presented in this paper with the N fertilizer replacement value (NFRV), apparent N recovery

(ANR) and mineral fertilizer equivalent (MFE) of the same materials from a field study (Li et al. 2015b)

Treatments	Net N mineralization (d.100, % of N input)	NFRV (%)	ANR (kg ha <sup>-1</sup> )	Net <sup>15</sup> N mineralization (d.100, % of <sup>15</sup> N input)	MFE (% of <sup>15</sup> N input)
S Red clover	31	31		29	34
S Winter vetch	42	39		42	47
S Ryegrass	18	9		23	26
S Fodder radish	17	2		25	29
R Red clover	27		21	28	27
R Winter vetch	26		2	34	11
R Ryegrass	1		-18	8	14
R Fodder radish	19		-1	23	15

Abbreviations: S shoot, R root

used in the field study by Li et al. (2015b), which also included root exudates and micro-roots. Root decomposition studies may thus not be representative of field conditions if roots isolated by washing do not have the same composition as the whole root system. In studies with intact roots (without root isolation), it is on the other hand impossible to quantify the exact size of the root N and C pools involved, unless these pools are labeled.

The mineral fertilizer equivalent was calculated by dividing  $^{15}\text{N}$  recovery in barley following CC incorporation by that after applying a mineral fertilizer reference in Li et al. (2015b) as a measure for the  $^{15}\text{N}$  in the CC residues available for crop uptake. This mineral fertilizer equivalent should be comparable with the net  $^{15}\text{N}$  mineralization in the incubation. However, the  $^{15}\text{N}$  mineralization from the incubated CC shoots was consistently lower than the corresponding mineral fertilizer equivalent (Table 4). This discrepancy may be attributable to higher mineralization of labeled CC N in the field than in the 100-d laboratory incubation, or a higher proportion of mineralized N being taken up in the harvested crop than from the applied mineral fertilizer reference in the field. It could also be due to the competition from plants that may have limited the microbial immobilization compared to that in the incubation when no plant is present to compete with the microbes (Korsaeth et al. 2001).

### Perspectives

Our results show that after the application of roots and shoots with low N concentrations, a significant amount of N immobilization occurred from the soil mineral N pool. After the application of shoots, this immobilization mainly occurred within the first 7–14 days followed by a period where around 50% of this N was re-mineralized. For roots, the immobilization of soil N was lower but persisted for a longer period with no detectable re-mineralization in most cases (Fig. 2). These differences in net N mineralization between roots and shoots were confirmed by a corresponding field study showing less crop N uptake from N supplied in roots compared with shoots (Li et al. 2015a). Nitrogen transformation in soil is largely driven by the presence and activities of microorganisms (Ye et al. 2018). The mechanisms behind these differences warrant further investigation since differences are linked with the C turnover of the respective residues.

The N input in residues that is not net mineralized within the first month will only be mineralized slowly over many years. This long-term mineralization occurs also in periods without crop growth, where N leaching can occur resulting in lower N utilization, whereas the N released when residues are applied/incorporated just before establishing a new crop shows a high N utilization (Li et al. 2015b). Therefore, the incorporation of CC residues with low C/N ratios is expected to improve synchronization of mineral N release with crop demand and thus increase N use efficiency, while residues with higher C/N ratios should be applied in combination with appropriate N fertilizer to avoid competition for N between the decay of plant residues and growth of the following crop.

### Conclusions

Our study with cover crops grown under North European conditions showed different C and N turnover rates after soil incorporation of different species and plant parts (i.e. shoots and roots) within the first 100 days of decomposition in soil at 10 °C. The net mineralization of N is significantly and negatively related to the C/N ratio of both shoots and roots, with the same relationship for both legumes and non-legumes. The effect of C/N ratio on N mineralization mainly occurs within the first 2 weeks. By contrast, the net C mineralization from CC residues is significantly reduced by the concentration of lignin in residues. The relationships between mineralization and these biochemical parameters are the same for both CC shoots and roots. For shoot residues with a low C/N ratio we found no priming effects on the release of N from the soil N pool, while materials with a C/N greater than 12 caused initial immobilization of soil N. The data of this study could be used to improve parameters in dynamic N and C models used in agroecosystems including cover crops. Further research should focus on utilizing microbial diversity to evaluate the C and N turnover of different species and plant parts using  $^{13}\text{C}$  or/ and  $^{15}\text{N}$  labeled plant residues.

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