Agronomy Research **15**(2), 594–601, 2017

Nitrogen and carbon release during decomposition of roots and shoots of leguminous green manure crops

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Abstract. In Nordic conditions, soils are frozen during winter, affecting the decomposition rates of crop residues. Hence, the decomposition rates of above- and underground biomass and the dynamics of the N and C released into the soil were studied in trials focused on green manure crops. The decomposition of the residue and N release from the residue varied among the five species of legume tested. There was a marked difference in decomposition rates between shoots and roots, which may also be explained by the differences in the chemical composition of the residue. The shoot residue decomposes rapidly and it serves as a source of N for the subsequent crop. The root residue decomposes more slowly and this had a positive effect in a crop rotation in the second year.

Key words: C:N ratio, residue decomposition, N and C release, green manure crops, legumes.

INTRODUCTION

Cultivation of green manure crops in crop rotations has been reintroduced into ecological farming in many European countries, as a substitute for mineral N fertilizers. Through efficient utilization of symbiotically fixed N by legumes in green manure, it is possible to achieve higher yields of subsequent crops. During decomposition, microorganisms use the organic carbon, while nutrients are mineralized and eventually returned to the soil (Brunetto et al., 2011) in forms that are available to plants.

The C and N mineralization rates depend on climatic and soil conditions such as temperature, moisture content, microbial activity, soil pH, aeration status and texture (Ha et al., 2008; Havstad et al., 2010). Mineralization rates also depend on the quality of organic matter (Tejada & Gonzalez, 2006). The main quality properties of the residue that affect mineralization include the C:N ratio, the content of cellulose and lignin (Chaves et al., 2004). A high C:N ratio (> 25) generally leads to immobilization: nitrogen is either immobilised by microorganisms during the decomposition of organic matter or mineralised into the soil as ammoniacal N.

Numerous studies of net N mineralization from decomposing legume residues have been carried out on aboveground biomass of various species in cool climates (Marstorp

& Kirchmann, 1991; Haynes, 1997; Wivstad, 1999; Trinsoutrot et al., 2000; Lupwayi et al., 2006), but there are few studies about N release to soil from roots, none of which is from a boreal or sub-boreal climate (i.e. Chaves et al., 2004; Osanai et al., 2012). The topic is important, because the proportion of plant nitrogen in roots is up to 40% of total plant N (Rochester at al., 1998; Talgre et al., 2009) and when used as fodder for animals aboveground plant parts are not returned to soil.

The objectives of this experiment were to determine the net N and C mineralization from the roots and aboveground residues of red clover (*Trifolium pratense* L.), bird's-foot trefoil (*Lotus corniculatus* L.), lucerne (*Medicago sativa* L.), large-leaved lupine (*Lupinus polyphyllus* Lind.) and white melilot (*Melilotus albus* Med), as typical pasture or green-manure legume crops for Nordic conditions. The data can be used to predict the influence of residue decomposition on soil fertility.

MATERIALS AND METHODS

The trials were carried out during 2007–2010 in the Department of Field Crop and Grassland Husbandry, Institute of Agricultural and Environmental Sciences, at the Estonian University of Life Sciences, (58° 23' N, 26° 44' E). The soil type of the experimental area was sandy loam *Stagnic Luvisol* according to the World Reference Base classification (FAO 2014). The mean characteristics of the humus horizon were as follows: C_{org} 1.1–1.2%, N_{tot} 0.10–0.12%, P 110–120 mg kg^{-1}, K 253–260 mg kg^{-1}, pH_{KCl} 5.9, soil bulk density 1.45–1.50 g cm^{-3}. The humus horizon was 27–29 cm. More detailed description of the experiment is presented in the article by Talgre et al. (2014).

The experimental area belongs to the South-Estonian upland agro-climatic region, where the mean annual temperature is 4.4 °C and total precipitation is 550–650 mm (Tarand, 2003). During the experimental period, rainfall and air temperature were recorded daily at the meteorological station located close (< 1 km) to the experimental area using the Metos Model MCR300 (Pessl Instruments GmbH, Weiz, Austria) weather station. Weather conditions during the study period (2007–2010) differed substantially (Table 1).

Table 1. Monthly precipitation and average temperature during the experimental period (2007–2010).

Month	Air temperatures, °C				Precipitation, mm			
	2007	2008	2009	2010	2007	2008	2009	2010
January	-7.1	-1.3	-3.4	-12.7	29	22	10	3
February	-6.6	0.6	-4.9	-7.9	23	34	7	5
March	-2.4	0.4	-1.5	-2.1	26	8	22	30
April	4.2	7.1	5.3	6.1	33	27	14	26
May	11.6	10.6	11.5	12.6	55	27	13	61
June	15.1	14.4	13.8	14.6	66	110	137	73
July	16.7	16.1	16.9	22.2	72	54	55	36
August	15.6	17.7	15.4	18.2	79	118	89	107
September	10.4	9.8	12.8	11.1	66	46	49	93
October	5.7	8.2	4.1	4.2	52	68	116	49
November	0.3	2.3	2.3	0.8	48	49	36	53
December	-4.2	-1.1	-3.8	-7.7	40	24	41	17

Two trials were carried out: experiment 1 in 2007–2009 and experiment 2 in 2008–2010. Plant species used in the study of experiment 1 were red clover, lucerne, bird's-foot trefoil, and white melilot. In experiment 2 there were red clover, white melilot and large-leaved lupine.

Pure crops were sown at the beginning of May (2007 and 2008). The length of their growing season was 6 months on average. The roots and shoots were collected from field-grown plants before the incorporation of green manure into the soil by ploughing at the end of October. Roots were sampled from a 0.25 m^2 frame, 0–30 cm depth (the main part of the roots being located in this soil layer), and soil was washed from the roots. Samples of aboveground biomass were taken from plot of 0.25 m^2. The roots and shoots were cut into 5 cm pieces. It is equivalent to the effect of the machinery used for green manure crushing before ploughing. Fresh plant residues (25 g) were placed in 20 × 20 cm nylon bags with 1 mm mesh. Corresponding samples were dried, milled and analysed as described below. The bags were buried in a completely randomized design at a depth of 22 cm with 0.3 m spacing at the end of October before ploughing (ploughing depth was 18–20 cm).

After 6, 12 and 24 months of decomposition, 4 bags (representing 4 replicates) for each species and plant fraction (roots and shoots) were selected for measuring the dry matter content and nutrient losses.

Plant materials remaining in the nylon bags at each sampling time were manually separated from soil and organic debris, oven-dried at 65 °C to a constant weight. The oven-dried samples were weighed separately to determine the dry matter losses. Subsequently the samples were ground to pass a 0.5 mm sieve for chemical analysis. Total N and C contents of oven-dried samples were determined by dry combustion method on varioMAX CNS elemental analyzer (Elementar, Germany).

The software STATISTICA 10 (Statsoft Inc., USA) was used for the statistical data analysis. The decomposition data were processed using descriptive statistics. Two-way ANOVA was applied to test the effect of plant species and tissue type (root or shoot). The means are presented with standard deviation (± SE). Tukey tests were used for testing significance of differences between means at $p < 0.05$. A correlation analysis was conducted to quantify the relationships between chemical composition of residue and N mineralization. The level of statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

At the beginning of decomposition process the total N content in green manure plants was 19.1–31.1 mg kg^{-1}. The N concentrations of roots (except in red clover roots in 2008) were significantly higher than those of aboveground plant parts. Carbon concentration of the residues showed also variation: 41–46% for aboveground residues and 38–44% for roots. The C:N ratio and N content of the residues have been suggested as indicators of decomposition rate. The C:N ratio varied considerably among species, while root residue had a lower C:N ratio than shoot residue in all species ($p < 0.05$) (Table 2). White melilot roots had the lowest C:N ratio. Our results about C:N ratio values differ from other studies: Chaves et al. (2004) and Osanai et al. (2012) found that shoot residue had a lower C:N ratio than root residue and shoots contained more N than roots.

Table 2. Dry matter (DM) (g kg^{-1}), C_{tot}, N_{tot} contents (mg kg^{-1}) and C:N ratio of legumes shoots and roots set for decomposition

Green manure crop	Plant part	DM	C_{tot}	N_{tot}	C:N
	2007 (Experiment 1)				
Red clover	Shoots	291 c	447 b	24.7 b	18.1 c
	Roots	342 b	429 c	27.1 ab	15.8 d
White melilot	Shoots	321 bc	465 a	19.1 d	24.3 a
	Roots	373 b	430 c	31.1 a	13.8 e
Bird's-foot trefoil	Shoots	316 bc	462 a	20.4 c	22.6 b
	Roots	402 ab	402 d	26.7 ab	15.1 de
Lucerne	Shoots	434 ab	457 b	21.2 c	21.6 b
	Roots	460 a	441 b	25.7 ab	17.1 c
	2008 (Experiment 2)				
Red clover	Shoots	251 b	415 a	21.1 ab	19.7 b
	Roots	258 b	399 b	19.3 b	18.7 bc
White melilot	Shoots	371 a	428 a	19.3 b	22.1 a
	Roots	216 b	395 b	24.1 a	16.4 c
Large-leaved lupine	Shoots	188 c	410 a	21.3 ab	19.2 b
	Roots	264 b	380 b	20.7 ab	18.5 c

Within each column and year, the mean values with different letters are significantly different (Tukey test, $p < 0.05$).

N release rate is usually considered to depend on the C:N ratio. For optimal growth of the degrading microorganisms, C:N ratios of 25–35 are usually suitable during the initial phase (Biddlestone et al., 1994). Legume residues generally have low C:N ratios, which is in accordance with our study: C:N ratio was 14–20 in roots and 18–24 in shoots. Low C:N ratio is generally associated with higher mineralization rates (Franzluebbers & Hill, 2005; Brunetto et al., 2011). The highest C:N ratio was observed in white melilot shoots. The residues with high C:N ratio are generally reported to have negative effect on the N availability (Trinsoutrot et al., 2000). Our research results confirm earlier results (Kumar & Goh 2000; 150), that C:N ratio of shoots is negatively correlated with N mineralization ($r = -0.75$, $p < 0.05$). Osanai et al. (2012) found no relationship between N mineralization and C:N ratio of residue despite the clear relationship observed between residue decomposition and C:N ratio of residue. Lupwayi et al. (2006) found that percentage of N released was positively correlated with N concentration, but our results did not confirm this. Our results also indicate a marked difference in decomposition rates between shoots and roots, which may be attributed to differences in the chemical composition of the residues. While the C:N ratio of aboveground biomass is higher than that of the roots, the nitrogen was also released from the aboveground biomass at a higher rate. Soon and Arshad (2002) found that the decomposition of roots was not controlled by the C:N ratio. Hence, the rate of crop residues decomposition cannot be explained only by the C:N ratio (Hofmann et al. 2009). This may be due to the fact that root material containing lignified tissues and other structural components, provides more recalcitrant material than shoots (Rasse et al., 2005). Moore et al. (1999) and Rasse et al. (2005) have reported that lignin:N ratio is an important factor influencing decomposition and it is lower in residues that are more easily biodegradable.

During experiment 2 (colder winter), the crop residues decomposed more slowly than in experiment 1. In experiment 1 55–59% of initial nitrogen and up to 48% of initial carbon remained in the residues after 6 months (Figs 1a and 2a). In experiment 2, the initial decomposition rate over the winter period was slower and depending on the species up to 75% of nitrogen and 57–68% (shoots) and 88% (roots) of carbon remained in the residues after 6 months (Figs 1b and 2b). The effect of climatic conditions on the decomposition rate of biomass and nutrient release was also seen by Soon & Arshad (2002). Legume roots decomposed more slowly than aboveground residue ($p < 0.001$). After 6 months the total C mineralization of the root residue was lower than that of shoot residue (Fig. 2).

Between 6 and 24 months, the nitrogen was released more slowly from the aboveground biomass of birds-foot trefoil and lucerne roots than from other species in trial ($p < 0.05$) (Fig. 1a). The slow decomposition rate of lucerne has been also observed by Harris & Hesterman (1990), whose results showed that lucerne residues did not provide significant amounts of N to a succeeding barley crop. It may be due to the lignification of root material, which led to slow decomposition and later N availability. De Neergaard et al. (2002) found that the lignin content in roots of clover is 2.5 times higher than in shoots.

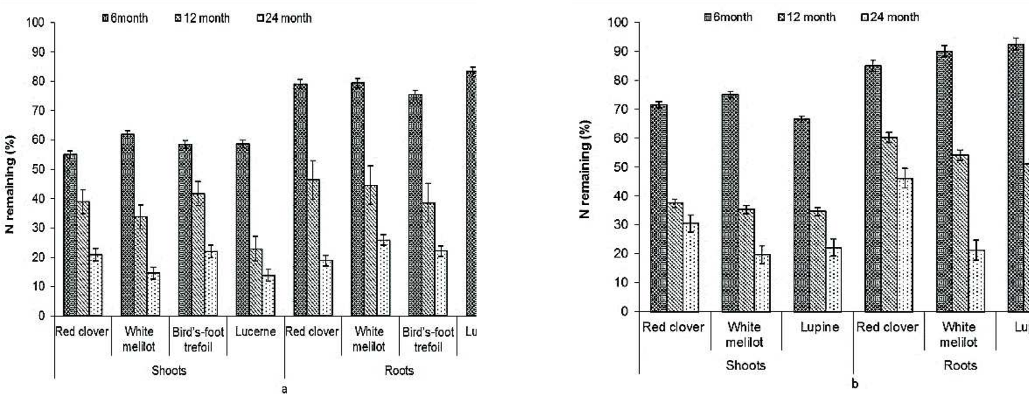

Figure 1. Nitrogen remaining in biomass (%) after 6, 12 and 24 months of decomposition in experiment 1 (a) and in experiment 2 (b). Vertical bars denote standard deviation (± SE).

In experiment 1 (Fig. 1a), 24 months after the beginning of decomposition, 20% of the initial N content remained in the shoots of red clover and birds-foot trefoil and 27% in roots of white melilot. C concentrations were still highest in residues of lucerne roots ($p < 0.05$). In experiment 2, where decomposition rate was lower, significantly more N remained in red clover residue compared to white melilot (Fig. 1b). At the end of the experiment there were still 12–21% of the initial C content remained in the shoots and 19–25% of initial C left in the roots.

During relatively warm winters (average temperature from February to March above 0 °C), crop residues decomposed faster than in cold winter conditions (average temperature from December to March below 0 °C), because little or no microbial activity takes place below 0 °C. Green manure should be ploughed into the soil in late autumn or early spring, in order to minimise the leaching of N. Lahti & Kuikman (2003) suggested that the incorporation of green manure crop should be at the onset of soil freezing to reduce the potential for N leaching.

Depending on biomass the green manure crops fixed up to 206 kg N ha^{-1} (Talgre et al., 2012) and up to 3.8 t ha^{-1} C (Talgre et al., 2009). As the legumes have an impact on almost all the chemical and physical properties of the soil, their effect is not limited to one year (Reeves, 1997). Our previous trials (Lauringson & Talgre, 2010) have shown that the higher decomposition rate of red clover has an effect on the yield of following crop. Thus we suggest that the effect of nitrogen provided to the soil with red clover would be higher after one year whilst the nitrogen left in the soil after lucerne and melilot would have a greater impact during the second year. Kanal (2005) found that the decomposition was influenced by the depth of ploughing and occurs faster in the top soil layers. Viil & Võsa (2005) found that the decomposition of green manure crops was faster in lighter and more fertile soils than in heavier and less fertile ones.

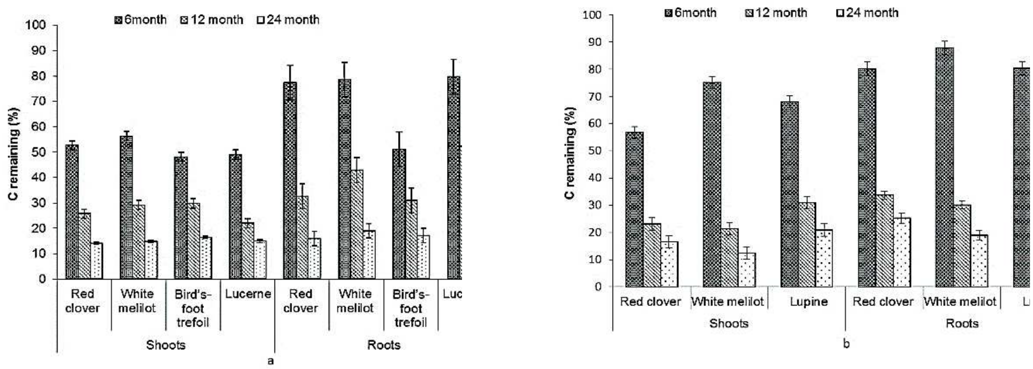

Figure 2. Carbon (%) remaining in aboveground biomass and roots after 6, 12 and 24 month decomposition in experiment 1 (a) and experiment 2 (b). Vertical bars denote standard deviation ± SE).

CONCLUSIONS

Our results showed that the rates of decomposition of residues and of N release from the residues were significantly different among plant species. After 12 months the overall N and C release from the lucerne roots was 35% of total N and 50% of total C, on average, which was lower than for the roots of red clover and birds-foot trefoil. N and C release is not only influenced by the chemical composition and decomposition time of the residues, but also by weather conditions. The root and shoot residues of green manure crops differed in their chemical composition and their N and C release patterns. We demostrated that shoot residue decomposes more rapidly than root residue.

ACKNOWLEDGEMENT. This research was supported by the Ministry of Agriculture of the Republic of Estonia within the framework of project 'Researching methods of regulating humus status and nutrient balance of soil and phytoproductivity of various green manure crops in conventional and organic farming'.

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