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REGULAR ARTICLE

Quantifying biological nitrogen fixation of different catch crops, and residual effects of roots and tops on nitrogen uptake in barley using in-situ ^{15}N labelling

Xiaoxi Li · Peter Sørensen · Fucui Li · Søren O. Petersen · Jørgen E. Olesen

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Abstract
Background and aims Contributions of legume-based catch crops (LBCCs) to succeeding cereals may be significant. We quantified biological N fixation (BNF) and residual N effects of contrasting CC tops and roots.
Methods BNF of three LBCCs (red clover, winter vetch, perennial ryegrass-red clover mixture) was quantified in microplots by ^{15}N labelling. Their residual effects on spring barley were tested against two non-LBCCs (perennial ryegrass, fodder radish) after spring incorporation of CC tops or roots in monoliths.
Results Total N accumulated in LBCCs was 153–226 kg N ha^{-1}, of which 62–66 % was derived from BNF in tops and 31–46 % in macro-roots (0–18 cm soil). Macro-roots represented 31–50 % of total plant N. LBCCs showed similar capacity for soil N extraction as non-LBCCs. After incorporation of LBCC residues, the dry matter and N yields of spring barley were comparable to the effect of 50 kg N fertilisation ha^{-1}, whereas no extra N uptake was derived from non-LBCCs. The ^{15}N-based N fertiliser values of LBCC tops were 34–47 % against 26–29 % for non-LBCCs.
Conclusions LBCC roots contributed substantial amounts of N to the system, a source that is usually underestimated. N immobilisation after incorporation of non-LBCCs may hamper the growth of following main crops especially after removing tops.

Keywords Legume-based catch crop · Biological N fixation · Soil N uptake · Residual N effect · ^{15}N isotope dilution · Spring barley

Abbreviations

N	Nitrogen
DM	Dry matter
CC	Catch crop
(Non-)LBCC	(Non-)legume-based catch crop
BNF	Biological N fixation
CL	Red clover
GC	Perennial ryegrass-red clover mixture
WV	Winter vetch
FR	Fodder radish
GR	Perennial ryegrass
CO	Control without catch crops in large plots

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X. Li (✉) · P. Sørensen · S. O. Petersen · J. E. Olesen
Department of Agroecology, Aarhus University, Blichers Allé 20, Tjele DK 8830, Denmark
e-mail: xiaoxi.li@agro.au.dk

X. Li
e-mail: lixiaoxi86@gmail.com

F. Li
College of Natural Resources and Environment, Northwest Agricultural and Forestry University, Yangling 712100 Shaanxi, China

N0	Control without residue or fertiliser addition during barley season
N1	Control without residue but fertiliser addition during barley season
EAF	Excess atom fraction ^{15}N
Ndfa	Percentage of CC-N derived from atmosphere
Nds	Amount of CC-N derived from soil plus applied ^{15}N-labelled fertiliser
Ndfr	Barley N derived from CC residues
N_{Res_BG}	Pool size of ^{15}N-labelled below-ground N in the CC root treatment
N_{Res_Top}	Amount of N in CC tops incorporated
ANR	Apparent N recovery
MFE	Mineral fertiliser equivalent (^{15}N-based)
NFRV	N fertiliser replacement value (non-^{15}N-based)

Introduction

Ecological intensification is crucial for the development of sustainable agriculture, which calls for higher and more stable crop yields while reducing undesirable environmental impacts (Cassman 1999; FAO 2009; Foresight 2011). Organic farming may contribute to such a goal via its core principles of restricting external input, and reliance on local and recycled resources, but productivity is often limited, which is usually attributed to lack of nitrogen (N) (Berry et al. 2002). Using catch crops (or cover crops, CCs) in cool seasons, e.g., from autumn to early spring, is a common practice to preserve soil N and decrease N leaching losses outside the main crop season in Western and Northern Europe (Askegaard and Eriksen 2008). Moreover, N in CC residues may be a significant source of N and contribute to the yield of succeeding main crops in N limited systems (Thorup-Kristensen et al. 2003; Li et al. 2015). A tendency of less annual variation in yields was observed in organic crop rotations with catch crops (Olesen et al. 2007).

Legume-based catch crops (LBCCs) can, besides taking up N from the soil, supply additional N through biological N fixation (BNF) from the atmosphere (Askegaard and Eriksen 2008; Amosse et al. 2014). However, the N fixation activities and the residual effects of LBCCs or green manure crops on the following cereal crops are variable and affected by species, soil type, local climate and management (Sparrow et al. 1995; Carlsson and Huss-Danell 2003; Doltra and Olesen 2013). In a 12-year organic crop rotation study, Doltra and Olesen (2013) observed an increase of 0.1–1.5 Mg ha^{-1} in grain yield of spring barley when including catch crops. Moreover, the benefit of including CCs tends to increase over time. Bergkvist et al. (2011) found that spring barley with a clover catch crop during winter had 1.9–2.4 Mg dry matter (DM) ha^{-1} higher yields than that without cover crops. Greater dry matter yields and N uptake of spring barley following legumes than non-legumes were also reported by Sparrow et al. (1995).

As shown by these examples, the residual effect can be simply evaluated by comparing the yields of a succeeding main crop with and without a catch crop. Nevertheless, more accurate information about N fixation and the recovery of residue N in the main crop is needed to help quantify and evaluate the potential of N supply and N losses in relation to catch cropping, especially under field conditions. The abilities of catch crops to "catch" soil N, symbiotically fix N from the atmosphere, and supply N to a new crop after residue incorporation, vary among catch crop species. In-situ ^{15}N labelling is a suitable approach to quantitatively study BNF in legumes, to estimate the below-ground N and to trace the fate of N in rotations (Chalk et al. 1993; Danso et al. 1993; Mueller and Thorup-Kristensen 2001; Carlsson and Huss-Danell 2003). Application of ^{15}N enriched fertiliser to soil enlarges the differences of ^{15}N abundance between the soil N and the atmospheric N_2. Legume ^{15}N pool will be diluted when N is assimilated both from atmosphere via BNF and soil uptake, while the non-legume (reference plant) ^{15}N pool will be more similar to the soil due to N derived solely from the artificially enriched soil pool (Huss-Danell and Chaia 2005). Therefore, differences in ^{15}N enrichment between N_2-fixing and non-fixing crops will allow precise estimation of the N fixation (Chalk 1985; Danso et al. 1993). Based on this principle of ^{15}N isotope dilution, N fixation of 8 to 177 kg N ha^{-1} by red clover in the sowing year was

reported for several studies in northern temperate regions (Carlsson and Huss-Danell 2003). In a study that compared the N fixation ability of various LBCCs, Mueller and Thorup-Kristensen (2001) observed the highest N fixation rate of 149 kg N ha^{-1} in hairy vetch (*Vicia villosa* Roth.) in a study where only the above-ground parts were analysed. Due to the ability for BNF, legumes were sometimes reported to be less effective in extracting soil N compared with non-legumes (Thorup-Kristensen et al. 2003; Bergkvist et al. 2011), leading to higher risks of N losses via leaching or denitrification. It is thus crucial to quantify and partition the N sources of LBCCs to justify their use as a catch crop and N provider for succeeding crops.

Catch crop tops may be harvested in autumn for use as winter fodder, silage or biogas digestion (Stinner et al. 2008; Jensen et al. 2012), and consequences of this for the N supply to succeeding crops also needs to be evaluated. Under some conditions, mineralisation from the catch crop cannot compensate for the effect of the catch crop N uptake, which reduces N supply for the succeeding crop and is termed pre-emptive competition (Thorup-Kristensen et al. 2003). Harvest of tops may exacerbate the negative impact. The below-ground parts of catch crops, especially of LBCCs, were reported to account for a considerable proportion of plant N (Danso et al. 1993; Askegaard and Eriksen 2007; Chirinda et al. 2012). Thomsen (1993) observed that a spring barley crop recovered 29–41 % of the ^{15}N from Italian ryegrass shoot residues, and less from the labelled roots. However, addition of physically recovered roots in this kind of study usually underestimates total carbon (C) and N in the below-ground part (McNeill et al. 1997; Wichern et al. 2008). In a field mezotron study using an in-situ ^{15}N labelling technique, Høgh-Jensen and Schjoerring (2001) showed substantial N rhizodeposition into the soil through the roots of clover and ryegrass, and in fine roots. Using undisturbed monoliths including the roots may be superior for on-site studies of the residual effects of root-derived N after removal of catch crop tops. Such monoliths retain not only N in the macro-roots, but also the N in fine roots and N deposited to the soil by the plants (Thomsen et al. 1996).

The objectives of the present field microplot study were: (1) to quantify and compare the uptake of soil N and biologically fixed N allocated in tops and roots (0–18 cm) of three types of LBCCs and two non-LBCCs, and (2) to quantify the contributions of incorporated CC tops and root-derived N to a succeeding spring barley crop, using a ^{15}N labelling method on a temperate loamy sand soil.

Materials and methods

Experimental site

The experiment was conducted at Research Centre Foulum, Aarhus University, Denmark (56°30′N, 9°34′E) as part of a field experiment to study nitrous oxide (N_2O) emissions as affected by different catch crop types and management (Li et al. 2015). The upper 30 cm soil is a loamy sand soil with 8.6 % clay, 12.0 % silt and 79.4 % sand. Furthermore, the topsoil contained 18 g kg^{-1} organic matter, 1.6 g kg^{-1} total N, 33 mg Olsen-P kg^{-1} and 120 mg kg^{-1} extractable K, and the soil pH was 6.4 ($CaCl_2$). The bulk density of the top 10 cm soil layer was 1.38 g cm^{-3}. Climate data were collected from a climate station adjacent to the field plots (Table 1). The 1-year period of this field study was characterised by a cold autumn and winter

Table 1 Monthly mean air temperature and precipitation at the study site

	Air temperature (°C)			Precipitation (mm)		
Month	2012	2013	Ref[a]	2012	2013	Ref
Jan	1.7	−0.6	−0.5	61	46	43
Feb	−1.0	−1.1	−0.5	28	19	34
Mar	5.6	−1.3	1.8	29	3	48
Apr	5.7	5.1	5.5	66	22	40
May	11.7	12.1	10.5	31	61	50
Jun	12.0	13.5	14.2	83	69	57
Jul	15.2	16.7	15.4	109	17	72
Aug	15.8	16.2	15.1	71	67	71
Sep	11.9	12.4	12.1	100	81	75
Oct	7.9	10.2	8.5	85	108	76
Nov	5.4	4.9	4.2	51	72	78
Dec	−0.6	5.1	1.1	52	74	61
Year	7.6	7.8	7.3	766	638	704

[a] *Ref* the monthly mean air temperature and precipitation at the study site during the reference period of 1961–1990 (Olesen et al. 2000)

(August 2012–March 2013) with above-average autumn precipitation and scarce precipitation during winter (Table 1). Rainfall in May and June 2013 was relatively high, and in July low, compared to the long-term average.

Catch crop establishment and labelling

Three LBCCs and two non-LBCCs were compared in this experiment, i.e., red clover (CL, *Trifolium pratense* L., cv. Rajah), winter vetch (WV, *Vicia villosa*, cv. Villana), and a mixture of perennial ryegrass (*Lolium perenne* L., cv. Foxtrot) and red clover (GC), *versus* perennial ryegrass (GR) and fodder radish (FR, *Raphanus sativus* L., cv. Lunetta). In accordance with normal practice, CL, GC and GR were undersown in preceding spring barley (*Hordeum vulgare* L.) main crop on 15 May 2012, while the WV and FR were sown on 10 August 2012, the day after harvest of the barley. The sowing rates were 4, 10, 12 and 50 kg ha^{-1} for CL, GR, FR and WV, respectively. Seeds of 3 kg ha^{-1} CL and 7 kg ha^{-1} GR were mixed and sown for the GC mixture.

After emergence of the FR and WV on 21 August 2012, the microplot experiment was set up within the respective treatments of the large-plot experiment (20 × 3 m per plot replicated in three blocks). Galvanised metal frames confining an area of 25 × 35 cm (0.0875 m^2) were driven 18 cm into the soil leaving 2 cm above the ground. Two rows of catch crops were covered in each microplot. The microplot was replicated twice per plot, and hence there were six replicates for each type of the five catch crops. A schematic representation of the microplot deployment within the large plots during catch crop season is available as Online Resource 1.

A ^{15}N enriched KNO_3 (11.58 % excess atom fraction ^{15}N) solution was applied as a tracer to all microplots on the soil surface along the crop rows using a pipette. The total application of 10 kg N ha^{-1} was split into three doses applied on 29 August, 7 September and 17 September 2012, respectively. These measures were taken to maximise crop uptake and minimise leaching losses of ^{15}N. Weeds emerging in and around the microplot area were on several occasions killed, but left on the soil surface. The field received 33 Mg ha^{-1} cattle slurry (80 kg ha^{-1} NH_4-N) on 15 March 2012, but no further application of manure or fertiliser in the catch crop treatments during 2012–2013 to emphasize the role of catch crops as N source for the spring barley crop in 2013. Table 2 shows a summary of all field operations.

Table 2 Summary of field operations

Date	Field operation
15/03/2012	Cattle slurry injection
19/03/2012	Moldboard ploughing (20–25 cm)
17/04/2012	Sowing of spring barley
15/05/2012	Sowing of catch crops: CL, GC and GR
09/08/2012	Harvest of spring barley
10/08/2012	Sowing of catch crops: FR and WV
21/08/2012	Setup of microplots
29/08/2012	1st $K[^{15}N]O_3$ solution application
07/09/2012	2nd $K[^{15}N]O_3$ solution application
17/09/2012	3rd $K[^{15}N]O_3$ solution application
26/10/2012	Harvest of catch crop tops
23/04/2013	Setup of new microplots, residue incorporation and sowing of spring barley
09/08/2013	Harvest of spring barley

Sampling of catch crops and chemical analyses

The catch crop tops in all microplots were harvested by hand-cutting at the soil surface on 26 October 2012, i.e., before the first frost arrived. Senescent leaves on the ground were collected. 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) and stored dry until spring. Meanwhile, a subsample was ball-milled for ^{15}N analysis.

One of each pair of microplots situated within the same large plot was excavated to 18 cm depth in early November 2012, while the other was left without disturbance until application of spring treatments. The excavated soil monoliths were kept at 2 °C until root washing, maximum of 3 weeks from the time of excavation. To separate out macro-roots, each monolith was transferred to a bucket and the soil washed away with tap water. The roots were further washed on a sieve (0.425 mm), and then transferred to a tray with water, where organic debris was removed based on appearance and colour as described by Chirinda et al. (2011). The recovered macro-roots were dried at 60 °C (48 h) for dry weight and then ball-milled for ^{15}N analysis.

The total N and atom fraction ^{15}N of plant samples were analysed at UC Davis Stable Isotope Facility, using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Establishment of spring barley following catch crops

In the spring 2013, new microplots with incorporation of catch crop residues were set up to test residual N effects on a succeeding spring barley crop (cv. a mixture of Anakin, Colombus and Simba). Before moldboard ploughing (20–25 cm), the intact monoliths which had been left overwintering in the field were excavated (0–18 cm). Then new microplot areas, unaffected by ^{15}N fertiliser, were located in the three fallow control plots (large plots named "CO" as shown in Online Resource 1) to form three blocks. These fallow plots had been treated with glyphosate, a commonly used herbicide, in September 2012 in order to minimise the influence of volunteer plants growing in autumn.

In order to establish a new microplot, a galvanised metal frame (25×35 cm) was driven 20 cm into ground at a designated site, inside which the soil was removed. The transferred soil from intact monoliths containing ^{15}N-labelled root residues was gently turned upside down into the excavated frames to simulate the turning of soil by ploughing. The uppermost 5 cm soil was gently harrowed and 30 seeds of spring barley sown in two rows.

For treatments incorporated with catch crop tops, galvanised metal frames were also driven 20 cm into the soil at pre-selected positions in the CO plots. The upper 15 cm soil was removed and catch crop tops were distributed evenly before the removed topsoil was returned. The amount of incorporated top materials was equal to the mean dry matter yield of the six replicates determined in October 2012 (Table 3). Prior to incorporation, the top materials from all six replicates, collected in late autumn and stored dry until spring, were cut into <5-mm pieces, mixed thoroughly and subsampled for analysis of N concentration and atom fraction ^{15}N. In the GC treatment, clover and ryegrass were not separated, and subsequent measurements and calculations for this treatment were based on the mixed sample. The topsoil was harrowed to 5 cm depth and spring barley sown the same as described above. Two reference treatments were included, one without any residue incorporation, but amended with 50 kg N ha^{-1} ($K[^{15}N]O_3$ solution, 2.08 % excess atom fraction ^{15}N) (N1) and one without residue or fertiliser application (N0). The soil in these two treatments was disturbed in the same way as the top treatments. Hence, there were 12 treatments in total (five catch crops times root / top + N0 + N1), which were randomised and replicated in three blocks. A schematic representation of the microplot deployment during the spring barley season in the field is available as Online Resource 2. Spring barley was also sown to the space between the microplots.

The total above-ground part of the spring barley was sampled at maturity in August 2013 by hand-cutting at the soil surface. The samples were dried at 60 °C (48 h), weighed and ball-milled for total N and ^{15}N analysis.

Calculations and statistical analyses

The recovery rate of the applied ^{15}N enriched fertiliser, Recovery (%), in the top or root of a catch crop was calculated using the following equation:

$$\text{Recovery}\ (\%) = \left[\left(\frac{\text{EAF of CC}}{\text{EAF of fertiliser}}\right) \times \text{CC-N / fertiliser N}\right] \times 100,$$

where, EAF is the excess atom fraction ^{15}N of a sample, which was taken as the atom fraction ^{15}N of the sample subtracting 0.366 %, i.e., the natural atom fraction ^{15}N in atmospheric N_2; the CC-N was the total N in tops or roots (kg N ha^{-1}) and the fertiliser N was 10 kg N ha^{-1}.

The mean EAF of the FR and GR was used as reference to calculate the N fixation by the LBCCs. Thus, the percentage of N in LBCC derived from the atmosphere, Ndfa (%), was calculated as (Huss-Danell and Chaia 2005):

$$\text{Ndfa}\ (\%) = \left[1-\left(\frac{\text{EAF of LBCC}}{\text{mean EAF of non-LBCC}}\right)\right] \times 100,$$

and the amount of biologically fixed N allocated in LBCC tops or roots, N fixation (kg N ha^{-1}), was:

$$\text{N fixation} = \text{Ndfa}\ (\%)/100 \times \text{CC-N}$$

The amount of plant N derived from the soil N pool, Nds (kg N ha^{-1}) reflects the ability of a catch crop to extract N from soil. Due to a small amount of ^{15}N enriched fertiliser applied as a tracer and to simplify the interpretation, the Nds in this study consisted of N

Table 3 Dry matter (DM), N concentration, C/N ratio, atom fraction ^{15}N and allocation of N in catch crop tops and macro-roots (0–18 cm) determined in late autumn 2012

CC	DM (Mg ha^{-1})			N concentration (%)		C/N		Atom fraction ^{15}N (%)		N allocated in tops (%)
	Top	Root	Total[a]	Top	Root	Top	Root	Top	Root	
CL	3.7 a	2.7 a	6.6 a	3.6 b	3.0 a	12	14	0.7748 b	0.6554 bc	63 a
GC	3.9 a	2.8 a	6.9 a	3.4 b	2.8 a	12	15	0.8086 b	0.6018 c	64 a
WV	2.6 b	1.7 b	4.2 b	4.5 a	2.7 a	9	14	0.8154 b	0.6669 bc	69 a
FR	2.2 b	2.3 ab	4.6 b	2.1 c	2.2 b	18	19	1.4436 a	0.7243 b	50 b
GR	2.0 b	1.7 b	3.7 b	2.2 c	1.6 c	19	25	1.6609 a	0.8778 a	60 ab

Different letters in each column indicate significant differences ($p<0.05$) among treatments (Replications: $n=6$ for tops and $n=3$ for roots)

[a] *Total* mean value for the entire plant calculated from replicates with measurements both on tops and macro-roots ($n=3$)

taken up from both the native soil N pool and the applied ^{15}N fertiliser. Therefore, Nds of the non-LBCC was identical to the total N uptake, while for LBCCs it can be calculated as follows:

$$\mathrm{Nds} = [1-\mathrm{Ndfa}\ (\%)/100] \times \mathrm{CC\text{-}N}$$

The fraction of spring barley N derived from the incorporated CC residues, Ndfr (%), was calculated according to:

$$\mathrm{Ndfr}\ (\%) = \left(\frac{\mathrm{EAF\ of\ barley}}{\mathrm{EAF\ of\ residue}}\right) \times 100,$$

where in the N1 treatment with fertiliser application Ndfr (%) of spring barley indicated the proportion of N derived from the fertiliser. In the CC top treatment, the EAF of residue was measured for the composite sample of the six replicates before soil incorporation. However, in the CC root treatment, two assumptions were made. First, we assumed there was no loss of the applied ^{15}N fertiliser from the system, i.e., that it was all taken up by the CC and either recovered in CC plant tissues or deposited through the roots (as N in exudates, in unrecoverable fine roots, or in decomposed roots). Second, all root-derived N in the soil was assumed to have the same atom fraction ^{15}N as the macro-roots recovered. With these assumptions, a diluted ^{15}N-labelled below-ground N pool in the root treatment, N_{Res_BG} (kg N ha^{-1}), could be approximated from the ^{15}N atom mass balance:

$$N_{Res\_BG} = \frac{\mathrm{EAF\ of\ fertiliser} \times \mathrm{residual\ fertiliser\ N}}{\mathrm{EAF\ of\ macro\text{-}roots}},$$

where the residual fertiliser N was calculated as the amount of applied fertiliser (10 kg N ha^{-1}) subtracting the amount of fertiliser N recovered and removed in catch crop tops. Then the amount of N in catch crop residues (or fertiliser N in N1 plots) recovered in the barley crop, Ndfr (kg N ha^{-1}) was:

$$\mathrm{Ndfr} = \mathrm{Ndfr}\ (\%)/100 \times \mathrm{barley\ N},$$

where the barley N is the N yield in the tops of spring barley measured in August 2013.

A recovery rate of residue N in the spring barley, Recovery (%), could be calculated for CC top treatments,

$$\mathrm{Recovery}\ (\%) = \left(\mathrm{Ndfr}/N_{Res\_Top}\right) \times 100,$$

where the N_{Res_Top} was the amount of N in CC tops (kg N ha^{-1}) that were incorporated.

And for root treatments:

$$\mathrm{Recovery}\ (\%) = (\mathrm{Ndfr}/N_{Res\_BG}) \times 100$$

For N1 plots with fertiliser application of 50 kg N ha^{-1}:

$$\mathrm{Recovery}\ (\%) = \left(\mathrm{Ndfr}/50\ \mathrm{kg\ N\ ha^{-1}}\right) \times 100$$

The mineral fertiliser equivalent, MFE (%), could be used to evaluate the N availability of organic residues in comparison to a mineral fertiliser. It was calculated based on ^{15}N recoveries in the barley crop according to Christensen (1996):

$$\mathrm{MFE}\ (\%) = \frac{\mathrm{Recovery}\ (\%)\ \mathrm{in\ CC\ treatment}}{\mathrm{Recovery}\ (\%)\ \mathrm{in\ N1\ treatment}} \times 100$$

The N fertiliser replacement value, NFRV, is equivalent to MFE (Jensen 2013). However, in order to compare ^{15}N-based and non-^{15}N-based approaches in calculating the N availability in the added residues, the NFRV (%) was defined as below in the present study:

$$\text{NFRV (\%)} = \frac{\text{ANR (\%) in CC treatment}}{\text{ANR (\%) in N1 treatment}} \times 100,$$

where the ANR (%) is the apparent N recovery, by subtracting the above-ground N in spring barley in N0 reference from that in treatments amended with catch crop residues (or fertiliser in N1) and expressed as a percentage of the total residue N incorporated (or fertiliser applied in N1).

All statistical analyses were carried out using R software (R Core Team 2014). Tukey's HSD were used for multiple comparisons of treatment effects using a significance level of α=0.05.

Results

Dry matter and N accumulation of catch crops

The three LBCCs tended to produce more biomass and accumulate more N than the non-LBCCs (Table 3, Fig. 1; no separation was made of clover and ryegrass in the GC treatment). By late autumn 2012, red clover and the ryegrass-clover mixture had 6.6 and 6.9 Mg DM ha^{-1} (tops + recovered macro-roots), which were significantly more than that of the winter vetch (4.2 Mg ha^{-1}), and the two non-LBCCs, 4.6 Mg ha^{-1} for fodder radish and 2.9 Mg ha^{-1} for perennial ryegrass (Table 3). LBCCs had higher N concentrations in both tops and roots than the two non-LBCCs, with highest concentrations of 4.5 % in the tops of winter vetch. The LBCCs contained 153–226 kg N ha^{-1} in tops + roots against 71 and 96 kg N ha^{-1} for non-LBCCs (Fig. 1). The macro-roots of LBCCs in the 0–18 cm soil layer accounted for 31–37 % of the plant N, while fodder radish had 50 % of its N in the roots and perennial ryegrass 40 % (Table 3).

^{15}N labelling of catch crops and N source partitioning

The ^{15}N isotope analysis showed generally higher atom fraction ^{15}N of the tops than of the recovered macro-roots, on average 0.800 and 0.641 % for LBCC tops and roots, respectively (Table 3). The values for non-LBCCs were 1.552 and 0.801 %, respectively (Table 3). The total recovery of labelled N in catch crops varied from 57 to 66 % (Table 4). Furthermore, on average 46 % of the fertiliser N was recovered in the tops and 15 % in the macro-roots as determined in late autumn 2012.

The separate determination of atom fraction ^{15}N for tops and roots allowed us to calculate the allocation of biologically fixed N in the two parts. There were no marked differences among the three LBCCs, and the average ratios of N derived from the atmosphere were 63 and 37 % in tops and roots, respectively, corresponding to an average N fixation rate of 55 % for the entire plant (Table 4). This means that 78, 125 and 128 kg N ha^{-1} was fixed by WV, CL and GC, respectively. Our calculations showed that 75–101 kg N ha^{-1} in LBCCs were from the native soil N pool and the fertiliser added, which was comparable to, and even greater than, the uptake by the non-LBCCs, with 71 kg N ha^{-1} taken up by GR and 96 kg N ha^{-1} by FR (Table 4).

Residual N effect of catch crops

The higher atom fraction ^{15}N of catch crop tops was reflected in the following barley crop. The atom fraction ^{15}N of harvested spring barley in treatments amended with tops was generally greater than that of the root treatments (Table 5). The percentages of barley N derived from incorporated LBCC tops or roots (29–44 %) significantly exceeded those from the non-LBCCs (10–26 %), except for the WV root treatment which was in the range of the non-LBCCs (Table 5). Calculating the fractions of residue N recovered in spring barley demonstrated a slightly higher recovery of LBCC top N (17–24 %) compared to GR (13 %) and FR (14 %) (Table 5). In the KNO_3 fertilised treatment (N1), the recovery of the fertiliser N in the barley crop was up to 51 %. According to our calculations for the treatments with root residues, the recovery of residue N in the barley crop was 6–14 %, which was generally lower than that in the corresponding top treatments (Table 5).

A tendency for greater dry matter and N yield of spring barley was observed in the LBCC top or root treatments compared to the non-LBCCs (Table 5, Fig. 2). However, it has to be noted that the amounts

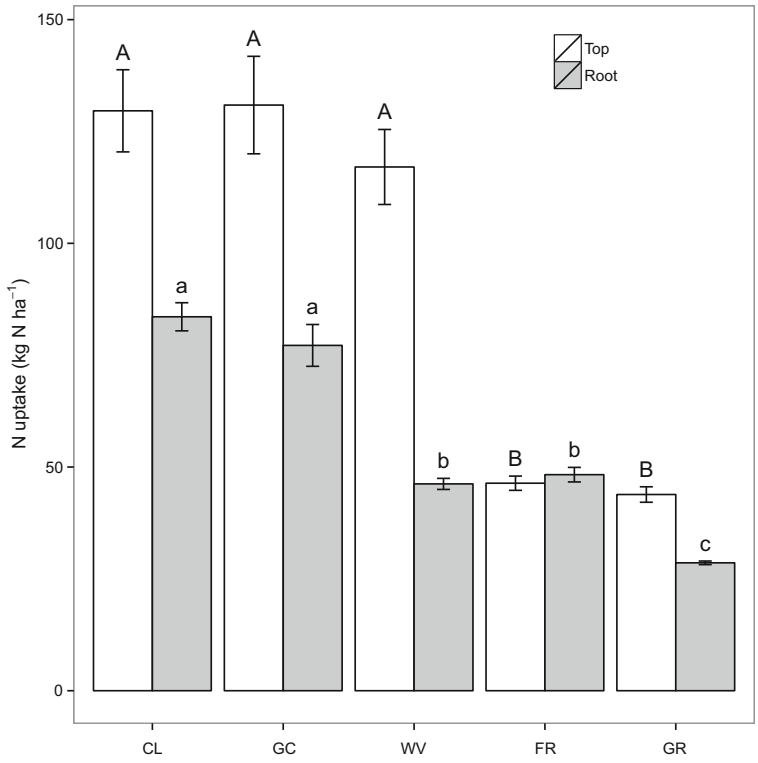

Fig. 1 N uptake in tops (*white column*, replications: $n=6$) and 0–18 cm macro-roots (*grey column*, $n=3$) of catch crops determined in late autumn 2012. *Bars* indicate standard errors. Different upper- / lower-case letters over bars indicate significant differences ($p<0.05$) among catch crop tops / roots, respectively

of dry matter and N in residues incorporated varied among treatments, since this was based on the average yields determined in late autumn 2012. Accordingly, there was a positive linear relationship between the total N in spring barley tops and the amount of residue N incorporated in the soil, $R^2=0.54$ ($p=0.001$) for CC top residues, while $R^2=0.63$ ($p=0.0002$) for the macro-roots (Fig. 3).

Discussion

Biological N fixation in LBCCs assessed by ^{15}N isotope dilution

As expected, a considerable amount of the N in LBCCs was derived from BNF, ranging from 78 to 128 kg N ha^{-1} including both tops and macro-roots. More than

Table 4 Source partitioning of catch crop N and the recovery of the ^{15}N tracer calculated by ^{15}N dilution determined in late autumn 2012

CC	Ndfa[a] (%)			N fixation (kg ha^{-1})			Nds[c] (kg ha^{-1})	Recovery[d] (%)		
	Top	Root	Total[b]	Top	Root	Total		Top	Root	Total
CL	66 a	34 a	55 a	86 a	28 ab	125 a	101 a	45 a	21 a	67 a
GC	63 a	46 a	59 a	84 a	35 a	128 a	87 ab	48 a	16 ab	62 a
WV	62 a	31 a	51 a	74 a	14 b	78 a	75 b	45 a	12 b	56 a
FR	–	–	–	–	–	–	96 a	43 a	15 b	58 a
GR	–	–	–	–	–	–	71 b	49 a	13 b	65 a

Different letters in each column indicate significant differences ($p<0.05$) among treatments (Replications: $n=6$ for tops and $n=3$ for roots)

[a] *Ndfa* the percentage of N derived from the atmosphere

[b] *Total* mean value for the entire plant calculated from replicates with measurements both on tops and macro-roots ($n=3$)

[c] *Nds* the amount of N derived from soil (including applied fertiliser N)

[d] *Recovery* the recovery rate in catch crops of the applied ^{15}N fertiliser (10 kg N ha^{-1})

Table 5 Dry matter yield (DM), atom fraction ^{15}N, residue N recovered in tops of spring barley and the mineral fertiliser value of different catch crop residues

CC	Part	DM (Mg ha^{-1})	Atom fraction ^{15}N (%)	Ndfr[a] (%)	Ndfr (kg ha^{-1})	Recovery[b] (%)	MFE[c] (%)	ANR[d] (kg ha^{-1})	ANR (%)	NFRV[e] (%)
CL	Top	8.5 ac	0.4912 ce	29 bc	23 a	17 cd	34 bc	23	17	31
GC	Top	7.3 ac	0.5287 b	37 ab	28 a	22 bc	43 ab	20	15	27
WV	Top	9.2 a	0.5264 bc	32 ac	25 a	24 b	47 a	23	22	39
FR	Top	5.4 cd	0.5085 bcd	14 de	8 bc	14 de	29 cd	1	1	2
GR	Top	5.7 bcd	0.5073 bcd	10 e	6 c	13 de	26 cd	2	5	9
CL	Root	8.3 ac	0.4776 de	39 ab	29 a	14 de	27 cd	21	–	–
GC	Root	8.0 ac	0.4676 e	44 a	30 a	11 ef	22 de	16	–	–
WV	Root	6.3 ad	0.4272 f	21 ce	12 bc	6 g	11 f	2	–	–
FR	Root	5.6 cd	0.4580 ef	26 bcd	14 b	8 fg	15 ef	−1	–	–
GR	Root	3.5 d	0.4673 e	20 ce	7 bc	7 fg	14 ef	−18	–	–
N0		6.2 ad	0.3683 g	–	–	–	–	–	–	–
N1		9.0 ab	0.9930 a	30 ac	25 a	51 a	100	28	56	100

Different letters in each column indicate significant differences ($p<0.05$) among treatments (Replications: $n=3$)

[a] *Ndfr* N derived from applied N (catch crop residues or fertiliser)

[b] *Recovery* the recovery rate of applied N (catch crop residues or fertiliser)

[c] *MFE* the mineral fertiliser equivalent of applied residues based on ^{15}N recoveries

[d] *ANR* the apparent N recovery in spring barley of applied N; the values presented were simply the differences of mean total above-ground N in spring barley between treatments with catch crop residues (or N1) and N0 control; the ANR in percentage was the ratio between the ANR in absolute amount and applied N (catch crop residues or fertiliser)

[e] *NFRV* the N fertiliser replacement value based on ANR

60 % of N in the tops and 31–46 % of N in the roots were derived from the atmosphere by late autumn in the present study (Table 4). The percentages for red clover tops were in the same range as observed in other studies from northern temperate regions using the isotope dilution method under conditions with <20 kg N fertilisation ha^{-1} (Carlsson and Huss-Danell 2003). We observed a slightly higher Ndfa in the grass-clover mixture than the red clover pure stands (59 % vs. 55 %), in accordance with previous studies (Carlsson and Huss-Danell 2003; Rasmussen et al. 2012). This may be due to elevated N fixation activity of the clover crop, which was stimulated by the competition for soil N from the companion grass (Rasmussen et al. 2012). In a 2-year field study comparing BNF in several LBCCs with ^{15}N isotope dilution in Denmark, it was reported that hairy vetch (*Vicia villosa*) had ca. 80 kg N fixed ha^{-1} in the tops in 1996, which increased to almost 150 kg N ha^{-1} in the following year (Mueller and Thorup-Kristensen 2001). Thus, inter-annual variation of weather and soil moisture conditions may be crucial for BNF by legumes, especially at the early stage of plant development and during the cool and variable conditions of autumn and spring, which affects the growth duration of the plants.

Studies that quantify N fixation in both tops and roots of LBCCs or forage legumes are rare, especially under field conditions. In northern Sweden (63°N), Huss-Danell and Chaia (2005) measured Ndfa of >81 % in the tops of field-grown red clover leys of 1-to-3-year old, and >60 % in the roots, both values higher than the first-year clover in our study. Higher atom fraction ^{15}N in tops than in roots of legumes was also observed in studies by McNeill et al. (1997) and Khan et al. (2002), but using ^{15}N labelling via shoots rather than via soil as in our work. Tops seemed to be a strong sink of fixed N (Table 4), in accordance with other studies (Warembourg et al. 1997; Huss-Danell and Chaia 2005). A likely reason is a decreasing allocation of N to roots with plant growth, and meanwhile, Ndfa is not constant but progressively increases during the growth of legumes as shown in Huss-Danell and Chaia (2005).

The amount of N fixation was positively correlated with the dry matter production of LBCCs (Table 6). The slope indicates a fixation of 24.0 kg N Mg^{-1} DM in tops.

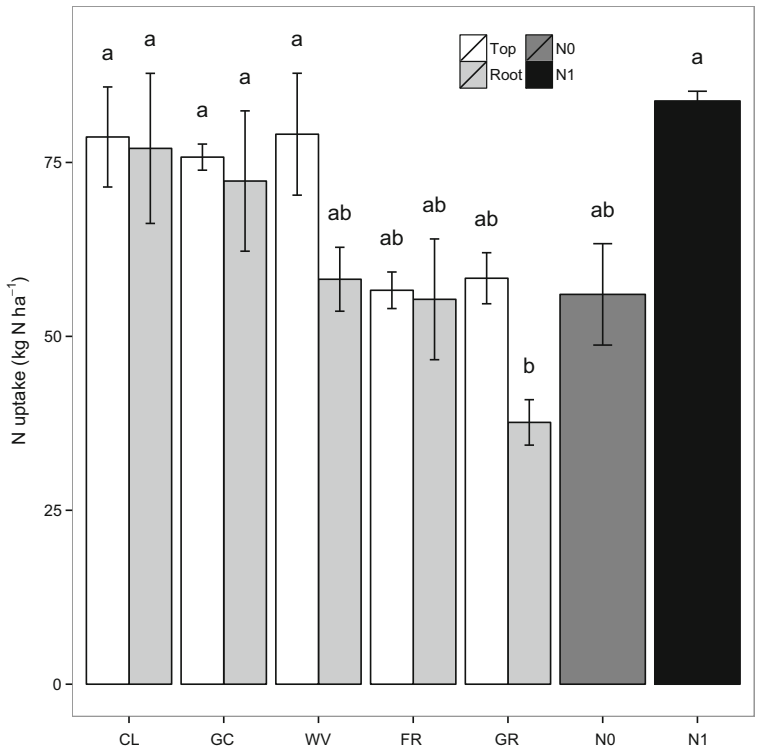

Fig. 2 Above-ground N yield in spring barley following top (*white column*) and 0–18 cm root (*light grey column*) residue incorporation, N0 control (without residue or fertiliser addition, *dark grey column*) and N1 control (without residue but 50 kg N ha^{-1} fertiliser addition, *black column*). Bars indicate standard errors. Different lowercase letters over bars indicate significant differences ($p<0.05$) among all treatments, including N0 and N1 references (Replications: $n=3$)

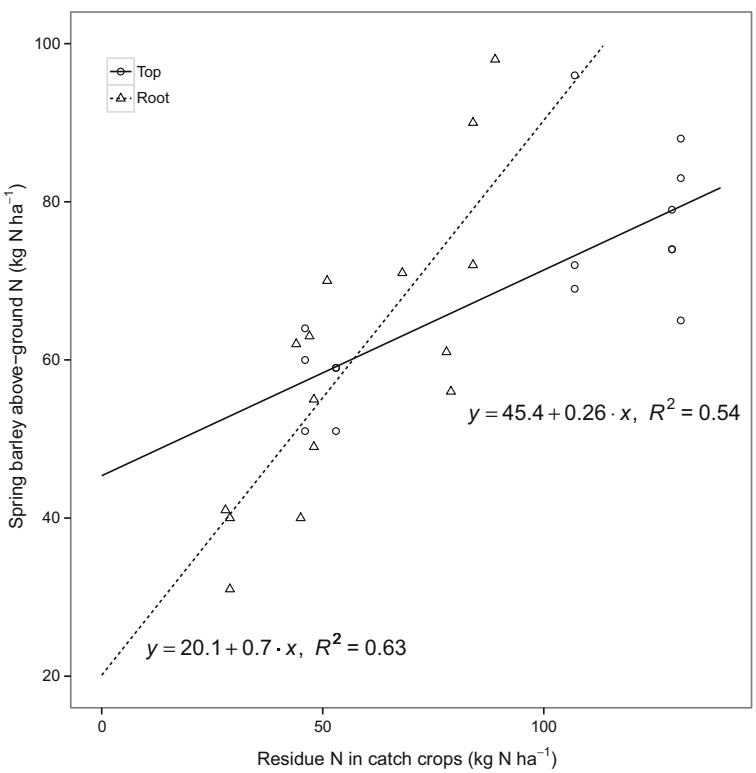

Fig. 3 Linear regression between above-ground N yield of spring barley and the amount of incorporated residue N (in tops or macro-roots) ($n=15$, $p<0.05$)

Table 6 Regression equation between the amount of BNF (Nfix, kg N ha^{-1}) in tops or tops + macro-roots of all three LBCCs and catch crop dry matter yield (DM, Mg ha^{-1})

	Regression equation	*n*	R^2	Significance
Tops	Nfix = 24.0×DM	18	0.98	$p<0.0001$
Tops + roots	Nfix = 18.8×DM	9	0.99	$p<0.0001$

This value agrees well with several reviews, e.g., 23 kg N fixed Mg^{-1} DM for red clover as a forage legume in northern temperate regions (Carlsson and Huss-Danell 2003), 20.2–24.3 for annual legumes and 18.7 for perennial lucerne on Australian pastures (Unkovich et al. 2010), and 15–25 kg shoot N from fixation for every Mg shoot DM accumulated in an attempt to estimate the global BNF input (Herridge et al. 2008). Hence, such a relationship allows for a rough but simple estimation of BNF based on the biomass production of LBCCs at field or farm scale.

Catch crop dry matter and N accumulation

The ability of LBCCs in organic crop rotations to fix N from the atmosphere, to decrease soil mineral N during the off-season and thereby preserve N over the winter, and to make N available to succeeding crops, is largely dependent on biomass production and N accumulation (Rinnofner et al. 2008). In late autumn 2012, red clover and the grass-clover mixture averaged 3.8 Mg DM ha^{-1} and 130 kg N ha^{-1} in the tops, respectively. The biomass was in the range of 1.5–9.6 Mg DM ha^{-1} reported by Carlsson and Huss-Danell (2003). In a recent study in France, Amosse et al. (2014) reported a production of 2.9 Mg DM ha^{-1} with 76.9 kg N ha^{-1} for a red clover catch crop undersown in a winter wheat crop across six organic farm sites, which was less than in the present microplot study. The observed DM and N yield of *Vicia villosa* was in accordance with Mueller and Thorup-Kristensen (2001), although N uptake varied from ca. 100 to 200 kg N ha^{-1} in the tops in their study. The fodder radish produced 2.2 Mg DM ha^{-1} and 47 kg N ha^{-1} in the tops in the present study, which was comparable to observations by Sapkota et al. (2012) over 2 years and two locations in Denmark with 2.0 Mg DM ha^{-1} and 45 kg N ha^{-1}, respectively. However, the dry matter of 1.05 Mg ha^{-1} and N uptake of 22 kg N ha^{-1} for ryegrass in the same study was much lower than that in ours (2.0 Mg DM ha^{-1} and 44 kg N ha^{-1}).

The recovered macro-roots accounted for 30–50 % of the plant N in catch crops (Fig. 1), which was a low estimate for the total below-ground N derived from roots due to unrecoverable fine roots, decomposed roots and root exudates. A sampling depth of 18 cm was also a potential source of error, since it was reported that roots of red clover-ryegrass at 0–20 cm accounted for only 75 % of the N content in roots at 0–60 cm soil depth (Høgh-Jensen and Schjoerring 2001). Fodder radish has been observed to reach 2.0 m depth and ryegrass 1.0 m depth (Thorup-Kristensen 2006). Rhizodeposited N may account for 4–71 % of total plant N (Wichern et al. 2008; Fustec et al. 2010). In fact, the calculated N_{Res_BG}, an approximation of root-derived N based on ^{15}N dilution in the present study ranged from 108 to 265 kg N ha^{-1} for all catch crops (data not shown), which exceeded the N accumulation in the top of the respective species. Therefore, a substantial root-derived N pool under catch crops would contribute to soil fertility, even if the tops are removed from the field (Chirinda et al. 2012).

Previous studies reported that non-LBCCs were generally more effective than LBCCs in reducing N leaching losses (Tonitto et al. 2006; Askegaard and Eriksen 2007). By partitioning the plant N of catch crops into Ndfa (BNF) and Nds (soil N uptake), we observed a comparable or even greater ability of LBCCs to exploit soil N than that of non-LBCCs (Table 4). The soil mineral N content, which may be an indicator of N leaching risk (Kankanen and Eriksson 2007), was not measured in our microplots. However, there were no dramatic changes in soil nitrate under catch crops before and during the winter 2012 in our large plots (Li et al. 2015). Similarly, Kankanen and Eriksson (2007) observed no increase of soil nitrate under red clover in late autumn and succeeding spring compared to bare soil fallow. Therefore, LBCCs are promising in terms of enhancing total N supply in cropping systems and lowering the risk of soil N leaching.

Compared to yields in the large plots, dry matter production and total N in the tops were considerably greater in the microplots. For LBCCs, the difference in total N of tops ranged from 50 to 72 kg N ha^{-1}, while for FR and GR it was 6 and 12 kg N ha^{-1}, respectively (cf. Table 2 in Li et al. 2015). A similar discrepancy was also reported by Mueller and Thorup-Kristensen (2001) using 2.5×3 m microplots to investigate N fixation of several green manure crops. A possible explanation offered by these authors was the difference in

microclimate between microplots and large plots. However, in our setup with no major above-ground obstacles, it is unlikely that there were major microclimatic differences. In contrast, another study quantifying BNF in soybean using both the isotope dilution method (in a microplot area of 0.0462 m^2) and the natural ^{15}N abundance method (Oberson et al. 2007), less biomass production was recorded in microplots than in the main plots. Here it was argued that the growth of soybean inside the microplots had been restricted. In our case, the difference in growth might be caused by several factors. In order to determine dry matter and N, tops were cut at the soil surface both in the microplots and the large plots, but senescent leaves were collected in microplots only. Bias might also have been introduced favouring for the better growing crops when we selected sites for the microplots. The sites were selected to include relatively similar crop stands in all replicates despite the use of small metal frames (with an area of 0.0875 m^2). Better growing legumes probably obtained more N via BNF (Carlsson and Huss-Danell 2003; Unkovich et al. 2010), which may have exaggerated the difference of total plant N between the microplots and the large plots for LBCCs in this study. Hence, the present microplot study indicates the potential effects of well-established catch crops, but in practice effects may be less due to poorer establishment in parts of a field.

Residual N effect of catch crops

Several field plot studies in Northwestern Europe have previously shown beneficial N effect from LBCCs on the following cereal crops, especially in low input farming systems (Askegaard and Eriksen 2008; Bergkvist et al. 2011; Doltra and Olesen 2013; Amosse et al. 2014), including also our large plot experiment (Li et al. 2015). In our microplot study, LBCCs resulted in slightly increased dry matter production and N uptake in the subsequent barley crop compared with non-LBCCs and the N0 control (Fig. 2). The statistically non-significant differences may be due to the separation of above- and below-ground parts of catch crops into different treatments in order to study the impact of removing tops. Moreover, the amount of residue added varied across treatments, since the addition was based on the observed dry matter yield of the catch crop. Although soil mineral N was not measured in microplots at the beginning of the barley season, 5.9 mg NO_3-N kg^{-1} (ca. 25 kg N ha^{-1}; 0–30 cm; cf. Fig. 4 in Li et al. 2015) was recorded in the CO large plots on 25 April 2013, which represented the initial soil mineral N level in the top / N0 microplot treatments. This value was close to that of the respective root treatments, except for the treatment with ryegrass root, which was significantly lower than the top / N0 treatments by 19 kg N ha^{-1} (cf. Fig. 4 in Li et al. 2015). Hence, it would be more relevant to compare among treatments the proportions of crop N derived from residues and the recovery rates of residue N in the barley crop. Calculations based on isotope dilution demonstrated that LBCC residues contributed more to the crop N with higher recoveries in comparison to non-LBCCs, except for the WV roots (Table 5). The recovery of residue N in cereals is a product of the net N mineralisation rate and N utilisation efficiency of the cereal (Chalk et al. 1993). Thus, the characteristics of the residues played a crucial role which influenced the net N mineralisation.

The residual N effect of a catch crop in the rotation is determined mainly by effects of pre-emptive competition (depletion of soil N) and the balance of subsequent mineralisation and immobilisation (Thorup-Kristensen et al. 2003). The N yields of spring barley following non-LBCCs (and WV roots) were close to the N0 reference, i.e., 55–58 vs. 56 kg N ha^{-1} (Fig. 2). This indicates that the net contributions of N in CC residues to the barley crop in these treatments were insignificant. A low level of N availability in the WV macro-roots may have determined its low N benefit to the barley (Fig. 1). The lowest barley N yield was observed with ryegrass root amendment, which was lower than in the N0 reference by 18 kg N ha^{-1} (Fig. 2). This was in line with their difference in initial soil mineral N of about 19 kg N ha^{-1}. It may have been caused by depletion of soil N during the ryegrass growing period (pre-emptive competition) combined with N immobilisation impeding crop N uptake. Before sowing of spring barley, the ryegrass removed 71 kg N ha^{-1} from the soil, of which 60 % was recovered in the tops. Incorporation of ryegrass with relatively high C/N ratio (Table 3), in particular for the roots, has been shown to immobilise N in soil and reduce unfertilised crop yield (Kumar and Goh 2002, and references within).

Using the N0 treatment as reference, the residual N effects of the three LBCCs (either top or root, except for WV root) on spring barley N yields were in the range of 16–23 kg N ha^{-1} (ANR in Table 5), which was similar to results from Rasmussen et al. (2012) showing residual N effects of 6–21 kg N ha^{-1}

on an unfertilised spring barley following incorporation of different grass-clover mixtures on a neighbouring study site. The calculation of ANR in main crops following organic residue amendment builds on the assumption that the additional crop N uptake is only derived from the sources added, and that the N uptake from native soil pools does not change with amendment (Jensen 2013). This assumption is not always held as demonstrated in the present study in particular for the non-LBCCs. The ANR values were slightly smaller than those calculated based on ^{15}N enrichment, 23–30 kg N ha^{-1} (Ndfr in Table 5). Furthermore, the ANR and the ^{15}N-based Ndfr values were comparable for the LBCC top treatments, while lower values of ANR than Ndfr were shown for the non-LBCCs. This implies that the addition of low quality residues, i.e., non-LBCCs, may have stimulated immobilisation of soil inorganic N, making it less available for crop uptake (Kumar and Goh 2002). Therefore, Ndfr calculated by ^{15}N isotope dilution represents only the contributions from the source of labelled material, while ANR represents a net effect of all N sources, which needs to be differentiated in future applications especially for non-LBCCs. The difference between Ndfr and ANR was magnified when calculating MFE (26–29 %) and NFRV (2–9 %) for FR and GR tops. MFE or NFRV is a measure of the N fertiliser value of organic residues, calculated as the fraction of total residue N that has the same availability to crops as a mineral fertiliser (Sørensen et al. 2003; Delin et al. 2012; Jensen 2013). NFRV of the incorporated LBCC tops ranged from 27 to 39 % (Table 6), which were similar with a previous study showing 32–48 % NFRV for grass-clover or lucerne silage used as mobile green manure at a neighbouring study site (Sørensen et al. 2013).

The N uptake by barley in the root treatments of red clover and grass-clover was comparable with that in the corresponding top treatments (Fig. 2), even though the N measured in macro-roots were much lower than in the tops (Fig. 1). This implies that a significant amount of below-ground N was unaccounted in our macro-root measurement, which still contributed substantially to the N supply of the following cereal crop after removal of the tops. In addition, relatively lower C/N ratios of tops compared to roots (Table 3) were expected to result in a greater slope of the linear regression between barley N and residue N for the top than for the root treatments in our Fig. 3. However, the actual observation of a greater slope in the root treatment (0.70 vs. 0.26) is also an indication that the amount of below-ground N derived from roots is under-estimated when measuring only the N in macro-roots (Fig. 3).

Conclusions

The present study, employing ^{15}N labelling in field microplots showed that well established LBCCs accumulated significantly more N both in the tops and roots compared with non-LBCCs. Biological N fixation contributed >60 % of the N in LBCC tops, and 31–46 % in the roots. The LBCCs showed similar capacity for taking up N from the soil as the non-LBCCs. Macro-roots accounted for 31–50 % of total plant N which, however, underestimated root-derived N in the soil. Incorporation of LBCC top or root residues tended to increase the dry matter and N yield of a succeeding spring barley crop, in comparison with non-LBCCs. The absence of a net N benefit (ANR) from the non-LBCCs despite of release of labelled N was probably due to immobilisation of soil N.

Based on the separate turnover of tops and retained roots, this study implies that LBCC, i.e., red clover or a mixture of red clover and perennial ryegrass, can increase N supply of a subsequent main crop on this soil type resulting in an extra N uptake of ca. 20 kg N ha^{-1} due to roots alone compared to a bare soil, and a similar effect of LBCC tops can be expected. Non-fixing catch crops may have insignificant or even negative effects on the following crop, in particular after autumn harvest of tops. The long-term efficiency of catch cropping relies on the ability of managers to continue to use suitable types of catch crops and smart management for the local climatic and soil conditions.

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