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REGULAR ARTICLE

In the land of plenty: catch crops trigger nitrogen uptake by soil microorganisms

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Abstract
Background and aims Catch crops (CC) reduce nitrate leaching, and may resolve a major concern in nitrogen (N) intensive agriculture. CC efficiency depends on N uptake ability, which is related to root development, biomass partitioning, and competition with soil microbes. We investigated the effect of N addition on this with three CC species.
Methods Three CC species were grown in pots with three N concentrations. Shoot and root biomass, C:N content, and specific root length were determined, whereas residual N, dissolved organic N (DON) and C, and microbial biomass N and C were measured.
Results Addition of N did not consistently effect plant biomass nor its partitioning, probably because of overall high N. However, CC did reduce residual N, and so did soil microorganisms, likely facilitated by C-release from roots. Moreover, plant presence reduced DON, likely through uptake by soil microorganisms, partly followed by plant uptake.
Conclusions CC not only take up residual N themselves, but also trigger considerable N uptake by soil microorganisms that thrive on C-release from roots. This plant-microbe-nitrogen interaction has to be considered when evaluating CC systems. It remains unclear to which extent soil microorganisms immobilise inorganic N and mineralise or take up DON.

Keywords Biomass partitioning · Cover crops · Nitrogen partitioning · Plant-soil feedback · Rhizodeposition · Root traits

Abbreviations

C	Carbon
CC	Catch crops
DON	Dissolved organic nitrogen
DOC	Dissolved organic carbon
NH_4^+	Ammonium
NO_3^-	Nitrate
N	Nitrogen
RE	Recovery efficiency
SRL	specific root length

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Introduction

Crop production is mainly limited by the acquisition of soil resources (Lynch 1998). Increases in yield have therefore been achieved by, amongst others, a high input of fertilizers. Many crops have, however, a low nutrient uptake efficiency and leave 50 to 70% of the applied nitrogen (N) in the soil after harvest (Tilman et al. 2002; Good et al. 2004; Nacry et al. 2013). The residual nitrate (NO_3^-) in these systems is soluble and thus prone to leaching to deeper soil layers, especially in autumn and winter under humid conditions when the agricultural fields lie fallow. This results in pollution of ground, surface and coastal waters and ultimately, N saturation of terrestrial ecosystems (e.g. Vance 2001; Tilman et al. 2002; Good et al. 2004; Nacry et al. 2013).

In 2009, 13% of the groundwater monitoring stations in Europe exceeded the European threshold for groundwater NO_3^- levels of 50 mg L^{-1}, and in several countries more than 20% of these monitoring locations reflected a threshold of concern (between 25 and 50 mg NO_3^- L^{-1}) (Eurostat 2012). As a result, fertilizer laws and regulations have been tightened in many European countries, increasing the demand for more efficient N use and strategies to capture unused N in agricultural soils. One of the means to reduce N losses by NO_3^- leaching and preserving N is the cultivation of catch crops grown in between successive main crops from late summer until spring (Thorup-Kristensen et al. 2001). The aim of catch cropping is to immobilise residual N in plant tissue, preventing it from leaching into deeper soil layers as NO_3^-. A meta-analysis showed that catch crop cultivation reduced NO_3^- leaching by 70% (Tonitto et al. 2006). Moreover, it appeared to be more effective than no tillage systems and/or a lowered N input, most probably due to the continuous uptake of newly mineralised N throughout the whole period in between the successive main crops (Constantin et al. 2010). Even though the plant species used as catch crops have been studied for their N acquisition capacity, quantification has mainly been based on assessing aboveground plant traits, such as leaf traits, aboveground biomass and shoot N content (e.g. Tribouillois et al. 2015). However, since N is taken up by the root system, it is most likely that the ability of catch crops to remove NO_3^- is more strongly related to root system characteristics. Thorup-Kristensen (2001) compared root growth of seven catch crop species and showed that species with a quickly developing deep root system with high root density remove more NO_3^- from the subsoil, and that this was not at the expense of NO_3^- removal in the topsoil. Sapkota et al. (2012) found comparable results and showed that deep rooting species decreased N leaching by up to 79%. An early developing deep root system with high root density therefore, appears to be optimal for high N acquisition rates (Thorup-Kristensen 2001).

Apart from storage in aboveground tissue, substantial amounts of N are stored in belowground biomass, which consists of roots and released organic and inorganic molecules and ions, referred to as rhizodeposition (Wichern et al. 2008). Kanders et al. (2017) showed that rhizodeposits may contribute strongly to N removal and has to be considered in quantification studies. Since the amount of rhizodeposition is closely related to root biomass (Shamoot et al. 1968; Wichern et al. 2008), investment in root biomass of catch crops should be considered when assessing their ability to remove NO_3^-, especially as roots respond to changing environmental conditions (e.g. Hodge 2004, 2009; Hodge et al. 2009; Semchenko et al. 2014; Belter et al. 2015).

To boost early development of catch crops, small to medium amounts (40–80 kg N ha^{-1}) of N fertilizer are often added, even under conditions of high residual N. However, it is unknown whether these additions of reactive N indeed trigger substantial increases in biomass development, especially belowground, and whether this leads to a higher N uptake. If so, the question remains whether N uptake improves enough to compensate for the added N or, alternatively, whether it results in higher net N losses compared to no N addition. From ecological studies it is well known that the plants' root systems reacts strongly to changing nutrient conditions (e.g. Hodge et al. 1999; Hodge 2004; Cahill and McNickle 2011; Bennett and Cahill 2016). Plants proliferate roots in nutrient-rich patches, especially in high-N, often with localized increase of root length and density, and often at the expense of root development outside the high nutrient patch (e.g. Hackett 1972; Drew 1975; Hodge 2004; Visser et al. 2008; in 't Zandt et al. 2015). This response occurs across a broad range of plant species (Robinson 1994; Cahill and McNickle 2011), but also depends on the individual nutrient being deficient (Drew 1975; Linkohr et al. 2002; Gruber et al. 2013). N fertilisation in the top soil may, thus, result in root development in this top soil and thus interfere with (early) deep root development of the catch crops. In addition, the optimal partitioning theory implies that plants invest more in aboveground biomass when

belowground resources are less limiting, even though allocation plasticity is highly ontogenetically influenced (McConnaughay and Coleman 1999; Gruber et al. 2013). N fertilisation may thus result in a faster shoot than root development. However, as N is often a strongly limiting factor in ecological studies, the question arises to which extent optimal partitioning takes place under the high nutrient conditions in intensive agriculture.

Not only plant responses may differ between systems with limiting and high nutrient conditions, but also the competition between plants and microbes for N may shift. Under N limiting conditions, competition for N is usually strong (Jackson et al. 1989; Jingguo and Bakken 1997; Kuzyakov and Xu 2013; Liu et al. 2016), while in high N catch crop systems this competition may be less. Liu et al. (2016) showed that microorganisms took up at least seven times more N than plants in temperate grassland. This is not always the case, as shown by Harrison et al. (2008) for a semi-natural grassland where microorganisms took up about one third N less than plants, but it indicates a potential important role for microbes in immobilising N from agricultural soil that is often overlooked. On the other hand, it is well known that plant roots release rhizodeposits (Wichern et al. 2008), which have been shown to contribute a substantial proportion of the total residual carbon (C) and N of plants in the soil (Wichern et al. 2007a; Wichern et al. 2007b), also specifically of catch crops (Kanders et al. 2017). These rhizodeposits are partly labile (e.g. exudates) and partly less easily available to microorganisms (e.g. root fragments, debris), depending on plant species (Wichern et al. 2007a, 2007b). Such rhizodeposits can function as C source for microbes and trigger both organic matter mineralisation and release of inorganic N, and immobilisation processes, likewise (Meier et al. 2017). These processes, however, also depend on availability of N and other nutrients for soil microorganisms (Fisk et al. 2015; Lloyd et al. 2016), which is likely high in agricultural soils. In this scenario, catch crops could thus stimulate microorganisms to release additional leachable N to the soil.

Here, we examine how fertilization of catch crops influences biomass partitioning between roots and shoots, total plant N uptake and if changes in root development affect the soil microbial biomass in a typical arable soil with relatively high amounts of residual N. We selected three commonly grown catch crops with varying rooting depth and traits: *Raphanus sativus* L., *Brassica rapa* L. and *Phacelia tanacetifolia* Benth. (Thorup-Kristensen 2001; Sapkota et al. 2012; Kanders et al. 2017). The objectives of our study were to evaluate (i) early root development, root system traits and how these relate to N immobilisation by catch crops and (ii) the effects of N addition on these root traits and catch crop immobilisation capacity. Finally, (iii) we determined the role of soil microorganisms in N immobilisation in catch crop systems with and without N addition.

Materials and methods

Plant growth and N treatments

Seeds of *Raphanus sativus* L. *ssp. oleiformis* (oil radish), *Brassica rapa ssp. oleifera* L. (winter turnip rape), and *Phacelia tanacetifolia* Benth. (hereafter referred to by their generic name alone) were incubated on wet filter paper in aluminium trays and placed in closed plastic bags in the dark at room temperature. After 3 d, five seedlings of the same plant species were transferred into 7 L pots (top diameter 20 cm; bottom diameter 16 cm; height 27 cm) filled with a loamy sand soil taken from an agricultural field with a winter cereal and silage maize dominated crop rotation. Pots were filled until 24 cm height resulting in, on average, 6.8 kg dry soil mass and a bulk density of 1.11 g cm^{-3}. Soil was collected in September and additionally in late October with oilseed radish on the field, and homogenised by sieving at 1 cm. One pot of each treatment received soil collected in October mixed into the top 10 cm of the soil, except for the fallow treatments for which this was done for two pots. Three N levels were applied to each plant species: 0, 40 and 80 kg N ha^{-1} by adding a calcium ammonium nitrate (27% N, w:w) solution with the given amounts as a single dose on the surface soil. Background NO_3^--N and NH_4^+-N levels were 10.8 and 0.6 μg g^{-1}, respectively. For NO_3^--N, the levels increased to 24.1 and 64.5 μg g^{-1} in the top soil layer with addition of 40 and 80 kg N ha^{-1}, respectively. NH_4^+-N levels were 0.6 and 4.0 μg g^{-1} with addition of 40 and 80 kg N ha^{-1}, respectively. Plants were grown in the greenhouse for a subsequent 39 d at around 25°C during the 14 h light period (approximately 105 μmol PAR m^{-2} s^{-2} at pot level) and 18°C during the 10 h dark period. Soil was covered with plastic foil with holes for the plants to grow through, and kept at 60–70% of its

water holding capacity by watering the pots according to their weight loss every 2–3 d. Every species-fertilizer combination and pots without plants (fallow), which were used as control, were implemented in five replicates each.

Harvest

After 39 d, the shoots of the plants were cut off at the base, weighed and dried at 60°C for at least 72 h after which dry weight was determined. Roots accumulated at the bottom of the pots were removed and the soil columns were cut horizontally into two parts at 11 cm from the top to divide the top from the bottom soil layer. Approximately 30 g of soil was sampled from the centre of the lower half of the top soil layer, and around 20 g of soil was sampled from the centre of the lower half of the bottom soil layer. The samples were stored in closed plastic bags at 4°C until further analysis (see plant and soil analyses). Roots were washed out from the two soil layers using tap water. Taproots from *Raphanus* and *Brassica* were removed from the root samples (*Phacelia* did not have taproots). The remaining washed roots were stained for at least 24 h in a 0.035% neutral red solution with 70% ethanol after which root length of all three plant species was determined as described by Bouma et al. (2000). In short, a subsample was taken, spread out in water in a transparent, plastic tray, and scanned at 400 dpi on a flatbed scanner with two-sided lighting system (EPSON Expression 11000XL, Seiko Epson Corporation, Suwa, Japan). Root length was determined using image analysis software (WinRhizo, Régent Instruments Inc., Quebec, Canada), and dry weight of the root subsample was determined based on which the specific root length (SRL) was calculated.

Plant and soil analyses

Based on dry weight, one representative shoot per pot was ground (Retsch ZM 200 centrifugal mill at 0.25 mm and Retsch MM 200 mixer mill) after which total shoot N was determined (Elemental Analyzer NA1500, Thermo Scientific, Waltham, MA, USA). Total root N was determined similarly, but with the distinction that tissue from a follow-up experiment with the same setup and comparable results was used. Root N in the current experiment was then calculated based on shoot N times the root:shoot N ratio of the species in the follow-up experiment.

Microbial biomass C and N of the soil samples taken at harvest were determined by chloroform-fumigation extraction as described by Brookes et al. (1985) and Vance et al. (1987). In short, 10 g fresh root-free soil was fumigated for 24 h at 25°C with ethanol-free chloroform. Following fumigant removal, soil was extracted with 40 mL 0.5 M K_2SO_4 by shaking for 30 min on a horizontal shaker at 200 rpm and collecting the supernatant after filtration. Next, organic C and total N were determined in the supernatant (multi N/C 2100 with Autosampler APG 60, Analytik Jena AG, Jena, Germany). During fumigation, 10 g of non-fumigated fresh soil was extracted as well, and organic C and total N were determined similarly. In addition, NO_3^--N and NH_4^+-N concentrations in the supernatant of the non-fumigated extracted soil was estimated using an AutoAnalyzer 3 HR (SEAL Analytical GmbH, Norderstedt, Germany).

Microbial C biomass was then calculated as E_C / k_{EC}, where E_c = (organic C extracted from fumigated soil)-(organic C extracted from non-fumigated soil) and $k_{EC} = 0.45$ (Wu et al. 1990). Microbial N biomass was calculated as E_N / k_{EN}, where E_N = (total N extracted from fumigated soil)-(total N extracted from non-fumigated soil) and $k_{EN} = 0.54$ (Brookes et al. 1985; Joergensen and Mueller 1996). Dissolved organic C (DOC) was defined as the extractable organic C from the non-fumigated sample extracted with 0.5 M K_2SO_4. Likewise, dissolved organic N (DON) was defined as the difference between total extractable N and extractable inorganic N ($NO_3^- + NO_2^- + NH_4^+$) (Jones et al. 2004).

Calculations and statistics

Recovery efficiency (RE, i.e. fertilizer uptake efficiency) was calculated as the difference between shoot N (mg pot^{-1}) of the fertilized treatment (40 and 80 kg N ha^{-1}) and the unfertilized treatment (0 kg N ha^{-1}) as a percentage of the added fertilizer (Baligar et al. 2001). Fertilizer uptake efficiency including the N in roots (RE_{sr}%; *s* = shoot, *r* = root) was calculated as the difference between N in shoot and root biomass of fertilized treatments (40 and 80 kg N ha^{-1}) and the unfertilized treatments (0 kg N ha^{-1}) in relation to the added fertilizer.

All statistical analyses were performed in R (R Core Team 2016) using the NLME (Pinheiro et al. 2014) and MULTCOMP packages (Hothorn et al. 2008). Root length and root dry weight were determined per pot

and divided by the number of plants in the pot. All parameters were analysed as dependent variables in a two-way type III ANOVA with catch crop species (*Raphanus*, *Brassica*, *Phacelia*, fallow) and N level (0, 40, 80 N ha^{-1}) as fixed factors. Soil collection (September, late October) was added as a random factor to avoid any potential effects resulting from the two soil batches. To explore responses of the three plant species and responses in pots without plants, data was split per plant species and a one-way type III ANOVA was performed with N level as fixed factor and soil collection as random factor, followed by a Tukey HSD test. To meet assumptions of ANOVA, data were log or square root transformed where necessary. In case of violation of homogeneity of variance, the data was subject to weighted analysis according to Zuur et al. (2009).

Results

Biomass effects of N addition

Three commonly cultivated catch crops were grown with addition of 0, 40 and 80 kg N ha^{-1}. A significant interaction between catch crop species and N addition occurred on aboveground biomass (Table 1). For *Raphanus*, N addition resulted in a significantly higher shoot dry weight of, on average, 40% without a difference between 40 and 80 kg N ha^{-1} (Fig. 1). For *Brassica*, the addition of 40 kg ha^{-1} N did, however, not improve shoot growth, while 80 kg N ha^{-1} did with 33%. In contrast to these two species, N addition did not significantly affect shoot biomass of *Phacelia* at all. Contrary to aboveground biomass, no interaction occurred for root biomass in the top 11 cm of the soil. In the bottom 11 cm this interaction was, however, found (Table 1). Root biomass of *Raphanus* in this layer was not affected by N addition, while root biomass of both *Brassica* and *Phacelia* was. *Brassica* produced significantly less roots when 40 kg N ha^{-1} was added compared to 80 kg ha^{-1} N, and *Phacelia* significantly less with the addition of 80 kg ha^{-1} N compared to the two other N treatments (Fig. 1). Root distribution between the top and bottom part of the soil was only slightly affected. The top:bottom ratio of root biomass showed an increase with increasing N addition for *Raphanus*, for *Brassica* this ratio was significantly higher with 40 kg N ha^{-1} added compared to 80 N ha^{-1} and for *Phacelia* this ratio was not significantly affected (Fig. S1). In addition, root:shoot ratios were not significantly different among the catch crop species nor affected by N addition (Table 1).

Shoot N concentration was not significantly affected by N addition, and instead, appeared to be most strongly affected by catch crop species (Table 2). *Phacelia* had a significantly higher N concentration of 47 mg g^{-1} compared to 21 mg g^{-1} in *Brassica* and 26 mg g^{-1} in *Raphanus* (Fig. 2I–K). The N stored in the aboveground biomass of *Brassica* at 80 kg N was only twice as high compared to *Phacelia* and on the same level for *Raphanus*. As a consequence, *Brassica* and *Raphanus* always took up comparable amounts of N at the three fertilizer levels with highest amounts at 80 kg N, even though differences in aboveground biomass were

Table 1 ANOVA results describing the effects of catch crop growth and nitrogen (N) addition on shoot and root growth, root: shoot ratio and specific root length (SRL)

		Shoot biomass		Root biomass				Root:shoot ratio		SRL			
				Top		Bottom				Top		Bottom	
Effect	df	F	P≤	F	P≤	F	P≤	F	P≤	F	P≤	F	P≤
Catch crop	2	10.32	**<0.001**	8.36	**0.0011**	5.40	**0.009**	3.26	0.0504	55.05	**<0.001**	27.01	**<0.001**
N	2	6.76	**0.003**	0.21	0.8077	1.55	0.225	0.65	0.5283	3.29	**0.049**	3.04	0.061
Catch crop x N	4	4.19	**0.011**	1.92	0.1284	4.01	**0.009**	0.33	0.8577	0.75	0.567	1.49	0.228

Root biomass and SRL were determined in both the top 11 cm and the bottom 11 cm of the soil column. Catch crop and N are fixed factors and soil collection is a random factor. Catch crop consists of four levels (fallow, *Brassica*, *Phacelia*, *Raphanus*), N out of three (0 N, 40 N, 80 N) and soil collection of two (Sept, Oct). Data on root biomass in the bottom layer and data on root:shoot ratios were sqrt-transformed. Bold values represent significant values of $P < 0.05$

df, degrees of freedom

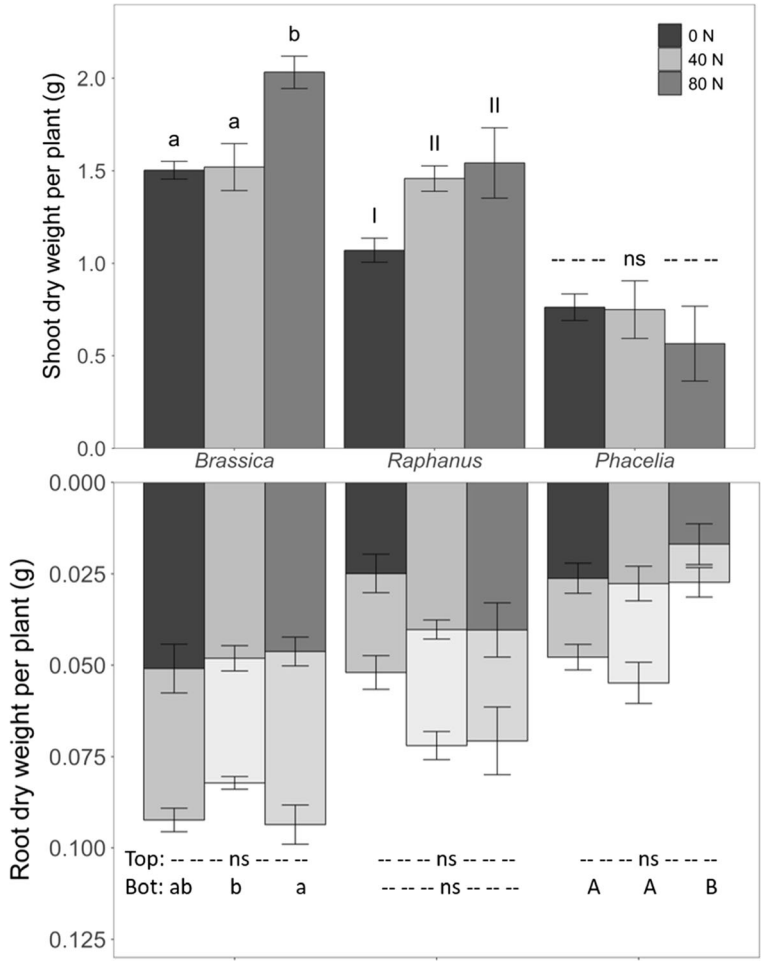

Fig. 1 Shoot and root dry weight per plant of the three catch crop species with addition of 0 (dark grey), 40 (light grey) or 80 (medium grey) kg N ha^{-1}. Root dry weight is given in both the top 11 cm (dark bars) and bottom 11 cm of the column (light bars). Bars represent means ± SE; $n = 5$. Different letters within species indicate significant differences ($P < 0.05$). Note that shoot and root y-axes differ in range

detected between the two species at 0 and 80 kg N (Fig. 4). This effect was not detected in the root biomass, where the N concentration was neither affected by plant species nor by N fertilizer addition, and was on average lower than in the shoot (Fig. 2L–N, Table 1). In contrast to the N concentrations, the C concentrations in

Table 2 ANOVA results describing the effects of catch crop growth and nitrogen (N) addition on residual nitrate (NO_3^-) in the soil, shoot and root N content, and soil microbial biomass N

		NO_3^- soil				Shoot N		Root N		Microbial N			
		Top		Bottom						Top		Bottom	
Effect	df	F	P≤	F	P≤	F	P≤	F	P≤	F	P≤	F	P≤
Catch crop	2	41.72	**<0.001**	33.25	**<0.001**	20.71	**<0.001**	3.25	**<0.001**	3.17	**0.034**	0.81	0.500
N	2	6.05	**0.005**	5.38	**0.009**	0.78	0.467	0.40	0.665	1.83	0.173	1.68	0.203
Catch crop x N	4	3.65	**0.005**	3.83	**0.005**	0.18	0.945	0.86	0.795	1.71	0.143	2.34	0.056

NO_3^- and microbial N were determined in both the top 11 cm and the bottom 11 cm of the soil column. Catch crop and N are fixed factors and soil collection is a random factor. Catch crop consists of four levels (fallow, *Brassica*, *Phacelia*, *Raphanus*), N out of three (0 N, 40 N, 80 N) and soil collection of two (Sept, Oct). NO_3^- in both the top and bottom soil and top microbial N were log-transformed and weighted. Bottom microbial N and shoot N were log-transformed. Bold values represent significant values of $P < 0.05$

df, degrees of freedom

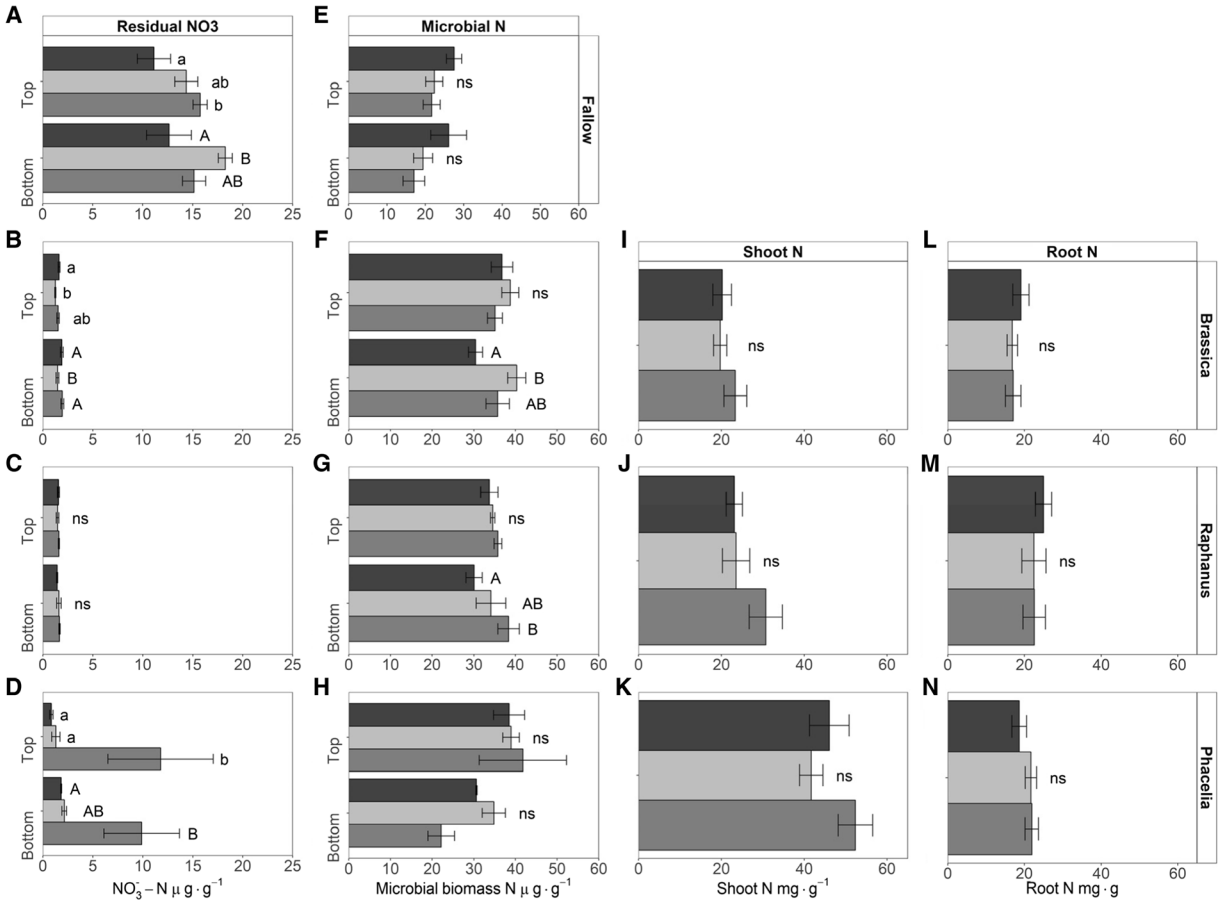

Fig. 2 Residual nitrate (NO_3^-; **A**–**D**), microbial biomass nitrogen (N; **E**–**H**) and catch crop shoot N (**I**–**K**) and root N content (**L**–**N**) with the addition of 0 (dark grey), 40 (light grey) or 80 (medium grey) kg N ha^{-1} for the three catch crops. Residual NO_3^- and microbial N are also given for the fallow treatment, and were measured in both the top 11 cm and the bottom 11 cm of the soil column. Bars represent means ± SE; $n = 3$–5. Different letters within species indicate significant differences ($P < 0.05$)

Phacelia were significantly lower compared to *Brassica* and *Raphanus* (data not shown). Together with the higher shoot N concentration of *Phacelia*, this resulted in a lower C:N ratio of 7.8 compared to 19.7 and 16.0 in *Brassica* and *Raphanus*, respectively (Table S1). Fertilizer addition did not significantly affect C:N shoot ratio's (Table S1).

Effects of N addition on specific root length

In both, the top and bottom 11 cm of the soil, SRL was mainly affected by the catch crop species and only little by N addition in the top 11 cm (Table 1). Yet, *Raphanus* and *Brassica* showed a slight decrease in SRL with increasing N addition in the top layer (Fig. 3). The same pattern occurred for all three plant species in the bottom 11 cm with a significantly lower SRL for *Raphanus* with the addition of 40 and 80 kg N ha^{-1} than with 0 kg N ha^{-1} added and for *Brassica* a significantly lower SRL in the 80 kg N ha^{-1} treatment than without N addition (Table 1).

Catch crops and residual inorganic N

Addition of N clearly resulted in different growth responses of the three catch crop species, which raises the question whether this also affected the amount of N that remained in the soil. Catch crop growth resulted in an overall decrease of, on average, 85 and 81% of residual NO_3^- in the top and the bottom soil layer, respectively (Fig. 2A–D). As expected, the level of NO_3^- left in both soil layers was significantly affected by the amount of N

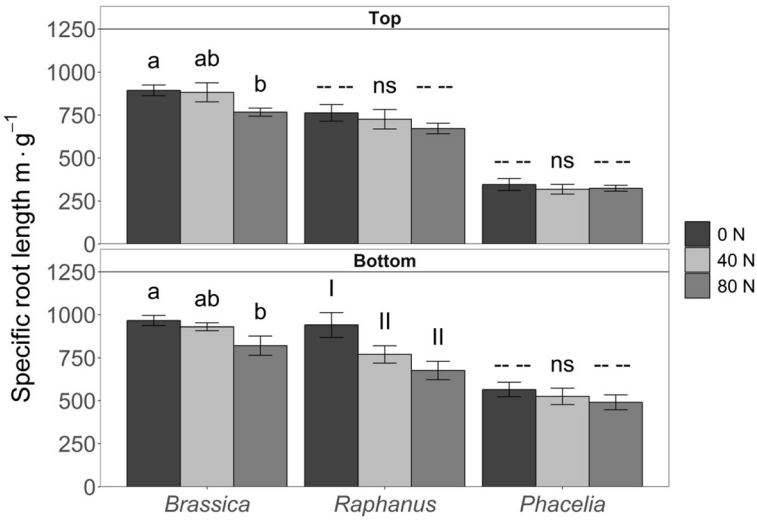

Fig. 3 Specific root length (SRL) in the top 11 cm (top panel) and bottom 11 cm (lower panel) of the soil for the three catch crop species with addition of 0 (dark grey), 40 (light grey) or 80 (medium grey) kg N ha^{-1}. Bars represent means ± SE; $n = 5$. Different letters within species indicate significant differences ($P < 0.05$)

added (Table 2). However, effects were treatment specific. Treatments without catch crops showed an increase in residual NO_3^- with increasing N addition. The same pattern occurred for soil where *Phacelia* was grown, but not for *Raphanus* and *Brassica* (Fig. 2B–D). For *Phacelia*, the addition of 80 kg N ha^{-1} resulted in significantly more NO_3^- left in the top soil layer than when no or 40 kg N ha^{-1} was added. This pattern also occurred in the bottom layer, but only the difference between 0 and 80 kg N ha^{-1} was statistically significant (Fig. 2C). *Brassica*, on the other hand, left slightly less NO_3^- in both the top and bottom layer with the addition of 40 kg N ha^{-1} compared to the other two N treatments (Fig. 2B).

Microbial N immobilisation

A decrease in residual N in the soil is expected to occur largely via plant N uptake and incorporation in the above-ground plant parts. However, besides catch crops, microbes may also immobilise N. In the top 11 cm, microbial N immobilisation was only significantly affected by catch crop growth and not N addition (Table 2). Here, catch crop growth increased microbial N biomass by about 55% (Fig. 2E–H). In the bottom 11 cm, no such effect was found (Table 2; Fig. 2E–H).

The average amount of N in different soil pools, namely microbial N, inorganic N and extractable organic N (DON), was calculated for all

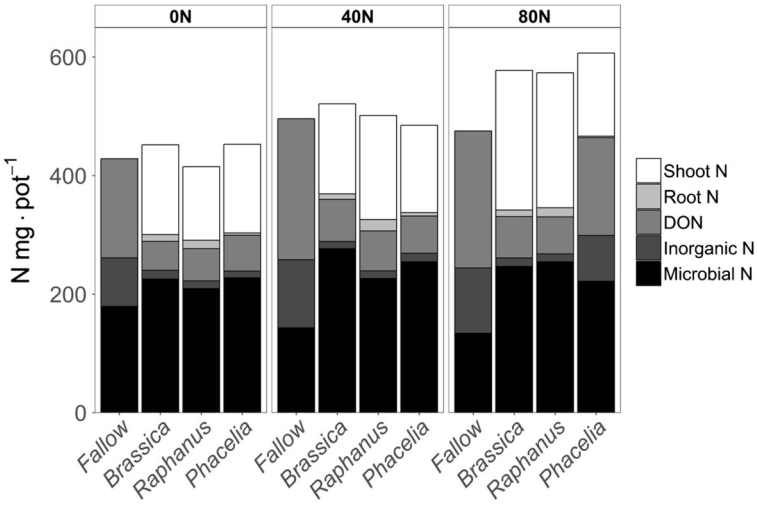

Fig. 4 Nitrogen (N) distribution (mg pot^{-1}) in fallow and three catch crop plant-soil systems with 0, 40 and 80 kg N ha^{-1} added. Bars represent means; $n = 5$. For figure readability, error bars are not shown. For variability of data and statistics, see Figs. 1 and 2 and Tables 1 and 2

treatments (Fig. 4). The amount of inorganic N in the soil decreased by, on average, 80% when catch crops were present. Not much difference occurred between the three catch crop species, except for *Phacelia* at 80 kg N ha^{-1} where substantially more inorganic N was left in the soil. As expected, a major part of the residual N was immobilised in the catch crop shoot. More striking is the 1.5 times higher microbial N biomass in the soil for all three catch crops compared to the fallow treatment. Despite this increase in microbial N, microbial biomass C remained unaffected by catch crop presence (Table S1; Fig. S2). Along with the microbial N increase and the decrease of inorganic N, we observed a substantial decrease of DON when catch crops were present, but no fertilizer effect (Fig. 4). This was less pronounced in *Phacelia* at 80 kg N ha^{-1}, where also the inorganic N levels remained higher and the microbial biomass N did not increase to the same extent as in the other treatments. In the fallow treatment, on the other hand, DON along with inorganic N was slightly enhanced after fertilizer addition. At the same time, the microbial biomass N was slightly lower compared to the 0 kg N ha^{-1} treatment (Fig. 4). Dissolved organic C (DOC), on the other hand, was hardly influenced by catch crop presence and fertilizer addition (Table S1; Fig. S3).

Fertilizer uptake efficiency

Depending on the crop species and the amount of fertilizer, fertilizer addition boosted early plant development (Fig. 1). This, however, raises the question whether the fertilizer uptake efficiency (RE), the difference in N uptake in the fertilized compared to the non-fertilized treatments as a percentage of the added fertilizer, is high enough to compensate for the added mineral N. Fertilizer uptake efficiency based on shoot N uptake (RE_s) was 35 and 44% for *Raphanus* at 40 and 80 kg N, respectively, whereas these values were 3 and 36% for *Brassica*, and −8 and −5% for *Phacelia*. For the latter species, this indicates no additional N uptake from the added fertilizer. Due to the small root N contribution to total plant N, considering root N in addition to shoot N hardly increased fertilizer uptake efficiency (data not shown).

Discussion

Nitrogen retention capacity of catch crops is species specific

Catch crop cultivation aims at reducing N leaching via immobilisation of residual N in plant material. High N uptake is therefore of critical importance for successful catch crop species. Moreover, an early developing, deep root system with high root density has previously been shown to aid in catch crop take up of leachable N from deeper soil layers (Thorup-Kristensen 2001). Furthermore, slow N release from catch crop residues reduces N leaching and gaseous N losses in late winter and/or early spring. N from plant tissue is mineralised slower with high C:N ratios due to stronger physical protection (Puget and Drinkwater 2001; Bodner et al. 2011), indicating that high C:N ratios of the plant tissue is another useful catch crop trait (Wendling et al. 2016).

In our experiment, all catch crops successfully reduced residual NO_3^- by 81–85% as previously observed by others (Thorup-Kristensen 2001; Tribouillois et al. 2015), even at high N levels. Only *Phacelia* at 80 kg N ha^{-1} was not able to cope with the high N loads and did not reduce NO_3^- levels. *Phacelia* plants even had a lower biomass development, potentially due to toxic levels of ammonium at 80 kg N ha^{-1} (reviewed by Esteban et al. 2016). *Phacelia* may thus be a species that cannot handle very high N concentrations.

Overall, our study shows species-specific N retention capacities, comparable to what Wendling et al. (2016) found. As biomass partitioning above- and belowground, and N concentrations in shoot material varied between species, the N retention capacity is a function of above- and belowground biomass and the respective N concentration. *Phacelia*, even though developing less biomass, removed larger amounts of N than would be expected based on biomass alone, due to high N concentrations in the shoot tissue. This is, however, in contrast to Wendling et al. (2016), where the shoot N concentration in *Phacelia* was not higher compared to *Raphanus* or *Brassica*. Root N immobilisation in our experiment was low, it can, however, be expected, that this process contributes much stronger to the N retention capacity under field conditions, where root biomass is often larger (Kanders et al. 2017). Moreover, in a previous study we showed that N rhizodeposition contributed substantially to the N retention capacity of catch crops (Kanders et al. 2017), indicating that these

capacities may thus be even higher than shown here, if rhizodeposits were included.

Shoot biomass mineralisation and the subsequent N release from plant tissue, is expected to occur faster in *Phacelia* compared to *Raphanus* and *Brassica* due to its high C:N ratio. *Brassica* is expected to be mineralised most slowly and is therefore expected to not only remove overall large amounts of N, but to also have a longer retention of N in its biomass reducing N losses late in its growing season, compared to *Raphanus* and especially *Phacelia.*

Aboveground performance of the catch crops did not reflect belowground effects. Root biomass of all species was not much affected by N addition and no significant effect on root:shoot ratio was observed, which may be related to the already high N concentrations in the initial soil. The addition of N affected root distribution of *Raphanus* and, to a lesser extent, of *Brassica*, indicating a decrease in root biomass in the lower layer of the pots with N addition. Since fertilizer is typically added on or into the top soil, N addition may thus result in catch crops that develop a more extensive root system in the top soil and less in the deeper soil layers as a response to the high nutrient conditions (e.g. Hackett 1972; Drew 1975; Hodge 2004; Visser et al. 2008; in 't Zandt et al. 2015). In turn, this may result in higher N losses, because the residual N can leach more quickly away from the rooting zone of the catch crops. Thorup-Kristensen and Rasmussen (2015) indeed showed a strong correlation between deep rooting and total N immobilisation by the catch crop. Moreover, the observed trend of a decreasing specific root length with increasing N in the bottom soil layer for both *Raphanus* and *Brassica* could indicate a less finely branched root system with increasing N addition. Fine rooting is another important catch crop trait as it is strongly linked to high N uptake (e.g. Jackson et al. 1997).

Fertilizer addition increases risk of N losses

N fertilizer addition indeed boosted early plant development despite the already high residual N in the agricultural soil. Nevertheless, from a resource use efficiency point of view, it is not recommended to fertilize catch crops, as fertilizer use efficiencies were in all cases far below 50%. For *Phacelia*, fertilizer use efficiencies were even negative and the plants showed a decreased growth with the highest N addition. *Phacelia* should, therefore, not be grown under these very high N availabilities and no additional fertilizers should be added. Although it can be assumed that over a longer period of time larger percentages of the added fertilizer would be taken up, more than 50% remains in the soil and becomes prone to leaching, since it is not immobilised by the catch crops' early growing days. More so, under field conditions where temporal anoxic conditions after heavy rainfall events are likely, additional N in the top soil can contribute to N losses as the potent greenhouse gas N_2O.

Furthermore, no clear effect of N addition on biomass partitioning was observed. This questions the applicability of the optimal resource partitioning theory to conditions of high nutrient availability such as intensive arable systems and in particular catch crop systems. Since N is plentiful available, plants may simply not have to invest in roots as much as under N limiting conditions, regardless of the addition of even more N, and do not show the expected, lowered root:shoot ratio with increasing N availability (e.g. Levin et al. 1989; Ågren and Franklin 2003; Bonifas et al. 2005).

Catch crops trigger microbial N immobilisation

Microbial N increased with catch crop presence. This increase indicates that soil microorganisms were C limited under fallow conditions, which was counteracted by the presence of catch crops. Here, microorganisms most likely made use of rhizodeposits, such as root exudates, lysates and other easily available C sources released from the catch crop roots, which are then used as an energy source to assimilate inorganic N (Farrell et al. 2014; Lloyd et al. 2016). As this inorganic N was plentiful available, the microorganisms did not rely on mineralisation of soil organic matter for N mining (Zang et al. 2016). This could indicate that under high N conditions, competition between soil microorganisms and plant roots might be limited (Schimel and Bennett 2004), while under lower N availability, competition may be high (e.g. Liu et al. 2016). In other words, due to a high resource availability catch crops stimulated microbial N immobilisation by providing them with easily available C sources.

At the same time, microbial biomass C did not increase with catch crop presence, which either reflects no growth or a fast turnover of the microbial biomass. Previous studies showed slight to strong increases of microbial biomass C when plants were present (Müller et al. 2009; Wichern et al. 2011), whereas other studies

showed no change in the microbial biomass C and N in soil, even though the proportion of the microbial biomass derived from plant roots increased (Wichern et al. 2007a, 2007b). This highlights the use of root derived C and N by soil microorganisms. The incorporation of plant derived C and N without an increase of the microbial biomass C, indicates an increased turnover of microbial cells, with part of the immobilised N being released as inorganic N and another part of the immobilised N being stored in microbial residues. Future studies have to shed further light on how plant roots influence microbial biomass C and N development at high and low nutrient availability, throughout the growth period of plants, and ^{15}N labelling studies may help to elucidate in which soil pools N fertilizers end up. This is particularly important in catch crop systems as the microbial immobilised N is usually not accounted for, but can be a substantial pool in the soil. Moreover, since this N will be re-mineralised at some stage and can cause positive nutrition effects for a subsequent crop or negative effects on the environment due to N leaching or gaseous N losses, it is crucial to improve our knowledge on microbial turnover processes and re-mineralisation of these microbial residues and the effect of plants on this.

Our results indicate a positive plant-soil feedback in catch crop systems caused by the release of C as a limiting resource for soil microorganisms (Fig. 5). The plentiful available N can be used by both plants and microorganisms at limited competition. Plants make use of inorganic N either present in the soil as residual inorganic N, added as fertilizer N or mineralized by soil microorganisms. The plants most likely make no or only limited use of DON as a source of N. On the other hand, the soil microorganisms make use of DON and other organic N sources in addition to inorganic N. These findings are in contrast to an earlier study (Jones et al. 2004), where the soil microbial biomass used low molecular weight DON as a source of C. In our experiment, plants provided sufficient amounts of C input, reducing the necessity for microorganisms to mineralise DON for C capturing. Furthermore, plants use the assimilated N mainly to develop shoot biomass and translocate parts of their C-assimilates below ground, where especially easily available C is used as energy source by the soil microorganisms (Paterson 2003; Jones et al. 2009). Consequently, microbial biomass N increases (Fig. 5; indicated by dotted frame), whereas the inorganic N and the DON pools show a net decrease (Fig. 5; indicated by dotted frames). Most likely, the microbial residues partly increase with increasing microbial N and microbial turnover. An increase in microbial N followed by a synchronous decrease of DON has been observed before, however, in the absence of plants (Wichern et al. 2004) indicating that DON was used as a N source, and potentially at the same time as a C source. Plant presence, the release of C from roots and the subsequent use of this C as an energy source by soil microorganisms is a well-known phenomenon (e.g. Paterson et al. 2007;

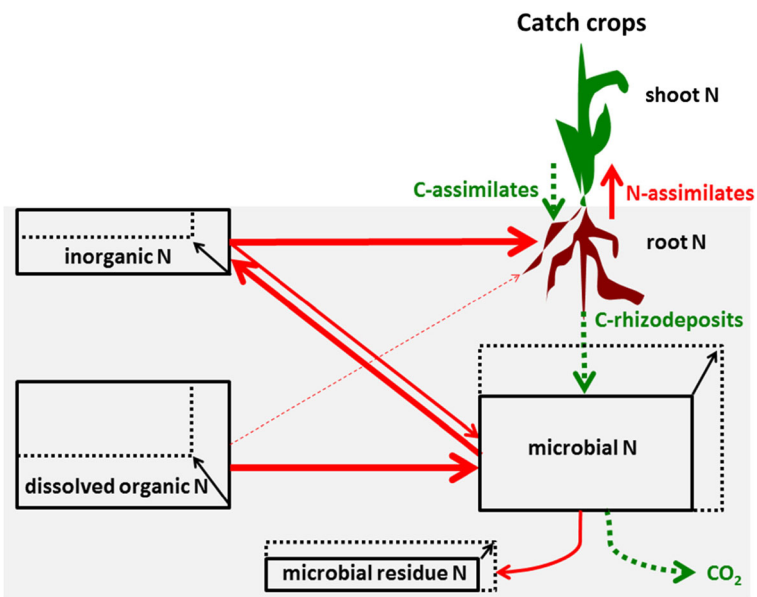

Fig. 5 Proposed changes in nitrogen (N) and carbon (C) fluxes (red and green arrows, respectively) between different N and C pools (boxes) in high N soils with catch crops present compared to high N soils without catch crops. Arrow thickness indicates flux size and dotted boxes indicate pool increase or decrease with black arrows illustrating the direction

Drigo et al. 2010; De Deyn et al. 2011; Pausch et al. 2016). Likewise, N released from roots in the process of N rhizodeposition, can be used as N source by soil microorganisms (Mayer et al. 2003; Wichern et al. 2007a, 2007b; Hupe et al. 2016) and has to be considered in future studies to elucidate the effect of catch crops on C and N assimilation by soil microorganisms.

Conclusions

Our study shows species-specific responses of catch crops to N availability with little root plasticity in response to the N added. It highlights that aboveground changes are not related to root system alterations in a nutrient-rich environment. Therefore, N fertilization should depend on the catch crop species and the residual N levels in the soil, and thus the legacy of the subsequent main crops. Since fertilizer uptake efficiencies are low, N addition should be avoided altogether in agricultural systems with high residual N levels to not further increase N losses. Moreover, N immobilisation by soil microorganisms, rhizodeposition, which is triggered by C released from roots, is an important pathway of N removal when N is plentiful available. The extent of this plant-soil feedback has to be evaluated in future studies. We conclude that N immobilisation by soil microorganisms has to be considered when assessing the effect of catch crops on residual N as an important ecosystem service of plant-soil-microbial systems.

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