


# Even flow? Changes of carbon and nitrogen release from pea roots over time

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## Abstract

**Background and aims** Soil nutrient dynamics are affected by root-microbe interactions and plant development. We investigated the influence of plant growth stage and arbuscular mycorrhiza fungi (AMF) on carbon (C) and nitrogen (N) rhizodeposition and the transfer into the microbial biomass (MB).

**Methods** Pea varieties (*Pisum sativum* L.) with (Frisson) and without mycorrhiza (P2) were  $^{13}\text{C}$ - $^{15}\text{N}$ -labelled and harvested at 45, 63, 71, and 95 days after sowing. Mycorrhization, MB, total C, N,  $^{13}\text{C}$ ,  $^{15}\text{N}$  were determined in plant and soil compartments to calculate C and N derived from rhizodeposition (CdfR, NdfR).

**Results** Total CdfR increased until pea maturity, NdfR until end of flowering. Their relative contribution steadily decreased over time, accounting for 4–10% of total plant C and N at harvest. Rhizodeposition contributed between 1 and 6% to MB C and N, although 20% of the rhizodeposits were discovered in the MB. Frisson released more NdfR than P2 but it was not possible to accurately estimate AMF effects on C and N due to differences in biomass partitioning.

**Conclusions** CdfR followed an even flow from early growth until senescence. NdfR flow ceased after flowering possibly due to N relocation within the plant. Rhizodeposits contribute very little to MB in our study.

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## Introduction

The amount of belowground plant biomass, consisting of roots and rhizodeposits, and its microbial utilization shape the carbon (C) sequestration potential of plants. Additionally, nitrogen (N) is also stored in the belowground biomass and is partly released by mineralisation and partly immobilized by soil microorganisms. The rhizodeposition of plants represents an essential C and N input into soil and has been repeatedly studied (Wichern et al. 2008; Jones et al. 2009). The calculation of rhizodeposition helps to improve N balances of legume based crop rotations (Mayer et al. 2003a, b) and to

estimate the C sequestration potential of plants, as this input source of plant biomass is often neglected, causing inaccurate C and nutrient budgets (Kanders et al. 2017). Rhizodeposition is the release process of rhizodeposits, which are all compounds released from roots of living plants. This comprises volatile gaseous compounds, all non-particulate compounds, i.e. ions, exudates, lysates, secretions, and all particulate compounds, i.e. root and cap cells, sloughed roots and tissues, root hairs, root fragments, decaying roots and nodules (Wichern et al. 2008; Uren 2001). It is the broadest definition of rhizodeposition, which is also used in the present study to consider the variety of C and N containing molecules released from roots ( $C_{dfR}$  = C derived from rhizodeposition;  $N_{dfR}$  = N derived from rhizodeposition). Moreover, rhizodeposition is a dynamic process, affected by biotic factors, such as growth stage, plant species or variety and mycorrhiza (Jones et al. 2004; Rees et al. 2005) and abiotic factors, like soil properties (e.g. soil compaction, nutrient availability) (Rees et al. 2005) or climatic factors such as drought stress (Salon et al. 2007).

During the vegetative period, the N concentration in various plant parts can vary substantially (Salon and Munier-Jolain 2010). The changes are caused by N availability and N translocation or remobilization processes during seed filling, probably also affecting the quality and quantity of rhizodeposition (Arcand et al. 2013b). In pea plants, C is mainly transported as sucrose in the phloem (Kühn and Grof 2010), whereas N is especially transported in the plant xylem, e.g. as nitrate, urea (Witte 2011) or as amino acids (Lalonde et al. 2003; Salon and Munier-Jolain 2010). As a consequence, xylem sap of peas has a low C/N ratio of 1.5–6, whereas the C/N ratio of the phloem sap is often higher (Salon and Munier-Jolain 2010). The phloem also delivers C compounds to the root system, where they are required for cell metabolism and division, leaving a large proportion of it respired and released as  $CO_2$  through root respiration, which is sometimes included in the definition of rhizodeposition. On the other hand, N is mainly taken up from the soil solution and transferred to plant sinks, where it is required to build storage or structural proteins. As the dominant movement of assimilates is downward while that of inorganic N compounds is upward, differences between C and N rhizodeposition patterns can be expected. To elucidate this, double labelling of plants with  $^{13}C$  and  $^{15}N$  isotopes in the form of a glucose-urea mixture is particularly suitable for

simultaneously measuring C and N rhizodeposition of peas and oats (Wichern et al. 2007b) or chickpeas (Yasmin et al. 2010). In these experiments, C rhizodeposition ranged from 25 to 31% of total plant C and N similarly from 28 to 36% of total plant N. However, temporal patterns of rhizodeposition have not been investigated in detail, despite some efforts to investigate  $C_{dfR}$  and  $N_{dfR}$  in peas throughout the vegetative period and at reproductive growth stages (Mayer et al. 2003b; Wichern et al. 2007a, b). Results investigating C and N partitioning at an early growth stage showed extraordinary high values for  $C_{dfR}$  and  $N_{dfR}$  (Wichern et al. 2007b), which are likely caused by non-homogeneous labelling of roots and root derived compounds resulting in an overestimation of  $C_{dfR}$  and  $N_{dfR}$  (Hupe et al. 2016; Kanders et al. 2017).

Quality and quantity of rhizodeposits are expected to change with time, depending on plant development as well as root growth and root senescence (Gavito et al. 2001). Arcand et al. (2013b) studied the temporal dynamics of  $N_{dfR}$  of peas and found that the amount increased over time. They concluded that in the vegetative stage, N rhizodeposition mainly consisted of root exudates, whereas at maturity the amount of N rhizodeposits was dominated by N from root senescence (Arcand et al. 2013b). As root exudates are likely to be metabolized faster by soil microorganisms, this would lead to the conclusion, that at an earlier growth stage a higher amount of rhizodeposits is incorporated into the microbial biomass and microbial metabolites, whereas the rhizodeposits at a later growth stage are less easily metabolized by soil microorganisms, as they consist of decaying roots. These differences would be reflected by changes in  $^{13}C$  and  $^{15}N$  enrichment of the microbial biomass, when labelling plants and tracing the  $^{13}C$  and  $^{15}N$ , which has not been done in many studies before.

Part of the microbial biomass consists of arbuscular mycorrhizal fungi (AMF) (Faust et al. 2017). AMF form a symbiosis with over 80% of land plant species, providing additional phosphorus, water and micronutrients for the host plants in exchange for assimilates (Smith et al. 2010), resulting in a strong C flux into the mycorrhizosphere, amounting to up to 20% of assimilated C (Parniske 2008). AMF also affect root architecture, improve soil aggregation and influence the quality and quantity of rhizodeposits (Rillig and Mummey 2006). Consequently, mycorrhization of plants will result in a stronger translocation of rhizodeposits into the microbial biomass and thus a stronger  $^{13}C$  signal when

labelling plants with  $^{13}\text{C}$ . A potentially useful, but not fully evaluated tool for investigating AMF effects on rhizodeposition is the use of AMF resistant pea (*Pisum sativum* L.) mutants and their symbiotic isolines (Kleikamp and Joergensen 2006; Jannoura et al. 2012).

To improve our understanding of the time course of C and N rhizodeposition and the influence of mycorrhiza on it, a pot experiment was carried out, using a mycorrhizal and a non-mycorrhizal pea variety, labelled with  $^{13}\text{C}$  and  $^{15}\text{N}$ , using a cotton wick stem feeding technique (Russell and Fillery 1996; Wichern et al. 2007b). The underlying hypothesis was that the rhizodeposition of C continues throughout plant development, whereas N rhizodeposition decreases after flowering caused by relocation of N from roots to shoots and seeds. Moreover, we assume that at an early growth stage of pea plants, incorporation of rhizodeposits into soil microorganisms is greater due to a higher proportion of easily metabolizable root exudates. After flowering root senescence contributes stronger to rhizodeposition, which is expected to be metabolized more slowly. Furthermore, the presence of mycorrhiza is expected to increase rhizodeposition with a larger C flux into the mycorrhizosphere until the end of flowering, when mycorrhization ceases.

## Materials and methods

### Soil

The soil used for the pot experiment was collected from an arable field at 0–30 cm depth from the research station of the University of Kassel in Neu-Eichenberg, Germany (51°23'N, 9° 55'E, 220 m above sea level). It was sieved (<10 mm) and stored for six weeks in boxes at 10 to 15 °C in the dark before the experiment started. The silty loam (13% clay; 83% silt; 3% sand) was classified as a Haplic Luvisol according to the WRB-FAO classification system, with a pH of 6.0, an organic carbon content of 12 mg g<sup>-1</sup> soil and total nitrogen content of 1.3 mg g<sup>-1</sup> soil.

### Experimental design and plant labelling

The experiment was carried out at the University of Kassel, Faculty of Organic Agricultural Sciences in Witzenhausen, Northern Hesse, Germany. *Pisum*

*sativum* L. cv. Frisson and the non-mycorrhizal (myc<sup>-</sup>) and non-nodulating (nod<sup>-</sup>) pea mutant of Frisson, P2, were cultivated under controlled conditions in the greenhouse with a relative humidity of 60% and an illumination intensity of 110 klxh d<sup>-1</sup>. Seeds were provided by the Institut National de la Recherche Agronomique (INRA), France. The mean temperature was 20 °C during the day and 15 °C during night. Pots of 8.5 l (28 cm in diameter and 20 cm long) were filled with 11 kg of soil, which was mechanically compressed to a bulk density of 1.3 g cm<sup>-3</sup>. Two plants per pot were cultivated with six replicates per treatment and pots were randomly distributed. One day before sowing, the soil was fertilized with 150 kg N as NH<sub>4</sub>NO<sub>3</sub> to exclude Frisson nodulation effects in the experiment. Besides this, KH<sub>2</sub>PO<sub>4</sub>, CaCO<sub>3</sub> and K<sub>2</sub>SO<sub>4</sub> were applied at a rate of 17 kg P, 43 kg K, 100 kg Ca and 9 kg S ha<sup>-1</sup> to provide sufficient nutrients also for the non-mycorrhizal and non-nodulating pea mutant P2. According to Mahieu et al. (2009), with a high N fertilization, no differences in plant dry matter of Frisson and P2 were expected. All pots were inoculated with 5 g rootgrow™ professional (PlantWorks, Sittingbourne, UK) placed directly below the seeds containing a range of mycorrhiza fungi species (but no rhizobial inoculant) to ensure AMF symbiosis. Once a week, the pots were weighed and watered with deionized water, to keep the water holding capacity between 60 and 80%.

For approximating continuous labelling, the plants were labelled with a 0.5%  $^{15}\text{N}$ -urea (95 atom%) and 2%  $^{13}\text{C}$ -glucose (99 atom%) solution, using the cotton wick stem feeding technique (Russell and Fillery 1996; Wichern et al. 2007b). The stem of each plant was drilled with a 0.5 mm diameter drill, approximately 3 cm above the ground. Then, a cotton wick was passed through the hole in the stem. The ends of the wick were passed through silicon tubes and through the lid of a 2 ml vial, which contained 1 ml of the labelling solution. During the labelling period, each plant received 2 to 5 ml of the labelling solution in total, depending on the harvest date (Table 1). To guarantee a sufficient distribution of the labelling solution within the plant, the time between the last labelling event and harvest was at least 6 days. To prevent evaporation losses, the connections between wick and plant or wick and lid were sealed with kneading

**Table 1** Labelling and harvest dates for Frisson and P2 (in days after sowing; DAS)

	Plant labelling:				
	14 DAS	29 DAS	42 DAS	57 DAS	70 DAS
Harvest date:					
Beginning of flowering (45 DAS)	X	X			
End of flowering (63 DAS)	X	X	X	X	
Green ripeness (71 DAS)	X	X	X	X	
Dry ripeness (95 DAS)	X	X	X	X	X

X represents the providing of 1 ml labelling solution (0.5%  $^{15}\text{N}$ -urea (95 atom %) and 2%  $^{13}\text{C}$ -glucose (99 atom %)) per pea plant

mass (Teroson, Henkel, Düsseldorf, Germany). The labelling solution was produced using deionized and then sterile filtered ( $< 0.2 \mu\text{m}$ ) water. All material used for labelling was steam sterilized for at least 20 min at a temperature of  $121 \text{ }^\circ\text{C}$ . The plants were labelled once every two weeks, five times during the experiment, beginning 14 days after sowing (DAS), with three leaves unfolded. Due to callus formation, the hole for the cotton wick had to be re-drilled before every labelling event. Solution uptake varied in time. After each solution uptake, the empty vials were filled with 0.5 ml deionized water to encourage complete isotope uptake. Pots were covered with mesh (1 mm) to prevent soil contamination from falling leaves. For measuring the natural abundance of  $^{13}\text{C}$  and  $^{15}\text{N}$  of the pea plants, as background values for calculating rhizodeposition, unlabelled control plants of Frisson and P2 were cultivated and randomized with the labelled pea plants. As Wichern et al. (2010) showed, the cotton wick system does not influence the plant development or dry matter. Therefore, control plants to determine background values of  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  had no labelling system and were cultivated under similar conditions along with the labelled plants.

#### Sampling and analyses

Frisson and P2 plants were harvested at four different growth stages, depending on plant development, beginning 45 DAS (first petals visible, flowers still closed; beginning of flowering), 63 DAS (end of flowering), 71 DAS (pods had reached typical size; green ripeness) and 95 DAS (fully ripe: all pods were dry and brown; seeds were dry and hard; maturity/dry ripeness). At every sampling date, 6

pots with labelled Frisson, 6 pots with labelled P2 and the same number of pots with unlabelled plants of Frisson and P2 were harvested. At harvest, labelling systems were carefully removed from the plants and stored at  $-18 \text{ }^\circ\text{C}$  for analyses of the remaining  $^{13}\text{C}$  and  $^{15}\text{N}$ . All pea plants were separated into roots, stem, leaves, pods and grain (the latter two at 63, 71 and 95 DAS). After weighing, plant parts were dried for at least 48 h at  $60 \text{ }^\circ\text{C}$  to estimate dry matter. The dry plant parts were ground with a ball mill, weighed into tin cups (1 mg) and analyzed for total C, total N and the isotope ratio of  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ . The mycorrhizal colonization of roots was determined by staining fresh roots with trypan blue and using the gridline intersection method (Kleikamp and Joergensen 2006). Also the nodulation of Frisson was examined (without counting or weighing the nodules) to evaluate the efficiency of nodulation inhibition in Frisson by N fertilizer addition. Therefore, the formation and the colour of the nodules was checked (pink = active = atmospheric nitrogen will be fixed through symbiosis of plant and rhizo bacteria).

At harvest, the soil was weighed and a subsample of 10 g fresh soil was dried for 24 h at  $105 \text{ }^\circ\text{C}$  to determine dry matter. Another subsample (300 g) was taken for analyzing total soil C and N content and the isotope ratio of  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ . For this, the soil was sieved ( $< 2 \text{ mm}$ ) and all visible roots were removed manually with tweezers, and the soil was dried, ground and weighed into tin cups (as described above for plant parts; 30 mg on average). In addition, two samples of acetanilide standard were included for every ten plant or soil samples. Total C and total N were analyzed with an elemental analyzer (Fisons-Instruments, Rodano, Milano,

Italy). The  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  isotope ratios were determined by isotope ratio mass spectrometry (Finnigan MAT, Bremen, Germany).

To calculate the total recovery of the isotopes, the vials and labelling systems were extracted with 200 ml of 0.05 M  $\text{K}_2\text{SO}_4$  and organic C and total N in the extracts were analyzed using a CN Analyzer (Multi N/C 2100S, Analytik Jena, Germany). The remaining amount of  $^{13}\text{C}$  and  $^{15}\text{N}$  in the extracts was calculated, assuming that the measured C and N in the extracts were solely derived from the isotope solution.

Microbial biomass C and N were determined by the fumigation extraction method (Brookes et al. 1985; Vance et al. 1987), including a pre-extraction step to remove living roots (Mueller et al. 1992). For the pre-extraction, 30 g of soil (after removing visible roots) and 70 ml 0.05 M  $\text{K}_2\text{SO}_4$  were horizontally shaken for 30 min at 200 rev  $\text{min}^{-1}$ . Then, the soil solution was centrifuged for 10 min at 4000 g and 6 °C. The supernatant was filtered to remove the floating living roots and frozen prior organic C, total N, inorganic N, and isotope ratio analysis. Then, 15 g of the pre-extracted soil was fumigated with  $\text{CHCl}_3$  for 24 h at 25 °C in the dark. After removal of the  $\text{CHCl}_3$ , the fumigated soil was extracted with 60 ml 0.05 M  $\text{K}_2\text{SO}_4$  (Potthoff et al. 2003). While fumigation commenced, 15 g of the pre-extracted soil was extracted with 0.05 M  $\text{K}_2\text{SO}_4$ , providing the non-fumigated samples. Organic C and total N in the extracts were analyzed using a CN Analyzer (Multi N/C 2100S, Analytik Jena, Germany). After freeze-drying, the  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  isotope ratios in the extracts (now dry salts) were also determined by isotope ratio mass spectrometry. Microbial biomass C was calculated as  $E_C/k_{\text{EC}}$ , where  $E_C$  = (organic C extracted from fumigated soils) – (organic C extracted from non-fumigated soils) and  $k_{\text{EC}} = 0.45$  (Wu et al. 1990). Microbial biomass N was calculated as  $E_N/k_{\text{EN}}$ , where  $E_N$  = (total N extracted from fumigated soils) – (total N extracted from non-fumigated soils) and  $k_{\text{EN}} = 0.54$  (Joergensen and Mueller 1996).

#### Calculation and statistical analyses

The percentage (Eqs. 1 and 2) and the amount of C and N derived from rhizodeposition (CdfR and NdfR – Eqs. 3, 4 and 5, respectively) were calculated with

an isotope mass balance approach as described by Hupe et al. (2016). Calculation details can be found in the [supplementary material](#).

$$^{13}\text{C}_{\text{tracerdfR}} (\text{in}\%) = \frac{^{13}\text{C}_{\text{tracer dfR}}}{^{13}\text{C}_{\text{tracer in total}}} \times 100 \quad (1)$$

$$^{15}\text{N}_{\text{tracerdfR}} (\text{in}\%) = \frac{^{15}\text{N}_{\text{tracer dfR}}}{^{15}\text{N}_{\text{tracer in total}}} \times 100 \quad (2)$$

$$^{15}\text{NdfR}(\text{mg}) = \frac{^{15}\text{N}_{\text{trace in soil}}(\text{mg}) \times ^{15}\text{N in plant}(\text{mg})^*}{^{15}\text{N}_{\text{tracer in plant}}(\text{mg})} \quad (3)$$

$$^{14}\text{NdfR}(\text{mg}) = \frac{^{15}\text{N in soil}(\text{mg}) \times ^{14}\text{N in plant}(\text{mg})^*}{^{15}\text{N in plant}(\text{mg})} \quad (4)$$

$$\text{NdfR}(\text{mg}) = ^{15}\text{NdfR}(\text{including } ^{15}\text{N}_{\text{tracerdfR}}) + ^{14}\text{NdfR} \quad (5)$$

\* The same calculation was used for the calculation of C derived from rhizodeposition (CdfR)

Briefly, for the calculations the following assumptions were made: (i) the distribution of the tracer in the plant corresponds to the distribution of total plant C and N, respectively, (ii) the isotopic enrichment of roots and rhizodeposits is equal in space and time (Hupe et al. 2016). For this reason, we assumed, that the ratio of  $^{13}\text{C}_{\text{tracer}}$  derived from rhizodeposition ( $^{13}\text{C}_{\text{tracerdfR}}$ ) or  $^{15}\text{N}_{\text{tracer}}$  derived from rhizodeposition ( $^{15}\text{N}_{\text{tracerdfR}}$ ) in soil in relation to  $^{13}\text{C}_{\text{tracer}}$  or  $^{15}\text{N}_{\text{tracer}}$  in total (in plant and rhizodeposition) is the same as the ratio of C or N derived from rhizodeposition (CdfR, NdfR) in soil in relation to C or N in total (in plant and rhizodeposition). As the total C and N content and thus the isotopic enrichment of plant organs differ because of nutrient and tracer relocation during plant development, the enrichment of grain, shoot and root was calculated separately and then summarized as total  $^{13}\text{C}_{\text{tracer}}$  or  $^{15}\text{N}_{\text{tracer}}$ , respectively.

Microbial C and N, extractable C and inorganic N derived from rhizodeposition were also calculated using the isotope mass balance approach (Hupe et al. 2016; see [supplementary material](#)).

Statistical analyses were performed using IBM SPSS Statistics 22. Differences of the means between the date of harvest (depending on plant development) were tested by a one-way analysis of variance

(ANOVA) using the Scheffé post-hoc test. Student's t-test was performed on pairs of means to determine differences between both the CdfR and NdfR of the pea varieties Frisson and P2, respectively. Data were normally distributed (tested with Q-Q Plot) and variances were homogeneous (Levene-test).

## Results

### Tracer uptake, plant enrichment and tracer recovery

Four weeks after the beginning of labelling (14 DAS), at beginning of flowering (45 DAS), pea plants had taken up between 4 mg (Frisson) and 6 mg (P2)  $^{13}\text{C}$  (Table 2) and 2 mg  $^{15}\text{N}$  (Table 3). At maturity (9 weeks after beginning of labelling), the  $^{15}\text{N}$  uptake increased to 9 mg (Frisson) and 7 mg (P2). At the same time, the  $^{13}\text{C}$  uptake increased to 21 mg (Frisson) and 17 mg (P2), indicating that the wick technique can be used for multiple pulse labelling of plants.

During plant growth, the multiple pulse labelling resulted in a high enrichment of  $^{13}\text{C}$  and  $^{15}\text{N}$  tracers in roots and shoots, with no significant changes in plant enrichment over time (Tables 2 and 3). Regardless of harvest time, the  $^{13}\text{C}$  and  $^{15}\text{N}$  enrichment of the P2 grain was in most cases twice as high in comparison with the Frisson grain. The  $^{13}\text{C}$  tracer recovery was similar for Frisson and P2 and varied between 39 and 57% of the

applied amount (Table 2). Approximately 75% of the recovered  $^{13}\text{C}$  amount was found in the plant, 5% in the soil and roughly 20% in the labelling systems (data not shown). In contrast, 80 to 100% of the  $^{15}\text{N}$  applied was recovered, with most of it found in the plant, about 4% in the soil, and roughly 30% in the labelling systems (Table 3). Assuming comparable proportions of  $^{13}\text{C}$  and  $^{15}\text{N}$  retained in the wick system, an additional loss of approximately 10% of the applied  $^{13}\text{C}$  as  $\text{CO}_2$  from the wick system is likely. This leaves another 30 to 50% of the applied  $^{13}\text{C}$  lost as  $\text{CO}_2$  by rhizo-respiration.

### Development of plant biomass and time course of rhizodeposition

Between beginning of flowering and maturity, C distribution among the different plant parts was similar to that of N (Figs. 1 and 2). In the shoots, C and N showed a roughly fourfold increase until green ripeness, followed by a 23 and 58% decrease, between green ripeness and maturity, respectively. Between green ripeness and maturity, the amounts of C and N in grains increased more than five times to 2370 mg C and 230 mg N  $\text{plant}^{-1}$ . The amount of root C and N roughly doubled until green ripeness. Then, the root C showed a 20% decrease, whereas root N remained constant between green ripeness and maturity. Despite the additional N added to the soil, Frisson produced active nodules until the end of flowering, which, however, was not further investigated.

**Table 2** Uptake (in  $\text{mg plant}^{-1}$ ), recovery (in plant, soil, wick system and total, in % of applied) and enrichment (of various plant parts and soil in  $\text{atom}\%_{\text{excess}}$ ) of  $^{13}\text{C}$  in pea (Frisson and P2,

harvested at beginning of flowering (45 DAS), end of flowering (63 DAS), green ripeness (71 DAS) and maturity (95 DAS)

$^{13}\text{C}$	$^{13}\text{C}$ -Uptake ( $\text{mg plant}^{-1}$ )	Recovery (% of applied $^{13}\text{C}$ )				$\text{Atom}\%_{\text{excess}}$			
		In plant	In soil	In wick system	In total	Soil	Root	Grain	Shoot
Frisson									
45 DAS	4 ± 0.8	25 ± 4.6	2 ± 0.9	12 ± 2.4	39 ± 6.5	0.001 c	0.5 a		0.9 a
63 DAS	13 ± 1.8	35 ± 5.8	3 ± 0.4	7 ± 1.0	46 ± 5.8	0.002 ab	0.6 a	0.2 a	0.8 a
71 DAS	11 ± 0.8	30 ± 2.2	2 ± 0.2	7 ± 0.5	39 ± 2.2	0.001 bc	0.4 a	0.1 b	0.4 a
95 DAS	21 ± 1.7	47 ± 4.1	3 ± 0.3	7 ± 0.3	57 ± 4.0	0.002 a	0.5 a	0.2 c	0.8 a
P2									
45 DAS	6 ± 1.1	32 ± 6.5	3 ± 0.2	8 ± 1.3	43 ± 5.6	0.001 a	0.6 a		0.9 a
63 DAS	12 ± 0.7	34 ± 2.1	2 ± 0.2	8 ± 0.8	43 ± 1.9	0.001 a	0.5 a	0.4 a	0.7 a
71 DAS	13 ± 2.3	35 ± 7.5	3 ± 1.0	8 ± 0.4	45 ± 6.7	0.001 a	0.5 a	0.2 b	0.5 a
95 DAS	17 ± 0.6	38 ± 1.5	2 ± 0.4	6 ± 0.7	47 ± 1.7	0.001 a	0.6 a	0.4 a	0.9 a

Values show means ± standard error of the mean (Frisson:  $n = 4$  for 45 and 63 DAS and  $n = 6$  for 71 and 95 DAS; P2:  $n = 6$ ; except 71 DAS:  $n = 5$ ). Different letters indicate significant differences between DAS (Scheffé,  $p < 0.05$ )

**Table 3** Uptake (in mg plant<sup>-1</sup>), recovery (in plant, soil, wick system and total, in % of applied) and enrichment (of various plant parts and soil in atom%excess) of <sup>15</sup>N in pea (Frisson and P2, harvested at beginning of flowering (45 DAS), end of flowering (63 DAS), green ripeness (71 DAS) and maturity (95 DAS))

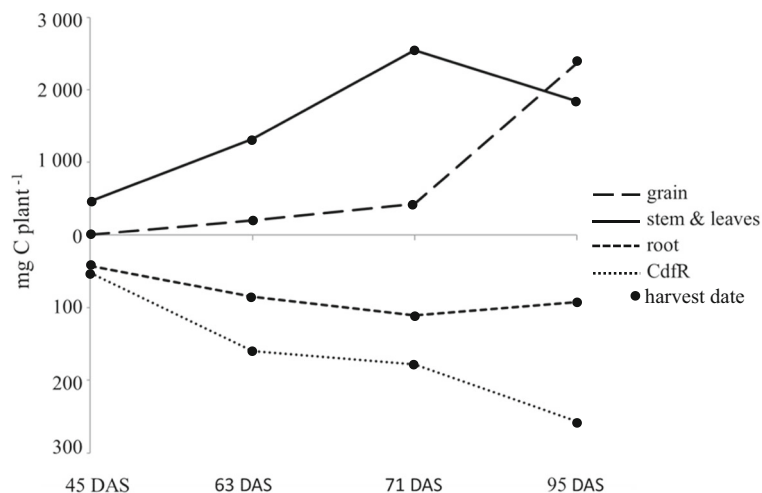
<sup>15</sup> N	<sup>15</sup> N-Uptake (mg plant <sup>-1</sup> )	Recovery (% of applied <sup>15</sup> N)				Atom% <sub>excess</sub>			
		In plant	In soil	In wick system	In total	Soil	Root	Grain	Shoot
Frisson									
45 DAS	2 ± 0.2	36 ± 3.8	4 ± 0.9	45 ± 8.3	86 ± 8.3	0.002 b	1.9 a		4.0 a
63 DAS	5 ± 0.2	50 ± 2.2	5 ± 0.8	29 ± 2.6	84 ± 2.9	0.005 a	2.5 a	2.9 a	3.8 a
71 DAS	6 ± 0.3	62 ± 3.3	3 ± 0.3	32 ± 2.4	97 ± 3.3	0.003 ab	1.7 a	1.7 b	2.7 a
95 DAS	9 ± 0.5	78 ± 4.4	3 ± 0.3	29 ± 2.5	110 ± 5.4	0.004 ab	2.2 a	2.4 a	4.1 a
P2									
45 DAS	2 ± 0.2	44 ± 4.9	4 ± 0.7	33 ± 4.7	81 ± 2.8	0.002 a	2.0 a		4.3 a
63 DAS	5 ± 0.3	49 ± 2.8	4 ± 0.2	32 ± 3.4	85 ± 3.2	0.004 a	1.9 a	4.7 ab	4.9 a
71 DAS	5 ± 0.4	54 ± 4.8	4 ± 0.9	33 ± 2.1	90 ± 4.3	0.004 a	1.8 a	3.3 a	3.6 a
95 DAS	7 ± 0.2	60 ± 1.9	3 ± 0.5	29 ± 1.2	93 ± 2.1	0.004 a	2.1 a	5.1 b	5.0 a

Values show means ± standard error of the mean (Frisson: *n* = 5 for 45DAS, *n* = 4 for 63 DAS and *n* = 6 for 71 and 95 DAS; P2: *n* = 6; except 71 DAS: *n* = 5). Different letters indicate significant differences between DAS (Scheffé, *p* < 0.05)

The amount of CdfR showed a significant five-fold increase between beginning of flowering and maturity (Table 4), whereas NdfR only doubled between beginning of flowering and end of flowering. Subsequently, the amount of NdfR remained roughly constant at 12 mg N plant<sup>-1</sup>. The contribution of BGP-C and BGP-N to total plant C and plant N significantly decreased with time from 15% to roughly 6% at maturity.

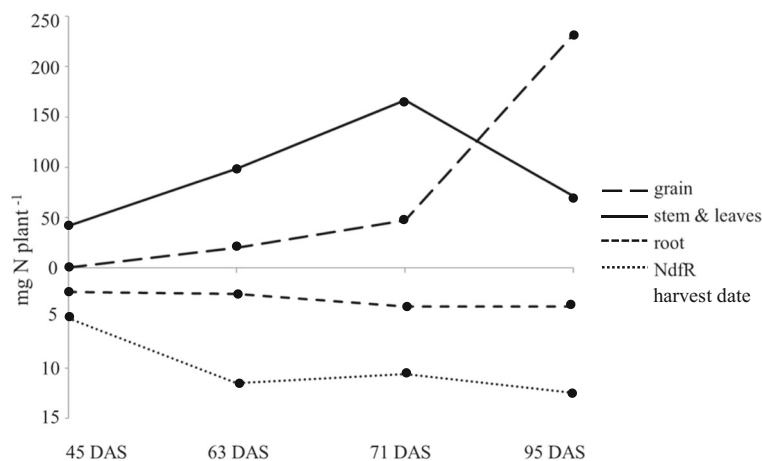
Transfer of rhizodeposits into microbial biomass, inorganic N and extractable C

The microbial biomass C and N content in soil increased until the end of flowering or green ripeness and decreased again afterwards, though not strongly significantly (Tables 5 and 6). At the beginning of flowering, about 20% of CdfR was incorporated into the microbial biomass, which remained roughly constant until maturity



**Fig. 1** Illustration of the distribution of C (in mg plant<sup>-1</sup>) in various plant parts of Frisson (grain, shoot and root) and C derived from rhizodeposition (in soil), harvested at beginning of flowering (45 DAS), end of flowering (63 DAS), green ripeness (71 DAS) and maturity (95 DAS). Due to high differences between the

amount of C in above and belowground biomass, two different scales were used. To maintain readability of the figure, no error bars or statistics are shown in this figure. Statistical data are given in Table 4



**Fig. 2** Illustration of the distribution of N (in mg plant<sup>-1</sup>) in various plant parts of Frisson (grain, shoot and root) and N derived from rhizodeposition (in soil), harvested at beginning of flowering (45 DAS), end of flowering (63 DAS), green ripeness (71 DAS) and maturity (95 DAS). Due to high differences between the

amount of N in above and belowground biomass, two different scales were used. To maintain readability of the figure, no error bars or statistics are shown in this figure. Statistical data are given in Table 4

(Table 5). Between 1% of microbial biomass C at the beginning of flowering and 6% at maturity derived from rhizodeposition, indicating a continuous increase with increasing plant development. At the beginning of flowering, about 17% of NdfR was incorporated into the microbial biomass, which insignificantly increased to 24% at maturity (Table 6). In total, between 1 and 3% of

the microbial biomass N originated from rhizodeposition and increased throughout the growth period. Roughly 9% of CdFR was recovered in the extractable organic C pool throughout plant growth (Table 5). The proportion of this C pool derived from rhizodeposition ranged from 1% at begin of flowering to 5% at maturity. At begin of flowering 11% of NdfR was recovered in the inorganic

**Table 4** Mycorrhization of Frisson (in % of total root numbers), N and C derived from rhizodeposition (NdfR & CdFR) in percentage of total plant N / C and in mg plant<sup>-1</sup> and below-ground plant (BGP) N / C (NdfR plus N in roots; CdFR plus C in roots) in

percentage of total plant N / C of Frisson and P2, harvested at beginning of flowering (45 DAS), end of flowering (63 DAS), green ripeness (71 DAS) and maturity (95 DAS)

	Mycorrhization (% of total roots)	NdfR		BGP-N	CdFR		BGP-C
		% of total N	mg plant <sup>-1</sup>	% of total N	% of total C	mg plant <sup>-1</sup>	% of total C
<b>Frisson</b>							
45 DAS	32 ± 5 a	10 ± 1.0 a	5 ± 0.7 a	16 ± 1.5 a *	8 ± 3.1 a	52 ± 25.7 a	15 ± 2.7 a
63 DAS	21 ± 4 ab	9 ± 1.3 a	12 ± 2.0 b	11 ± 1.5 b	9 ± 2.1 a	160 ± 46.8 ab	12 ± 2.2 ab
71 DAS	17 ± 2 b	5 ± 0.3 b	11 ± 1.1 b	6 ± 0.4 bc	6 ± 0.6 a	179 ± 30.7 ab	7 ± 0.6 b
95 DAS	17 ± 3 b	4 ± 0.3 b	13 ± 1.3 b *	5 ± 0.4 c	6 ± 0.7 a	257 ± 40.5 b	7 ± 0.8 b
<b>P2</b>							
45 DAS	–	8 ± 1.0 a	4 ± 0.5 a	12 ± 1.0 a	9 ± 1.2 a	54 ± 7.4 a	14 ± 1.2 a
63 DAS	–	7 ± 0.4 a	7 ± 0.7 a	9 ± 0.4 ab	6 ± 0.7 a	107 ± 19.9 a	8 ± 0.7 a
71 DAS	–	7 ± 2.2 a	9 ± 2.4 a	9 ± 2.3 ab	9 ± 3.2 a	240 ± 77.5 a	11 ± 3.3 a
95 DAS	–	4 ± 0.8 a	6 ± 1.4 a	6 ± 0.8 b	6 ± 1.0 a	162 ± 36.1 a	8 ± 1.0 a

Values show means ± standard error of the mean (N in Frisson:  $n = 5$  for 45 DAS,  $n = 4$  for 63 DAS and  $n = 6$  for 71 and 95 DAS; C in Frisson:  $n = 4$  for 45 and 63 DAS and  $n = 6$  for 71 and 95 DAS; N and C in P2:  $n = 6$ ; except 71 DAS:  $n = 5$ )

Different letters indicate significant differences between DAS (Scheffé,  $p < 0.05$ ). Values with \* indicate significant differences between the means of Frisson and P2 (t-test, pairwise comparison,  $p < 0.05$ )



**Table 5** C in microbial biomass (MBC) and in extractable C in  $\mu\text{g g}^{-1}$  soil, their percentage derived from rhizodeposition [dfR] and the percentage of CdFR found in MBC and extractable C,

respectively, when plants were harvested at beginning of flowering (45 DAS), end of flowering (63 DAS), green ripeness (71 DAS) and maturity (95 DAS).

	Microbial biomass C		% of CdFR in MBC	Extractable C		% of CdFR in extr. C
	$\mu\text{g g}^{-1}$ soil	dfR (%)		$\mu\text{g g}^{-1}$ soil	dfR (%)	
Frisson						
45 DAS	165 a	0.9 a	20 a	92 a	0.8 a	9 a
63 DAS	205 ab	3.5 a	20 a	104 ab	3.2 a	9 a
71 DAS	217 b	3.5 a	22 a	115 b	3.0 a	10 a
95 DAS	187 ab	5.6 a	22 a	104 ab	4.7 a	10 a

Different letters indicate significant differences between DAS (Scheffé,  $p < 0.05$ ). Values show means  $\pm$  standard error of the mean ( $n = 4$  for 45 and 63 DAS and  $n = 6$  for 71 and 95 DAS)

N pool and this percentage increased to 17% at maturity (Table 6). Nevertheless, only between 0.2 and 0.4% of the inorganic N pool in soil resulted from rhizodeposition throughout plant growth. Overall, about 30% of CdFR and 30–40% of NdfR present in soil after harvest was recovered in soil microbial biomass C or N, extractable organic C or inorganic N, leaving up to 70% of the C and N rhizodeposition left in the soil, probably incorporated in cellular root material that is yet to be decomposed or may partly in microbial products and microbial residues (necromass) derived from fast turnover of rhizodeposits.

### Mycorrhization and rhizodeposition

AMF colonization of Frisson roots significantly decreased with time, from 32% at beginning of flowering to 17% at green ripeness and maturity (Table 4). No AMF colonization of P2 roots was detected and also no nodulation, as

**Table 6** N in microbial biomass (MBN) and in inorganic N in  $\mu\text{g g}^{-1}$  soil, their percentage derived from rhizodeposition [dfR] and the percentage of NdfR found in MBN and inorganic N,

expected. The NdfR of Frisson ( $13 \text{ mg plant}^{-1}$ ) was significantly higher than that of P2 ( $6 \text{ mg plant}^{-1}$ ) only at maturity. CdFR in  $\text{mg plant}^{-1}$  or  $\text{mg g}^{-1}$  plant dry matter did not differ between Frisson and P2, regardless of plant development or root mycorrhization (Fig. 3). As Frisson produced more total plant dry matter than P2, NdfR in  $\text{mg g}^{-1}$  plant dry matter differed significantly between Frisson and P2 at begin of flowering, i.e. the period of highest mycorrhization (Fig. 4). This difference disappeared with decreasing mycorrhization of Frisson.

### Discussion

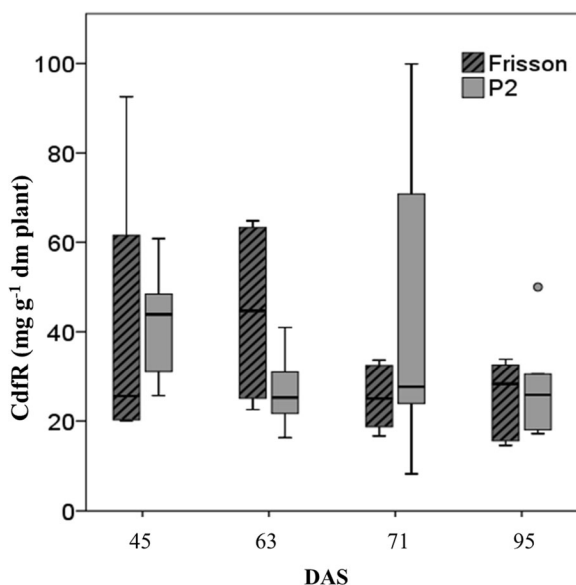
#### Tracer uptake, plant enrichment and tracer recovery

The strategy of multiple pulse labelling plants as an approximation of continuous labelling using the wick method, provides a tool for labelling plants with

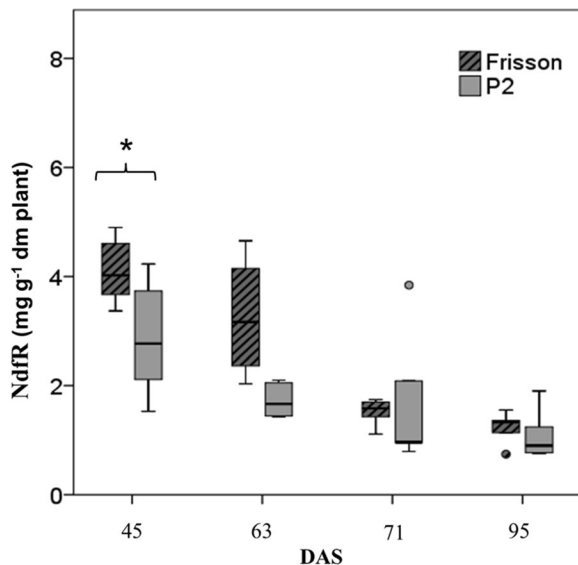
harvested at beginning of flowering (45 DAS), end of flowering (63 DAS), green ripeness (71 DAS) and maturity (95 DAS).

	Microbial biomass N		% of NdfR in MBN	Inorganic N		% of NdfR in inorg. N
	$\mu\text{g g}^{-1}$ soil	dfR (%)		$\mu\text{g g}^{-1}$ soil	dfR (%)	
Frisson						
45 DAS	20 a	0.8 a	17 a	95 a	0.2 a	11 a
63 DAS	29 b	1.4 a	18 a	123 a	0.3 a	17 a
71 DAS	25 ab	1.8 a	22 a	91 a	0.3 a	12 a
95 DAS	20 a	3.2 a	24 a	105 a	0.4 a	17 a

Different letters indicate significant differences between DAS (Scheffé,  $p < 0.05$ ). Values show means  $\pm$  standard error of the mean ( $n = 5$  for 45 DAS,  $n = 4$  for 63 DAS and  $n = 6$  for 71 and 95 DAS)



**Fig. 3** C derived from rhizodeposition (CdfR) in  $\text{mg g}^{-1}$  dry matter (dm) per plant of Frisson and P2, harvested at beginning of flowering (45 DAS), end of flowering (63 DAS), green ripeness (71 DAS) and maturity (95 DAS). There were no significant differences between the means (t-test, pairwise comparison,  $p < 0.05$ ). Values show means (Frisson:  $n = 4$  for 45 and 63 DAS and  $n = 6$  for 71 and 95 DAS; P2:  $n = 6$ ; except 71 DAS:  $n = 5$ )



**Fig. 4** N derived from rhizodeposition (NdfR) in  $\text{mg g}^{-1}$  dry matter (dm) per plant of Frisson and P2, harvested at beginning of flowering (45 DAS), end of flowering (63 DAS), green ripeness (71 DAS) and maturity (95 DAS). Values with \* indicate significant differences between the means (t-test, pairwise comparison,  $p < 0.05$ ). Values show means (Frisson:  $n = 5$  for 45 DAS,  $n = 4$  for 63 DAS and  $n = 6$  for 71 and 95 DAS; P2:  $n = 6$ ; except 71 DAS:  $n = 5$ )

multiple tracers (e.g.  $^{13}\text{C}$ ,  $^{15}\text{N}$ ) at relatively high concentrations, as shown earlier (Wichern et al. 2007a, b). As shown by the enrichment values (Tables 2 and 3), we were able to homogeneously label plant roots over time, which is an important prerequisite for estimating rhizodeposition quantitatively (Janzen and Bruinsma 1989). A homogeneous labelling of plant roots meant that the ratio of  $^{13}\text{C}$  tracer to total C or  $^{15}\text{N}$  tracer to total N in the roots is equal during plant development. This can be seen in the Atom%excess values (Tables 2 and 3). Differences between the  $^{13}\text{C}$  or  $^{15}\text{N}$  enrichment of root, shoot and grain are caused by plant development, isotope discrimination and nutrient relocation within the plant, which has also been observed for natural abundance of stable isotopes (e.g. Yoneyama et al. 2003) but are not relevant for estimating rhizodeposition, as long as roots and rhizodeposits have the same enrichment (Russell and Fillery 1996). This, however, remains unclear as it currently can not be measured in situ. Kanders et al. (2017) showed that different root size classes were differently enriched with  $^{15}\text{N}$ , which was, in relation to plant species, more or less consistent. Differences in grain enrichment between end of flowering and green ripeness in our study as well as between green ripeness and maturity show that  $^{13}\text{C}$  and  $^{15}\text{N}$  provided via the wick system is preferentially transferred into the sink organs of the plant during the reproductive phase. Additionally, some of the previously assimilated  $^{13}\text{C}$  and  $^{15}\text{N}$  might also have been relocated within the plant (e.g.  $^{15}\text{N}$  from roots). However, C and N rhizodeposition were calculated with a mass balance approach to prevent over- or underestimation caused by heterogeneous label distribution due to tracer relocation processes. Tracer relocation during plant development may lead to differences between the enrichment of roots and compounds released by living roots, i.e. volatile, gaseous compounds, ions, exudates, lysates, and secretions. Therefore, it is important to label plants continuously or as often as possible, using a multiple pulse labelling approach to keep the differences in enrichment as small as possible.

The formation of fungi and algae within the labelling system was observed during plant labelling, disturbing tracer uptake and causing C losses by microbial respiration in the labelling system. Consequently, the labelling system had to be replaced four times in total to facilitate multiple pulse labelling and reach high plant

enrichment. The recovery of  $^{13}\text{C}$  varied between 40 and 60% of the applied  $^{13}\text{C}$  tracer. Between 13 and 30% of the recovered  $^{13}\text{C}$  were found in the labelling system. A possible reason for the low recovery of  $^{13}\text{C}$  is the loss of  $^{13}\text{C}$  as  $\text{CO}_2$  by rhizo-respiration (Jones et al. 2009). Losses of up to 30% of the applied  $^{13}\text{C}$  tracer were observed by Yasmin et al. (2010). Even higher amounts of between 26 and 52% of the recovered  $^{15}\text{N}$  were found in the labelling system. The gaseous loss of  $^{15}\text{N}$  from soil, plant and labelling system is expected to be small under the experimental conditions. However, some gaseous losses of  $^{15}\text{N}$  by  $\text{N}_2$ ,  $\text{N}_2\text{O}$ ,  $\text{NO}_x$  or  $\text{NH}_3$  release might have occurred, in addition to analytical errors, contributing to the losses of recovered  $^{15}\text{N}$ . Overall, the recovery of the applied tracer is similar to other publications (Mayer et al. 2003b; Wichern et al. 2007b; Fustec et al. 2010; Arcand et al. 2013a).

### Belowground plant C and N

The contribution of both BGP-C and N to total plant C and N varied around 15% at beginning of flowering and around 6% at maturity. Compared with other studies, this value seems to be very low. Fustec et al. (2010) found that between 14 and 71% of total plant N of peas was BGP-N. For Frisson at maturity, Mahieu et al. (2007) found a contribution of 26% BGP-N to total plant N under greenhouse conditions. Wichern et al. (2007b) found a contribution of BGP-C to total plant C of 34% for peas at maturity under field conditions in mesocosms. The low percentages in the current study were probably due to a shift in the ratio of aboveground plant C (AGP-C) to root C in comparison with field conditions (Mayer et al. 2003b). N fertilization and pot size are the likely reasons for this observation, with pot size probably being more important in intensively used agricultural soils as also shown by Kanders et al. (2017). Wichern et al. (2007a) achieved an AGP-N to root N ratio of 17. In the current study, an AGP-N to root N ratio of 20 was observed in a similar vegetation period. Due to the high N fertilization in our experiment, pea plants developed a smaller root system, leading to a larger AGP-N to root N ratio (Nguyen 2003). The amount of rhizodeposition largely depends on the size of the root system (Shamoot et al. 1968; Kanders et al. 2017), considering the fact that rhizodeposition consists to a large extent of sloughed root cells and decomposed root material (Uren 2001). The effects of N fertilization on rhizodeposition are

often inconsistent. Arcand et al. (2013b) observed no effects on the amounts of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  released by pea roots, whereas Nguyen (2003) found that N fertilization increased C rhizodeposition, contradicting the results of Kuzyakov and Domanski (2000), who observed a decrease of C rhizodeposition.

Limitation of root growth due to lower soil volume caused by pot size can also influence the amount of rhizodeposition (Poorter et al. 2012; Kanders et al. 2017). Pot experiments can lead to a lower root to shoot ratio in comparison with field experiments, leading to a reduction in rhizodeposition (Wichern et al. 2008; Kanders et al. 2017). Anglade et al. (2015) found significantly less NdfR as a percentage of BGP-N in pot experiments (56%) than in field trials (82%). Consequently, the results from our study cannot be directly transferred to field conditions. Nevertheless the ratio of rhizodeposited C (or N) and root C (or N) can be used to predict rhizodeposition under field conditions as proposed by Pausch et al. (2013), using root biomass data (Kanders et al. 2017). However, as roots are very plastic in their response to nutrient availability (Hodge 2004), it remains questionable whether experimental conditions under controlled conditions can resemble field conditions.

### Amounts, time course and fate of rhizodeposition

Independent of the uncertainties related to the methods and the experimental conditions as discussed above, we were able to elucidate the belowground C and N partitioning of peas over time. The amount of N rhizodeposition per plant (cv. Frisson) increased until flowering and remained constant afterwards until harvest. This may indicate an increasing release of root exudates until flowering. It is already known that the quality and quantity of root exudates changes over the course of time (Grayston et al. 1997). For many annual crops it is known that the root system of plants increases until flowering and, due to cell death, decreases thereafter (Jakobsen 1986; Pritchard and Rogers 2000). Therefore, N rhizodeposition probably increases until the end of flowering by enhanced root exudation of the growing root system. Grayston et al. (1997) found a decreasing release of amino acids by plants under N deficient conditions, whereas increasing  $\text{NO}_3^-$  contents led to an increased release of carboxylates. On the other hand, an increasing  $\text{NH}_4^+$  content caused a decline in the release of carboxylates (Neumann and Römheld 2007).

Therefore, the high N fertilization in the current study may have resulted in an increased release of root exudates, probably carboxylates, until flowering. The C rhizodeposition constantly increased during the vegetation period and differed significantly between beginning of flowering and maturity. After flowering, the decomposition of root material and the reduction in AMF most likely increased C rhizodeposition (Engels and Körschens 2010), which was by then mostly derived from root fragments, sloughed root cells and fungal hyphae.

No significant differences could be determined for the incorporation of rhizodeposition into the microbial biomass, the extractable organic C or inorganic N pool in soil over the course of time. The C and N rhizodeposition was similarly incorporated into these three pools at beginning of flowering and at maturity. In total, between 11 and 17% of total NdfR was found in the inorganic N pool, which is similar to the roughly 12% observed by Arcand et al. (2013b). However, this is only half the levels obtained by Mayer et al. (2003b); Wichern et al. (2007b). Up to 24% of total NdfR was incorporated into the microbial biomass, somewhat above the 18% measured by Mayer et al. (2003b); Wichern et al. (2007b). The 20% of total CdfR in the microbial biomass was similar to Wichern et al. (2007b). In the current study, more labile N compounds, e.g. amino acids, peptides, may have been released by the pea roots and incorporated into the microbial biomass, resulting in the enhanced microbial uptake.

It is assumed that root exudation (including lysates, exudates, mucilage, etc.) is an important process of rhizodeposition only up to the reproductive growth phase of plants and that senescence of roots becomes a more important part of rhizodeposition after flowering, as more roots die. This is probably true for C, as the steady increase of rhizodeposition in our study supports this and no translocation within the plant took place compared with N. Consequently, when aiming at quantifying the plant derived C input, root senescence as part of rhizodeposition has to be considered. This questions the reliability of the quantitative results of the many studies on C rhizodeposition done with plants in the vegetative growth stage (see Jones et al. 2009 for a very good summary). Based on this and the above mentioned methodical constraints, more studies have to be done over the whole growth period of plants and under field conditions to be able to quantify total belowground plant C input. This is also true for NdfR studies, even though many of

those investigated a longer growth period (Jones et al. 2009). Overall, our findings support our first hypothesis of a different time course of C and N rhizodeposition, most likely being the result of changing contribution of root exudates and root decay to the rhizodeposits.

In addition to the time course of rhizodeposition, we were interested in the availability of rhizodeposits to soil microorganisms, as this influences nutrient availability and C dynamics. We observed that microbial biomass increased until the end of flowering or green ripeness, which represents peak plant biomass. One would assume that this increase would also be reflected by an increased incorporation of  $^{13}\text{C}$  and  $^{15}\text{N}$  into the microbial biomass. However, this was not the case, negating our second hypothesis, that more easily metabolizable rhizodeposits during the vegetative growth period result in an increased  $^{13}\text{C}$  and  $^{15}\text{N}$  enrichment of the soil microbial biomass. Throughout plant growth rhizodeposition is obviously a minor source of C and N for the soil microbial biomass, as indicated by the small part of the microbial biomass derived from rhizodeposition (less than 6%, Tables 5 and 6), which is in the range of an earlier study (Mayer et al. 2003a) but substantially lower compared with Wichern et al. (2007a, b). Moreover, in our study, the proportion of the microbial biomass derived from rhizodeposition increased. These observations indicate that rhizodeposits can not be claimed an easily metabolizable substrate for soil microorganisms, a concept derived from the misuse of glucose proposed to be a representative rhizodeposit. This however, is not the case, as shown here and by Lloyd et al. (2016). On top of this, only about 20% of the rhizodeposits ended up in the microbial biomass, as observed in earlier studies (Wichern et al. 2007a, b), leaving about 50 to 70% of the rhizodeposited C and N unaccounted for. This may represent slowly available C and N in root fragments (McNeill and Fillery 2008), which will be mineralised later. However, the unaccounted C and NdfR in the soil can may partly reflect microbial products and microbial residues (necromass) derived from fast turnover of rhizodeposits. However, this was not investigated in our study and should be investigated in future studies looking at recent microbial residues (e.g.  $^{13}\text{C}$  in amino sugars).

#### Influence of mycorrhiza on rhizodeposition

It is well known that arbuscular mycorrhiza fungi (AMF) can influence the rhizodeposition of plants

(Jones et al. 2009; Rillig and Mummey 2006). In our study, however, AMF did not affect C rhizodeposition when comparing the mycorrhiza variety Frisson with the non-mycorrhiza variety P2. Nevertheless, the observed differences in the amount of C rhizodeposition after the end of flowering may be caused by an additional C input from dying AMF hyphae (Engels and Körschens 2010). On the other hand, Frisson had significantly higher N rhizodeposition than P2 at the time of the highest AMF colonization. With decreasing mycorrhization during pea maturation (Zhang et al. 1995), these differences in N rhizodeposition decreased too. This may be caused by differences in root exudation between mycorrhizal and non-mycorrhizal plants. It has been shown that hyphal exudation of mycorrhizal fungi can increase root respiration (Jones et al. 2004).

The highest mycorrhization of Frisson at the beginning of flowering may caused an increased exudation of various N compounds from roots to soil. Mada and Bagyaraj (1993) mentioned that plants with mycorrhizal fungi release less potassium, phosphate and glucose, but more N, especially as protein. This is further supported by the observation that pea roots and pea root nodules are highly active during flowering (Dupont et al. 2012). Moreover, AMF support the N transfer from N<sub>2</sub> fixing plants to non-fixing plants (Hamel et al. 1991). These differences in plant morphology and physiology may also cause increased N rhizodeposition (Fustec et al. 2010). Despite the N fertilization, Frisson roots formed nodules in our study. Therefore, we cannot estimate whether differences in N rhizodeposition and biomass development were caused by mycorrhiza or N-fixation through nodulation, when comparing Frisson and P2. Thus, using the pea variety Frisson and its isoline mutant P2 as comparison to elucidate the effect of mycorrhization on C and N rhizodeposition was not overly successful and has to be evaluated in future studies, where environmental conditions are even more controlled compared with our pot experiment, to guarantee a comparable partitioning of both pea varieties.

## Conclusions

Rhizodeposition of C and N followed different patterns over time, with a steady increase of CdfR, most likely initially dominated by root exudation of the growing root system and a later increase caused by continuous senescence of roots. On the other hand, N was most likely

relocated within the plant during maturation and partly removed from the root system to allow protein synthesis during grain filling, leaving similar amounts of NdfR during the generative phase and thus showing a decreasing relative contribution to total plant N. Obviously rhizodeposition as a whole was not an easily metabolizable source of C and N for soil microorganisms. Besides root exudates, rhizodeposition also contains particular components, such as root fragments, that are only slowly degraded. Consequently, when investigating C and N dynamics in agricultural systems quantitatively, the term rhizodeposition has to be used in its broad definition, including all compounds released by living roots and root fragments. Especially during the reproductive phase, root senescence made a greater contribution to rhizodeposition than during vegetative growth, which is relevant when assessing total belowground plant C and N input. Mycorrhiza tended to increase N rhizodeposition at flowering, indicating the significance of mycorrhiza to nutrient transfer. However, due to the nodulation of Frisson, we cannot give a final statement of the impact of mycorrhiza on rhizodeposition. Future experiments aiming at estimating total C and N rhizodeposition thus should be conducted under field conditions, without a limitation of root growth, and have to focus on the whole growth period.

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