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REGULAR ARTICLE

Carbon input and partitioning in subsoil by chicory and alfalfa

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Abstract
Background and Aims Input of organic matter into soil creates microbial hotspots. Due to the low organic matter content in subsoil, microbial hotspots can improve nutrient availability to plants. Therefore, carbon (C) input of root biomass and rhizodeposition and the microbial utilization of root C by alfalfa and chicory, both deep-rooting taprooted preceding crops, was determined.
Methods Three replicate plots of alfalfa and chicory grown on a Haplic Luvisol were $^{13}CO_2$ pulse labeled after 110 days of growth. ^{13}C was traced in plant biomass, rhizosphere, bulk soil and in microbial biomass after 1 and 40 days. C stocks and $\delta^{13}C$ signature were quantified in 15 cm intervals down to 105 cm depth.
Results Alfalfa plant biomass was higher and root biomass was more homogeneously distributed between top- (0–30 cm) and subsoil (30–105 cm) compared to chicory. C input into subsoil by alfalfa, including roots and rhizodeposited C, was 8 times higher (3820 kg C ha^{-1}) into subsoil compared to chicory after 150 days of growth. Microbial biomass in subsoil increased with alfalfa but decreased with chicory.
Conclusions Despite their general ability to build biopores, taprooted preceding crops differ in creating microbial hotspots in subsoil. Higher C input and microbial growth in subsoil under alfalfa cultivation can improve physico-chemical and biological properties, and so enhance root growth and consequently the water and nutrient uptake from subsoil compared to chicory.

Keywords Microbial hotspots · Plant-soil-microorganism interactions · Rhizosphere · Subsoil · C input · $^{13}CO_2$ pulse labeling

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Introduction

Crops with a taproot system form vertical stable macropores extending from topsoil into subsoil (Mitchell et al. 2008; McCallum et al. 2004). These biopores can be used by subsequent crops to easily grow into the subsoil, due to lower mechanical impedance, higher oxygen and water availability, and higher soil organic matter (SOM) content compared to bulk soil (Böhm and Köpke 1977; Stewart et al. 1999; Rasse and Smucker 1998). The increased SOM in biopores compared to bulk soil mainly results from rhizodeposition, root litter and leaching of organics from topsoil SOM (Kaiser and Kalbitz 2012; Kautz et al. 2013). Higher resource availability enables

increased organic matter turnover and microbial nutrient mobilization in biopores compared to bulk soil due to higher microbial activity and abundance (Cheng 2009; Kuzyakov 2010). Decreasing SOM content and nutrient availability with increasing soil depth make biopore conditions especially relevant for nutrient acquisition from subsoil. Nutrient uptake from arable subsoil, i.e. the soil below the plough layer, can be relevant for plant nutrition (Marschner 1995). It is especially important under dry or nutrient-poor topsoil conditions and during drought periods (Fleige et al. 1983; Kuhlmann and Baumgärtel 1991). Therefore, crop sequences using taproot preceding crops can enhance the exploration of subsoil resources for the subsequent crops. In turn, better knowledge of biopore characteristics and input of SOC into arable subsoil is needed.

Rhizodeposits translocated by plant roots into the soil are of ecological importance as they affect nutrient availability for plant growth (Dakora and Phillips 2002; Dilkes et al. 2004). Rhizodeposits are one of the preferred substrates for microorganisms (Blagodatskaya et al. 2009), which are responsible for most biochemical reactions that mobilize nutrients from SOM. More heterogeneous distribution (Rumpel and Kögel-Knabner 2011) and lower content of SOM in subsoil (Salomé et al. 2010) strengthen the contrast between the rhizosphere and bulk soil. Therefore, the importance of rhizodeposits for microbial nutrient mobilization is assumed to be higher in subsoil compared to topsoil (Kautz et al. 2013). Knowledge of the amounts of organic substances added by plant roots into the soil and especially into subsoil is crucial for evaluating mobilization of nutrients. Nearly all previous studies estimated carbon (C) input within the top 30 cm of the soil (Kuzyakov and Domanski 2000; Amos and Walters 2006). Despite various studies on root depth distribution (Böhm 1979; Jackson et al. 1996), C input by rhizodeposition into deeper soil horizons remains largely unconsidered.

To determine the input of photosynthetically fixed C into soil by roots, both root biomass and rhizodeposition need to be considered (Johnson et al. 2006; Pausch et al. 2013). Up to 50 % of photosynthetically fixed C by grasses including cereals is allocated belowground, whereof approximately 50 % is invested into root growth and 30 % is rhizodeposited (Kuzyakov and Domanski 2000; Kuzyakov 2002; Johnson et al. 2006). However, there are few studies that include rhizodeposition of agricultural crops to assess C input into soil, especially into subsoil.

The aims of our study were (1) to estimate the amount of photosynthetically fixed C invested in building up root biomass and released into soil by rhizodeposition down to 105 cm depth and; (2) to compare C input into top- and subsoil between two common taprooted preceding crops chicory (*Cichorium intybus* L.) and alfalfa (*Medicago sativa* L.). Root biomass of alfalfa and chicory plants was determined down to 105 cm depth, 110 and 150 days after sowing. The partitioning of recently assimilated C between plant and soil C pools was determined by in situ $^{13}CO_2$ pulse labeling of alfalfa and chicory plants. Pulse labeling enabled the amount of photosynthetically fixed C invested into root biomass and rhizodeposition to be compared between alfalfa and chicory.

Material and Methods

Site description

The agricultural field site is located at the Klein Altendorf experimental station of the University of Bonn (50°37′21″N, 06°59′29″E). The climate is maritime temperate (Cfb Köppen climate classification) with a mean annual precipitation of 625 mm and a mean annual temperature of 9.6 °C (Gaiser et al. 2012).

The soil at the experimental site developed from loess and is classified as loamy Haplic Luvisol WRB (IUSS-ISRIC-FAO 2006) having an A_p horizon of 30 cm, followed by an E/B horizon down to 45 cm. Accumulation of clay was found from 45 cm down to 95 cm (Gaiser et al. 2012).

Alfalfa (*Medicago sativa* L.) and chicory (*Cichorium intybus* L.) were sown on the 15th of April 2011 with a seeding density of 25 kg ha^{-1} (alfalfa) and 5 kg ha^{-1} (cichory) (Gaiser et al. 2012). The plots for alfalfa and chicory were 60 m^2 each. Neither the alfalfa nor the chicory plots were fertilized before or during the experiment.

$^{13}CO_2$ pulse labeling

The $^{13}CO_2$ pulse labeling of chicory and alfalfa was performed after 110 days of growth, on the 1st of August 2011 (alfalfa) and on the 2nd of August 2011 (chicory) (Riederer et al. 2015; Hafner et al. 2012). Three replicate plots (1 m^2 each) of chicory and alfalfa were pulse labeled. The $^{13}CO_2$ pulses for each crop

replicate were applied simultaneously. The chambers were 1 m long, 1 m wide and 0.5 m high. 100 ml of the labeling solution containing 15 g sodium carbonate ($Na_2{}^{13}CO_2$) enriched with ^{13}C to 99 atom% was placed inside the chamber. After closing the chamber, 80 ml of 5 M sulphuric acid (H_2SO_4) was injected into the labeling solution from the outside, using a syringe. A 12-V fan ensured a uniform distribution of $^{13}CO_2$ inside the chamber. The temperature inside the chamber was measured during labeling. The CO_2 concentration inside the chamber was monitored by a CO_2 sensor (GM 70, Vaisala, Helsinki, Finland). Plants assimilated the label for 5 h before the chamber was removed.

Sampling and sample preparation

Samples were taken 1 and 40 days after labeling, which corresponded to 110 and 150 days of plant growth, respectively. The partitioning of assimilated C was determined as ^{13}C in shoots, roots, rhizosphere, bulk soil and microbial biomass. Alfalfa and chicory shoots were sampled by cutting 2 plants directly at the soil surface at each of the three replicate plots. The shoot samples of each plot were combined thereafter. To sample soil and roots a root auger with a diameter of 84 mm was used. Soil cores with a length of 15 cm were taken successively from the soil surface down to 105 cm depth. At each replicate plot, soil cores were sampled: 1) exactly at the place where the shoot was cut (including the main root biomass of the taproot crops) after 1 and 40 days and; 2) between rows (after 40 days). Roots were manually removed from the soil cores and carefully shaken to separate bulk soil from rhizosphere soil. Roots and the attached rhizosphere soil were put into a beaker containing deionized water. To improve separation, the beaker was put into an ultrasonic bath for five minutes (35 kHz, 320 W, 3 L). After removing the roots and rhizosphere soil, the bulk soil was sieved to 2 mm. Shoots, roots, rhizosphere and bulk soil were freeze dried, weighed and ball milled (ball mill, Retsch MM2). Before the bulk soil was freeze dried, gravimetric water content was determined for each soil depth in three replicates and soil for the determination of microbial biomass (see below) was removed. Soil respiration and the amount of recent assimilates recovered in soil respiration 1 day after labeling was determined by the static alkali absorption method (Lundegardh 1921; Kirita 1971; Singh and Gupta 1977). $SrCl_2$ was added to the NaOH to precipitate $SrCO_3$. The extracts were freeze dried and $\delta^{13}C$ signature was determined in $SrCO_3$.

Reference samples

To determine ^{13}C assimilation during the $^{13}CO_2$ pulse labeling period two replicate samples of shoot, root, rhizosphere and bulk soil samples down to 50 cm depth were taken directly after removing the labeling chamber from all three replicate plots of alfalfa and chicory, respectively. Sampling and sample preparation was done according to the procedure described above. The sum of the ^{13}C recovered directly after removing the chamber was used as a reference for the samplings after 1 and 40 days.

Microbial biomass carbon

Microbial biomass C (C_{mic}) was determined by the chloroform fumigation-extraction method modified after Brookes et al. (1985) and Vance et al. (1987), in each case using 10 g of fresh bulk soil (sieved to <2 mm) from every depth interval. Samples were fumigated in a chloroform atmosphere for one week. For the extraction of the fumigated and non-fumigated samples, 30 ml of 0.05 M K_2SO_4 was used. Extractable organic carbon (EOC) was measured by catalytic oxidation (Multi N/C 2100 S, Analytik Jena, Germany). The difference in EOC between fumigated and non-fumigated samples was divided by the k_{EC} (0.45) value, defining the extractable part of microbial biomass C, after Joergensen (1996), to estimate total C_{mic}.

To measure the $\delta^{13}C$ signature of C_{mic}, the K_2SO_4 extracts of both fumigated and non-fumigated samples were freeze dried. Dried extracts were weighed into tin capsules (> 15 μg C per capsule) for $\delta^{13}C$ analysis.

Natural abundance samples

To determine the natural abundance of ^{13}C in shoots, roots, rhizosphere, bulk soil and microbial biomass down to 105 cm depth, these C pools were sampled once before the $^{13}CO_2$ pulse labeling. For the natural abundance samples the same sampling and sample preparation was performed as for the enriched samples described before.

C stock calculation

To compare the above- and belowground C stocks in top- and subsoil between alfalfa and chicory, C stocks (kg C ha^{-1}) of shoots, roots, rhizosphere, bulk soil and microbial biomass were calculated. Shoot C stocks were calculated by the following equation:

$$\mathrm{ShootC} = P \cdot \frac{S}{2} \cdot C \cdot 10$$

where P is the number of plants on a plot (1 m^2), S (g) is the dry weight that was divided by 2 because the dry weight was measured on two plants and C (%) is the C content of the shoots.

C stocks of roots, rhizosphere, bulk soil and microbial biomass were calculated for each soil layer using the following equations:

$$RootC = \frac{R}{V} \cdot z \cdot C \cdot 1000$$
$$RhizosphereC = \frac{RS}{V} \cdot z \cdot C \cdot 1000$$
$$BulksoilC = z \cdot \rho \cdot C \cdot 1000$$
$$MBC = C_{mic} \cdot z \cdot \rho \cdot C \cdot 1000$$

where R is the dry weight of root biomass (g), V (cm^3) is the volume of the root auger, z (cm) is the length of the soil core, C (%) is the C content, ρ (g cm^{-3}) is the bulk density and C_{mic} (mg g^{-1}) is the microbial biomass C content.

The planting of alfalfa and chicory in rows results in differing C stocks between the rows and the interrows. The ratio of plant-covered to interrrow C stocks in every soil depth determined after 150 days was used to calculate interrow C stocks after 110 days of growth. To calculate total C stocks, the plot area was divided into (1) the area covered with plants and (2) the interrow area. The area covered with plants was calculated by multiplying the diameter of the root auger by the number of plants per plot, giving 52 %. Total C stocks were calculated as area-weighted averages of plant-covered and interrow C stocks.

$\delta^{13}C$ analysis and stable isotope calculations

The $\delta^{13}C$ signature and C content of shoots, roots, rhizosphere soil and bulk soil and the $\delta^{13}C$ signature of EOC of the fumigated and non-fumigated samples ($\delta^{13}C$ signature of C_{mic}) and of natural abundance control samples were determined with an isotope ratio mass spectrometer (Thermo Fischer, Bremen, Germany) coupled with an elemental analyzer (Eurovector, Milan, Italy) via a ConFlo III interface (Thermo-Fischer, Bremen, Germany) at the Centre for Stable Isotope Research and Analysis, University of Göttingen.

The ^{13}C excess in a C pool (% of total C atoms) caused by the $^{13}CO_2$ pulse labeling was determined as ^{13}C excess compared to the natural abundance samples

$$^{13}C_{\mathrm{atom\ \%\ excess}} = {}^{13}C_{\mathrm{atom\ \%\ sample}} - {}^{13}C_{\mathrm{atom\ \%\ NA}}$$

The ^{13}C excess in a C pool was used to estimate the amount of ^{13}C (g ^{13}C m^{-2}) that was incorporated into that pool (g C m^{-2}).

$$^{13}C_{\mathrm{amount}} = \frac{{}^{13}C_{\mathrm{atom\ \%\ excess}}}{100} \cdot C_{\mathrm{pool}}$$

The sum of the ^{13}C recovered in shoots, roots, rhizosphere and bulk soil of the reference samples ($^{13}C_{ref}$) was used as 100 % of ^{13}C assimilated by plants. To calculate the percentage of ^{13}C recovery in a C pool ($^{13}C_{rec}$) at time t (1 and 40 days) after labeling, the ^{13}C amount was related to the reference ^{13}C amount ($^{13}C_{ref}$).

$$^{13}C_{\mathrm{rect}} = \frac{{}^{13}C_{\mathrm{amount\ }t}}{{}^{13}C_{ref}} \cdot 100$$

According to C stock calculations, total ^{13}C recoveries in C pools were calculated as area-weighted averages of plant-covered and interrow ^{13}C recoveries.

Estimation of net rhizodeposition

To estimate net rhizodeposition $netC_E$ (kg C ha^{-1}) into top- and subsoil, the ratio between C released into soil and C retained in root biomass was calculated. The sum of ^{13}C recovered in rhizosphere soil $^{13}C_{RS}$ and in bulk soil $^{13}C_{BU}$ was divided by the ^{13}C recovered in roots. This ratio was calculated for topsoil (0–30 cm) and for subsoil (30–105 cm) 1 day after labeling. The topsoil ratio was multiplied by the measured root C stocks C_{root} (kg C ha^{-1}) 110 or 150 days after sowing in 0–15 cm and 15–30 cm depth. The subsoil ratio was multiplied

by the measured root C stocks C_{root} (kg C ha^{-1}) in every sampling interval from 30 to 105 cm depth.

$$netC_E = \frac{\left({}^{13}C_{RS} + {}^{13}C_{BU}\right)}{{}^{13}C_{root}} \cdot C_{root}$$

Statistics

All results are presented as means of 3 field replicates ± standard error of the mean (SEM). Only the significant differences between crops or between depths are described in the text.

We tested if root C stocks, rhizosphere C stocks, microbial biomass C, or the distribution of ^{13}C between roots, rhizosphere and microbial biomass differed between the soil depths or between the preceding crops and if there were interactions between these effects. The test was a 2 × 7 factorial analysis of variance (ANOVA) (2 cultivars × 7 soil depths) at a significance level of $p < 0.05$, using R version 3.0.2 (R Core Team 2013). Normal distribution of the residuals was tested using the Shapiro-Wilk normality test. Levene's test was conducted to test for homogeneity of variances using the R package car (Fox and Weisberg 2011). The 2 × 7 ANOVA was calculated using log-transformed data. The residuals of the ANOVA model for all variables were then normally distributed and homoscedasticity was improved.

Kruskal-Wallis ANOVA was conducted to test for significant differences in shoot C stock, top- and subsoil root, rhizosphere and microbial biomass C stock between alfalfa and chicory ($p < 0.05$) and between the sampling times ($p < 0.05$). Kruskal-Wallis ANOVA was also applied to test for significant differences in ^{13}C recovery in shoots, top- and subsoil roots, rhizosphere soil and microbial biomass between alfalfa and chicory ($p < 0.05$) and between the sampling times ($p < 0.05$).

Results

Above- and belowground carbon stocks

Alfalfa and chicory shoot C stocks were equal 110 days after sowing (Table 1). The increase in aboveground biomass of alfalfa during the following 40 days was higher than of chicory, resulting in the alfalfa shoot C stock being more than twice that of chicory after 150 days of growth.

The average alfalfa root C stock from 0 to 105 cm depth was lower than the chicory root C stock at the beginning of the observation period, but higher at the end. (Fig. 1 a, Table 1). The increase in alfalfa root biomass resulted in equal C amounts being stored in alfalfa topsoil roots and three times more C being stored in alfalfa subsoil roots compared to chicory after 150 days (Table 1). Root C stock was highest in the upper 15 cm and decreased with soil depth at both observation dates (Fig. 1 a, Table 1).

Microbial biomass decreased throughout the entire profile with depth and was higher under chicory than under alfalfa after 110 days (Fig. 1 c). In contrast, the microbial biomass C stock was higher under alfalfa compared to that under chicory after 150 days (Table 1). In topsoil, microbial biomass was equal between the chicory and alfalfa cultivation and between the beginning and end of the observation period (Table 1). In subsoil, however, microbial biomass decreased under chicory from day 110 to day 150, resulting in lower microbial biomass under chicory than under alfalfa.

In summary, the increase in alfalfa above- and belowground plant biomass over 40 days resulted in higher plant C stocks than for chicory. The main differences were found in subsoil root C stocks and microbial biomass.

Isotopic signature after $^{13}CO_2$ labeling and of natural abundance samples

The isotopic signature of roots, rhizosphere soil and microbial biomass indicated strong ^{13}C enrichment after the $^{13}CO_2$ pulse labeling of alfalfa and chicory plants (Fig. 2 a-c). The ^{13}C enrichment was found for roots, rhizosphere soil and microbial biomass in every depth down to 105 cm, 1 day and 40 days after labeling. This ^{13}C enrichment allowed the recently assimilated C to be partitioned between shoots, roots, rhizosphere soil and microbial biomass. Bulk soil was excluded from the calculations, due to the low ^{13}C enrichment relative to the natural abundance reference samples (Fig. 2 d).

The $\delta^{13}C$ values of roots, rhizosphere soil and microbial biomass under chicory tended to decrease with depth 1 and 40 days after labeling (Fig. 2 a-c). In contrast, $\delta^{13}C$ values of roots, rhizosphere soil and

Table 1 Above- and belowground C stocks (kg C ha^{-1}) 110 and 150 days after sowing of alfalfa and chicory. Belowground C stocks are presented for topsoil (0–30 cm) and subsoil (30–105 cm)

C pool	C stock (kg C ha^{-1}) [e]			
	Alfalfa 110 d	Chicory 110 d	Alfalfa 150 d	Chicory 150 d
Shoot	528.4 ± 32.9 a [f] * [g]	468.1 ± 4.3 a *	1961.3 ± 194.9 a **	817.8 ± 126.0 b **
Soil respiration (kg C $ha^{-1}d^{-1}$) [e]	204.8 ± 22.6 a	93.3 ± 8.3 b		
0–30 cm				
Root	662.6 ± 66.3 a *	1902.9 ± 350.6 b	2640.9 ± 814.9 a **	2238.3 ± 436.7 a
Rhizosphere	64.8 ± 17.9 a	301.1 ± 221.2 a	81.9 ± 19.6 a	116.2 ± 36.3 a
Microbial biomass	92.5 ± 15.3 a	101.6 ± 13.2 a	76.0 ± 6.4 a	80.5 ± 5.4 a
Bulk soil	36,975.6 ± 2005.8	47,275.9 ± 2873.8		
30–105 cm				
Root	109.1 ± 44.1 a *	67.3 ± 7.4 a *	662.9 ± 40.2 a **	211.1 ± 48.9 b **
Rhizosphere	27.3 ± 3.8 a	56.5 ± 35.6 a	36.8 ± 1.7 a	29.6 ± 5.4 a
Microbial biomass	81.6 ± 9.6 a	128.7 ± 12.5 a *	114.5 ± 16.5 a	76.0 ± 2.9 b **
Bulk soil	56,718.0 ± 337.0	57,016.7 ± 1397.6		

[e] Values are given as means and standard errors of the mean

[f] Different letters indicate significant differences between alfalfa and chicory 1 day after labeling or 40 days after labeling (Kruskal-Wallis test; $p < 0.05$)

[g] Asterisks indicate significant differences between 110 and 150 days after sowing for alfalfa or chicory (Kruskal-Wallis test; $p < 0.05$)

microbial biomass under alfalfa increased with depth. Increasing ^{13}C enrichment with soil depth indicated that the percentage of recently assimilated C in total C present was higher under alfalfa.

Budget of assimilated ^{13}C

The recovery of ^{13}C in the reference samples amounted to 69 ± 5 % and 76 ± 13 % of the applied ^{13}C in the alfalfa and chicory plots, respectively.

^{13}C recovery in shoots of alfalfa was higher compared to chicory one day after labeling (Table 2). However, chicory allocated half of the assimilated C belowground, compared to only one third allocated by alfalfa. At the end of the 40-day chase period, 29 % and 22 % of assimilated ^{13}C was incorporated into shoots of alfalfa and chicory, respectively. Equal ^{13}C amounts incorporated into shoots, but lower ^{13}C incorporation into alfalfa belowground C pools indicated that ^{13}C losses by shoot and soil respiration within the chase period were higher under alfalfa (Table 2).

In topsoil, five times less ^{13}C was recovered in alfalfa roots compared to chicory after one day (Table 2, Table A2). Despite an increase of ^{13}C in alfalfa topsoil roots during the chase period, total ^{13}C incorporation remained lower after 40 days. Similar to roots, the ^{13}C recoveries in topsoil rhizosphere soil and microbial biomass were lower under alfalfa than those of chicory.

At the end of the chase period, 4.1 % of assimilated ^{13}C was incorporated into alfalfa subsoil roots. In contrast, only 1.2 % was incorporated into chicory subsoil roots (Table 2, Table 3). A higher incorporation of assimilated ^{13}C into alfalfa subsoil roots was found at every soil depth after 40 days (Fig. 3, Table A3). Despite the higher recovery in alfalfa subsoil roots, the incorporation of ^{13}C into the microbial biomass remained lower.

The ^{13}C budget indicated that the allocation of assimilated C to belowground C pools was faster in chicory than in alfalfa. Despite the higher ^{13}C incorporation into belowground C pools under chicory, more than twice as much ^{13}C was incorporated into subsoil C pools under alfalfa.

Differences in C stocks and assimilate partitioning between top- and subsoil

Root C stocks in topsoil were 8 times higher for alfalfa and 28 times higher for chicory than in subsoil 110 days

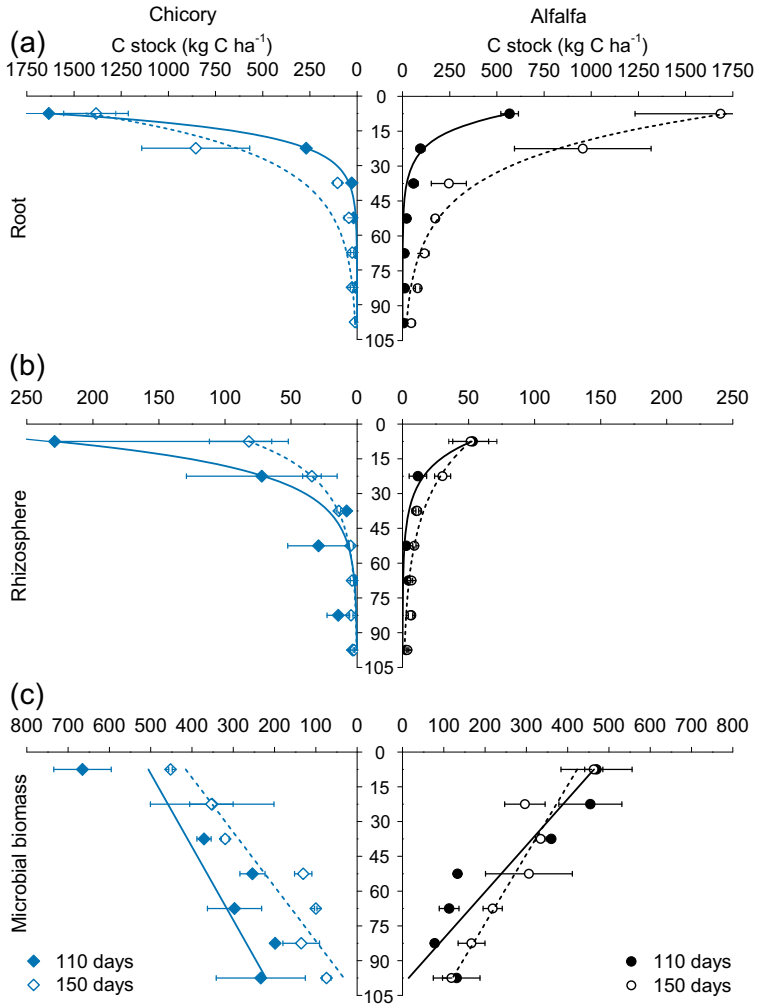

Fig. 1 Root, rhizosphere and microbial biomass C stocks under alfalfa and chicory, measured in 15 cm intervals down to 105 cm soil depth, 110 and 150 days after sowing. *Error bars* represent standard errors of the mean ($n = 3$)

after sowing (Table 3). Over the 40-day observation period, the ratio of root C stock in topsoil to root C stock in subsoil decreased. At the end of the observation period, the alfalfa root C stock in topsoil was only 4 times higher than in subsoil, whereas for chicory plants it was still 12.5 times higher than in subsoil. The ^{13}C recovery in topsoil roots of chicory was 67 times higher than in subsoil roots, indicating that chicory plants allocated and incorporated more assimilated C into topsoil roots compared to alfalfa plants (Table 3). Smallest differences between top- and subsoil were found for microbial biomass C stocks and microbial biomass ^{13}C recoveries under both plants. Microbial biomass C stocks under alfalfa were even higher in subsoil compared to topsoil 150 days after sowing.

Estimation of rhizodeposition

To estimate net rhizodeposition down to 105 cm depth, we assumed that ^{13}C recovered in rhizosphere and bulk soil reflects assimilated C released into soil. Our estimation therefore excludes the amount of assimilated C that was respired by roots or microorganisms during the first day. The ratio of ^{13}C released into soil to ^{13}C recovered in roots was smaller in topsoil (0.5 and 0.1 for alfalfa and chicory, respectively) than in subsoil (4.8 and 1.2 for alfalfa and chicory, respectively). To estimate the amount of assimilated C released into soil, we assumed that the ratio of ^{13}C released into soil and ^{13}C recovered in roots is constant over time. This ratio, determined 110 days after sowing, was multiplied with the root C stock at 110 and 150 days. We estimated that alfalfa released 325 kg

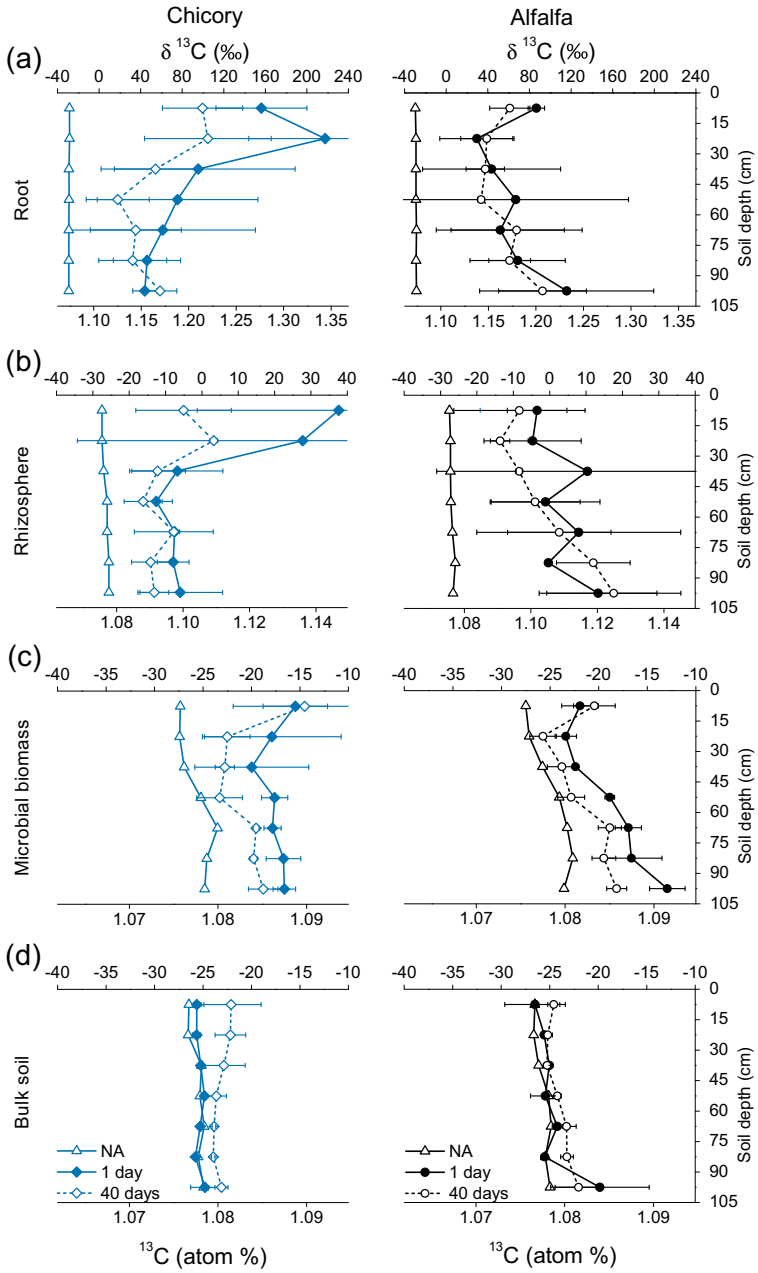

Fig. 2 ^{13}C enrichment (atom%^{13}C) and the corresponding isotopic signature $\delta^{13}C$ (‰) of roots, rhizosphere, bulk soil and microbial biomass down to 105 cm depth. Values are given for the natural abundance control samples (*white triangles*) and for the samples taken at the 1st and 40th day after the in situ $^{13}CO_2$ pulse labeling of chicory and alfalfa. *Error bars* represent standard errors of the mean ($n = 3$)

C ha^{-1} into topsoil and 521 kg C ha^{-1} into subsoil during 110 days of growth (Fig. 4b). The higher alfalfa root biomass 150 days after sowing resulted in 1294 kg C ha^{-1} released into topsoil and 3166 kg C ha^{-1} into subsoil (Fig. 4a,b). Chicory rhizodeposition was lower. We estimated 203 kg C ha^{-1} and 82 kg C ha^{-1} into top- and subsoil, respectively, during 110 days of growth, and 239 kg C ha^{-1} and 256 kg C ha^{-1} into top- and subsoil, respectively, during 150 days of growth.

Discussion

C input into top- and subsoil by alfalfa and chicory

The comparison of C input into top- and subsoil between alfalfa and chicory revealed higher C input, including roots and net rhizodeposition, under alfalfa during 150 days of growth. The partitioning of photosynthetically fixed C revealed that alfalfa invested more into

Table 2 Partitioning of assimilated ^{13}C between C pools, 1 and 40 days after labeling

C pool	^{13}C recovery (% of assimilated ^{13}C) [e]			
	Alfalfa 1 d	Chicory 1 d	Alfalfa 40 d	Chicory 40 d
Shoot	66.9 ± 2.3 a [f] * [g]	38.1 ± 5.5 b *	29.4 ± 3.9 a **	21.5 ± 2.6 a **
Soil respiration	11.6 ± 1.1 a	5.9 ± 1.6 b		
0–30 cm				
Root	6.0 ± 0.6 a *	28.2 ± 4.0 b	18.0 ± 5.8 a **	28.0 ± 12.7 a
Rhizosphere	0.15 ± 0.05 a	0.7 ± 0.2 b	0.1 ± 0.01 a	0.2 ± 0.1 a
Microbial biomass	0.45 ± 0.08 a	1.25 ± 0.3 b	0.49 ± 0.05 a	0.89 ± 0.25 a
30–105 cm				
Root	0.9 ± 0.6 a *	0.5 ± 0.2 a *	4.1 ± 0.6 a **	1.2 ± 0.1 b **
Rhizosphere	0.07 ± 0.03 a	0.05 ± 0.02 a	0.07 ± 0.003 a	0.03 ± 0.004 b
Microbial biomass	0.26	0.69 ± 0.11	0.52 ± 0.15 a	0.59 ± 0.15 a

[e] Values are given as means and standard errors of the mean

[f] Different letters indicate significant differences between alfalfa and chicory 1 day after labeling or 40 days after labeling (Kruskal-Wallis test; $p < 0.05$)

[g] Asterisks indicate significant differences between 1 and 40 days after labeling for alfalfa or chicory (Kruskal-Wallis test; $p < 0.05$)

building up subsoil roots compared to chicory (Table 2). As a consequence, alfalfa root biomass was more evenly distributed between top- and subsoil compared to chicory (Table 3). Root distribution is affected by plant species, period of growth and environmental factors (Lamba et al. 1949), causing varying distribution of root biomass throughout the soil profile. Previous studies of alfalfa root distribution reported a fast development of deeply penetrating taproots, which agrees with the increase in alfalfa subsoil root C stock in the current study (Upchurch and Lovvorn 1951; Bell 2005). Chicory, however, incorporated 26 times more assimilated C into topsoil roots than subsoil roots (Table 3). The higher investment into topsoil root biomass found in the current study is related to the developmental stage, as chicory was reported to develop a deep root system after 2 years of growth (Perkons et al. 2014).

In addition to root biomass, C released into soil needs to be determined in order to estimate total C input into soil (Johnson et al. 2006; Pausch et al. 2013). However, the quantification of rhizodeposition is difficult, as rhizodeposits are easy to decompose (Johnson et al. 2006; Pausch et al. 2013). To estimate rhizodeposition at the field scale, Pausch et al. (2013) determined the ratio of rhizodeposited C to root C in a lab study, which was then applied to root C determined in the field. To estimate net rhizodeposition in the current study, we determined the rhizodeposited-C-to-root-C ratio in top-

Table 3 Topsoil (0–30 cm) to subsoil (30–105 cm) root, rhizosphere and microbial biomass C stock ratio and ^{13}C recovery ratio for alfalfa and chicory plots

	C pool	Ratio Topsoil/Subsoil [e]			
		Alfalfa 1d	Chicory 1d	Alfalfa 40 d	Chicory 40 d
^{13}C recovery	Root	17.6 ± 7.2	67.4 ± 25.1	4.2 ± 0.7	25.6 ± 11.8
	Rhizosphere	3.3 ± 1.4	15.7 ± 5.4	1.4 ± 0.1	7.9 ± 3.0
	Microbial biomass	1.7 ±	1.9 ± 0.5	1.1 ± 0.3	1.5 ± 0.3
C stock	Root	7.8 ± 2.5	28.2 ± 4.0	3.9 ± 1.1	12.5 ± 4.1
	Rhizosphere	2.6 ± 1.0	4.7 ± 0.7	2.3 ± 0.6	4.2 ± 1.4
	Microbial biomass	1.4 ±	0.8 ± 0.1	0.7 ± 0.1	1.1 ± 0.1

[e] Values are given as means and standard errors of the mean

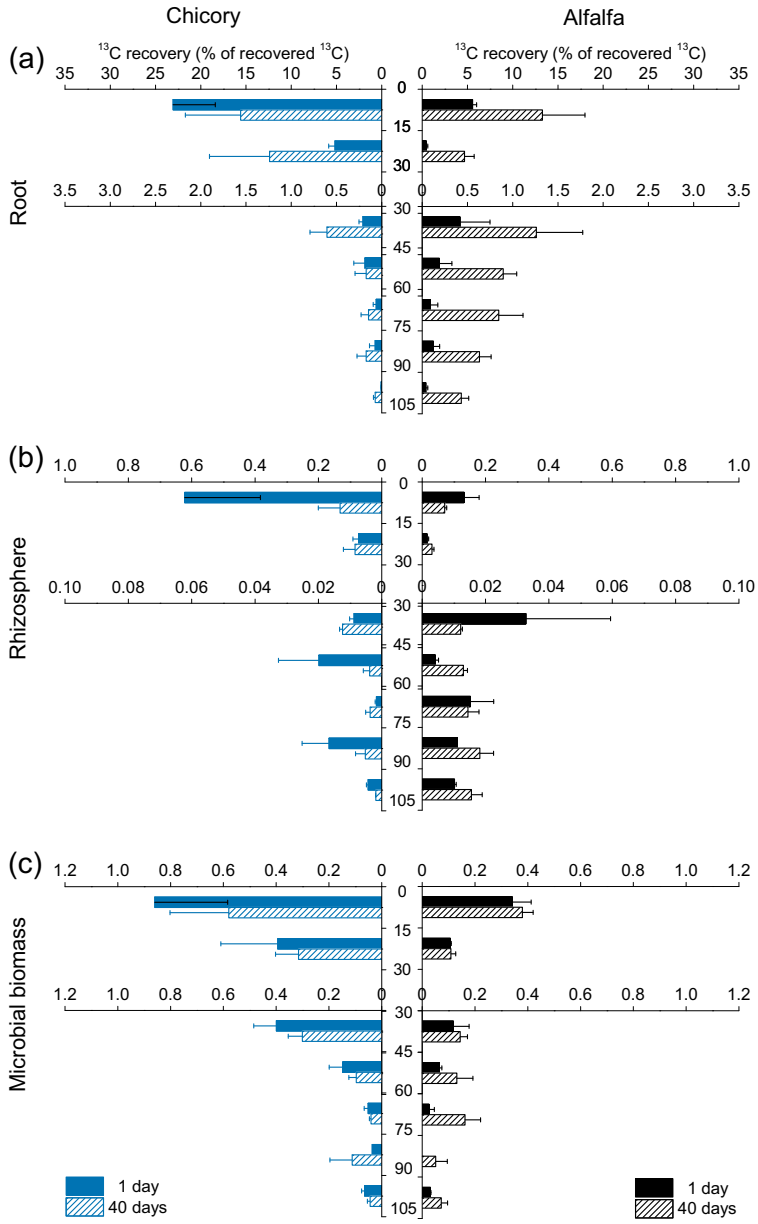

Fig. 3 ^{13}C recovery in root biomass, rhizosphere soil and microbial biomass in top- and subsoil of the alfalfa and chicory plots, 1 and 40 days after the $^{13}CO_2$ pulse labeling. Topsoil and subsoil are separated by horizontal lines. Note much higher resolution of the x-axis for the subsoil (**a**, **b**). *Error bars* represent standard errors of the mean ($n = 3$)

and subsoil. C lost by soil respiration was excluded from our calculation due to experimental difficulties in determining in situ respiration down to 105 cm depth. Therefore, the actual ratio of rhizodeposited C to root C would be higher. Net rhizodeposition of alfalfa was estimated to be 1290 kg C ha^{-1} and 3160 kg C ha^{-1} into top- and subsoil, respectively, during 150 days of growth, and therefore much higher than of chicory. Rhizodeposition increases the activity of microorganisms in the rhizosphere (De Nobili et al. 2001). This, in turn, increases organic matter turnover and nutrient mineralization (Cheng 2009). The requirement of nutrients, i.e. P and micronutrients, for N_2 fixation of alfalfa (O'Hara 2001) and the higher increase in above- and belowground plant biomass of alfalfa than chicory resulted in higher investment of recent C into soil (Tables 1 and 2 and Fig. 4). Moreover, N_2 fixation from the atmosphere leads to a higher C demand by rhizosphere microorganisms (Vance and Heichel 1991; Herridge et al. 2008) under legumes compared to non-leguminous herbs. This is another reason why alfalfa allocated more C into the soil than chicory. In

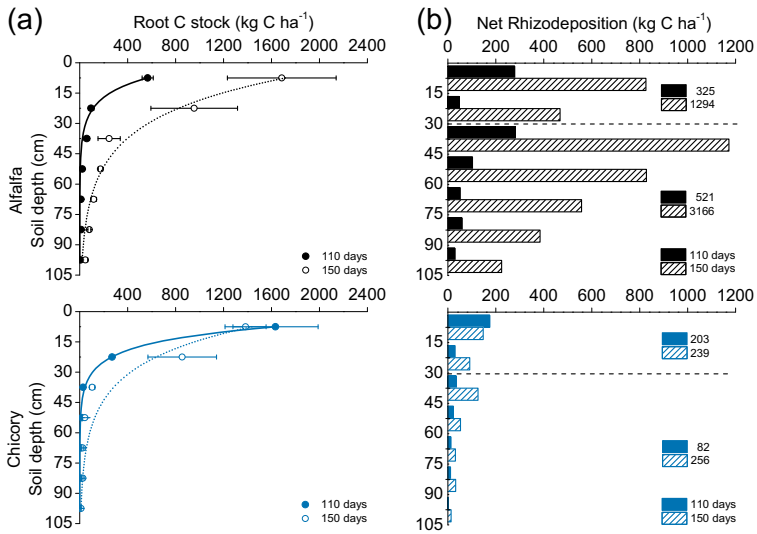

Fig. 4 Estimation of alfalfa and chicory net rhizodeposition from 0 to 105 cm depth in 15 cm intervals, 110 and 150 days after sowing (**b**). The numbers of the filled (110 days) and dashed (150 days) boxes represent the sum of rhizodeposited-C (kg C ha^{-1}) into top- or subsoil. Root C stock 110 and 150 days after sowing (**a**) was multiplied by the ratio of recent C (^{13}C) recovered in soil to recent C recovered in roots, calculated for top- and subsoil separately

conclusion, total C input including root biomass and net rhizodeposition was estimated to be 3940 kg C ha^{-1} into topsoil and 3830 kg C ha^{-1} into subsoil by alfalfa and only 2480 kg C ha^{-1} into topsoil and 470 kg C ha^{-1} into subsoil by chicory within 150 days of growth.

The estimation of net rhizodeposition into subsoil could be improved by a series of $^{13}CO_2$ pulse labelings accompanied by root biomass determination throughout the vegetation period. It has been shown that C input into soil as root biomass and exudation depends on the developmental stage of the crop (Swinnen et al. 1994; Kuzyakov et al. 1999; Kuzyakov et al. 2001). A series of $^{13}CO_2$ pulse labelings throughout the vegetation period of alfalfa and chicory would account for changes in the partitioning of recently assimilated C between root biomass and soil depending on depth and developmental stage of the plants. Furthermore, the time necessary for plant roots to explore deeper soil would be considered.

The response of microorganisms to C input into subsoil

Microbial biomass in subsoil is limited in energy due to a lower supply of fresh C (Fontaine et al. 2007) than in topsoil. Easily available C that is released into soil via rhizodeposition stimulates microbial activity (De Nobili et al. 2001). Especially in subsoil, rhizodeposits are important for microorganisms due to the usually scarce substrate supply. The amount of released C taken up by microorganisms in subsoil was similar to that in topsoil under alfalfa and chicory. Although C input into roots and C released into soil strongly decreased with depth, the uptake of C by microorganisms was only slightly affected (Fig. 3). This suggests that in subsoil, microorganisms used a higher proportion of the substrate supplied by rhizodeposition, as a result of C limitation. In contrast, continuous input of plant litter ensures substrate availability for microorganisms in topsoil. Sufficient substrate availability caused higher mineralization of rhizodeposits, resulting in similar uptake of released C into topsoil compared to subsoil microbial biomass.

During the observation period, the microbial biomass in subsoil increased under alfalfa but decreased under chicory (Table 1, Fig. 1 c). However, the absolute incorporation of released ^{13}C into microbial biomass under chicory was higher after 40 days (Fig. 3 c). The increase in alfalfa root biomass and associated rhizodeposition indicates a continuous supply of substrate for microorganisms, enabling their growth (De Nobili et al. 2001). This suggest that the higher and sustained availability of easily available C under alfalfa caused an accelerated turnover of microbial biomass C (Dorodnikov et al. 2009; Blagodatskaya et al. 2011). In contrast, an insufficient substrate supply under chicory could not even maintain microbial biomass. In conclusion, accelerated turnover of microbial C resulted in lower total ^{13}C incorporation into the microbial biomass in subsoil under alfalfa compared to chicory.

Furthermore, lower ^{13}C incorporation into the subsoil microbial biomass under alfalfa plants could have been

affected by root distribution. Alfalfa taproots can branch up to the fifth order, whereas chicory taproots can branch up to the fourth order (Kutschera et al. 2009). The increase in alfalfa root biomass and lateral root development could have caused a more dispersed root distribution, leading to rhizodeposition of ^{13}C throughout a larger volume of subsoil. Due to the low levels of microbial biomass in the subsoil, a greater proportion of the rhizodeposited C of the alfalfa roots did not come into contact with the microbial biomass and was therefore not incorporated.

Relevance of carbon input into subsoil

Generally, SOM content and microbial biomass decrease exponentially with soil depth (Fierer et al. 2003; Castellazzi et al. 2004). The SOC stock of the chicory and alfalfa plots decreased exponentially down the entire soil profile (data not shown) but we found a slower decrease of microbial biomass that followed a linear rather than exponential decline (Fig. 1). We think that the distribution of the microbial biomass was a function of the root distribution over the soil profile. In particular, the exploration of the subsoil by alfalfa taproots and the release of easily available C enabled microbial growth and the development of microbial hotspots (Kuzyakov and Blagodatskaya 2015; Spohn and Kuzyakov 2014). The input of a diversity of organic compounds, including both low and high molecular weight organic substances maintains a broad capability in microbial decomposition functions (De Nobili et al. 2001). This biochemical ability to decompose various substrates also enables decomposition of various SOM compounds and thus the opportunity to access immobilized nutrients.

Conclusions

C input into soil, including root biomass and net rhizodeposition, by two taprooted preceding crops, alfalfa and chicory, was determined over 150 days of growth down to 105 cm depth. C input into the topsoil (0–30 cm) by alfalfa was 1.6 times higher (3940 kg C ha^{-1}) than by chicory (2480 kg C ha^{-1}) and C input into subsoil (30–105 cm depth) by alfalfa was 8.2 times higher (3830 kg C ha^{-1}) than by chicory (470 kg C ha^{-1}). The higher C input into soil resulted from a larger increase in alfalfa above- and belowground biomass during the vegetation period. Especially the C input into subsoil was higher under alfalfa. The root system of alfalfa was more equally distributed between top- and subsoil, whereas the main part of chicory root biomass was found in the topsoil. The in situ $^{13}CO_2$ pulse labeling of alfalfa and chicory plants enabled the determination of assimilated C partitioning between above- and belowground C pools down to 105 cm depth. In accordance with the increase in subsoil root biomass of alfalfa, the labeling revealed higher incorporation of assimilated C into subsoil roots. Tracing of ^{13}C in roots and soil enabled the estimation of net rhizodeposition into top and subsoil down to 105 cm. Net rhizodeposition of alfalfa was 5 times higher into topsoil and 12 times higher into subsoil compared to chicory. To account for the variability of root biomass and rhizodeposition during the vegetation period and at different soil depths, several pulse labelings would enable a more precise estimation of C input.

Although C allocation to roots and rhizodeposition decreased strongly from top- to subsoil, the uptake by microorganisms was similar in top- and subsoil. Our results suggest that subsoil microorganisms incorporated a higher proportion of released C due to scarce substrate supply, whereas in the topsoil, sufficient substrate availability caused higher mineralization of released C to CO_2. Because alfalfa invested more C into building up subsoil root biomass and into rhizodeposition during the observation period, microbial turnover was accelerated. Therefore, total ^{13}C incorporation by microorganisms was lower in subsoil under alfalfa than under chicory.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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