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Cover crop root morphology rather than quality controls the fate of root and rhizodeposition C into distinct soil C pools

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Abstract
Cover crops increase carbon (C) inputs to agricultural soils, and thus have the potential to mitigate climate change through enhanced soil organic carbon (SOC) storage. However, few studies have explored the fate of belowground C inputs associated with varying root traits into the distinct SOC pools of mineral-associated organic carbon (MAOC) particulate organic carbon (POC). Therefore, a packed 0.5 m column trial was established with 0.25 m topsoil and 0.25 m subsoil with four cover crops species (winter rye, oilseed radish, chicory, and hairy vetch) known to differ in C:N ratio and root morphology. Cover crops were 14CO₂-labeled for 3 months, and then, half of the columns were sampled to quantify root and rhizodeposition C. In the remaining columns, plant shoots were harvested and the undisturbed soil and roots were left for incubation. Bulk soil from both sampling times was subjected to a simple fractionation scheme, where 14C in the <50 and >50 μm fraction was assumed to represent MAOC and POC, respectively. The fast-growing rye and radish produced the highest root C. The percentage loss of C via rhizodeposition (%ClvR) showed a distinct pattern, with 22% for the more branched roots (rye and vetch) and 6%–8% for the less branched roots (radish and chicory). This suggests that root morphology plays a key role in determining rhizodeposition C. After 1 year of incubation at room temperature, the remaining MAOC and POC were positively correlated with belowground inputs in absolute terms. However, topsoil MAOC formation efficiencies (cover crop-derived MAOC remaining as a share of belowground inputs) were higher for vetch and rye (21% and 15%, respectively) than for chicory and radish (9% and 10%, respectively), suggesting a greater importance of rhizodeposition (or indirectly, root morphology) than solely substrate C:N ratio for longer term C stabilization.

KEYWORDS
14C, cover crops, MAOC, POC, rhizodeposition, root carbon, root morphology, SOC
INTRODUCTION

The use of winter cover crops to increase annual photosynthesis and soil organic carbon (SOC) sequestration has been proposed as an effective tool for mitigating net greenhouse gas emissions from agriculture (Poeplau & Don, 2015), a sector that accounts for 10%–12% of global emissions (Smith et al., 2007). It has been suggested that cover crops increase SOC stock at an annual rate of 0.32 ± 0.08 Mg ha⁻¹ year⁻¹ (Poeplau & Don, 2015) and offer the total net mitigation potential of 1.4 Mg CO₂ equivalents ha⁻¹ year⁻¹ when nitrous oxide and the albedo effect, for example, are also accounted for (Kaye & Quemada, 2017).

However, there has been limited investigation into how different cover crops and their respective carbon (C) allocation to aboveground and belowground C pools, including rhizodeposition, translate into soil C pools that have different retention times, which is of great relevance in a climate change mitigation context.

Over the past decade, there has been increasing support of conceptualizing SOC into distinct C pools of particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) to explain how SOC is formed and stabilized (Lavallee et al., 2020; Lehmann & Kleber, 2015). Concurrently, theories of long-term stabilization through “humification” and “selective preservation” have been rejected alongside a shift in methodology; Earlier litter mass loss (or teabag) experiments have been replaced with state-of-the-art experimental designs using isotopic tracers and a wide range of fractionation protocols to isolate C fractions based on physical (size, density) and chemical (oxidation, extraction) properties, depending on the scientific goal (Cotrufo & Lavallee, 2022; Lehmann & Kleber, 2015; Poeplau et al., 2018; von Lützow et al., 2007). According to the emerging theory, POC and MAOC are formed via two distinct pathways (Cotrufo et al., 2015): MAOC is formed when less structural or so-called labile compounds are rapidly and efficiently transformed by microbial metabolism (in vivo) and extracellular modification (ex vivo; Liang et al., 2017) into low molecular weight compounds of both microbial and plant origin (Angst et al., 2021) that associate with mineral surfaces (Lavallee et al., 2020). In contrast, POC is mainly plant derived, and results from the fragmentation or polymerization of more structural (e.g., more lignified) compounds (Cotrufo et al., 2013; Lavallee et al., 2020). MAOC is protected by sorption to mineral surfaces or occlusion in fine aggregates and has proved to be less sensitive to environmental change, with a proposed mean residence time in the soil of decades to centuries (Cotrufo & Lavallee, 2022). POC is offered either no protection as “free POC” (IPC) or protection via occlusion in aggregates (“occluded POC”, oPOC), with the latter giving rise to a residence time of years to decades (Lavallee et al., 2020).

Taking this theoretical framework into consideration, it is clear that cover crops could result in greater MAOC formation efficiency as they can be considered young plants, and are typically associated with a lower C:N ratio and a lower amount of lignocellulose C structures than the typical residues (e.g., straw) left on fields from cereal production. Furthermore, different cover crop functional types, for example, grasses, brassicas, and legumes (Zhang et al., 2022), represent different C allocation patterns, biomass recalcitrance, root morphology, and associations with symbionts (e.g., mycorrhiza and rhizobium), which are all identified as the main parameters governing C stabilization mechanisms (Adkins et al., 2016; Poirier et al., 2018; Rossi et al., 2020).

In particular, rhizodeposition, which is defined as the C lost by the living plant via roots, has been shown to be effectively stabilized, with a proposed MAOC formation efficiency of 46% compared with 7% for shoot C and 9% for root C (Villarino et al., 2021). Rhizodeposition, more specifically, is always operationally defined, and includes root exudates, decomposed root parts, and finer roots such as root hairs, as it is practically impossible to distinguish between rhizospheric compounds (Rasmussen, 2011; Wichern et al., 2008).

In order to determine and model the mitigation potentials of plant C inputs to soil, it has been suggested that rhizodeposition-to-root C ratios are species independent (Pausch & Kuzyakov, 2018). Yet, as different species vary greatly in their root response to pedoclimatic conditions, for example, with changes in root morphology and turnover (Hupe et al., 2019), it can be expected that net rhizodeposition also varies with both species and soil depth. Similarly, an increasing number of root traits have been linked to SOC stabilization processes (Poirier et al., 2018), although there has as yet been little exploration of how variations in root morphology govern C dynamics (Adkins et al., 2016; Wu et al., 2007), and even less so in the scarce literature regarding cover crops specifically (Austin et al., 2017; De Notaris et al., 2020; Engedal, Karlsson, et al., 2023; Mortensen et al., 2021). Lastly, as deep soil C inventories are notoriously lacking, so are studies on both rhizodeposition and C stabilization below 0.25 m.

Therefore, a column trial was established with the aim of investigating: (i) how the quantity and quality (e.g., C:N ratio) of C inputs (roots and rhizodeposition) at two soil depths varies between four cover crop species known to have different root quality and morphology, and (ii) how belowground C inputs translate into the distinctive soil C pools of MAOC and POC. The following hypotheses were tested: hypothesis (I) Rhizodeposition C is positively correlated with root C, with a constant net rhizodeposition-to-root C ratio across species; hypothesis (II) the relative loss of C to rhizodeposition changes with depth; and hypothesis (III) MAOC formation from decomposition of belowground cover crop C increases with increasing substrate quality (i.e., low C:N ratio).

MATERIALS AND METHODS

2.1 Experimental design

Soil was collected in January 2020 from a sandy loam soil on the research farm of the University of Copenhagen in Taastrup, Denmark (55°40′31.3″ N 12°17′18.0″ E). Soil from the A₀ horizon (generally 0–30 cm) and the Bₗ horizon (generally 30–70 cm) was collected, air-dried, sieved to <4 mm, homogenized, and used as topsoil and subsoil, respectively, in the trial (Table 1). Thirty-two PVC tubes (d = 10.2 cm, h = 50 cm) were packed with 25 cm subsoil and 25 cm topsoil at 60% of water-holding capacity (WHC) to achieve a bulk density close to measured in the field (1.5–1.6 g cm⁻³). The
columns were equipped with a seeding mat attached to a wick at the bottom to allow drainage. Before packing, nutrients (N, S, P, K, Ca, Mg, Fe, Mn, Zn, B, Mo) were added to dry soil at standard nutrient ratios in equal amounts to both topsoil and subsoil to ensure subsoil root growth. The N target was 100 and 25 mg N kg−1 soil for nonlegumes and legumes, respectively. Forty-nine days after germination (DAG), each nonlegume column received additional N (target of 50 mg N kg−1 soil) with the irrigation water to avoid senescence and leaf loss, especially from oilseed radish, which was rapidly ripening.

Winter rye, oilseed radish, chicory, and hairy vetch were chosen to represent distinct cover crop functional types (grass, brassica, legume, and Asteraceae; Zhang et al., 2022) with known differences in root morphology (Hudek et al., 2022), which were also observed in the trial (Table 2). Eight replicate columns of each species were seeded at certain depths and thinned to certain plant densities (Table 2). Vetch was inoculated with rhizobium shortly before seeding. The columns were placed in a climate chamber, kept at 80% humidity with 12 h of light at 17°C and 12 h of darkness at 12°C. The columns were irrigated immediately after seeding, and then three times a week by weighing and watering to 50% WHC at the start of the experiment, rising gradually to 75% WHC throughout the experimental period.

Raw data have been deposited in the Zenodo repository (Engedal, Magid, et al., 2023).

### 2.2 | 14CO2 labeling

To enable quantification of C input to the soil from the living root during growth (rhizodeposition) and cover crop-derived C remaining in different soil C fractions, the cover crops were subjected to a multiple pulse-labeling scheme. Labeling was initiated at 20 DAG (27 DAG for chicory), repeated twice a week with increasing activity following the development of cover crop leaf area, resulting in 16 labeling events where each column received a total of 1.7 mega becquerel (MBq; Table S1). The 14CO2 labeling was performed by attaching a transparent plastic bag to each column, creating a temporary closed atmosphere around the plants. A sodium 14C-carbonate solution (in 1 M NaOH) in a falcon tube was placed inside, and 14CO2 was released upon injecting a surplus of HCl into the beaker with a syringe (Figure S1). Immediately afterward, the perforation was sealed with transparent tape. After 1–2 h, the plastic bag was removed.

### 2.3 | Plant and soil sampling

Columns were sampled at two different times. All eight replicate columns were terminated after a growing season of 77–81 DAG by shoot harvesting at the point clearly identifying the change from stem to root. Four of the eight replicate columns for each species were sampled for root and rhizodeposition determination directly afterward at t1. The remaining four replicate columns were left undisturbed for decomposition of belowground C inputs for 1 year before sampling at t2. The 1-year decomposition occurred at room temperature (20–22°C), corresponding to several years of decomposition compared with the annual Danish average of about 8°C (Wildung et al., 1975; Zhang et al., 2021), and with soil moisture kept at 30% WHC.

All the columns were opened longitudinally (the PVC tubes had been cut before the experiment and taped together). Topsoil and subsoil were separated based on a clear color difference. At t2, the roots were first gently collected separately by hand, including any soil adhering to the roots. Additional smaller roots were isolated from the remaining soil by gently sieving all the soil, first through a 10 mm sieve, followed by a 2 mm sieve for the topsoil and a 4 mm sieve for the subsoil, with small roots continuously being removed from the sieves during the process. The remaining topsoil and subsoil (later referred to as ”bulk soil”) that had passed through the sieves was mixed and representative subsamples taken for 14C analysis and determination of amount of root fragments >250 μm. For the latter, a 100 g subsample was stirred in water and carefully decanted over
a 250 μm sieve, recovering only floating material, referred to as “root fragments.” The recovered root fragments were analyzed for 14C and subtracted from bulk soil 14C for a reliable estimation of rhizodeposition (Section 2.5). The collected roots and root fragments are reported together unless otherwise noted.

The majority of soil adhering to the roots (later referred to as “rhizosphere soil”) was sampled by brushing the roots gently, and passed through a 1 mm sieve. Additional rhizosphere soil adhering tightly to the root surfaces after this procedure was removed by root washing, collected, and dried for estimation on a weight basis, assuming the same 14C activity per gram soil as the directly collected rhizosphere soil. Due to the small sample size, decantation for estimation of remaining root fragments in the rhizosphere soil was not carried out. Instead, it was assumed that the rhizosphere soil had the same absolute amount of root fragments per gram of soil as the bulk soil. This was chosen in order to account more accurately for the potentially higher rhizodeposition in the rhizosphere soil.

At t2, after 1 year of incubation, the remaining four replicate columns of each species were opened longitudinally (as described above). Topsoil and subsoil were separated, passed through a 10 mm sieve, and homogenized. Only a few roots remained on the sieve and only in the case of radish. As all roots would be interpreted as POM in the following fractions, recovered roots at t2 were kept in the soil after gently being broken up into smaller pieces and mixed with ~100 g of bulk soil before mixing thoroughly with the whole bulk soil.

Soil, shoot, and root samples were dried at 60°C, ball-milled, and analyzed for total C and N in a MACRO elementary analyzer (Elementar). To measure the activity of 14C, samples were first combusted for 2–3 min at 900°C using a sample oxidizer (Hidex 600OX Oxidizer; Hidex), thereby releasing 14CO2 which was trapped in 20 mL vials in a combined base trap and scintillation cocktail (600 OX Radiocarbon cocktail). Then 14C activity was analyzed on a liquid scintillation counter (Tri-Carb® 2910TR; PerkinElmer), where the disintegrations per minute (DPM) were determined for 10 min per sample.

2.4 | Soil fractionation by size

Bulk soil from both sampling times was subjected to simple soil C fractionation by size (adjusted from Cotrufo et al., 2019). Bulk soil at t1 included rhizodeposition and root fragments as the roots were removed, and bulk soil at t2 included all the remnants after the intact 1-year incubation. Then, 12 g of bulk soil was dispersed in 35 mL 0.5% sodium hexametaphosphate (NaHMP), and shaken in an end-over-end shaker (113 rpm) for 18 h with eight glass beads. The sample was then poured over a 50 μm sieve to isolate the coarse fraction (>50 μm) from the fine fraction (<50 μm), using approximately 250 mL of water. In a pre-trial, the heavy fraction of the >50 μm fraction (coarse mineral) was isolated by repeated decantation. As no 14C activity was measured in the heavy (coarse mineral) fraction alone, the >50 μm fraction was analyzed without density fractionation, assuming that all 14C could be ascribed to the particulate fraction (POC). The <50 μm fraction was examined under a stereomicroscope, and as no or only negligible pieces of root fragments were found, the 14C found in the fine fraction was assumed to be mainly MAOC. While it is acknowledged that small amounts of fine POC and dissolved organic carbon (DOC; presumably <1%–2% of total cover crop-derived C; Cotrufo & Lavallee, 2022) are recovered in the <50 μm fraction, this does not change the overall interpretation or conclusions of the results (Cotrufo et al., 2019). POC and MAOC fractions were prepared for 14C analysis as described above.

The mass recovery of the soil C fractionation averaged 99.2% with no variation across sampling time and depth, while 14C recovery averaged 64% and 72% at t1 and 86% and 145% at t2, for topsoil and subsoil, respectively.

In order to support that distinct C pools are isolated in the fractionation procedure, four control soils were fractionated, and the C:N ratio of the different fractions of four control soils was analyzed for total C and N analysis with a Pyro Cube elemental analyzer interfaced to an Isoprime 100 mass spectrometer (Elementar). The C:N ratios of the MAOC fraction were significant lower (10 and 11) than the POC fraction (15 and 16) in the control topsoil and subsoil, respectively (Table 1, statistics not shown).

2.5 | Calculations

The tracer mass balance approach described by Rasmussen et al. (2019) and De Notaris et al. (2020) was used to determine the net C lost via rhizodeposition (ClvR), that is, what remains in the soil after partial microbial turnover at the time of harvest (t1). Below, 14C data are expressed in the unit of disintegrations per minute (DPM) and total C data as mg C col−1. The percentage loss of 14C to the soil (%ClvR) is expressed as a share of the total 14C found in the below-ground pools:

\[
\% \text{ClvR} = \left( \frac{14C_{\text{soil}} - 14C_{\text{root fragments}}}{14C_{\text{soil}}} \right) \times 100. \quad (1)
\]

where 14Csoil and 14Croot fragments are based on both bulk soil and rhizosphere soil, based on the assumption that rhizosphere soil contains the same absolute amount of root fragments per gram as the bulk soil (Section 2.3). 14Croot fragments is subtracted from the 14Csoil pool in order to avoid overestimation of rhizodeposition C (Rasmussen, 2011). It should be noted that 14Croot fragments is part of the total 14C expressed in the denominator; hence, it is only subtracted from the numerator. Croot includes roots and root fragments.

Based on the %ClvR, the quantitative ClvR (qClvR) can be calculated from the total C found in recovered root biomass (mg C col−1):

\[
q\text{ClvR} \left( \text{mg C col}^{-1} \right) = \% \text{ClvR} \times C_{\text{root}} / (100 - \% \text{ClvR}), \quad (2)
\]

where carbon contributions from root fragments are estimated based on the assumption that root fragments have the same specific activity as roots.

As the recovery of 14C from the fractionation deviated from 100%, estimations of cover crop-derived MAOC and POC were
based on the relative distribution of $^{14}$C in these pools (%MAOC, %POC) and scaled by the total $^{14}$C found in a larger representative ball-milled sample of bulk soil. The recovered rhizosphere soil offered insufficient amounts for fractionation; therefore, the same distribution between MAOC and POC was assumed as in the bulk soil.

Cover crop-derived MAOC at t$_2$ was calculated by multiplying the relative share of MAOC and POC by the cover crop-derived C estimated in the bulk soil at t$_1$ after removing the roots (rhizodeposition C and root fragments C):

$$\text{MAOC}_{t_1} \left( \text{mg C mol}^{-1} \right) = \frac{\% \text{MAOC} \times (C_{\text{fragments}} + qClvR_t)}{100}$$  \hspace{1cm} (3)

In order to calculate MAOC$_{t_2}$, first the percentage $^{14}$C remaining in MAOC at t$_2$ relative to $^{14}$C input at t$_1$ (root and rhizodeposition) was calculated, assuming that $^{14}$C inputs measured for t$_1$ columns were, on average, the same for t$_2$ columns. The percentage of $^{14}$C remaining in MAOC could also be referred to as the MAOC formation efficiency:

$$\text{MAOC}_{t_2} \left( \% \right) = \frac{14C_{\text{MAOC}} - 14C_{\text{root}}}{14C_{\text{MAOC}}} \times 100$$  \hspace{1cm} (4)

Then, the cover crop-derived MAOC at t$_2$ was calculated by multiplying %MAOC$_{t_2}$ by the total average C input at t$_1$ (root C and rhizodeposition C):

$$\text{MAOC}_{t_2} \left( \text{mg C mol}^{-1} \right) = \text{MAOC}_{t_1} \times (C_{\text{root}} + qClvR_t) / 100$$  \hspace{1cm} (5)

All calculations and modeling were performed separately for each depth. POC$_{t_1}$, %POC$_{t_2}$, and POC$_{t_2}$ were calculated in the same way as MAOM.

### 2.6 Statistical analysis

The figures and tables present means and standard errors from raw data unless otherwise stated, while the letters and asterisks showing significant differences are derived from the statistical models. Statistical analyses were carried out in R (R Core Team 2021, version 4.1.2). The significance level was set at $p = .05$. One-way analysis of variance (ANOVA) with species was carried out for shoot data and when data were not separated into depths. Linear mixed models (LMMs) with plant ID as a random effect was used for analyses involving both topsoil and subsoil. LMMs for time-specific (t$_1$ or t$_2$) analyses included species, depth, and their interaction as fixed effects; LMMs involving data from both t$_1$ and t$_2$ also included time and all interactions between time, species, and depth. Pairwise comparisons of the means were conducted with the post hoc Tukey HSD test, using the functions emmeans and clm (compact letter display) from the “emmeans” (Lenth, 2022) and “multcomp” (Hothorn et al., 2008) packages, respectively. The assumptions of normality and homogeneity of variance were validated by quantile–quantile plots and residual plots, respectively. To comply with these assumptions, response variables were square root-transformed or log-transformed when appropriate and determined by a Box-Cox analysis. The numerator and denominator in Equation (4) stem from t$_2$ and t$_1$, respectively. As measurements from t$_1$ and t$_2$ were not paired, the ratio (Equation 4) could not be computed and analyzed for each plant ID. Therefore, an LMM on a log-scale was fitted to the numerator and denominator separately (as explained above). Estimates and variances from the LMMs were then combined to estimate standard errors, and pairwise comparisons for the ratio in Equation (4) (R code available in Data S2). Holm adjustment of $p$-values was applied for comparisons of ratios.

### 3 RESULTS

#### 3.1 Cover crop characteristics and quality

As the experimental conditions were designed to ensure optimal growing conditions for the four species in the climate chamber, the biomass production in the columns was relatively high (Table 3). If scaled from column surface area to hectare level, these productivity levels greatly exceed what is typically found for field-grown cover crops of the same species over a similar growing period. Therefore, results are given per column (col$^{-1}$), that is, with the diameter of 10 cm and soil depth of 25–25 cm (Section 2.1).

| Cover crop shoot C, shoot C:N ratio, root:shoot ratio (based on C data), root C (including root fragments), root N, and root C:N ratio (excluding root fragments). Values are presented as means and standard errors (n=4). Upper and lowercase letters denote significant differences between species for either the whole column (0–50 cm) or for topsoil (0–25 cm) and subsoil (25–50 cm), respectively. One and two asterisks denote significant differences between depth within species at $p < .05$ and $p < .01$, respectively. |
|---|---|---|---|---|---|
| **Shoot C (g C col$^{-1}$)** | **Shoot C:N ratio** | **Root:shoot ratio** | **Root C (g C col$^{-1}$)** | **Root N (mg N g C$^{-1}$)** | **Root C:N ratio** |
| **0–50 cm** | **0–25 cm** | **25–50 cm** | **25–50 cm** | **0–25 cm** | **25–50 cm** |
| Rye | 16.9 (0.5)$^b$ | 28.1 (0.9)$^a$ | 0.44 (0.02)$^c$ | 7.4 (0.2)$^b$ | 131 (8)$^b$ | **45 (8)$^a$** | 33.8 (0.2)$^b$ | **42.8 (2.1)$^a$** |
| Radish | 12.0 (1.6)$^b$ | 29.1 (2.8)$^a$ | 0.98 (0.13)$^b$ | 11.1 (0.4)$^a$ | 418 (28)$^a$ | **13 (1)$^a$** | 24.7 (1.2)$^b$ | **21.4 (1.3)$^a$** |
| Chicory | 2.9 (0.4)$^c$ | 20.4 (0.5)$^b$ | 1.90 (0.14)$^c$ | 5.5 (0.4)$^c$ | 79 (13)$^c$ | **17 (4)$^c$** | 59.0 (5.6)$^a$ | **50.7 (5.1)$^b$** |
| Vetch | 6.3 (0.5)$^c$ | 10.6 (0.3)$^c$ | 0.36 (0.03)$^c$ | 2.3 (0.2)$^b$ | 112 (12)$^{bc}$ | **35 (4)$^{bc}$** | 12.6 (0.2)$^b$ | **14.2 (0.5)$^b$** |
The four species were chosen for being representative of different cover crop functional groups of species with varying qualitative traits, for example, differing in biomass production, C:N ratio, and specific root length (Hudek et al., 2022; Zhang et al., 2022). The different traits are apparent from Table 3: Rye and radish produced substantially higher shoot C, with a higher C:N ratio than chicory and, in particular, vetch. The typical thick taproot for radish and chicory translated into higher root:shoot ratios than for rye and vetch. Although vetch roots are considered taproots, they showed branching more similar to rye (Table 2). Root C:N ratio for vetch was significantly lower than for the other species at both depths. Root C:N ratio increased with depth for rye and vetch, while it declined with depth for radish and chicory. Radish displayed the highest total N accumulation in root biomass in the topsoil, while chicory showed the lowest, primarily reflecting low biomass production.

3.2 | Root and rhizodeposition C

Root C (including root fragments) ranged from 1.5 to 10.6 g C col⁻¹ in the topsoil and from 0.5 to 2.5 g C col⁻¹ in the subsoil (Figure 1a). Radish accumulated the highest root C in the topsoil and the lowest root C in the subsoil, reflecting the large storage organ mainly placed in the topsoil. Chicory showed a similar distribution between depths, although it was less pronounced. Rye showed similar root C in the topsoil as chicory, but significantly higher root C than the other species in the subsoil. Vetch displayed the overall lowest root C in the topsoil, and similar root C in the subsoil as radish and chicory. The relative share of root fragments to the total root C pool was substantially higher in the subsoil than for the topsoil (Figure 1a,b).

The proportion of C lost from root via rhizodeposition (%ClvR) ranged between 6% and 39% (Figure 1c). In the topsoil, rye and vetch showed similar %ClvR of 22%, which was significantly higher than radish and chicory (6%–8%; p < .0001). In the subsoil, different patterns were exhibited, with the highest %ClvR for radish (39%) and the lowest for chicory (17%).

The absolute amount of rhizodeposition C (qClvR) was significantly higher for rye at both depths (Figure 1e). Interestingly, qClvR correlated with root C in two distinct patterns for species with a more branched (rye and vetch, R² = .82) and a less branched (radish and chicory, R² = .89) root system (Figure 2a). The pattern, which was only pronounced in the topsoil, was given from the distinct %ClvR (Figure 1b) between the groups. A more and less branched root system was associated with larger (rye and vetch) and smaller (radish and chicory) contributions from rhizosphere soil to the estimated percentage and quantitative C lost via rhizodeposition in the topsoil, respectively (Figure 1d,f). In the absence of measurements of specific root surface of the investigated species, a test was performed to identify whether the amount of rhizosphere soil (which was operationally defined as soil adhering to the roots) was related to rhizodeposition C. Indeed, the rhizosphere soil correlated well with qClvR across all species (Figure 2b, R² = .82).

All nonlegumes showed significantly higher %ClvR in the subsoil than in the topsoil (p = .046 for rye, p = .0003 for chicory, and p < .0001 for radish), while the vetch remained at 21%–22% throughout the profile (Figure 1e). Despite higher %ClvR in the subsoil for nonlegumes, the substantially lower subsoil root C (Figure 1a) resulted in a significantly lower quantity of C lost via rhizodeposition (qClvR) in the subsoil (130–800 mg C col⁻¹) compared with the topsoil (370–1220 mg C col⁻¹) for all species (Figure 1e).

3.3 | Cover crop-derived MAOC and POC and their formation efficiencies

In order to validate the results from the simple fractionation protocol, the correlations between ¹⁴C found in different C pools were examined. When sampled at the end of the growing period (t₁), roots were isolated, leaving bulk soil for fractionation consisting of cover crop C inputs of rhizodeposition and root fragments (RF). Figure 3 shows how MAOC and POC correlated with bulk soil and isolated root fragments (measured) and bulk soil without root fragments (calculated from the other two, cf. Equation 1). At t₁, the amount of ¹⁴C in cover crop-derived MAOC correlated well with qClvR (Figure 3b, R² = .73, i.e., to ¹⁴C in bulk soil without root fragments), while ¹⁴C in POC showed no relationship with qClvR (Figure 3e, R² = .15). In contrast, ¹⁴C in POC correlated well with the isolated root fragments (>250 μm; Figure 3f, R² = .53), while no correlation was found between ¹⁴C in MAOC and that in root fragments (Figure 3c, R² = .13). In combination with the significantly lower C:N ratio for MAOC than for POC (Table 1, statistics not shown), the simple fractionation protocol applied was considered appropriate for distinguishing between C pools with different origins.

After decomposition of belowground inputs for 1 year at room temperature in undisturbed columns (t₂), the vast majority of the roots had decomposed, leaving bulk soil for fractionation with decomposition products of cover crop inputs (there was no isolation of roots at t₁). This presumably accounts for the relative increase in POC compared with MAOC at t₂ (Figure S2). Thus, the bulk soil samples from t₁ and t₂ express inherently different C pools. Any comparison of fractions between t₁ and t₂ should be undertaken in the knowledge that roots had partly been removed by sieving at t₁, but allowed to decompose in intact columns at t₂. Bearing this in mind, Figure 4 shows the cover crop-derived MAOC and POC at t₁ and t₂. As described above, MAOC followed the pattern of qClvR, while POC followed that of root fragments at t₁ (Figure 3b,f). Between t₁ and t₂, the decomposition of root C generally resulted in higher POC, especially in the case of the high root input from radish (Figure 4b), and higher MAOC at t₂ than t₁ in the topsoil, except for rye (Figure 4b). In the subsoil, however, MAOC was lower at t₂ than at t₁ for all species, with pronounced and significant decreases for rye and radish (p = .001 and p = .003, respectively).

The total belowground cover crop-derived C remaining after 1 year of decomposition of roots and rhizodeposition inputs in the intact soil columns (at t₂ relative to t₁) ranged between 20% and 44% in topssoils and between 33% and 74% in subsoils (Table 4). The estimated MAOC and POC formation efficiencies (MAOCFE and POCFE) by belowground C inputs showed that vetch displayed the highest MAOCFE in the
topsoil, which was significantly higher than radish \((p = .026)\) and closer to rye \((p = .504)\) than to chicory \((p = .078)\). In the subsoil, there were no significant differences between species, but radish and vetch tended to show higher \(\text{POC}_{\text{FE}}\) than rye and chicory (Table 4).

In the topsoil, \(\text{POC}_{\text{FE}}\) were, like for \(\text{MAOM}_{\text{FE}}\), lower for the two taproots (radish and chicory) compared to rye and vetch. Radish and chicory also showed significantly lower \(\text{POC}_{\text{FE}}\) in the topsoil than in the subsoil, while vetch and rye showed similar \(\text{POC}_{\text{FE}}\) across depths.

## 4 | DISCUSSION

Cover crop root quality and morphology potentially determine soil C inputs and stabilization (Cotrufo et al., 2013; Stewart et al., 2017). This study investigated how the quantity and quality of C inputs varied between cover crops species with known differences in root morphology, and how these inputs translated into the distinctive soil C pools of MAOC and POC. The discussion of
the main results is structured in accordance with the study’s three hypotheses.

4.1 | Rhizodeposition C: A matter of root C? (hypothesis I)

Belowground C allocation was highly species dependent, as shown by the very different root:shoot ratios of the cover crop species studied here (Table 3). However, it has been proposed that further partitioning between root C and rhizodeposition C is species independent (Pausch & Kuzyakov, 2018), corresponding to constant %ClvR (hypothesis I). The results of the present study revealed a distinct pattern, with substantially higher %ClvR (Figure 1c) or rhizodeposition-to-root C ratios (Table S2) for the more branched (rye and vetch) root systems than for the less branched (radish and chicory) root systems in the topsoil. These results thus suggest a strong effect of root morphology on rhizodeposition—at least in the topsoil.

Although mainly studied for trees and shrubs, C exudation has been found to be higher for fine roots than for thick roots (Herms et al., 2022). Finer roots have a larger surface area for exudation (Guo et al., 2004; Nguyen, 2003), a shorter lifespan (Guo et al., 2008), and a faster turnover (Xia et al., 2010), as characterized by higher concentrations of N and labile C compounds than root systems with thicker roots rich in slowly decomposing cellulose and lignin (Guo et al., 2004). As rhizodeposition by definition consists of both exudates and decomposition products of finer root parts of the living plant (Rasmussen, 2011), it is highly reasonable to suggest that root morphology is a determinant of rhizodeposition C.

Albeit root C is an important indicator of root growth, it fails to provide insights into the processes at the root-soil interface, such as exudation and decomposition patterns. While root C, in the current study, was unable to explain the variation in qClvR, a rather coarse isolation of the soil adhering to the root system (rhizosphere soil) proved a better predictor (Figure 2b). Here, the amount of rhizosphere soil could either be interpreted as an indirect measure of the root surface area available for exudation processes, and/or simply as an indirect measure of rhizodeposition due to its adhesive properties (Galloway et al., 2020).

Based on the present findings, the hypothesis of a constant rhizodeposition-to-root ratio was rejected. An alternative hypothesis is proposed that the variation in rhizodeposition across species is instead a matter of root morphology (e.g., specific root length or root surface).

4.2 | Rhizodeposition C with depth: An N effect? (hypothesis II)

The percentage of belowground C lost via rhizodeposition (%ClvR) was found not only to vary by cover crop species but also by depth. In the topsoil, the %ClvR ranged between 6% and 22%, which is similar to or higher than the reported range in field trial-based literature (Austin et al., 2017; De Notaris et al., 2020; Engedal, Karlsson, et al., 2023; Mortensen et al., 2021). The %ClvR in the subsoil was, for nonlegumes, significantly higher than in the topsoil, ranging between 17% and 39%. Hirte et al. (2018) also show significant increases in %ClvR for the 25–50 cm soil layer compared with topsoil for maize and wheat, and ascribe the pattern to downward-moving dissolved organic matter and a higher relative abundance of finer roots in the subsoil interpreted as rhizodeposition. Angst et al. (2018) report increased fine root mortality in more nutrient-poor soils. All three explanations could be relevant in the present study. In contrast, Peixoto et al. (2020) found higher %ClvR in the topsoil (0–25 cm) than in the subsoil (1–1.2 m) and deep subsoil (1.2–3.6 m), and ascribe it to topsoil C investments in plant-microbial interactions supporting nutrient mobilization.

The results of the present study showed that nonlegumes had significantly higher %ClvR in the subsoil than in the topsoil, whereas that of vetch remained at 21%–22% throughout the profile (Figure 1c), suggesting an N effect of rhizodeposition with depth. Possible N limitations for nonlegumes in the subsoil could induce higher C expenditure as rhizodeposition to spur N mobilization.
In the subsoil, plant-available N was lower than in the topsoil due to lower inherent soil nitrogen (Table 1) and because the application of mineral N during the growth was applied from the soil surface with the irrigation water. N scarcity was hence more likely in the subsoil. Low N availability has been reported to cause greater root hair production (Robinson & Rorison, 1987), increasing the specific surface area, both of which could lead to higher relative rhizodeposition (Nguyen, 2003; Section 4.1). N availability in the topsoil has been shown to be negatively correlated with %ClvR (Engedal, Karlsson, et al., 2023; Mortensen et al., 2021). As thoroughly reviewed in Bowsher et al. (2018), many factors are at play in regulating root (exudation) response to soil N availability. While suggested mechanisms do include higher (active) root exudation for N mobilization, as well as higher (passive) C loss from the...
root due to a higher solute concentration gradient combined with a compromised root membrane integrity at low N availability, the literature remains inconclusive in terms of both the direction and magnitude of the N effect on rhizodeposition. A more readily applicable explanation could be that suboptimal conditions (low C and/or N) in the subsoil hamper microbial activity and cause a (s)lower turnover of rhizodeposition (Button et al., 2022). Based on the findings of the present study, hypothesis II that the contribution of decomposing roots, showed increments in MAOC in the topsoil, except for rye, possibly reflecting low microbial carbon use efficiency in the decomposition of the large amount of low quality (high C:N ratio) rye root. In contrast, root inputs to the subsoil were not sufficient to make up for the loss in MAOC between t1 and t2 for any of the species. Seemingly, microbial maintenance respiration exceeded the C input from decaying roots, leading to a net loss of MAOM. This was most pronounced for rye and radish (Table 4), for example, the contribution of decomposing roots, showed increments in MAOC in the topsoil, except for rye, possibly reflecting low microbial carbon use efficiency in the decomposition of the large amount of low quality (high C:N ratio) rye root. In contrast, root inputs to the subsoil were not sufficient to make up for the loss in MAOC between t1 and t2 for any of the species. Seemingly, microbial maintenance respiration exceeded the C input from decaying roots, leading to a net loss of MAOM. This was most pronounced for rye and radish (Table 4).

4.3 MAOC formation efficiency: A matter of root quality? (hypothesis III)

Given the recent shift in theoretical paradigm, a growing body of literature is currently investigating the effect of litter quality (e.g., C:N ratio) on the formation of C fractions with distinct turnover times and the relative importance of different C stabilization mechanisms (Cotrufo et al., 2013; Cotrufo & Lavallee, 2022). In the current study, the C remaining in the MAOC (<50 μm) and the POC (>50 μm) fraction was quantified, and the MAOC and POC formation efficiencies (MAOC FE, POC FE) were estimated by relating these fractions to the total belowground C input.

First, the development from t1 to t2 (Figure 4), for example, the contribution of decomposing roots, showed increments in MAOC in the topsoil, except for rye, possibly reflecting low microbial carbon use efficiency in the decomposition of the large amount of low quality (high C:N ratio) rye. In contrast, root inputs to the subsoil were not sufficient to make up for the loss in MAOC between t1 and t2 for any of the species. Seemingly, microbial maintenance respiration exceeded the C input from decaying roots, leading to a net loss of MAOM. This was most pronounced for rye and radish, indicating that SOC formation is primarily quantity driven. However, MAOC and POC formation efficiencies (Table 4)

<table>
<thead>
<tr>
<th>MAOC FE by belowground C input (%)</th>
<th>POC FE by belowground C input (%)</th>
<th>Total remaining C of belowground C input (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topsoil Subsoil</td>
<td>Topsoil Subsoil</td>
<td>Topsoil Subsoil</td>
</tr>
<tr>
<td>Rye</td>
<td>15.2 (3.0)AB 9.8 (2.0)*</td>
<td>20.6 (3.3)a</td>
</tr>
<tr>
<td>Radish</td>
<td>8.5 (1.7)B * 15.4 (3.0)b</td>
<td>14.9 (2.4)AB ** 58.3 (9.3)a</td>
</tr>
<tr>
<td>Chicory</td>
<td>10.0 (2.0)AB 10.7 (2.1)a</td>
<td>9.4 (1.5)b ** 21.9 (3.5)b</td>
</tr>
<tr>
<td>Vetch</td>
<td>21.1 (4.1)A 14.1 (2.7)a</td>
<td>22.4 (3.6)a</td>
</tr>
</tbody>
</table>

Based on the findings of the present study, hypothesis II that the relative loss of C to rhizodeposition changes with depth is accepted. It is proposed that %ClivR increases with depth governed by N limitation, but further investigation is needed.
seemed to be affected by both root C:N ratio and the pattern seen for the relative C allocation to rhizodeposition (%ClvR; Section 4.1). Overall, root C:N ratio seemed to be negatively correlated with both MAOC and POC-formation efficiencies at both depths, suggesting that high-quality (low C:N ratio) input results in higher microbial carbon use efficiency, as proposed in the Microbial Efficiency-Matrix Stabilization (MEMS) framework (Cotrufo et al., 2013). Based on a long-term cover crop trial, Zhang et al. (2022) also found higher MAOC under legume cover crops than under nonlegume cover crops, despite significantly lower shoot and root biomass by legumes. The higher formation efficiency under legumes could be linked to exudation of high-quality organic N compounds (Wichern et al., 2007) leading to greater relative microbial stabilization (Peixoto et al., 2022).

Interestingly, and more pronounced than for the C:N ratio, MAOCFE and POCFE seemed to closely follow the %ClvR (or qClvR-to-root ratio) in the topsoil, suggesting a direct link between the proportion remaining as rhizodeposition at t1 and the proportion remaining in SOC pools at t2. In terms of MAOCFE, this is in agreement with Villarino et al. (2021) who, based on experiments with decomposing roots with or without rhizodeposition (a so-called living plant experiment vs. incubation of isolated roots), suggest that rhizodeposition has a much higher MAOCFE (46%) than roots (9%). This is, again, supposedly due to the high quality of the rhizodeposits that are readily available for microbial assimilation (Cotrufo et al., 2013).

The pattern in MAOCFE could also be a direct effect of differences in root morphology. Indeed, studies on switchgrass ecotypes with varying specific root length (SRL) show increased root-derived microbial C from finer rooted ecotypes (Stewart et al., 2017), and with varying specific root length (SRL) show increased root-derived MAOCFE indirectly through higher C allocated as rhizodeposition, and directly as providing a surface area for in vivo and ex vivo transformation of fresh C. However, the possible effect of root morphology was only apparent in the topsoil, suggesting a need for further investigation of subsoil C input and cycling. The results also support the need for root structural data to be incorporated into research designs to improve the understanding of the impact of root spatial configuration on C cycling (Adkins et al., 2016; Wu et al., 2007).

4.4 | Methodological considerations

The most critical aspect of this study was the reliance throughout on size-based, operationally defined soil C fractions. The results found in this study could arise simply from a greater abundance of finer roots for rye and vetch, which would partially end up in the <250 μm fraction in terms of rhizodeposition and in the <50 μm fraction, and be interpreted as mineral-associated although they are “particulate.” However, Figure 3 gives a strong indication of the origin of the material recovered in the different fractions: 14C recovered in the root fragments (RF) pool (<250 μm) correlated with 14C in the >50 μm fraction (interpreted as POC, which was not the case for the <50 μm fraction (interpreted as MAOC). Meanwhile, 14C found in the bulk soil without root fragments (interpreted as rhizodeposition) correlated with the <50 μm fraction (MAOC) rather than with the >50 μm fraction (POC). Given the simple
fractionation scheme, at least some actual POC of a size below 50 μm will end up in the operationally defined MAOC pool. Yet, such a small POC has been found to be a relatively minor pool, especially in sandy soil (Angst et al., 2018; Witzgall et al., 2021), and, if occluded in aggregates, equally protected in the long term as MAOC (Angst et al., 2017). Thus, the overall interpretations of these results do not change. Also, the C:N ratio signature of the two C pools was found to be significantly different (p < 0.0001, Table 1), with MAOC being lower (10–11) than POC (15–16), which is in the same range as for MAOC and oPOC in Zhang et al. (2022). Again, this supports the differentiated origins of the pools as more microbial-based (MAOC) and plant-based (POC; Lavallee et al., 2020). Thus, this confirms the suitability of the simple fractionation scheme for the purposes of this study. In future studies, it is recommended that different soil C pools are examined rather than total SOC to improve understanding of C dynamics.

Other critical methodological choices that might lead to biases arise from studying four different species that reach plant developmental stages and degrees of turnover at very different points in time. This may be further accentuated when grown at close to optimal conditions, and where the maturity of the fastest growing species determines the timing of termination (t1). For instance, when the plants were terminated, the fast-growing radish and rye had started flowering and ripening, respectively, while the vetch and especially chicory were still at an earlier phenological stage. The phenological stage is closely linked to root responses, altering growth, morphology, and turnover (Poorter et al., 2012), and thus has implications for the estimation of net rhizodeposition C at any point in time (Hupe et al., 2019; Pausch & Kuzyakov, 2018). Similarly, the rhizodeposition from vetch might be of a higher quality (lower C:N ratio; Wichern et al., 2007), and hence decompose at a faster rate than for the other species, resulting in lower estimated net C for vetch at t1, while constituting a more stable C pool. Furthermore, the simple fractionation scheme used in the present study does not allow any differentiation between free and occluded POC. This hampers the interpretation of POC and POCFE due to the different decomposition rate of the four species. For example, 74% of the belowground C input from radish remained in the subsoil at t2. Most of this was found in the POC pool (Figure 4b) and is likely to be due to the subsoil roots not being fully decomposed at this time. Such biases present challenges for the analytical interpretation of studies across plant species, and more studies are therefore needed to further establish the relative ranking of cover crops in terms of C input and stabilization.

Lastly, estimation of rhizodeposition made in pot or column trials cannot readily be transferred to field-based situations, as not only root-to-shoot but also rhizodeposition-to-root ratios are significantly different (Hupe et al., 2019). Therefore, future trials should aim to investigate the impact of root morphology on rhizodeposition in field conditions.

5 | CONCLUSIONS

This study identified the highest root C input from the fast-growing rye and radish with the presence of thick taproots of radish and chicory mainly in the topsoil. The percentage of C lost via rhizodeposition revealed a distinct pattern, with 22% for the more branched roots (rye and vetch) and 6%–8% for the less branched roots (radish and chicory) in the topsoil, suggesting that root morphology plays a key role in determining rhizodeposition C. Soil adhering to the roots, as a suggested indirect proxy for root surface area, showed a clear correlation with the amount of C lost via rhizodeposition across all species and depths (R² = 0.82), while root C did not. After a 1-year incubation of the intact soil columns, the remaining cover crop-derived MAOC and POC were positively correlated with belowground inputs in absolute terms (root C + rhizodeposition C), suggesting that both pools are largely quantity driven. However, topsoil MAOC formation efficiencies (MAOC remaining as a share of belowground inputs) were higher for vetch and rye (21% and 15%, respectively) than for chicory and radish (9% and 10% respectively), suggesting the greater importance of rhizodeposition (or root surface area) than solely substrate C:N ratio for longer term C stabilization. Interestingly, the patterns appearing in the topsoil did not apply to the subsoil, suggesting that other mechanisms are at play at depth. The implications of these findings call for an acknowledgement of root morphology, among several other root traits, as an important determinant in soil C dynamics.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Zenodo repository at https://doi.org/10.5281/zenodo.8090465.

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