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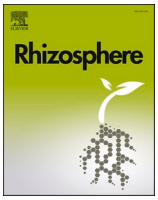

Simulated soil erosion predominantly affects fungal abundance in the rapeseed rhizosphere

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ABSTRACT

Eroded agricultural soils have reduced soil organic carbon (SOC) levels that may affect the plant-microbiome interactions in the rhizosphere. We explored the impact of simulated erosion on major microbial groups in a pot experiment with rapeseed (*Brassica napus* L.) grown on arable soil with the potential to capture SOC. An erosion gradient was simulated by admixture (0%, 12%, 24%) of subsoil horizon (Bt) to topsoil (Ap) material. Rapeseed plants were pulse-labeled with $^{14}CO_2$ at three growth stages and two soil compartments (bulk and rhizosphere soil) were sampled. Fungal ITS copy numbers were consistently higher in the rhizosphere and decreased with progressing plant growth stages. A significant increase of bacterial 16S rRNA gene copies in the rhizosphere only occurred at flowering. A response of fungal abundance to subsoil admixture was found detectable based on fungi:Bacteria and fungi:Archaea ratio at flowering. Archaea were neither affected by soil compartment nor subsoil admixture. ^{14}C activity of microbial biomass, an indicator for relative input of freshly assimilated C into soil microbiome, was impacted by growth stage and compartment and decreased with ongoing growth stage. During the rosette growth stage, the ^{14}C activity of the microbial biomass was elevated in the rhizosphere of the eroded soil indicating a plant response to the erosion factor. Our experiment revealed a compositional separation of the fungal community along the simulated erosion gradient and a selection of fungi for the two different soil compartments at flowering. *Olpidimycetes*, *Fusarium* and *Rhizopus* and putative pathogens were enriched in the rhizosphere at flowering. Fungi may have a competitive advantage in the rhizosphere of strongly eroded and nutrient diluted soils due to ecological adaptation and morphological traits i.e. hyphae that can bypass soil areas with low nutrient availability.

1. Introduction

Agricultural soils are essential for food production while also storing a significant share of the 3.500–4.800 Pg of soil organic carbon (SOC) worldwide (Lehmann and Kleber, 2015). Croplands exhibit 30–40% lower SOC levels compared to soils under natural vegetation, which may be mitigated with carbon (C) sequestration efforts such as the 4 per 1000 initiative (Poeplau and Don, 2015; Rumpel et al., 2018, 2020). C saturation, which is a potential challenge in SOC sequestration efforts, is currently a contentious issue with an ongoing debate about its occurrence (Stewart et al., 2007; Georgiou et al., 2022; Begill et al., 2023; Wenzel et al., 2022). Both the high potential and the uncertainty of arable soils to act as C sinks, illustrate the need to identify potential target soils for SOC sequestration (Shukla et al., 2019). Increased SOC not only stores atmospheric CO_2, but also improves nutrient and water availability, thereby bolstering the resilience of eroded soils to sustain food production (Jansson et al., 2021). Eroded arable soils are a potential target for SOC sequestration efforts, erosion itself has already offset one third of human-induced C losses in croplands, it is unlikely to globally drive C sequestration (Wang et al., 2017; Lugato et al., 2018; Doetterl et al., 2016). However, on a landscape level, tillage-induced erosion has been demonstrated to form a temporary C sink (Harden et al., 1999). Dynamic replacement of C occurs when reduced net primary production is compensated by reduced decomposition of SOC at depositional sites, reduced efflux of mineralized C and increased belowground allocation of crops (Harden et al., 1999). The role of the

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microbiome in this phenomenon however is largely unresolved.

The dynamic replacement of C is tangled to the interactions between crops and the soil microbiome in the rhizosphere, a hotspot of plant-microbe and microbe-microbe interactions, shaped by C-rich root exudates and interaction with its abiotic environment (Kuzyakov and Razavi, 2019). Especially in early growth stages, plant roots and the associated rhizosphere constitute a temporally dynamic environment characterized by root exudation and metabolite exchange, modulating the microbial community (Handakumbura et al., 2021; Kuzyakov and Domanski, 2000). Plant growth parameters (i.e. root:shoot ratio, root biomass) can be used as proxy for the C released from roots as rhizosdeposts, as root growth and biomass is linked to passive C release (Nguyen, 2009). Consequently, isotopic labelling approaches can be used to quantify the retention of C in the small yet highly active pool of microbial biomass (C_{mic}) pool resulting from rhizodepositions (Pausch and Kuzyakov, 2018; Pausch et al., 2016)

The soil microbial carbon pump concept underscores the pivotal role of the soil microbiome as a key actor in root exudate and plant litter turnover, leading to SOC formation (Liang et al., 2017; Zhu et al., 2020; Lehmann and Kleber 2015). These microbial transformations in soils are predominantly driven by Bacteria and fungi, which constitute the majority of microbial biomass and microbial diversity to soils around the globe (Six et al., 2006). Bacteria exhibit the highest diversity and species richness in soils, allowing them to thrive on a wide range of organic substrates, forming biofilms on nutrient rich patches (Killham and Prosser, 2015). Fungi play a crucial role in soil structure formation at various of spatial scales via entanglement and enmeshment mechanisms, which lead to the stabilization of fungal-derived organic matter in soil aggregates (Six et al., 2004; Angst et al., 2021). Fungi perform the initial steps in the degradation of cellulose, lignin and other complex macromolecules and contribute to improved plant nutrient uptake and resistance to abiotic and biotic stress factors (Francioli et al., 2021; Setälä and McLean, 2004; Begum et al., 2019; Wieczorek et al., 2019). Mycorrhizal fungi are prominent symbionts that provide nutrients to plants in exchange for photosynthate C compounds. However, they do not form in Brassicaceae crops (Sharma et al., 2023). Archaea, a microbial group thought to be predominantly found in extromophilic soil environments, may also have a role in some agroecosystems due to their involvement in nutrient cycles and due to the plant growth-promoting traits recently detected in some Archaea (Naitam and Kaushik, 2021; Gubry-Rangin et al., 2010; Alori et al., 2020; Kemnitz et al., 2007).

Changes in SOC content due to erosion and altered temporal dynamics of root exudation by host plants likely influence the abundance and composition of the microbial community in the rhizosphere. A pot experiment employing ^{14}C pulse labeling of rapeseed as model crop was conducted to elucidate the impact of simulated erosion states (admixture of subsoil) and growth stage on the relative C allocation into the microbial biomass and the abundance of major microbial groups (Bacteria, Archaea, fungi) in the rhizosphere and bulk soil of an agricultural soil. We anticipated an increased abundance of Bacteria and fungi in the rhizosphere compared to bulk soil due to root exudate utilization. In the rhizosphere, no difference in abundance between admixture treatments is expected due to an adaption mechanisms of the host plant under reduced SOC. We furthermore hypothesize that the highest ^{14}C activity in the microbial biomass (C_{mic}) can be recovered at the earliest growth stage and expected a pronounced decline with ongoing plant growth. Due to increased belowground allocation of the host plant under simulated erosion, we did not expect a decrease of ^{14}C signature of the microbial biomass in the rhizosphere under lower SOC. On top of that, we aimed to assess differences in the fungal community composition via ITS metabarcoding and to identify marker species in bulk and rhizosphere during flowering. Therefore, we hypothesized that disparities in the fungal community composition at flowering arise from subsoil admixture and that distinct marker groups can be identified in bulk and rhizosphere soil.

2. Material and methods

2.1. Study site and experimental conditions

A pot experiment with soil from the Carbo-D ZALF research site in the Uckermark region, NE Germany (N53.379, E 13.785) was conducted (Sommer et al., 2016). The soil from a mid-slope position that was eroded by tillage operations was classified as a Nudiargic Luvisol (FAO). Topsoil (0–30 cm) consists of an Ap horizon (61% sand, 26% silt, 13% clay) with a 0.78% SOC, followed by a Bt horizon (30–60 cm) with 0.16% SOC (54% sand, 27% silt, 19% clay), soil characteristics and nutrient status were measured in the ZALF headquaters central lab (Tabel S1). Soil was transferred air dry to ZALF headquarters and sieved with a 5 mm sieve. The erosion gradient simulates subsoil admixture into the topsoil horizon through tillage operations, the driving force of erosion at the research site. Three soil treatments with increasing Bt admixture were used: 0% Bt admixture (control) (0.78% SOC), 12% Bt admixture (0.72% SOC) and 24% Bt admixture (0.65% SOC) (Fig. 1). Dry soil was mixed thoroughly before rewetting and basal fertilization with 1g of Wopil (Siegfried W. Arnold e.K., Halle, Germany) (14% N (NH4 and NO3), 6% P (P2O5), 24% K (K2O) 3% Mg (MgO) and trace elements (B, Fe, Cu, Mn, Mo,Zn) and 0.1 g Kieserit (25% MgO, 20% SO_3) dissolved in 100 ml H_2O per pot.

Rewetted soil (3513g dry weight soil) was transferred to polypropylene pots (104.6 mm inner diameter) and filled without compaction to a height of 31.5 cm to reach 1.3 g/cm^3 bulk density, the remaining water to reach 60% WHC was added. Three pre soaked, uncoated seedlings of rapeseed (cultivar CAMPINO, NPZ KG) were placed in unfertilized soil to a depth of ~15 mm in a reduction tube (212 g dry weight soil, 45.5 mm inner diameter, 100 mm height). Seedlings emerged 2–5 days after sowing, two weeks after sowing a single plant was selected and the others removed, later the reduction tubes were placed on top of the large pots and sealed with a tailored lid to allow irrigation and soil respiration as described by Remus et al. (2022). Plants were cultivated in growth chambers under simulated early spring to summer conditions. Light settings were set to 12h of light with an intensity of 350 μmol m^{-2} s^{-1} on top of the pots, the floor was regularly lowered to adjust for plant height. Initially, temperatures were set to 8 °C during light and 5 °C during darkness. After 40 days temperatures were increased to 18 °C and 15 °C respectively and after 90 days temperatures were increased to 28 °C during the light phase and 25 °C at darkness, while relative humidity was kept at 60% throughout the growth period.

Three growth stages with distinct characteristics were sampled on four consecutive days according to individual plant development determined by EC development stage of rapeseed (Fig. 1). Plants were labeled 21 days prior to harvest on four consecutive days according to plant development, each plant was pulse labeled individually in a sealed bag for 6 h with 0.5 MBq (rosette and flowering) and 1 MBq (ripening) of $^{14}CO_2$ according to the procedure described in detail by Remus et al. (2016). The following growth stages were sampled: (i) rosette stage: labeling ~40 days (EC: 15–16), harvest and sampling~60 days after seedling emergence (EC 21–23); (ii) flowering stage: labeling ~60 days (EC:30–32), harvest and sampling ~80 days after seedling emergence (EC: 65–69); (iii) ripening stage: labeling ~120 days (EC:72–76), harvest and sampling ~140 days after seedling emergence (EC: 85–89). Plants that were grown to flowering and ripening were additionally fertilized with above mentioned solution after 60 days and plants grown to ripening were fertilized with 0.25 g of NH_4NO_3 during flowering to maintain plant growth. Five biological replicates were used for each growth stage and admixture treatment.

2.2. Sample collection

21 days after ^{14}C labeling the plants were harvested. Aboveground and belowground dry biomass was determined separately by drying

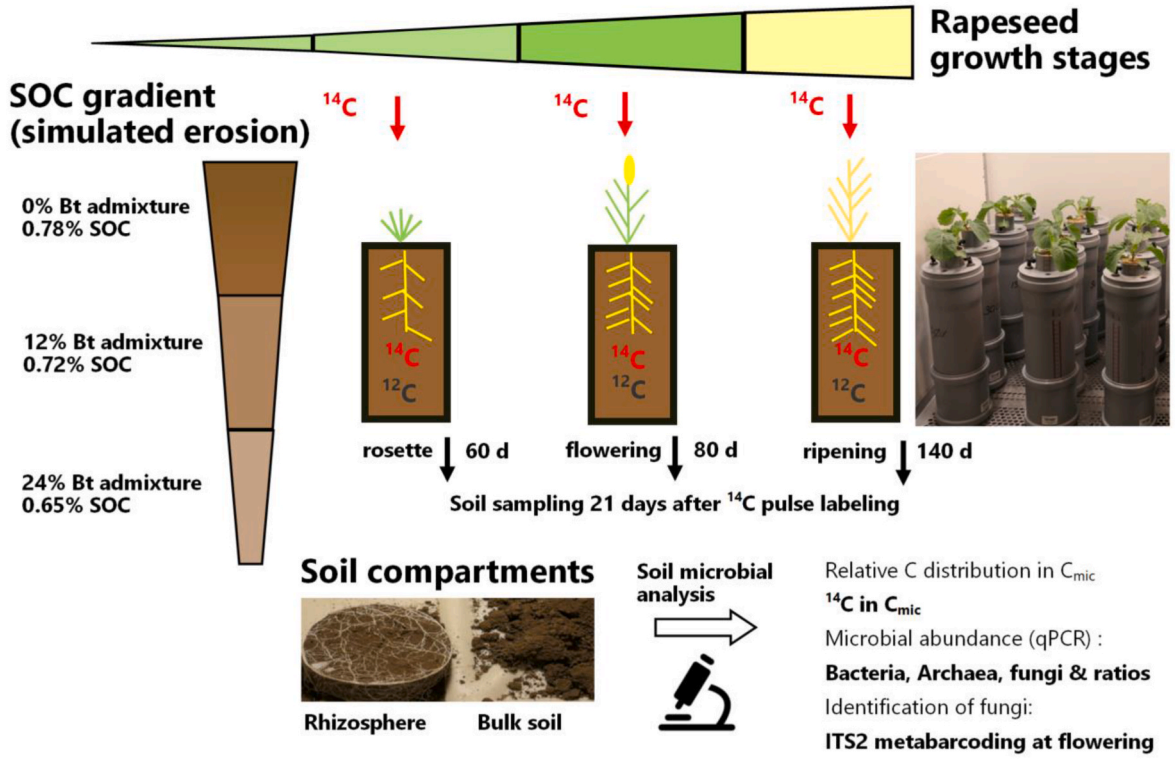

Fig. 1. Research design with simulated erosion (subsoil admixture) leading to SOC gradient, rapeseed growth stages reflecting distinct characteristics of the host plant and sampled soil compartments. Microbial analyses were conducted on both rhizosphere and bulk soil. ^{14}C labelling 21 day before soil sampling is highlighted in red.

plants at 105 °C. Rhizosphere was collected by removing large aggregates off the root ball and remaining fine aggregates attached to roots, together with soil attached to fine roots were defined as rhizosphere soil. Fine root selection with soil attached was done immediately after harvest by hand. The soil that was not attached to roots was defined as bulk soil. Rhizosphere soil was washed off the selected roots with 600 ml deionized water in a flask until roots were submerged and then put on rotary shaker at 150 rpm for 15 min. Afterwards roots were removed and the soil suspension was centrifuged in polypropylene bottles for 30 min at 4700×*g*. The supernatant was removed and the soil stored at 4 °C for CFE and at −20 °C for other analysis. Dry mass of the soil was determined at 105 °C using 30g of fresh soil for bulk and 1g of rhizosphere soil respectively.

2.3. Soil carbon measurement

SOC was determined individually for each pot after sampling in bulk soil to confirm the Total C (TC), SOC and Total Inorganic C (TIC) gradient due to admixture treatment (Table S2). The bulk soil was defrosted and sieved with a 2 mm mesh before visible root fragments were removed. Samples were then dried at 105 °C for at least two days. For total C determination in soil, 2 g of soil was weight out and samples were combusted in an Multi EA 4000 (Jena Analytiks, Germany) at 1200 °C with 20 mg of $CaCO_3$ as standard. Organic C was determined by weighting 2g of samples, destruction of carbonates of dry samples was performed by covering the sample with 1.25 ml of 10% HCl before evaporation for two days and drying at 35 °C for 5 h. Samples were combusted afterwards as described above. The following equation was used to calculate TIC from the samples TIC = TC – SOC.

2.4. Microbial biomass determination and ^{14}C activity of CFE extracts

Soil was stored at 4 °C before microbial biomass was determined with a modified protocol of the chloroform fumigation extraction (CFE) method (Brookes et al., 1985). Briefly, 5 g of soil (2.5 g for rhizosphere soil samples with low amounts of soil) was used per fumigated and non-fumigated sample. Fumigation was performed with ethanol-free chloroform (CAS No. 67-66-3, Carl Roth) for 24 h. Extraction of fumigated and non-fumigated samples was done with 20 ml (10 ml for samples with 2.5 g) of 0.05M K_2SO_4 for 30 min while horizontally shaking at 250 rpm, followed by centrifugation at 4700*g for 10 min. The supernatant was stored at −20 °C before further analysis. Total C in the samples was determined with a multi N/C 3100 (Analytik Jena, Germany). Carbon in microbial biomass (C_{mic}) was calculated by subtracting the non-fumigated sample from the fumigated and division by the correction factor of 0.45 (Vance et al., 1987).

^{14}C activity in the CFE extracts was determined with liquid scintillation counting. 4 ml of CFE extract was mixed with 16 ml of Ultima Gold™ uLLT (Perkin) and measured on a liquid scintillation counter (TriCarb 2800 TR, PerkiElmer, Rodgau, Germany). A sample with CFE extraction buffer was used to adjust for background radiation. To account for the higher amount of applied ^{14}C during ripening the Bq values of this growth stage were multiplied by 0.5. ^{14}C activity was calculated in Bq per g dw soil for fumigated and non-fumigated samples. ^{14}C activity of non-fumigated sample was subtracted from the respective sample to determine the ^{14}C activity of C_{mic} in Bq g^{-1}dw soil. Hereafter, ^{14}C of Cmic was divided by the respective Cmic values (in µg C_{mic} g^{-1} dw soil) to determine the specific ^{14}C activity per µg C_{mic}. Dry mass of rhizosphere soil and bulk was multiplied with the respective Bq g^{-1}dw soil and summed up to obtain the total Bq in Cmic in both soil compartments per pot. Total Bq in C_{mic} per pot was used to calculate the total % of ^{14}C that was recovered from the applied ^{14}C.

2.5. DNA extraction and marker gene quantification

Samples were stored at −20 °C before extraction with the DNeasy®

PowerSoil® Kit (Qiagen, Venlo, Netherlands). DNA concentrations for subsequent quantitative PCR (qPCR) were determined using a Qubit 2.0 (ThermoFisher Scientific) using the dsDNA-BR kit for calibration. Samples were normalized before qPCR. Bacterial 16S rRNA gene abundance was determined by qPCR using the 314F and 515R primer pair (López-Gutiérrez et al., 2004) (Table S3). One 20 µl reaction contained 10 µl of 2x Luna® Universal qPCR Master Mix, 500 µM of each primer and 4 ng of DNA template. PCR conditions were 2 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C, with a final elongation step at 72 °C for 5 min, followed by a melting curve analysis. Archael 16S rRNA gene abundance was determined with the primers 519F and 915R and 10 ng of template DNA (Herfort et al., 2009) (Table S3). PCR conditions were as above with following alterations: 12.5 µl of Luna® Universal qPCR Master Mix and annealing at 58 °C for 30 s (Table S3). ITS fungal copy numbers were quantified using ITS4R and ITS86F primers, 10 ng template DNA and conditions as described above besides 12.5 µl of Luna® Universal qPCR Master Mix per 20 µl reaction (White et al., 1990; Turenne et al., 1999) (Table S3). All qPCR's were performed on a qTOWER^{3}G (Analytik Jena, Germany) in duplicates. Ct values were calculated with qPCR SOFT 4.1. Efficiency and R square of the qPCR runs are shown in Table S3.

2.6. ITS2 metabarcoding

The characterization of the fungal community in the bulk and rhizosphere soil samples at flowering was performed using the same primers (with universal Illumina adapters) and PCR protocol employed for the qPCR as described above. The amplicons were purified with the MSB Spin PCRapace (INVITEK) and sent to LGC Genomics GmbH (Berlin, Germany) for barcoding and paired-end sequencing on Illumina MiSeq v3 platform. Demultiplexing was performed using Illumina bcl2fastq 2.17.1.14 software following clipping of barcode and sequencing adapters. Primers were removed using Cutadapt v3.4 (Martin, 2011) following sequence processing using QIIME 2 v2022.2 (Bolyen et al., 2019). Denoising was performed by using the build-in method for DADA2 (Callahan et al., 2016). ASVs produced by DADA2 were assigned to taxonomy using the naïve bayesian classifier (Wang et al., 2007) against the Unite 9.0 reference database (Nilsson et al., 2019). Alpha diversity metrics were calculated from the normalized sequence library, which was rarefied to 30,000 reads per sample. All raw sequences were deposited in the NCBI repository (SRA Accession: PRJNA993853).

2.7. Statistical analyses

Statistical analyses were performed with R version 4.2.1 (2022-06-23 ucrt) (R Core Team, 2022).

One way ANOVA aov() was performed to see if admixture significantly impacted ^{14}C activity among admixture treatments., Residuals were checked for normality with the shapiro.wilk() function and homogeneity of variance was checked with leveneTest(), followed by the Tukey-HSD test (TukeyHSD() function) for grouping. For non-normally distributed data, the Kruskal Walis test kruskal.walis() function was used, followed by the Pairwise Wilcox test (pairwise.wilcox.test(p.adjust.method = "fdr")) to determine the grouping of treatments.

qPCR copy numbers were calculated per g dw soil and ratios of microbial groups (fungi:Bacteria, fungi:Archaea and Archaea:Bacteria) were determined as described by Fierer et al. (2005) and subsequently calculated as % of copies encountered. Kruskal Walis test with the kruskal.walis() function followed by Pairwise Wilcox test (pairwise.wilcox.test(p.adjust.method = "fdr")) function was used to determine significant differences between treatments. Plant shoot, root and shoot:root ratio were analyzed with the Kruskal Wallis Test followed by the Pairwise Wilcox test as described above.

Differences in fungal richness between the samples investigated were estimated using ANOVA followed by Tukey's HSD post hoc test. Differences in the fungal community structure were determined across soil compartments and admixture treatment. First Bray-Curtis dissimilarities using Hellinger transformation were calculated (square root transformation of relative abundances; Legendre and Gallagher, 2001). Permutational multivariate analysis of variances (PERMANOVA) based on Bray-Curtis dissimilarity was performed to analyze the effect of the abovementioned experimental factors on the fungal community structure using 999 permutations for each test. Fungal biomarker taxa were identified by explaining differences between the soil compartments and between admixture treatments at flowering growth stage by employing a linear discriminant analysis effect size (LEfSe) (Segata et al., 2011). Graphs were made with the ggplot2 package.

3. Results

3.1. Response of rapeseed, C_{mic} and ^{14}C activity in C_{mic} to simulated erosion and growth stage

The shoot biomass consistently increased with growth stage and no significant differences were found between the admixture treatments (Fig. S1). The rapid development of the summer rapeseed variety is reflected by the approximate threefold increase in dry shoot biomass from rosette to flowering growth stage, while from flowering to ripening the shoot biomass further increased by about 75% in all admixture treatments. Root biomass was significantly lower at rosette growth stage than flowering for all subsoil admixture treatments. Root:shoot ratios (Table S4) decreased for all admixture treatments with consecutive growth stage, indicating highest belowground allocation of biomass in early growth stages. For the 24% treatment, the root:shoot ratio was significantly higher ($p = 0.019$) at flowering than for the other admixture treatments, indicating higher relative belowground allocation of C at this growth stage.

C in microbial biomass (C_{mic}) was affected by growth stage, compartment and admixture (Fig. S2) and significantly elevated in the rhizosphere compared to bulk soil ($p < 0.0001$).

In the rhizosphere, admixture significantly affected C_{mic}, the 24% treatment was lower when compared to the 0% treatment ($p = 0.047$). In this compartment growth stage significantly affected C_{mic}, during rosette C_{mic} was higher compared to ripening ($p = 0.004$); interaction of admixture and growth stage was non-significant. In the bulk soil compartment growth stage did not impact C_{mic}, but admixture was highly significant different ($p < 0.0001$). C_{mic} in 24% treatment was lower than the control treatment (0% subsoil, $p < 0.0001$) and 12% treatment ($p = 0.0086$), and lower in the 12% treatment compared to 0% ($p = 0.027$).

When ^{14}C activity of the sample was related to the microbial biomass of the sample (Bq*µg C_{mic}) a very pronounced effect of soil compartment and growth stage became apparent (Fig. 2). The activity per C_{mic} was highest at rosette growth stage and decreased in later growth stages. Furthermore, a strong effect of compartment was observed, the rhizosphere exhibited a much higher activity relative to C_{mic}. A significant impact of subsoil admixture was only found at the rosette growth stage for both compartments. The 12% and 24% admixture treatment had a higher ^{14}C signature than the control treatment in the rhizosphere ($p = 0.002$). In the bulk soil the 24% treatment had higher ^{14}C signature than both the 0% and 12% treatment ($p = 0.048$).

The total recovery of ^{14}C in C_{mic} for each pot showed a highly significant effect of growth stage ($p < 0.0001$), but no significant impact of admixture (Table 1). During rosette growth stage 0.82% of the ^{14}C activity was recovered, this decreased by more than half during flowering 0.30% and further decreased at ripening 0.15% (mean across admixture treatments). The total amount of activity recovered from the rhizosphere soil (mean across admixture treatments) also decreased from 12.5% at the rosette growth stage to 4.5% and 3.9% at flowering and ripening, respectively.

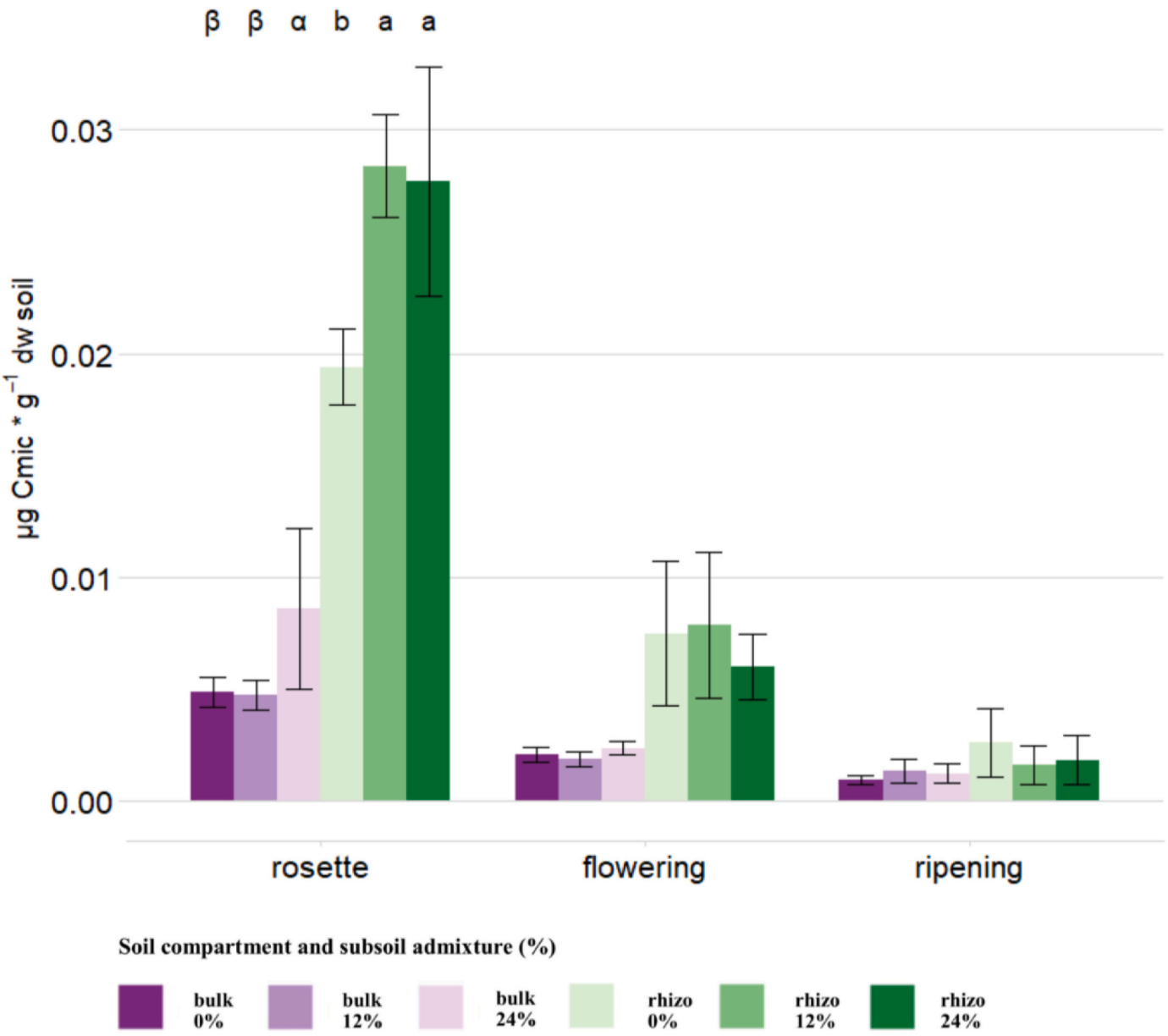

Fig. 2. ^{14}C activity per C_{mic} for different growth stages and soil compartments. Letters indicate grouping according to Tukey HSD test performed separately for growth stage and compartment combinations, greek letters indicate grouping in pairwise Wilcox test. Non-significant results are not shown. Error bars indicate standard deviation.

Table 1
Percentage of applied ^{14}C recovered in C_{mic} (rhizosphere and bulk soil combined) for different growth stages and admixture treatments. The values in brackets indicate the contribution of the rhizosphere soil (R) and bulk soil (B) respectively in percent to the overall ^{14}C activity per pot. Standard deviation is shown after percent values. Letters indicate grouping in pairwise Wilcox test for combination of all admixture treatments of a given growth stage.

	0% admixture	12% admixture	24% admixture	mean all admixtures
rosette	**0.83%** ± 0.11 (R: 13.8%, B: 86.2%)	**0.69%** ± 0.07 (R: 12.5%, B: 87.5%)	**0.94%** ± 0.40 (R: 11.3%, B: 88.7%)	**0.82% a** ± 0.25 (R: 12.5%, B: 87.5%)
flowering	**0.33%** ± 0.05 (R: 4.2 %, B: 95.8%)	**0.27%** ± 0.04 (R: 4.7%, B: 95.3%)	**0.30%** ± 0.03 (R: 3.9%, B: 96.1%)	**0.30% b** ± 0.04 (R: 4.5%, B: 95.5%)
ripening	**0.14%** ± 0.03 (R: 6.1%, B: 93.9%)	**0.18%** ± 0.07 (R: 2.3%, B: 97.7%)	**0.13%** ± 0.04 (R: 3.4%, B: 96.6%)	**0.15% c** ± 0.05 (R: 3.9%, B: 96.1%)

3.2. Abundance of microbial groups and microbial ratios

Fungal ITS gene copy numbers were significantly higher in the rhizosphere compared to the bulk soil at all plant growth stages and across the admixture treatments (Fig. 3A), particularly, for flowering ($p = 0.0005$) and ripening ($p = 0.0002$). Moreover, we observed that fungal ITS copy numbers decreased over plant growth which was highly significant in both rhizosphere and bulk soil ($p < 0.0001$). In both compartments the highest ITS copy numbers were observed at the rosette growth stage and they decreases steadily in the later growth stages. Fungal ITS copy numbers increased along the admixture gradient without reaching statistical significance (Fig. 3A). Only in the rosette growth stage a trend for increased ITS copy numbers in the rhizosphere was observed ($p = 0.075$).

The bacterial 16S rRNA gene decreased with increased subsoil admixture at flowering and ripening, which was however not statistically significant (Fig. 3B). We only found significantly higher abundance

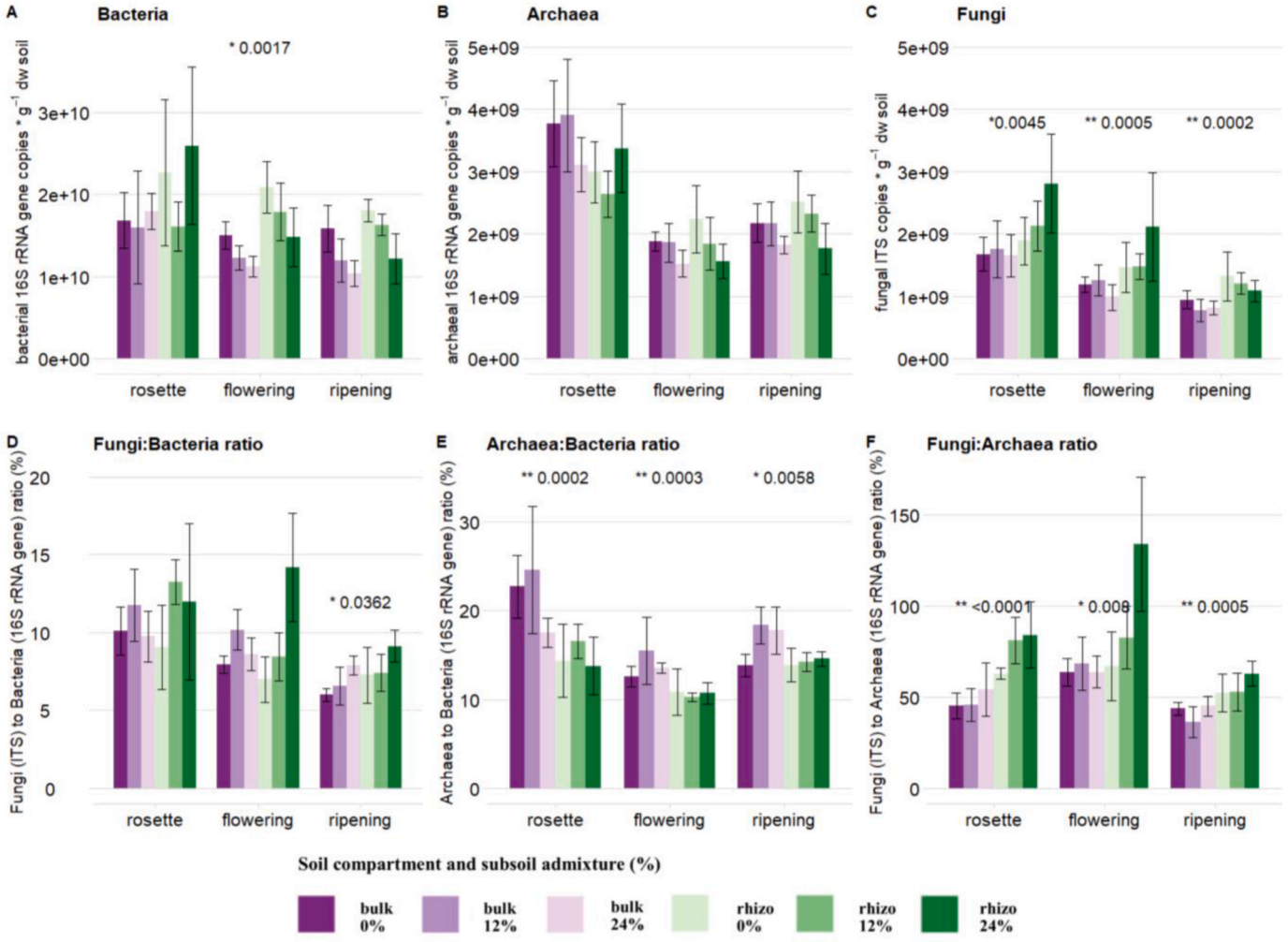

Fig. 3. Abundance of the fungal ITS marker (A), 16S rRNA gene of Bacteria (B), and Archaea (C), the ITS fungal marker (C), the ratio of fungi:Bacteria (D), Archaea: Bacteria (E) and Fungi:Archaea gene copies (in %). Bulk soil is shown in purple and rhizosphere soil in green, significant results in Kruskal Wallis test between bulk and rhizosphere soil are indicated above the growth stage with respective p-value, *$p < 0.05$, **$p < 0.001$, non-significant results are not shown. Error bars indicate standard error.

of the 16S rRNA gene of bacteria in the rhizosphere during the flowering stage ($p = 0.0017$). Growth stage only affected 16S rRNA gene copy numbers of Bacteria in the bulk soil ($p = 0.009$), with higher abundances found at rosette than for the other growth stages. The abundance of 16S rRNA genes of Archaea followed a similar trend as the 16S rRNA genes of Bacteria at flowering and ripening (Fig. 3C). The admixture gradient led to a decrease of Archaea without reaching significance. Moreover, the abundance of Archaea was not affected by soil compartment, but the impact of growth stage was highly significant in both rhizosphere and bulk soil ($p < 0.0001$) with highest abundances found in rosette growth stage.

Comparative analysis of the rhizosphere with bulk soil revealed a higher fungi:Bacteria ratios only at ripening ($p = 0.0362$), not for the other growth stages (Fig. 3D). Based on fungi:Bacteria ratios, fungi became increasingly abundant in the rhizosphere of some treatments with admixture, whereas the bulk soil samples did not exhibit a clear trend. In the rhizosphere, a significant ($p = 0.023$) higher fungi:Bacteria ratio was observed for the 24% subsoil admixture compared to the 0% treatment across all growth stages. Such differences were observed particularly at rosette for the 12% treatment ($p = 0.041$) and at flowering for the 24% treatment ($p = 0.041$), but not at ripening. In the bulk soil, the fungi:Bacteria ratio was only affected by the growth stage ($p < 0.0001$) and was highest in rosette and lowest at ripening.

The Archaea:Bacteria ratio (Fig. 3E) was significantly affected by soil compartment ($p < 0.001$) and growth stage ($p < 0.001$) but not by the subsoil admixture treatment. The Archaea:Bacteria ratio was consistently higher in bulk than rhizosphere soil (rosette $p = 0.00017$, flowering $p = 0.00028$, ripening $p = 0.0058$) indicating that Archaea are more abundant in bulk soil. For the fungi:Archaea ratio a greater value was found consistently in the rhizosphere (rosette $p < 0.0001$, flowering $p = 0.0084$, ripening $p < 0.0001$) again indicating an increased importance of fungi in the rhizosphere (Fig. 3F). In the rhizosphere, a significant ($p = 0.0021$) higher fungi:Archaea ratio was observed for the 24% subsoil admixture compared to the 0% treatment across all growth stages and a trend when 12% admixture was compared to 0% ($p = 0.068$). Effects of subsoil admixture on fungi:Archaea ratios were particularly observed for 12% treatment compared to 0% during rosette and flowering and for 24% compared to 0% ($p = 0.048$).

3.3. *ITS metabarcoding*

We characterized the fungal community structure at flowering due to the highest difference in abundance between ITS copy number in bulk and rhizosphere and the strongest effect of subsoil admixture observed at this growth stage. A total of 1,533,159 ITS high-quality reads were recovered from 24 soil samples (3 admixture treatments × 2 soil

compartments x 4 replicates), which clustered in 578 fungal ASVs. Overall, fungal sequences were assigned to six phyla, 23 classes, 49 orders, 109 families, 191 genera and 257 species. Ascomycota (73.4% of reads) was the dominant fungal phylum, followed by Basidiomycota (22.5%), while the other phyla represented a marginal fraction of the mycobiota, each accounting for less than 3% of the total fungal reads (Fig. S3). At a finer taxonomic level, *Solicoccozyma* (Basidiomycota) was the most abundant genera detected (21.3 % of fungal reads), followed by the Ascomycota genera *Talaromyces* (20.8%), *Gibellulopsis* (12.1%) and *Fusarium* (9.6%).

Fungal richness was not affected by neither compartment nor admixture, although we found a significant interaction between these two experimental variables (Table S5), suggesting a differential response of fungal richness to admixture across soil compartments. Indeed, fungal richness was significantly higher in the samples associated with bulk 24% admixture (highest richness) compared to bulk 0% and rhizosphere 24% (lowest richness) (Fig. S4).

Permutational multivariate analysis of variances (PERMANOVA) revealed that subsoil admixture was the main factor driving fungal community structure and captured 13.7% of the fungal community variation, whereas compartment accounted for 11.9% of variance (Table S6). We detected a significant interaction between the two experimental factors, which explained an additional 9.2% of fungal community variation. This interaction further indicated a differential response of the fungal community to soil compartment and subsoil admixture. Principal coordinate analysis (PCoA) corroborated these findings (Fig. 4). In the individual soil compartments (Fig. 4A & B), subsoil admixture significantly shaped the fungal community in bulk and rhizosphere soil along the gradient, accounting for 25.2% and 26.9% of fungal community variation, respectively (Tables S7a and S7b). Accordingly, it revealed a clear separation of the fungal community samples in the two soil compartments as observable along the first coordinate, while the second coordinate separated samples based on subsoil admixture treatments (Fig. 4C).

We used linear discriminant analysis (LDA) effect size (LEfSe) to explore which fungal taxa were responsible for the significant differences in community structures between bulk and rhizosphere soil (Fig. 5). On a higher level Leucanoromycetes, Chaetothyriales (Ascomycota), Mortierellomycetes (Mucoromycota) and Agaricales (Basidiomycota) were significantly ($p < 0.05$) enriched in the bulk soil. On a finer taxomic resolution some biomarker taxa of the bulk soil were *Podospora*, *Niesslia*, *Ophiosphaerella* (all Ascomycota), Psathyrellaceae (Basidiomycota), *Absidia* and *Mortierella* (both Mucoromycota). In the rhizosphere, a large proportion of fungal taxa associated with orders Glomerellales, Thelebolales and Xylariales (all Ascomycota) and with the class Olpidiomycetes (Olpidiomycota) were found. Furthermore the genera *Fusarium, Gibberella* (both Ascomycota) and *Truncatella* (Mucoromycota) and some unclassified Basidiomycota genera were marker for the rhizosphere.

Within bulk soil (Fig. 6a) and rhizosphere soil (Fig. 6b) LEfSe revealed different marker for the admixture treatments. In the 0% treatment of bulk soil multiple clades of Ascomycota were enriched, in the 12% treatment the genera *Cladosporium* and *Plectosphaerella* and in the 24% treatment the genera *Podospora*, *Phialophora* and *Neosetophoma* were found as markers (Fig. 6a). In the 0% treatment of the rhizosphere (Fig. 6b) the Basidiomycota *Solicoccozyma, Saitozyma,* Tremellomycetes and the Mucoromycota Mucoromycocetes were enriched, as well as the Ascomycota genera *Sagenomella* and *Phalophora.* In the treatments with admixture Xylariales and Thelebolales were significantly ($p < 0.05$) more abundant in both 12% and 24% treatment. Unique markers for the 12% treatment were the genera *Clonostachys*, *Geomyces* (Ascomycota) and *Morteriella* (Mucoromycota), whereas *Dendryphion*, *Cladosporium* and *Pseudogymnoascus* were unique markers of the treatment with 24% in the rhizosphere (Fig. 6b).

4. Discussion

4.1. ^{14}C signature of C_{mic} and plant response

^{14}C pulse labeling can be used to determine the relative C distribution in the plant-root-soil system at the time point of labeling. Thus, the relative C distribution of freshly assimilated C into C_{mic} at the time point of labeling is reflected in our study (Remus et al., 2016). Both relative proportion of ^{14}C as percentage recovered in C_{mic} and ^{14}C activity expressed in $Bq*\mu g^{-1}$ C_{mic} were highest at the rosette growth stage and sharply declined with ongoing growth stage. Relative allocation of C into rhizodeposition and belowground biomass is generally higher at early growth stages of many crops (Keith et al., 1986; Kuzyakov and Domanski 2000). This growth stage effect is particularly pronounced in annual crops compared to grasslands, underscoring the pivotal role of growth stage in crop-microbe interactions in arable soils (Pausch and Kuzyakov, 2018). Rhizodeposition of cereals crops such as spring rye peakes 40 days after plant emergence, which corresponds to the rosette growth stage of rapeseed in our experiment (Remus et al., 2016b). Absolute rhizodeposition of winter rapeseed is highest for inflorescence emergence in another study, which corresponds to sampling at flowering in our experiment (Remus et al., 2022). While rhizodepositions shape the rhizosphere soil of young plants, the results from ^{14}C labelling cannot be extrapolated to total belowground C allocation or C-fluxes, due to mass dilution effects and changing photosynthesis rates caused by plant growth stage (Remus et al., 2016; Swinnen et al., 1994).

For the rosette growth stage, the recovery of ^{14}C in C_{mic} (rhizosphere and bulk soil) is similar (1.2–0.86% of ^{14}C in C_{mic}) to various rice cultivars that were ^{14}C labeled 35 days after plant emergence (Tian et al., 2013), which are likely typical values across several plant species and developmental stages (Pausch and Kuzyakov, 2018). Higher ^{14}C activity of the C_{mic} pool in the rhizosphere during this growth stage suggests an increased rhizodeposition, to simulated erosion, that affects the microbial biomass. The soil microbial biomass is a small, yet active pool, and the relative small percentage of photosynthate C retained, does not reflect the much higher flux of C passing through the microbial biomass (Pausch et al., 2016; Pausch and Kuzyakov, 2018). The root-to-shoot ratio was highest at the rosette stage and decreased with ongoing plant development, hinting towards highest root growth rate in early plant development. Root growth is linked to root C deposition, which is often a passive diffusion from the cytoplasm of fine roots into the rhizosphere and a release of mucilage and senescent root cells (Nguyen, 2009). Senescent roots are another, albeit less important energy source for rhizosphere microorganisms during the growth period and root-derived litter was shown to contribute to the formation of new MAOC in eroded soils by microbial transformations (Kaštovská et al., 2024). High relative C rhizodepostion rates at early plant growth stages and limited extent rhizosphere likely explain why the highest ^{14}C activity C_{mic} values were recovered in the rhizosphere at the earliest growth stage in our experiment.

4.2. Effect of soil erosion and compartment on fungal, bacterial and archaeal abundances

In line with our hypothesis, our study demonstrates an increased abundance of fungi in the rhizosphere, the microbial hotspot for transformation of root exudates, for bacteria this was only shown at flowering. The high abundance of fungi in the rhizosphere is likely due to a competitive advantage of the encountered soil-borne fungi over Bacteria in the utilization of freshly assimilated C as rhizodeposits and senescent roots. Fungi are adaptable and mobile primary decomposers that traverse soils with sponge-like hyphal networks with the capability to utilize and degrade a wide variety of substrates reaching from low-molecular root exudates to complex polymers (Begum et al., 2019; Taylor and Sinsabaugh, 2015). Another competitive advantage of fungi is a reduced metabolic coefficient compared to Bacteria, which was

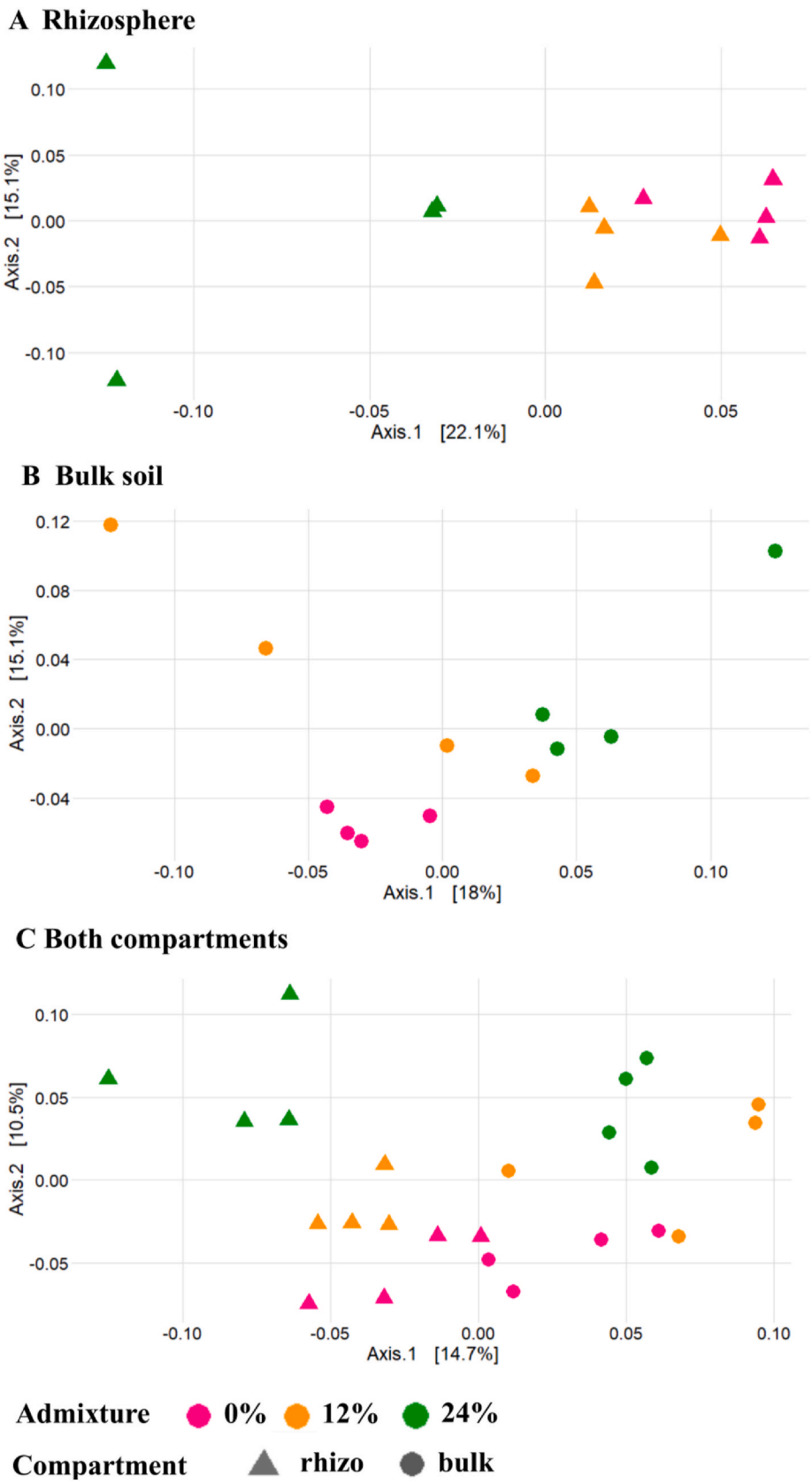

Fig. 4. PCoA2. Principal coordinates analysis (PCoA) of the fungal community structure of the rhizosphere (A), bulk soil (B) and both compartments (C) at the flowering growth stage.

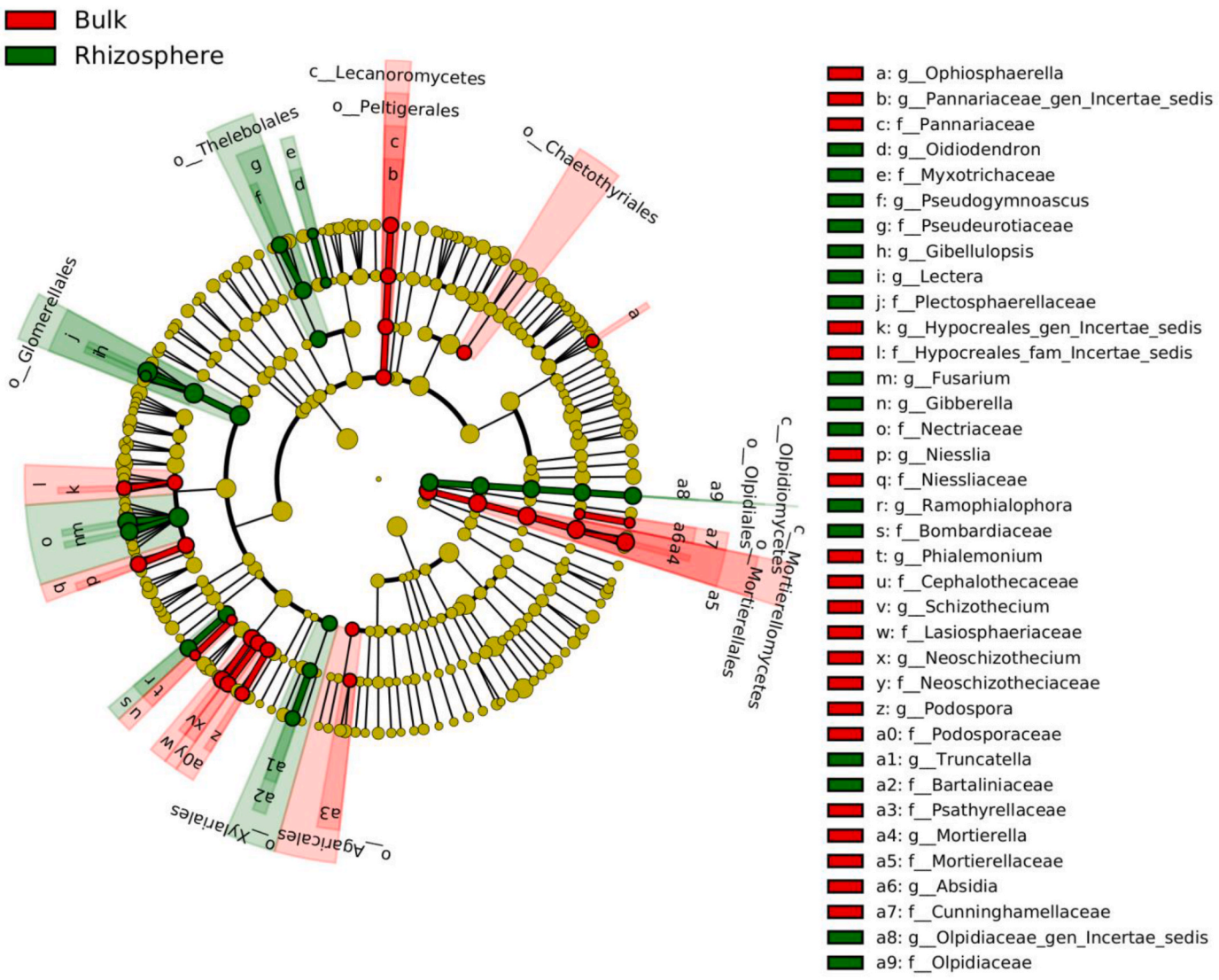

Fig. 5. LeFSe cladogram illustrating the taxonomic groups explaining the most variation among the fungal taxa between the bulk soil and the rhizosphere samples detected in the samples at the flowering growth stage. Each ring represents a taxonomic level, with phylum (p_), class (c_), order (o_), family (f_), and genus (g_) emanating from the centre to the periphery. Each circle is a taxonomic unit found in the dataset, with circles or nodes shown in colors (other than yellow) indicating where a taxon was significantly more abundant.

linked to reduced CO_2 losses and increased SOC sequestration in soils (Blagodatskaya and Anderson, 1998; Six et al., 2006). Lower SOC content of the eroded soil and response of the host crop appear to have further increase the prevalence of fungi. Increased fungi:Bacteria ratios as found in our experiment are also linked to a higher C storage potential and reduced respiration of C from freshly added litter (Malik et al., 2016). Especially the subsoil is characterized by large areas that lack either water or nutrients necessary for microbial growth. Many fungi are adapted to low nutrient and water limited conditions and mycelia stimulate the growth of Bacteria due to the transfer of nutrients and water to other soil areas (Worrich et al., 2017; Guhr et al., 2015; Begum et al., 2019; Taylor and Sinsabaugh, 2015). Soil Bacteria can synergistically grow in the direct vicinity of fungi and can thrive along the growth direction of hyphae in the soil (Meidute et al., 2008; Warmink and Van Elsas, 2009). In summary, the many ecological adaptions and traits of fungi allow for competitive growth at low nutrient conditions and their broad growth substrate spectra may explain the observed increased fungal abundance in the rhizopshere and higher fungi:Bacteria ratio under simulated erosion.

Bacterial 16S rRNA gene-based abundances were overall about five to ten times higher than the ITS marker of fungi, which does not necessarily reflect their relative importance in terms of biomass or activity in the investigate soil system. The fungal cell size is more variable than that of Bacteria, fungal cells may have no nucleus or multiple nuclei in a single cell (Strickland and Rousk, 2010). The abundance of rRNA genes is also variable for different species and life stages of filamentous Fungi (Black et al., 2013). This makes it difficult to solely imply a dominance of Bacteria in this soil based on the used markers. The relative C turnover rates of fungi are often much higher than that of Bacteria in arable soils (Pausch et al., 2016). Bacterial 16S rRNA copy numbers were about 10 times more abundant than for the same gene of Archaea. Hence, Bacteria were the predominant prokaryote in group our soil, which is consistent with the high prevalence of Bacteria in many top soils, while Archaea only contribute a small fraction (less than 10%) of the total prokaryotes (Bengtson et al., 2012; Kemnitz et al., 2007). While the relative abundance of Archaea may increase in subsoil horizons (i.e. Bt subsoil) (Kemnitz et al., 2007), our results did not show an increase of archaeal copy numbers with increased subsoil admixture. This seemingly discrepancy may be attributed to the lower adaptability and larger core microbiome of Archaea, which accounts for more than 50% in some

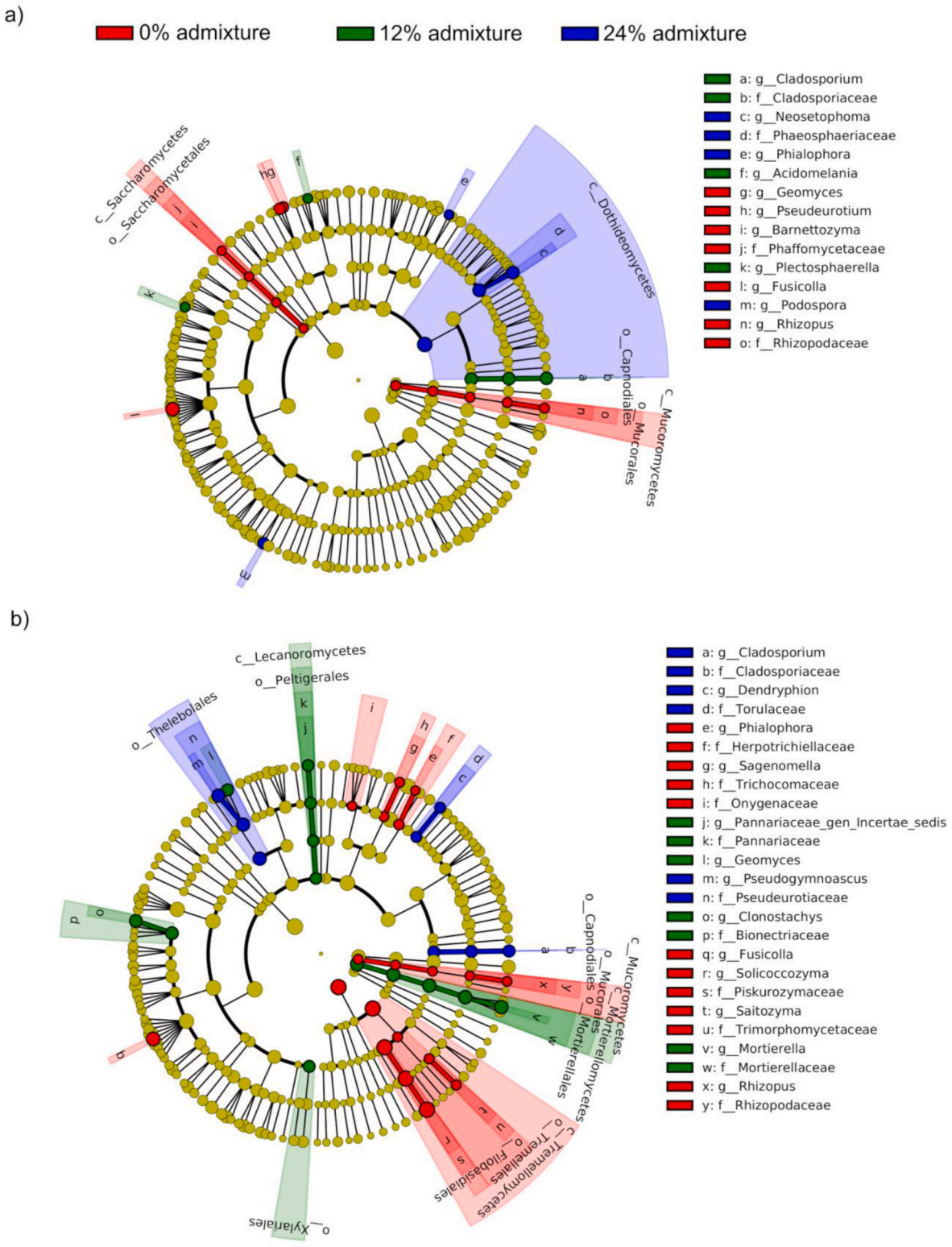

Fig. 6. LeFSe cladogram illustrating the taxonomic groups explaining the most variation among the fungal taxa between the admixture treatments detected in the (a) bulk and (b) rhizosphere soil at the flowering growth stage. Each ring represents a taxonomic level, with phylum (p_), class (c_), order (o_), family (f_), and genus (g_) emanating from the center to the periphery. Each circle is a taxonomic unit found in the dataset, with circles or nodes shown in colors (other than yellow) indicating where a taxon was significantly more abundant.

subsoils (Uksa et al., 2015). The quantification of Archaea in soils is less common and many published primers targeting Archaea have low coverage of known archaeal diversity. The primers used in our study cover more than 85% of known archaeal 16S rRNA genes (Tahon et al., 2021). Hence, our study supports (a) the general notion that Archaea are of minor subordinate importance in agricultural soils of temperate climate and (b) demonstrates that admixture of subsoil does not increase their abundance.

RNA based approaches (i.e. metatranscriptomics or RT-qPCR) may offer greater insights in the dynamic interactions of fungi with Bacteria in the rhizosphere as opposed to the DNA-based methods that we employed. The analysis of RNA in soils mitigates the impact of relic DNA of dead or inactive microorganisms, providing a more accurate representation of the active microbiome at the time of sampling. A combination of nucleic acid analysis with ^{13}C stable isotope probing and metabolomics, albeit technically challenging, may help to unravel highly complex rhizosphere interactions between host crop, fungi and bacteria, which currently remain unknown (Carvalhais et al., 2013; Karaoz et al., 2024).

4.3. Potential functional roles and biodiversity of the detected fungi

Research of plant-fungi interactions in the rhizosphere often focuses on mycorrhizal fungi, obligate endo-symbionts in plant roots and particularly arbuscular mycorrhizal fungi (AMF) in arable soils. Although rapeseed belonging to the non-mycorrhizal plant family Brassicaceae, does not form this symbiosis AMF have been shown to prevail in soil and rhizosphere of monocropped rapeseed for decades (Floc'h et al., 2020a,b). In our study, we did not find evidence for the occurrence of AMF but, the rhizosphere was shaped by putative pathogens and saprotrophic fungi. Some observed differences of the microbial community structures across the treatments might be caused by different initial fungi abundance and species composition of subsoil (Bt) caused by the different amounts of subsoil added to the Ap topsoil. For the compartment effect we found that members of Olpidiomycytes, a family of putative pathogenic fungi were enriched in the rhizosphere. The species *Olpidium brassicae (O. brassicae)* was frequently encountered in the rapeseed rhizosphere and is considered a pathogen and a core species of its rhizosphere microbiome (Lay et al., 2018; Floc'h et al., 2020a,b). However, there are contradicting observations on the pathogenicity and external factors contributing that contribute to the pathogenicity of *O. brassicae* in rapeseed. No association between canola yield and abundance of the supposed pathogen has been found in one study (Lay et al., 2018), while another study observed reduced plant growth with high infestation rates of *O. brassicae* (Hilton et al., 2013). We did not detect a decrease of shoot biomass for the 12% and 24% subsoil admixture treatments at any growth stage despite lower nutrient conditions in the eroded treatments.

The class *Mucoromycota* and the genus *Rhizopus* were enriched in the control treatment in the bulk soil and rhizosphere. *Mucuromycota* are important fungi in early cellulose degradation in soils (Koechli et al., 2019). Thus, the higher amount of plant-derived SOC and cellulose-rich substrate in the control treatment may explain the high abundance of *Mucoromycota*. The genera *Rhizopus* and *Fusarium,* enriched in the rhizosphere, were previously shown to occur mainly in the rhizosphere and as endophytes of Brassicacaea plants (Ishimoto et al., 2000; Poveda et al., 2022). Both genera are likely well adapted to glucosinulates, sulfur-rich Brassicaceae rhizodeposits which is reflected by a high myrosinase activity in cultivated *Fusarium* and *Rhizopus* strains from the rhizosphere (Ishimoto et al., 2000). *Fusarium* species are largely saprotrophic fungi that may shift to pathogenicity, e.g *Fusarium oxysporum* is a known pathogen of rapeseed. Non-pathogenic *Fusarium* species may protect host plants against *Fusarium oxysporum*-caused wilt and can decompose senescent plant roots or plant debris, and thus may also contribute to SOC formation (Yuan et al., 2020). Generally, the role of saprotrophic fungi in the degradation and subsequent stabilization of SOC has been acknowledged (Six et al., 2006; Fontaine et al., 2011). Thus, these species may be of importance for the formation of fungal derived SOC in agricultural soils, which has been shown to make up to 50% of MAOC and SOC in aggregates (Angst et al., 2021).

5. Conclusions

Our study identified fungi as the most responsive microbial group affected by soil compartment and simulated erosion, while ^{14}C activity of the microbial biomass was mostly affected by the growth stages of rapeseed, a modulating effect of simulated erosion occurred at the rosette growth stage. Non-mycorrhizal fungal taxa that were enriched in the rhizosphere (*Olpidiomycetes, Rhizopus* and *Fusarium)* may also foster activity and growth of Bacteria alongside. Overall, bacteria have the highest abundance assessed by qPCR across all treatments and archaea only play a minor subordinate role in the investigated arable soil. Our results suggest that the rhizosphere microbiota was predominantly shaped through belowground C allocation and rhizodepositions at early to mid plant growth stages, which is an accordance with previous studies. Further studies of the interactions of fungi with Bacteria and their host plant are required to reveal their specific functions and temporal dynamics in the rhizosphere. Metagenomics or metatranscriptomics of the rhizosphere microbiome allow the simultaneous exploration of functional and taxonomic changes mediated by environmental changes and host crop. Insights from these studies can contribute to microbial mechanisms of SOC formation in agricultural soils and help optimizing agricultural management measures to sequester C in soils.

CRediT authorship contribution statement

Julian Ruggaber: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Ayten Pehlivan:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Rainer Remus:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis. **Davide Francioli:** Writing – original draft, Validation, Formal analysis. **Stephan Wirth:** Writing – review & editing, Supervision, Funding acquisition. **Jürgen Augustin:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Steffen Kolb:** Writing – original draft, Supervision, Project administration, Conceptualization.

Declaration of competing interest

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

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2024.100912.

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