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Faba Bean Genotype, Mixed Cropping and Soil Fauna Jointly Influence Plant Roots and Rhizosphere Microbial Communities

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Abstract: Integrating belowground biodiversity into agricultural systems is key to enhancing agricultural sustainability, yet the role of soil decomposer animals in mediating genotype-specific root and rhizosphere responses remains largely unknown. Using a full-factorial microcosm experiment, we examined how two faba bean genotypes with contrasting root strategies (resource-acquisitive S-62 vs. resource-conservative S-69), mixed cropping with wheat and soil decomposer fauna (earthworms, collembola) jointly shape plant root characteristics and rhizosphere interactions. We quantified root architecture, microbial phospholipid fatty acids (PLFAs), litter carbon incorporation by microorganisms and soil fauna and fauna abundance/biomass. Bean root characteristics differed between genotypes, with higher specific root length and lower root mass density in genotype S-62 than in genotype S-69, reflecting their different resource strategies. Bean genotype S-69 increased fungal/bacterial and Gram⁺/Gram[−] PLFA ratios, indicating stronger microbial resource limitation. This was accompanied by reduced earthworm biomass, which shifted their resource use toward litter-derived carbon. Mixed cropping uniformly decreased bean root mass density, but increased microbial basal respiration and biomass in beans while elevating fungal dominance in wheat. Collembola enhanced bean specific root length and increased the fungal/bacterial PLFA ratio and microbial metabolic quotient in the bean rhizosphere, suggesting grazing-induced fungal dominance and stress. Earthworms increased wheat root mass density, while collembola reduced wheat root diameter, and in combination they increased microbial activity and reinforced fungal carbon use in wheat. Further, earthworms reduced the saturated/monoenic fatty acid ratio in bulk soil and increased the use of litter carbon by Gram[−] bacteria, indicating microbial stress mitigation. Overall, the results document that plant genotypes with different root strategies fundamentally drive the structure of rhizosphere microbial communities, their interactions with decomposer animals and plant litter dynamics. These findings highlight the importance of considering root phenotypic variation and belowground biodiversity when designing sustainable legume–cereal intercropping systems.



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1. Introduction

Modern agriculture faces the dual challenge of increasing productivity to feed a growing population while minimising environmental impact. Intensive practices often depend on monocultures and high input use; however, these approaches may compromise long-term sustainability, soil health and biodiversity [1]. Leveraging belowground biodiversity—particularly the complex interactions between plants and soil organisms—offers significant potential for improving sustainable management of arable systems [2,3].

Earthworms and collembola are vital components of the decomposer food web, driving essential processes such as organic matter decomposition, nutrient cycling and soil structure formation [4–6]. Thereby, they alter plant growth and resource allocation via multiple pathways, including enhanced nutrient availability, altered root architecture and the induction of plant physiological responses [7–9]. However, the effects of soil fauna on plants are highly context-dependent, varying with plant species identity, functional traits and environmental conditions [10,11]. Recent studies have begun to investigate how intraspecific genetic variation in crops modulates plant responses to soil decomposer animals, with genotype-dependent effects documented in both crop productivity and resource allocation [12,13]. However, the specific mechanisms by which differences in root architecture and resource allocation strategies between genotypes mediate plant–decomposer interactions remain poorly understood. Furthermore, while initial evidence suggests that genotype–fauna interactions are context-dependent and can be modulated by mixed cropping system [12,13], the interactive effects of legume–cereal mixtures on genotype-specific responses to decomposer animals have not been systematically explored.

Legume–cereal intercropping is a cornerstone strategy for ecological intensification. This intercropping strategy leverages complementary resource use (e.g., light, water and nutrients) and biological nitrogen fixation to enhance yield stability, reduce fertiliser inputs and improve soil fertility compared to monocultures [14,15]. The success of these systems hinges significantly on belowground interactions, particularly root plasticity and rhizosphere processes [16,17]. Breeding efforts are increasingly focusing on developing crop genotypes specifically adapted to mixed cropping systems, which often possess root and shoot traits distinct from those optimised for monoculture performance [18].

Despite this progress, the role of soil decomposer communities in mediating plant–plant interactions and resource dynamics in mixed cropping systems is poorly understood. Earthworms and collembola are known to differentially affect plants—earthworms primarily via nutrient mobilisation and soil structuring, and collembola through alterations in microbial community composition and root morphology [19,20]—potentially influencing plant performance in a complementary manner [4,21]. However, whether and how these faunal effects vary across different genotypes of the same crop species, and how this variation is modulated by the competitive environment in mixed cropping systems, remains unknown. A crucial knowledge gap therefore exists at the intersection of crop genotype, decomposer fauna and cropping system—a gap that limits our ability to predict the efficacy of diversification strategies and to harness soil biodiversity for sustainable agroecosystem management [13]. Understanding these genotype $\times$ decomposer fauna $\times$ cropping system interactions is essential for predicting the efficacy of diversification strategies and for designing breeding programmes that effectively harness soil biodiversity to enhance agroecosystem sustainability [12].

To address these knowledge gaps, we investigated three core hypotheses: (1) Bean genotypes with contrasting resource allocation strategies exhibit divergent responses to decomposer animals. (2) Earthworms and collembola exert complementary effects on root traits and soil processes, with their combined presence resulting in synergistic outcomes. (3) The influence of decomposer animals on bean genotypes is modified under mixed cropping with wheat. Together, these hypotheses target a mechanistic understanding of how soil biodiversity interacts with crop genotypes and cropping systems to shape root architecture and rhizosphere processes.

2. Material and Methods

2.1. Experimental Design

The study employed a full-factorial microcosm experiment with the factors plant genotype (bean, *Vicia faba*, genotypes S-62 and S-69), cropping system (intercropping with or without wheat, *Triticum aestivum* cv. Genius), collembola (presence or absence of *Heteromurus nitidus*) and earthworms (presence or absence of a mixture of *Lumbricus terrestris* and *Aporrectodea caliginosa*). The two faba bean genotypes were selected to represent contrasting root economic strategies: S-62 is a resource-acquisitive genotype characterised by high specific root

length and low root mass density, whereas S-69 is a resource-conservative genotype with lower specific root length and higher root mass density. The number of treatment combinations is 16 for beans, 12 for wheat and 80 microcosms in total. Specifically, for wheat, treatments included wheat grown alone (wheat monoculture) and wheat intercropped with each of the two bean genotypes, crossed with the two fauna factors (collembola and earthworms, each present or absent). The experiment was designed to assess their effects on root traits and soil microbial communities. The microcosms consisted of PVC tubes with 15 cm diameter and 30 cm height, and were filled with Luvisol soil taken from an arable field south of Göttingen (0–20 cm depth, passed through a 4-mm sieve) mixed with expanded clay (1:1 v/v) to enhance porosity. ^{13}C - and ^{15}N -labelled maize litter was used as mulching material to follow the incorporation of litter-derived carbon and nitrogen into soil microorganisms and soil fauna. To trace the incorporation of plant litter carbon into soil microbial communities and decomposer animals, ^{13}C - and ^{15}N -labelled maize litter ($\delta^{13}\text{C} = 130\text{‰}$, $\delta^{15}\text{N} = 7578\text{‰}$) was applied to the soil surface at the start of the experiment. Maize litter was chosen because it is a common crop residue in legume–cereal rotations and provides a well-defined, isotopically enriched substrate that allows unambiguous detection of carbon flow from residue to soil biota. To simulate mulching and chisel ploughing, 5 g dry weight of labelled maize residues was cut into pieces <5 mm and mixed into the upper 10 cm of soil. In treatments with collembola, 70 individuals of *H. nitidus* were added per microcosm. Treatments with earthworms received approximately 4.0 g of earthworm biomass per microcosm. Four replicates were established per treatment, resulting in a total of 80 microcosms. The microcosms were incubated for 6 months at 20 °C and 70% relative humidity with a photoperiod of 14 h light: 10 h dark and the photon flux density of $460 \pm 80 \mu\text{mol m}^{-2} \text{s}^{-1}$. The microcosms were irrigated with deionised water three times per week. Microcosms were randomised biweekly throughout the experiment. More details on the experimental design are given in Wang et al. [12].

2.2. Soil Sampling and Root Trait Measurement

After 6 months, rhizosphere and bulk soil were collected from 0–10 and 10–25 cm depths. The soil was sieved and homogenised by passing it through a 2-mm mesh. For obtaining rhizosphere soil, we followed the procedure described by Bulgarelli et al. [22,23]. Briefly, roots with adhering rhizosphere soil were collected in 50 ml Falcon tubes, mixed with 10 ml PBS-S buffer (130 mM NaCl, 7 mM Na_2HPO_4 , 3 mM NaH_2PO_4 , pH 7.0, 0.02% Silwet L-77) and incubated for 20 min at 180 rpm on the shaking platform. Subsequently, the suspensions were centrifuged at $4000\times g$ for 20 min. The soil pellet was taken as rhizosphere soil and stored at -20°C .

Similar to bulk and rhizosphere soil sampling, roots were harvested from 0–10 cm and 10–25 cm depths and washed over a 2-mm mesh. Subsequently, roots of the two crop species were manually separated using tweezers based on colour and texture. Fine roots—defined as first- and second-order roots responsible for water and nutrient uptake—were separated from coarse roots, rinsed with deionised water and stored in 75% ethanol. Root images were scanned at 600 dpi using a flatbed scanner (EPSON Perfection V700/V750, Portland, Or, USA) and analysed with WinRHIZO 2013e (Regent Instruments Inc., Sainte-Foy, Canada) to determine average root diameter and specific root length (cm g^{-1} dry weight). Root mass density (g cm^{-3}) of the two crop species was calculated as the total root dry weight (65 °C, 48 h) divided by soil volume, where the dry weight and volume are the sum of the upper and lower soil layers.

2.3. Microbial Basal Respiration, Specific Respiration and Biomass

Basal respiration and microbial biomass were measured by O_2 consumption using an automated electrolytic microrespirometer [24]. Frozen samples were thawed and incubated overnight at 4 °C, and 4 g fresh weight was loaded into small glass vessels. Following system connection, microbial basal respiration was quantified as mean O_2 consumption ($\mu\text{g O}_2 \text{g}^{-1} \text{C h}^{-1}$) measured 10–23 h after vessel attachment to the respirometer system and incubation at 22 °C in a water bath. Thereafter, to measure microbial biomass via substrate-induced respiration, glucose equivalent to 8 mg g^{-1} dry soil—dissolved in 400 $\mu\text{L H}_2\text{O}$ —was added [24,25]. The computer-controlled system uses KOH for CO_2 absorption and CuSO_4 electrolysis to replenish O_2 . Microbial biomass carbon (C_{mic}) was calculated from the maximum initial respiratory response (MIRR; $\mu\text{g O}_2 \text{g}^{-1} \text{soil h}^{-1}$) recorded 4–7 h after glucose addition, using the formula: $C_{\text{mic}} (\mu\text{g C}_{\text{mic}} \text{g}^{-1} \text{soil}) = 38 \times \text{MIRR}$ [26]. Microbial specific respiration was calculated as the basal respiration-to-biomass ratio ($q\text{O}_2$; $\mu\text{g O}_2 \mu\text{g}^{-1} C_{\text{mic}} \text{h}^{-1}$).

2.4. Phospholipid Fatty Acids

Soil phospholipid fatty acids (PLFAs) were extracted from 2 g fresh soil using a modified Bligh–Dyer method in 96-well plates [27,28]. Lipids were fractionated into neutral lipids, glycolipids and phospholipids by sequential elution through silica acid columns using chloroform, acetone and methanol, respectively (0.5 g silicic acid, 3 ml; HF BOND ELUT-SI, Varian Inc., Darmstadt, Germany). This was followed by alkaline methanolysis to generate fatty acid methyl esters (FAMES). FAMES were analysed by GC-FID (Clarus 500, PerkinElmer Corporation, Norwalk, CT, USA) using an Elite 5 column (0.32 mm × 30 m, 0.25 µm film) and temperature programming. Identification relied on chromatographic retention times against standards (FAME CRM47885, C11 to C24; BAME 47080-U, C11 to C20; Sigma-Aldrich, Darmstadt, Germany).

Stress indicators and microbial markers were derived from PLFA mole percentages [29,30]. The ratio between cyclopropyl PLFAs (cy17:0 and cy19:0) and their monoenoic precursors (16:1ω7 and 18:1ω) (cy/pre) was taken as physiological stress indicator. The ratio of saturated (14:0, 15:0, 16:0, 17:0 and 18:0) to monounsaturated PLFAs (16:1ω7, 17:1 and 18:1ω9/7)—referred to as the sat/mono ratio—was used as an indicator of nutritional stress. The ratio of Gram⁺ (i/a15:0, i16:0 and i17:0) to Gram[−] fatty acid markers (cy17:0, cy19:0, 16:1ω7 and 18:1ω7)—the Gram⁺/Gram[−] ratio) was taken as marker for carbon availability. Linoleic acid (18:2ω6,9) was used as fungal marker, and the sum of Gram⁺ and Gram[−] fatty acids as marker for total bacteria [31]. The total amount of PLFAs included all identified PLFAs (n = 35; nmol g^{−1} dry weight) and was used to calculate PLFA proportions. All stress indicators and PLFA markers were analysed based on proportional data.

2.5. Stable Isotope Analysis of Fatty Acids and Soil Animals

Isotope ratios (¹³C/¹²C) of FAMES were measured using a Thermo Finnigan Trace GC coupled via a GP interface to a Delta Plus mass spectrometer (Finnigan, Bremen, Germany). To obtain isotope ratios of the fatty acids, the δ¹³C values of FAMES were corrected for the isotopic contribution of the methyl moiety derived from methanol using the formula: δ¹³C_{FA} = [(C_n + 1) × δ¹³C_{FAME} − δ¹³C_{MeOH}]/C_n, where δ¹³C_{FA} is the δ¹³C of the fatty acid, C_n is the number of carbon atoms in the fatty acid, δ¹³C_{FAME} is the δ¹³C of the FAME and δ¹³C_{MeOH} is the δ¹³C of the methanol (−52‰) used for methylation. δ¹³C_{FA} was then converted to atom% ¹³C_{FA} using the formula: ((¹³C/¹²C)_{standard} × (δ¹³C + 1000) × 100)/((¹³C/¹²C)_{standard} × (δ¹³C + 1000) + 1000), with δ¹³C calculated as ((¹³C/¹²C)_{sample} − (¹³C/¹²C)_{standard})/((¹³C/¹²C)_{standard} × 1000); V-PDB was used as standard.

The ¹³C amount of each PLFA (e.g., cy17:0) was calculated using the equation: ¹³C concentration = Atom% ¹³C_{FA} × FA concentration × C proportion. After determining the total ¹³C content of each PLFA, the total ¹³C contents of PLFA components representative of Gram⁺ bacteria (i15:0, a15:0, i16:0 and i17:0), Gram[−] bacteria (cy17:0, cy19:0, 16:1ω7 and 18:1ω7) and fungi (18:2ω6,9) were calculated. This total was then divided by the total carbon content of these components to yield the ¹³C atom percent excess, which was further converted to the corresponding δ¹³C value.

At the end of the experiment, collembola and earthworms were sampled without differentiating soil layers. Collembola were extracted by heat from soil cores, and the number was counted (inner diameter 5 cm, depth of 6 cm) [32]. The remaining soil material was washed through a 500-µm screen, and earthworms were collected and weighed. The change in earthworm biomass was expressed as the difference between the original and final fresh biomass. Subsequently, earthworms and collembola were dried at 60 °C. Aliquots of the dried material were weighed into tin capsules for stable isotope measurement. Stable isotope ratios were analysed using a coupled system comprising an elemental analyser (NA 1500, Carlo Erba, Milan, Italy) and an isotope ratio mass spectrometer (Delta Plus, Finnigan MAT) [33]. Isotope ratios were expressed in δ notation, with δ¹³C calculated as ((¹³C/¹²C)_{sample} − (¹³C/¹²C)_{standard})/((¹³C/¹²C)_{standard} × 1000) and δ¹⁵N calculated analogously as ((¹⁵N/¹⁴N)_{sample} − (¹⁵N/¹⁴N)_{standard})/((¹⁵N/¹⁴N)_{standard} × 1000). V-PDB was used as standard for ¹³C and atmospheric N as primary standard for ¹⁵N and acetanilide for internal calibration.

2.6. Statistical Analysis

Data for the two crop species were analysed separately. For beans, root mass density, δ¹³C values of PLFAs, δ¹⁵N and δ¹³C values of soil animals, collembola abundance and changes in earthworm biomass were analysed using a four-factor analysis of variance (ANOVA). The factors included genotype (S-62 and S-69), mixed cropping (with and without wheat), collembola (present and absent), earthworms (present and absent) and their interactions. For wheat, root mass density, δ¹³C values of PLFAs, δ¹⁵N and δ¹³C values of soil animals, collembola abundance and changes in earthworm biomass were analysed using a three-factor ANOVA. The factors included mixed cropping (without bean, with bean genotype S-62 and with bean genotype S-69), collembola (present and absent), earthworms (present and absent) and their interactions.

Data on average root diameter, specific root length, rhizosphere PLFA, bulk soil PLFA, microbial biomass and basal respiration were analysed using linear mixed-effects models (LMMs). The LMMs for bean treatments included genotype (S-62 and S-69), wheat (present and absent), collembola (present and absent), earthworms (present and absent) and their interactions as fixed factors. For wheat treatments, the models included mixed cropping (without bean, with bean genotype S-62 and with bean genotype S-69), collembola (present and absent), earthworms (present and absent) and their interactions as fixed factors. Microcosm identity and, if applicable, sampling depth (0–10 and 10–25 cm) were included as random effects to account for non-independence of samples from the same microcosm and sampling depth, respectively.

All analyses were done in R 4.4.3 [34]. We used the “nlme” package to fit LMMs. We checked model residuals using Q-Q plots and residual-vs-fitted plots; no obvious violations were found. Consequently, all mixed models met the assumptions of normality of residuals and homogeneity of variance.

3. Results

3.1. Root Characteristics

Beans. None of the four examined factors significantly affected the average diameter of bean fine roots as main effect; however, root diameter varied between bean genotypes depending on mixed cropping (Table 1a). In monoculture, bean genotype S-69 had slightly lower average diameter (0.41 ± 0.01 mm) than S-62 (0.43 ± 0.01 mm; Figure 1a). Bean genotype and collembola significantly influenced specific root length, while genotype and mixed cropping affected root mass density. Bean genotype S-62 showed higher specific root length (10.43 cm g^{-1}) than S-69 (9.77 cm g^{-1}), along with a lower root mass density (1.72 vs. 2.18 g cm^{-3}). Mixed cropping with wheat reduced root mass density in both genotypes on average from 2.13 g cm^{-3} in monoculture to 1.75 g cm^{-3} in mixture. Generally, collembola increased specific fine root length on average from 9.66 ± 0.27 to $10.53 \pm 0.26 \text{ cm g}^{-1}$; however, the effect varied with mixed cropping and earthworms (significant collembola $\times$ mixed cropping $\times$ earthworm interaction; Table 1a). In bean monoculture, the increase was more pronounced in treatments without earthworms, whereas in mixed cropping systems with wheat, it was more pronounced in treatments with earthworms (Figure S1).

Table 1. F- and p-values from linear mixed-effects models assessing the effects of genotype (S-62, S-69; beans only), mixed cropping (with and without wheat for beans and with and without beans for wheat), collembola (present vs. absent) and earthworms (present vs. absent) on average fine root diameter, specific fine root length and root mass density of beans (a) and wheat (b). Significant effects ($p < 0.05$) are shown in bold.

Factor	Df	F	p	F	p	F	p
(a) Beans		Fine Root Diameter		Specific Fine Root Length		Root Mass Density	
(Intercept)	1,61	2,9926.77	<0.001	1,1512.11	<0.001	/	/
Genotype (G)	1,47	0.59	0.448	11.31	0.002	11.23	0.002
Mixed cropping (M)	1,47	0.88	0.353	0.21	0.648	14.32	<0.001
Collembola (C)	1,47	3.43	0.070	21.27	<0.001	0.79	0.379
Earthworms (E)	1,47	0.01	0.925	1.48	0.230	3.74	0.059
G $\times$ M	1,47	5.33	0.025	3.51	0.067	0.03	0.871
G $\times$ C	1,47	0.56	0.460	0.91	0.346	0.00	0.966
M $\times$ C	1,47	1.22	0.276	2.28	0.138	0.33	0.566
G $\times$ E	1,47	0.15	0.703	0.04	0.842	1.04	0.314
M $\times$ E	1,47	0.14	0.712	1.01	0.319	0.05	0.819
C $\times$ E	1,47	0.39	0.536	0.38	0.542	1.43	0.238
G $\times$ M $\times$ C	1,47	0.09	0.762	1.54	0.221	0.17	0.682
G $\times$ M $\times$ E	1,47	0.43	0.518	1.67	0.203	0.01	0.912
G $\times$ C $\times$ E	1,47	1.38	0.247	3.72	0.060	0.10	0.754
M $\times$ C $\times$ E	1,47	3.33	0.074	6.54	0.014	0.79	0.378
G $\times$ M $\times$ C $\times$ E	1,47	0.28	0.600	0.04	0.835	1.87	0.178
(b) Wheat							
(Intercept)	1,45	1,7316.9	<0.001	8503.5	<0.001	/	/
Mixed cropping (M)	2,35	15.6	<0.001	17.7	<0.001	7.14	0.003
Collembola (C)	1,35	8.53	0.006	7.38	0.012	0.37	0.548
Earthworms (E)	1,35	0.38	0.539	2.67	0.111	8.36	0.007
M $\times$ C	2,35	4.61	0.017	0.99	0.383	0.33	0.721
M $\times$ E	2,35	0.17	0.842	0.27	0.792	3.24	0.051
C $\times$ E	1,35	1.91	0.176	7.54	0.009	0.32	0.577
M $\times$ C $\times$ E	2,35	0.63	0.539	0.81	0.454	0.14	0.866

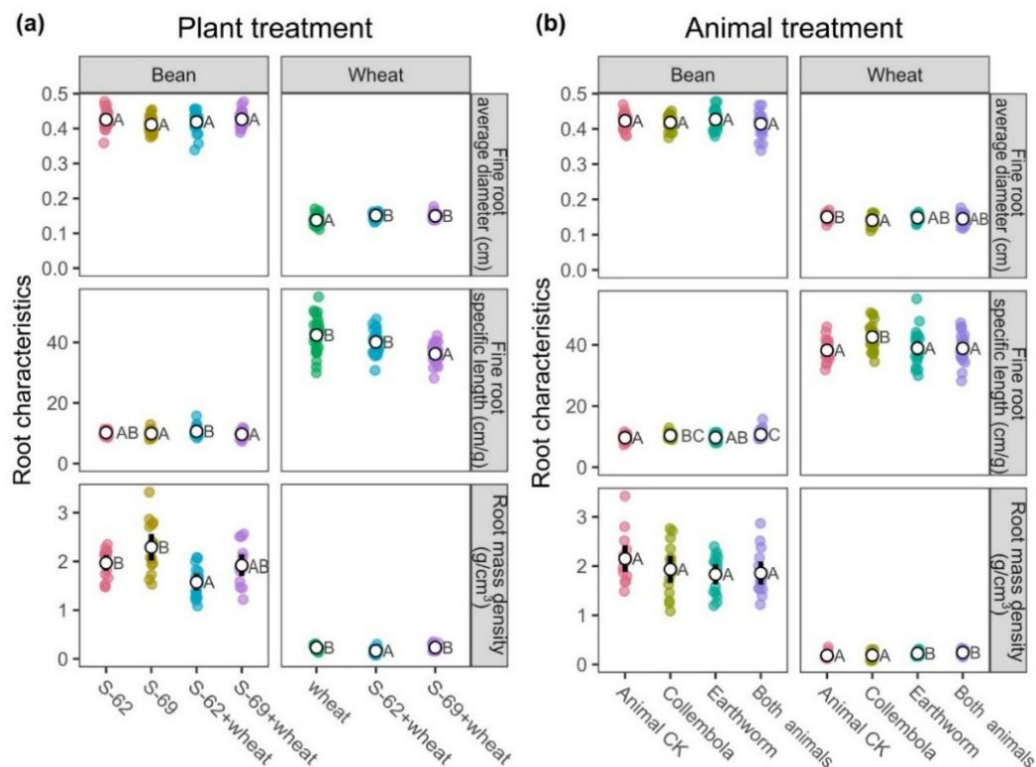


Figure 1. Effect of bean genotype (S-62, S-69) and mixed cropping (with and without wheat for beans and with and without beans for wheat) on root characteristics—average fine root diameter, specific fine root length and root mass density—of beans and wheat (data pooled across animal treatments) (a), and modifications by collembola and earthworms (Ctr, without collembola and earthworms; Collembola, with collembola; Earthworm, with earthworms; Both animals, with both collembola and earthworms) (data pooled across plant treatments) (b). Means $\pm$ 95% confidence intervals; means sharing the same letter do not differ significantly (Tukey's post hoc pairwise comparison, $p < 0.05$). Note that the two plant species (bean and wheat) were analysed separately; see Table 1 for statistical details.

Wheat. The diameter and specific root length of wheat fine roots were significantly affected by mixed cropping; however, the effect on root diameter varied with the presence of collembola, as indicated by substantial mixed cropping $\times$ collembola interaction (Table 1b). Furthermore, wheat root mass density varied significantly with mixed cropping with beans and the presence of earthworms. Compared with monocultures (0.138 cm), wheat fine root diameter was higher in mixed cropping systems with legumes (0.150 cm) (Figure 1b). Conversely, specific root length was significantly lower in mixtures with bean S-69 (36.1 cm g⁻¹) than in monoculture (42.5 cm g⁻¹) and mixtures with bean S-62 (40.1 cm g⁻¹). Furthermore, the root mass density of wheat in mixture with bean S-62 (0.370 g cm⁻³) was significantly lower than that in monoculture and mixtures with bean S-69 (0.512 and 0.503 g cm⁻³, respectively). Collembola reduced root diameter but only in wheat monoculture (from 0.145 to 0.130 cm; Figure S2). Moreover, collembola increased specific root length but only in treatments without earthworms (from 37.9 to 42.6 cm g⁻¹; significant earthworm $\times$ collembola interaction; Table 1b). In contrast to collembola, earthworms did not significantly affect fine root diameter and specific root length but increased root mass density from 0.407 to 0.516 g cm⁻³.

3.2. Microbial Biomass in Bulk Soil

Beans. Microbial indicators in bulk soil of beans were predominantly influenced by mixed cropping with wheat but were generally unaffected by bean genotype or earthworms (Table 2a). However, collembola significantly affected each of the three microbial indicators, but the effect on basal respiration and microbial biomass depended on earthworms. Microbial basal respiration (from 3.34 ± 0.20 to 3.74 ± 0.21 $\mu\text{g O}_2 \text{ g}^{-1} \text{ C h}^{-1}$) and microbial biomass (from 4.28 ± 0.35 to 5.28 ± 0.37 $\mu\text{g C}_{\text{mic}} \text{ g}^{-1} \text{ C}$) were increased by mixed cropping with wheat, whereas the metabolic quotient decreased (from 0.81 ± 0.04 to 0.73 ± 0.04 $\mu\text{g O}_2 \mu\text{g}^{-1} \text{ C}_{\text{mic}} \text{ h}^{-1}$, Figure 2a). Collembola increased microbial basal respiration (from 3.25 ± 0.29 to 4.00 ± 0.29 $\mu\text{g O}_2 \text{ g}^{-1} \text{ C h}^{-1}$) and metabolic quotient (from 4.56 ± 0.50 to 5.31 ± 0.50 $\mu\text{g O}_2 \mu\text{g}^{-1} \text{ C}_{\text{mic}} \text{ h}^{-1}$) but only in presence of earthworms (Figure 2b).

Table 2. F- and p-values from linear mixed-effects models assessing the effects of genotype (S-62, S-69; beans only), mixed cropping (with and without wheat for beans and with and without beans for wheat), collembola (present vs. absent) and earthworms (present vs. absent) on microbial basal respiration, biomass and metabolic quotient in the bulk soil of bean (a) and wheat (b) treatments. Significant effects ($p < 0.05$) are shown in bold.

Factor	Df	F	p	F	p	F	p
(a) Beans		Basal Respiration		Microbial Biomass		Metabolic Quotient	
(Intercept)	1,62	2363.85	<0.001	1434.64	<0.001	3312.58	<0.001
Genotype (G)	1,47	1.65	0.205	1.43	0.238	0.00	0.952
Mixed cropping (M)	1,47	7.57	0.008	15.35	<0.001	7.98	0.007
Collembola (C)	1,47	7.46	0.009	0.13	0.718	6.07	0.017
Earthworms (E)	1,47	1.43	0.238	1.77	0.190	0.78	0.381
G × M	1,47	0.15	0.700	0.89	0.349	0.65	0.425
G × C	1,47	1.07	0.306	0.33	0.570	0.83	0.368
M × C	1,47	0.02	0.881	0.13	0.718	0.26	0.609
G × E	1,47	0.80	0.377	0.14	0.713	0.39	0.533
M × E	1,47	1.39	0.244	0.05	0.827	2.23	0.142
C × E	1,47	5.95	0.019	6.97	0.011	2.17	0.148
G × M × C	1,47	0.54	0.465	0.01	0.943	1.52	0.224
G × M × E	1,47	1.34	0.253	0.02	0.886	3.23	0.079
G × C × E	1,47	2.97	0.092	2.15	0.149	0.58	0.448
M × C × E	1,47	2.78	0.102	1.80	0.186	0.06	0.802
G × M × C × E	1,47	0.10	0.747	0.04	0.837	1.29	0.262
(b) Wheat		Basal Respiration		Microbial Biomass		Metabolic Quotient	
(Intercept)	1,47	1453.97	<0.001	947.57	<0.001	3619.17	<0.001
Mixed cropping (M)	2,35	3.37	0.046	1.63	0.211	1.28	0.290
Collembola (C)	1,35	7.17	0.011	0.23	0.636	8.94	0.005
Earthworms (E)	1,35	2.72	0.108	0.82	0.371	0.59	0.446
M × C	2,35	0.48	0.624	0.28	0.760	0.67	0.516
M × E	2,35	0.06	0.939	0.11	0.896	0.53	0.591
C × E	1,35	7.38	0.010	5.52	0.025	0.36	0.554
M × C × E	2,35	1.04	0.365	0.74	0.486	0.67	0.519

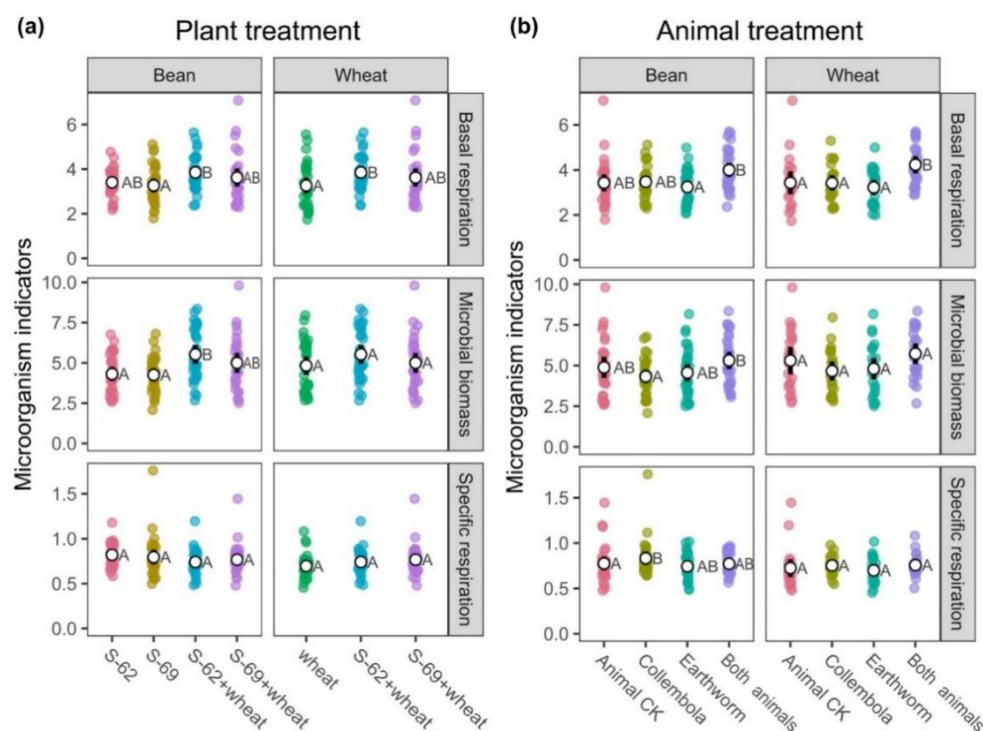


Figure 2. Effect of bean genotype (S-62, S-69) and mixed cropping (with and without wheat for beans and with and without beans for wheat) on microbial indicators—microbial basal respiration, biomass and metabolic quotient—in the bulk soil of beans and wheat (data pooled across animal treatments) (a), and modifications by collembola and earthworms (Ctr, without collembola and earthworms; Collembola, with collembola; Earthworm, with earthworms; Both animals, with collembola and earthworms) (data pooled across plant treatments) (b). Means $\pm$ 95% confidence intervals; means sharing the same letter do not differ significantly (Tukey's post hoc pairwise comparison, $p < 0.05$). Note that the two plant species (bean and wheat) were analysed separately; see Table 2 for statistical details.

Wheat. In wheat bulk soil, mixed cropping with beans affected microbial basal respiration but not microbial biomass and metabolic quotient (Table 2b). Furthermore, collembola significantly affected basal respiration and metabolic quotient; however, the effect on basal respiration depended on the presence of earthworms, and this was true for microbial biomass. Microbial basal respiration was increased by mixed cropping with beans from 3.27 ± 0.32 to $3.63 \pm 0.34 \mu\text{g O}_2 \text{ g}^{-1} \text{ C h}^{-1}$ in bean genotype S-62 and to $3.85 \pm 0.32 \mu\text{g O}_2 \text{ g}^{-1} \text{ C h}^{-1}$ in bean genotype S-69 (Figure 2b). Collembola generally increased the metabolic quotient, on average from 0.682 ± 0.035 to $0.755 \pm 0.034 \mu\text{g O}_2 \mu\text{g}^{-1} \text{ C}_{\text{mic}} \text{ h}^{-1}$, but microbial basal respiration was only increased in the presence of earthworms from 3.22 ± 0.38 to $4.23 \pm 0.38 \mu\text{g O}_2 \text{ g}^{-1} \text{ C h}^{-1}$. Similarly, microbial biomass was increased from 4.67 ± 0.67 to $5.73 \pm 0.67 \mu\text{g C}_{\text{mic}} \text{ g}^{-1} \text{ C}$ in presence of both collembola and earthworms.

3.3. PLFA Stress Indicators in Bulk Soil

Beans. The fungi/bacteria and Gram⁺/Gram⁻ bacteria ratio in bulk soil significantly varied with bean genotype, while the sat/mono ratio was significantly affected by mixed cropping with wheat (Table 3a). Furthermore, collembola significantly affected the sat/mono ratio, while earthworms significantly affected the fungi/bacteria ratio. The fungi/bacteria ratio (from 0.065 ± 0.003 to 0.058 ± 0.004) and the Gram⁺/Gram⁻ bacteria ratio (from 0.696 ± 0.024 to 0.651 ± 0.025) were lower in bean genotype S-62 than in S-69 (Figure 3a). Mixed cropping with wheat reduced the sat/mono PLFA ratio from 0.713 ± 0.016 to 0.684 ± 0.015 . By contrast, collembola increased the sat/mono ratio from 0.681 ± 0.017 to 0.716 ± 0.016 and earthworms increased the fungi/bacteria ratio from 0.058 ± 0.004 to 0.065 ± 0.004 .

Table 3. F- and p-values from linear mixed-effects models assessing the effects of genotype (S-62, S-69; beans only), mixed cropping (with and without wheat for beans and with and without beans for wheat), collembola (present vs. absent) and earthworms (present vs. absent) on PLFA-based stress indicators—fungi/bacteria ratio, sat/mono PLFA ratio and Gram⁺/Gram⁻ ratio—in the bulk soil of bean (a) and wheat (b) treatments. Significant effects ($p < 0.05$) are shown in bold.

Factor	Df	F	p	F	p	F	p
(a) Beans		Fungi/Bacteria Ratio		Sat/Mono Ratio		Gram ⁺ /Gram ⁻ Bacteria Ratio	
(Intercept)	1,60	2429.13	<0.001	1,5798.17	<0.001	6086.03	<0.001
Genotype (G)	1,47	7.73	0.008	3.79	0.058	7.04	0.011
Mixed cropping (M)	1,47	0.06	0.805	6.89	0.012	2.01	0.163
Collembola (C)	1,47	1.83	0.183	9.99	0.003	0.45	0.506
Earthworms (E)	1,47	7.49	0.009	2.57	0.116	2.61	0.113
G × M	1,47	0.35	0.558	1.86	0.179	0.64	0.427
G × C	1,47	0.96	0.331	0.42	0.522	0.00	0.978
M × C	1,47	0.00	0.948	0.26	0.611	0.02	0.893
G × E	1,47	2.43	0.125	0.10	0.749	0.00	0.964
M × E	1,47	0.56	0.458	0.06	0.812	0.50	0.483
C × E	1,47	3.03	0.088	0.70	0.406	2.74	0.105
G × M × C	1,47	0.86	0.357	0.11	0.741	0.68	0.414
G × M × E	1,47	0.25	0.617	0.13	0.719	0.19	0.666
G × C × E	1,47	1.20	0.278	0.78	0.382	0.04	0.846
M × C × E	1,47	0.78	0.382	0.85	0.360	0.22	0.642
G × M × C × E	1,47	0.74	0.395	1.19	0.282	0.13	0.720
(b) Wheat							
(Intercept)	1,44	2179.72	<0.001	1,0242.12	<0.001	5930.43	<0.001
Mixed cropping (M)	2,35	35.42	<0.001	23.97	<0.001	5.66	0.007
Collembola (C)	1,35	0.88	0.354	3.72	0.062	0.92	0.344
Earthworms (E)	1,35	4.97	0.032	3.29	0.078	4.25	0.047
M × C	2,35	1.47	0.244	0.40	0.674	0.23	0.795
M × E	2,35	0.45	0.639	0.44	0.645	0.17	0.847
C × E	1,35	5.02	0.032	2.25	0.143	4.04	0.052
M × C × E	2,35	0.44	0.649	0.95	0.396	0.03	0.966

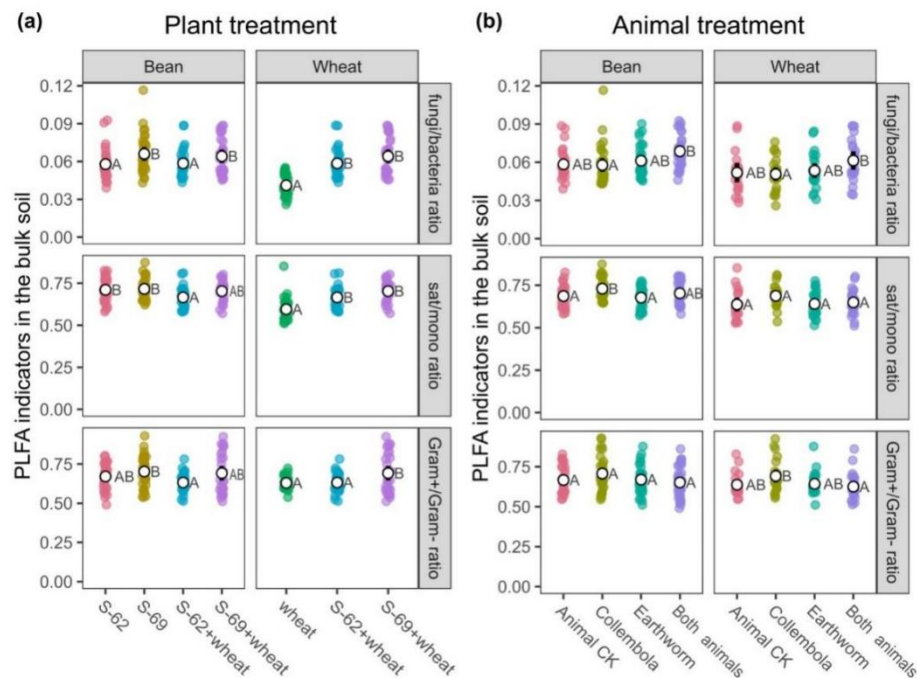


Figure 3. Effect of bean genotype (S-62, S-69) and mixed cropping (with and without wheat for beans and with and without beans for wheat) on the fungi/bacteria ratio, sat/mono PLFA ratio and Gram⁺/Gram[−] bacteria ratio in bulk soil of beans and wheat (data pooled across animal treatments) (a), and modifications by collembola and earthworms (Ctr, without collembola and earthworms; Collembola, with collembola; Earthworm, with earthworms; Both animals, with collembola and earthworms) (data pooled across plant treatments) (b). Means $\pm$ 95% confidence intervals; means sharing the same letter do not differ significantly (Tukey's post hoc pairwise comparison, $p < 0.05$). Note that the two plant species (bean and wheat) were analysed separately; see Table 3 for statistical details.

Wheat. Each of the three PLFA indicators in bulk soil of wheat varied significantly in response to mixed cropping with beans (Table 3b). Furthermore, the fungi/bacteria ratio and the bacteria sat/mono ratio were significantly affected by earthworms, with earthworm effect on the fungi/bacteria ratio depending on the presence of collembola. Mixed cropping with beans increased the fungi/bacteria ratio from 0.041 ± 0.004 (no beans) to 0.058 ± 0.004 (bean genotype S-62) and 0.064 ± 0.004 (bean genotype S-69). Similarly, the sat/mono PLFA ratio increased from 0.595 ± 0.022 to 0.665 ± 0.022 and 0.703 ± 0.040 across the same treatments (Figure 3b). Furthermore, the Gram⁺/Gram[−] ratio was increased by mixed cropping with beans but only in the S-69 treatment (from 0.629 ± 0.029 to 0.691 ± 0.031). Earthworms decreased the sat/mono ratio from 0.667 ± 0.024 to 0.634 ± 0.025 and increased the fungi/bacteria ratio, but only if collembola were also present (from 0.053 ± 0.003 to 0.061 ± 0.003).

3.4. PLFA Stress Indicators in Rhizosphere Soil

Beans. Bean genotype significantly affected each of the PLFA biomarkers in the bean rhizosphere (Table 4a). Furthermore, the Gram⁺/Gram[−] ratio varied significantly with mixed cropping with wheat. In addition, collembola significantly influenced the fungi/bacteria and sat/mono PLFA ratios with the latter being significantly affected by earthworms. Interaction effects were generally not significant. Each of the three biomarkers was higher in the rhizosphere of bean genotype S-69 than in that of S-62 (0.165 vs. 0.112 , 0.720 vs. 0.671 and 0.681 vs. 0.602 , respectively) (Figure 4a). Mixed cropping with wheat reduced the Gram⁺/Gram[−] bacteria ratio from 0.682 to 0.600 . Collembola increased the fungi/bacteria ratio from 0.118 to 0.158 and the sat/mono ratio from 0.677 to 0.714 (Figure 4b). Conversely, earthworms decreased the sat/mono ratio from 0.710 to 0.680 .

Wheat. In the wheat rhizosphere, each of the three PLFA biomarkers was significantly influenced by mixed cropping with beans (Table 4b). Furthermore, earthworms significantly affected the sat/mono ratio and fungi/bacteria ratio, but the latter depended on collembola. The fungi/bacteria and sat/mono PLFA ratios increased in mixed cropping systems with beans—from 0.048 to 0.083 and 0.105 and from 0.643 to 0.706 and 0.717 , respectively—in the wheat-only treatment and mixtures with bean genotypes S-62 and S-69 (Figure 4a). Conversely, the Gram⁺/Gram[−] bacteria ratio decreased under mixed cropping with beans but only in the S-62 treatment (from 0.759 to 0.690). Earthworms reduced the sat/mono ratio from 0.704 to 0.673 , whereas they increased the fungi/bacteria ratio but only if collembola were present (from 0.063 to 0.096) (Figure 4b).

Table 4. F- and p-values from linear mixed-effects models assessing the effects of genotype (S-62, S-69; beans only), mixed cropping (with and without wheat for beans and with and without beans for wheat), collembola (present vs. absent) and earthworms (present vs. absent) on PLFA-based stress indicators—fungi/bacteria ratio, sat/mono PLFA ratio and Gram⁺/Gram[−] ratio—in the rhizosphere soil of bean (a) and wheat (b) treatments. Significant effects ($p < 0.05$) are shown in bold.

Factor	Df	F	p	F	p	F	p
(a) Beans		Fungi/Bacteria Ratio		Sat/Mono Ratio		Gram ⁺ /Gram [−] Bacteria Ratio	
(Intercept)	1,60	563.79	<0.001	9363.75	<0.001	1596.45	<0.001
Genotype (G)	1,47	21.00	<0.001	10.48	0.002	6.07	0.017
Mixed cropping (M)	1,47	0.03	0.857	0.76	0.389	6.61	0.013
Collembola (C)	1,47	10.82	0.002	7.73	0.008	2.91	0.095
Earthworms (E)	1,47	0.26	0.615	4.59	0.037	1.76	0.191
G × M	1,47	0.71	0.404	0.28	0.602	0.07	0.790
G × C	1,47	0.33	0.569	0.10	0.756	0.06	0.806
M × C	1,47	0.16	0.691	0.64	0.429	1.60	0.212
G × E	1,47	0.19	0.669	0.35	0.559	0.46	0.501
M × E	1,47	1.63	0.208	0.51	0.480	1.35	0.250
C × E	1,47	0.69	0.411	0.50	0.485	0.59	0.447
G × M × C	1,47	0.11	0.737	0.50	0.483	0.00	0.986
G × M × E	1,47	2.63	0.111	0.21	0.646	0.02	0.880
G × C × E	1,47	0.16	0.693	2.70	0.107	0.06	0.810
M × C × E	1,47	0.47	0.498	0.56	0.457	0.81	0.372
G × M × C × E	1,47	0.66	0.422	0.28	0.602	0.50	0.483
(b) Wheat							
(Intercept)	1,44	501.21	<0.001	10335.01	<0.001	5764.05	<0.001
Mixed cropping (M)	2,35	21.64	<0.001	11.55	<0.001	7.48	0.002
Collembola (C)	1,35	3.8	0.059	0.12	0.730	1.71	0.200
Earthworms (E)	1,35	0.07	0.793	4.93	0.033	0.26	0.616
M × C	2,35	1.33	0.277	1.60	0.216	1.32	0.279
M × E	2,35	0.07	0.931	0.99	0.383	1.28	0.290
C × E	1,35	8.58	0.006	0.96	0.335	0.59	0.447
M × C × E	2,35	2.11	0.136	0.36	0.697	0.39	0.682

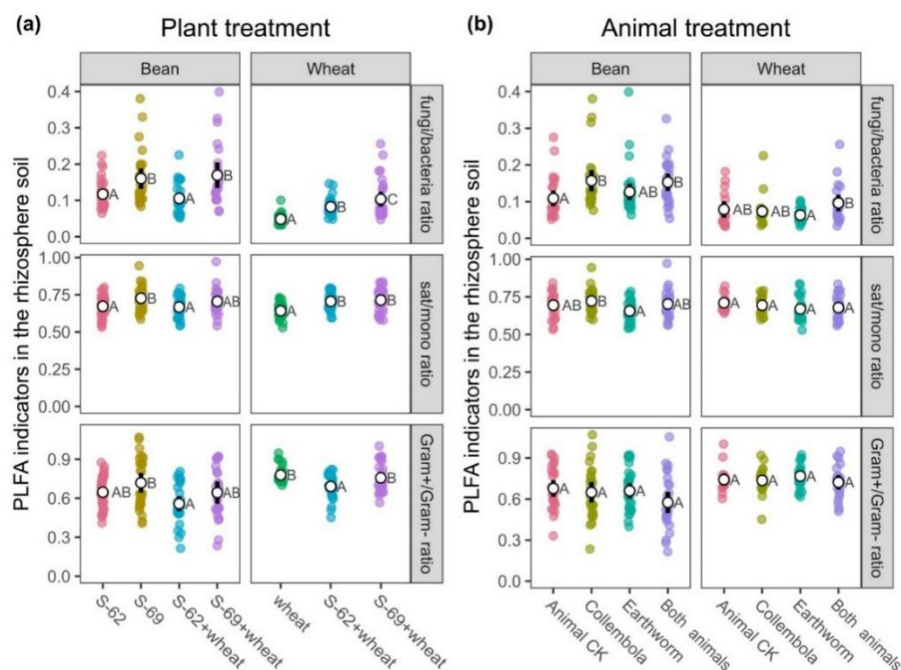


Figure 4. Effect of bean genotype (S-62, S-69) and mixed cropping (with and without wheat for beans and with and without beans for wheat) on the fungi/bacteria ratio, sat/mono PLFA ratio and Gram⁺/Gram[−] bacteria ratio in the rhizosphere soil of beans and wheat (data pooled across animal treatments) (a), and modifications by collembola and earthworms (Ctr, without collembola and earthworms; Collembola, with collembola; Earthworm, with earthworms; Both animals, with collembola and earthworms) (data pooled across plant treatments) (b). Means $\pm$ 95% confidence intervals; means sharing the same letter do not differ significantly (Tukey's post hoc pairwise comparison, $p < 0.05$). Note that the two plant species (bean and wheat) were analysed separately; see Table 4 for statistical details.

3.5. Microbial Incorporation of Litter Carbon

Beans. Bean genotype and mixed cropping did not significantly affect the incorporation of carbon from labelled litter into microorganisms as the main effect; however, incorporation varied significantly with earthworms and collembola (Table 5a). Generally, collembola decreased the incorporation of litter-derived carbon into Gram[−] bacteria, as indicated by lower $\delta^{13}\text{C}$ values in treatments with collembola than in those without (−16.1‰ vs. −14.2‰). Conversely, earthworms increased incorporation, as reflected by higher $\delta^{13}\text{C}$ values in treatments with earthworms than in those without (−13.9‰ vs. −16.0‰; Figure 5a). Additionally, the incorporation of litter carbon into Gram⁺ bacteria and fungi depended on the interaction between collembola and earthworms. Without earthworms, $\delta^{13}\text{C}$ values of Gram⁺ bacteria were higher in treatments with collembola (−14.1‰) than in those without (−15.6‰). By contrast, the opposite pattern was observed in treatments with earthworms, where $\delta^{13}\text{C}$ values were lower with collembola (−18.5‰) than without (−14.3‰). Likewise, for fungi, $\delta^{13}\text{C}$ values were higher with collembola (−14.3‰) than without (−15.8‰) in the absence of earthworms, whereas the reverse was true in the presence of earthworms (−15.8‰ vs. −12.4‰).

Wheat. Similar to the bean treatments, the incorporation of litter-derived carbon into microorganisms did not vary significantly with mixed cropping as a main effect in wheat treatments. However, it was significantly influenced by earthworms and by the interaction between collembola and mixed cropping (Table 5b). Generally, the incorporation of litter carbon into fungi was not significantly affected by any of the factors studied. By contrast, earthworms tended to reduce the incorporation of litter carbon into Gram⁺ bacteria, as indicated by lower $\delta^{13}\text{C}$ values in treatments with earthworms (−16.8‰) than those without (−13.0‰; Figure 5b). Furthermore, the incorporation of litter-derived carbon into Gram[−] bacteria varied with the presence of collembola, depending on mixed cropping with beans. In treatments without collembola, incorporation was highest in the mixture with bean genotype S-62, as indicated by higher $\delta^{13}\text{C}$ values (−11.7‰) than those with wheat only (−19.0‰) and with bean genotype S-69 (−17.0‰). By contrast, $\delta^{13}\text{C}$ values in treatments with collembola were more similar across cropping treatments, with respective values of −16.8‰, −16.9‰ and −14.3‰.

Table 5. F- and *p*-values from linear models assessing the effects of bean genotype (S-62, S-69), mixed cropping (with and without wheat for beans and with and without beans for wheat), collembola (present vs. absent) and earthworms (present vs. absent) on $\delta^{13}\text{C}$ values of Gram⁺ and Gram[−] bacteria and fungi in the bulk soil of bean (a) and wheat (b) treatments. Significant effects (*p* < 0.05) are shown in bold.

Factor	Df	F	<i>p</i>	F	<i>p</i>	Df	F	<i>p</i>
(a) Beans		$\delta^{13}\text{C}$ of Gram ⁺ Bacteria		$\delta^{13}\text{C}$ of Gram [−] Bacteria		$\delta^{13}\text{C}$ of Fungi		
Genotype (G)	1,47	0.49	0.488	0.09	0.764	1,32	1.72	0.198
Mixed cropping (M)	1,47	0.50	0.485	0.33	0.567	1,32	0.70	0.409
Collembola (C)	1,47	1.42	0.239	4.13	0.048	1,32	0.26	0.613
Earthworms (E)	1,47	1.96	0.168	5.04	0.030	1,32	1.90	0.178
G × M	1,47	0.01	0.990	0.59	0.447	1,32	0.17	0.683
G × C	1,47	0.22	0.639	2.93	0.094	1,32	3.05	0.090
M × C	1,47	1.91	0.173	0.53	0.471	1,32	2.96	0.095
G × E	1,47	3.17	0.082	0.08	0.779	1,32	0.35	0.558
M × E	1,47	3.70	0.061	0.33	0.569	1,32	0.67	0.419
C × E	1,47	5.49	0.023	2.13	0.151	1,32	11.40	0.002
G × M × C	1,47	2.48	0.122	3.34	0.074	1,32	0.15	0.702
G × M × E	1,47	1.18	0.284	0.17	0.683	1,32	0.23	0.632
G × C × E	1,47	0.81	0.374	3.05	0.087	1,32	3.94	0.056
M × C × E	1,47	0.33	0.566	0.01	0.990	1,32	0.03	0.855
G × M × C × E	1,47	0.16	0.695	1.66	0.204	1,32	2.83	0.102
(b) Wheat								
Mixed cropping (M)	2,35	0.30	0.740	2.89	0.069	2,21	0.19	0.830
Collembola (C)	1,35	0.24	0.625	0.03	0.854	1,21	0.09	0.774
Earthworms (E)	1,35	7.80	0.008	1.27	0.267	1,21	0.77	0.391
M × C	2,35	0.71	0.499	3.57	0.039	2,21	0.11	0.893
M × E	2,35	0.08	0.923	0.09	0.914	2,21	0.75	0.485
C × E	1,35	2.79	0.104	1.18	0.285	1,21	0.01	0.973
M × C × E	2,35	0.88	0.425	0.05	0.955	2,21	2.52	0.107

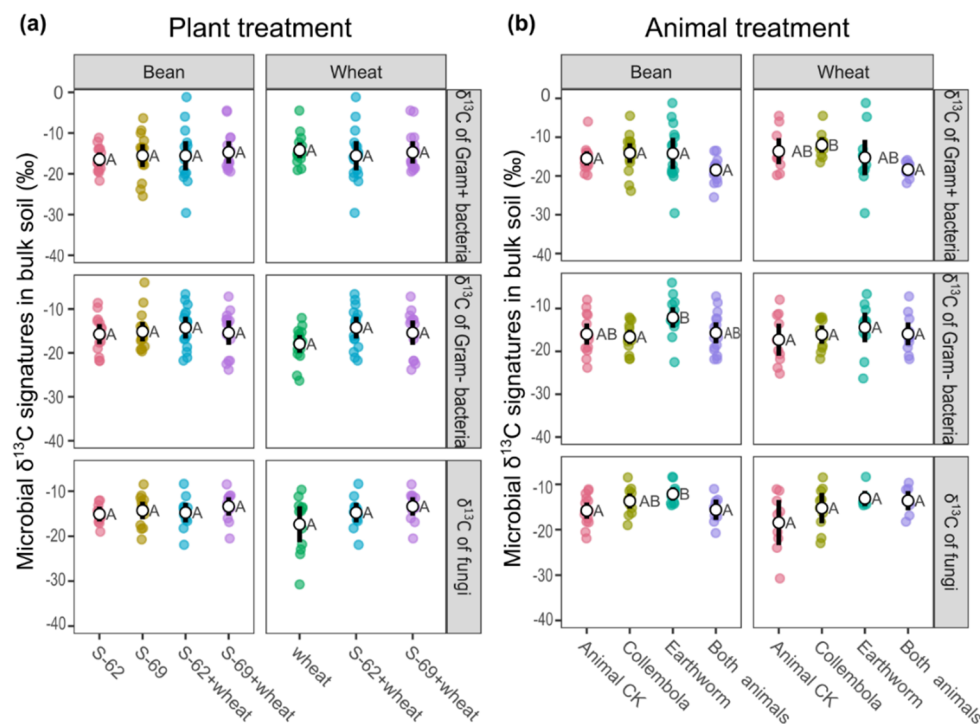


Figure 5. Effect of bean genotype (S-62, S-69) and mixed cropping (with and without wheat for beans and with and without beans for wheat) on $\delta^{13}\text{C}$ values of Gram⁺ and Gram⁻ bacteria and fungi in bulk soil of bean and wheat treatments (measured as $\delta^{13}\text{C}$ values, ‰; data pooled across animal treatments) (a), and modifications by collembola and earthworms (Ctr, without collembola and earthworms; Collembola, with collembola; Earthworm, with earthworms; Both animals, with collembola and earthworms) (data pooled across plant treatments) (b). Means $\pm$ 95% confidence intervals; means sharing the same letter do not differ significantly among plant types (Tukey's post hoc pairwise comparison, $p < 0.05$). Note that the two plant species (bean and wheat) were analysed separately; see Table 5 for statistical details.

3.6. Collembola Abundance, Earthworm Biomass and Incorporation of Litter-Derived Carbon and Nitrogen into Collembola and Earthworms

Collembola. The numbers of collembola at the end of the experiment varied significantly with bean genotype, however, the effect depended on mixed cropping with wheat (Table 6a). In mixed cropping systems with bean genotype S-69 and wheat, collembola numbers were significantly increased (from 170 to 1187), while this was not the case in mixed cropping systems with bean genotype S-62 and wheat (significant genotype $\times$ mixed cropping interaction; Table 6a). As indicated by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, the incorporation of carbon and nitrogen from maize litter into collembola varied significantly with bean genotype (Table 6a). In treatments with bean genotype S-69, $\delta^{15}\text{N}$ values of collembola (86.4‰) exceeded those in treatments with bean genotype S-62 (72.1‰), suggesting enhanced nitrogen transfer from labelled maize litter to collembola in S-69 treatments—consistent with fungal-mediated nitrogen channelling from decomposing litter, whereas $\delta^{13}\text{C}$ values were slightly lower in S-69 (−25.3‰) than in S-62 treatments (−24.6‰), indicating a greater contribution of root-derived carbon to collembola body tissue in S-69 treatments (Figure 6).

Earthworms. Changes in earthworm biomass during the experiment varied with plant genotype, but the effect depended on mixed cropping with wheat and the presence of collembola (Table 6b). Generally, across all treatments, the biomass of earthworms decreased. However, the decline in earthworm biomass was mitigated if collembola were present except in treatments with only bean genotype S-62 (Figure S3). Furthermore, as indicated by $\delta^{13}\text{C}$ values, the incorporation of nitrogen—but not carbon—from maize litter into earthworms varied significantly with bean genotype. $\delta^{13}\text{C}$ values of earthworms were higher in treatments with bean genotype S-69 (−17.1‰) than in those with S-62 (−18.4‰; Table 6b, Figure 6). Given that earthworms are large and directly consume litter, the higher $\delta^{13}\text{C}$ values in treatments with bean genotype S-69 indicates that earthworms in this treatment incorporated a greater proportion of ^{13}C -enriched maize litter—indicating enhanced litter carbon availability. By contrast, lower $\delta^{13}\text{C}$ values of earthworms in treatments with bean genotype S-62 point to a higher proportion of root-derived carbon in their diet.

Table 6. F- and *p*-values from three-way ANOVA assessing the effects of genotype (S-62, S-69) and mixed cropping (with and without wheat) on collembola abundance at the end of the experiment (a) and changes in earthworm biomass during the experiment (b), as well as on the incorporation of carbon and nitrogen from ^{13}C - and ^{15}N -labelled maize litter into collembola (a) and earthworms (b), as indicated by animal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Note that only treatments with beans were analysed. Significant effects ($p < 0.05$) are shown in bold.

Factor	Df	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
(a) Collembola		Numbers		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
Genotype (G)	1,23	0.05	0.826	5.46	0.029	7.46	0.012
Mixed cropping(M)	1,23	3.75	0.065	0.10	0.751	0.30	0.590
Earthworm (E)	1,23	3.76	0.065	3.64	0.069	1.56	0.224
G $\times$ M	1,23	4.41	0.047	0.32	0.576	1.12	0.301
G $\times$ E	1,23	0.01	0.932	0.42	0.525	0.05	0.825
M $\times$ E	1,23	0.62	0.441	2.12	0.159	0.04	0.834
G $\times$ M $\times$ E	1,23	2.89	0.102	1.65	0.212	0.85	0.366
(b) Earthworms		Change in biomass		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
Genotype (G)	1,24	4.96	0.036	4.83	0.038	1.39	0.250
Mixed cropping(M)	1,24	1.21	0.283	1.53	0.228	2.06	0.164
Collembola (C)	1,24	2.11	0.159	0.23	0.639	0.28	0.601
G $\times$ M	1,24	0.22	0.642	0.01	0.920	0.02	0.886
G $\times$ C	1,24	3.87	0.061	0.01	0.920	0.00	0.962
M $\times$ C	1,24	4.13	0.053	0.04	0.852	0.00	0.985
G $\times$ M $\times$ C	1,24	8.82	0.007	0.68	0.417	1.22	0.280

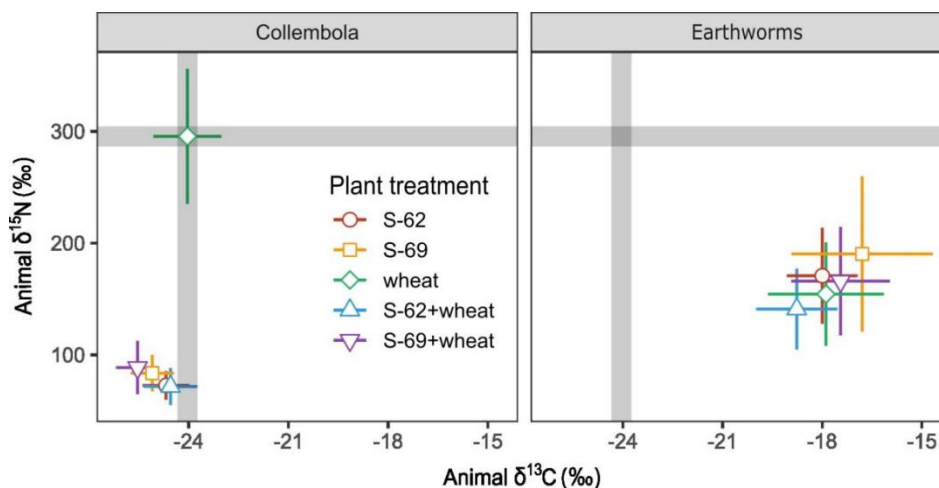


Figure 6. Shift in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of collembola (**left**) and earthworms (**right**) resulting from the incorporation of carbon and nitrogen from ^{13}C - and ^{15}N -labelled maize litter ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of 130‰ and 7578‰, respectively), as influenced by bean genotype (S-62, S-69) and mixed cropping (with and without wheat for beans and with and without beans for wheat). Means $\pm$ 95% confidence intervals; see Table 6 for statistical analyses. Means, confidence intervals and Tukey's post hoc pairwise comparisons are provided in Table S1.

4. Discussion

We demonstrated that plant genotypes, cropping systems and soil fauna interact to influence rhizosphere processes and feedbacks to plant roots, mainly including three mechanisms: (i) genotype-specific nitrogen routing pathways to soil fauna, (ii) amplification of fungal-mediated nitrogen transfer by conservative root traits and (iii) intensification of genotype-decomposer feedback loops by interspecific plant competition. Taken together, our results support a hierarchical plant–soil feedback framework in which root phenotypic traits (specific root length, root mass density) determine rhizosphere microbial community composition (fungal vs. bacterial dominance), which in turn shapes decomposer animal abundance and resource use ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values). In respect to the latter, resource-conservative genotypes strengthen the pathway of nitrogen transfer from litter to collembola via fungi. Critically, the cropping environment (monoculture vs. intercropping) modulates this hierarchy by intensifying or attenuating genotype-specific effects, demonstrating that plant genotype effects on belowground processes are not fixed but context-dependent. This framework extends the root economics spectrum from plants alone to the whole decomposer food web, revealing how conservative root traits create a “slow” carbon–nitrogen economy favouring fungal pathways and fungi–collembola coupling, in contrast to the “fast” bacterial-dominated cycling under resource-acquisitive genotypes.

Our results demonstrate that the selected faba bean genotypes fundamentally shape root architecture and associated rhizosphere microbiome characteristics, leading to distinct interactions with soil decomposers and co-cultivated plant species in mixed cropping systems—supporting our first hypothesis. The genotype-specific root traits reflect divergent resource-use strategies [35,36]. Bean genotype S-62 exhibited a resource-acquisitive phenotype, characterised by higher specific root length and lower root mass density than S-69. This morphology favours rapid soil exploration and nutrient uptake [37]. By contrast, genotype S-69 represents a resource-conservative strategy, which was particularly pronounced in mixed cropping with wheat, where fine root diameter further increased—suggesting that this strategy is intensified under interspecific competition [38]. This divergence in resource allocation, with S-62 being shoot-biased and S-69 root-biased, strongly influenced interactions with soil biota and their feedbacks to plant performance [39]. The uniform reduction in root mass density across faba bean genotypes when intercropped with wheat suggests a consistent response to interspecific competition. This aligns with earlier studies showing that legumes in cereal–legume mixtures often reduce root biomass investment to mitigate competition with cereals for soil space and nutrients [40,41].

Collembola consistently enhanced the specific root length of beans, which aligns with earlier findings that collembola stimulate the proliferation of fine roots [42]. However, this effect was context-dependent. In bean monoculture, the collembola-mediated increase in specific root length was most pronounced in the absence of earthworms, whereas in mixed cropping systems, collembola effects were amplified by their presence. This underscores that interactions between decomposer animals can vary markedly depending on plant species and their interactions, as previously observed in grassland systems [21]. Conversely, earthworms increased root mass density, presumably through bioturbation, improved soil structure and enhanced nutrient mobilisation [43].

The contrasting root architecture of the two bean genotypes resulted in distinct rhizosphere microbial communities. Bean genotype S-69 with higher fungi/bacteria ratios, harboured microbial communities indicative of greater environmental stress or resource limitation than genotype S-62 [44,45]. Also, higher sat/mono and Gram⁺/Gram[−] ratios suggest increased microbial stress, likely due to cell membrane rigidification under low substrate availability or harsher environmental conditions [28,46]. This pattern aligns with the resource-conservative strategy of bean genotype S-69 and may suggest the release of specific root exudates that shape microbial communities adapted to—or indicative of—a more resource-limited rhizosphere compared to the resource-acquisitive genotype S-62 [47,48]. By contrast, genotype S-62, characterised by higher specific root length, prioritises rapid nutrient foraging, fostering bacterial-dominated communities with a higher prevalence of Gram[−] bacteria [49]. This is consistent with the “root economics spectrum” paradigm, which posits that high specific root length enhances resource capture efficiency [37]. Mixed cropping with wheat modulated these characteristics and reduced the Gram⁺/Gram[−] bacteria ratio in the bean rhizosphere, potentially suggesting altered competitive dynamics or root exudate profiles under interspecific competition [50]. Supporting this conclusion, mixed cropping with beans increased the fungi/bacteria and sat/mono PLFA ratios in the wheat rhizosphere, suggesting that wheat-associated bacteria experienced greater microbial stress or responded to altered root exudation in the presence of beans—supporting our third hypothesis.

In wheat, mixed cropping with beans resulted in divergent effects depending on bean genotype. In mixtures with bean genotype S-69, wheat exhibited reduced specific root length, suggesting that the resource-conservative faba bean genotype intensified belowground competition [41]. Furthermore, collembola reduced wheat root diameter, with this being most pronounced in monoculture, indicating fauna-induced root thinning that may enhance foraging efficiency [51]. Notably, this effect was restricted to treatments without earthworms, suggesting that earthworms may mitigate collembola-mediated changes in root architecture—presumably by altering soil structure [39]. Earthworms consistently increased root mass density without affecting fine root morphology, underscoring their role in enhancing root system robustness and nutrient foraging capacity in monoculture and mixed cropping systems [52].

PLFA stress indicators as indirect proxies provided insights into how soil decomposers shaped microbial community structure and physiological state, and how these patterns depended on crop species, bean genotype and cropping system. Collembola generally increased the dominance of fungi in the bean rhizosphere, aligning with their role in stimulating saprotrophic fungi [51,53]. Furthermore, collembola increased the bacterial sat/mono PLFA ratio in the bean rhizosphere, indicative of increased microbial stress [28,46]. Conversely, earthworms reduced this ratio, pointing to enhanced nutrient mineralisation and alleviation of microbial stress [28,54]. In summary, while both detritivores influenced the rhizosphere microbiome, their pathways to enhancing ecosystem functioning differed and were based on contrasting mechanisms: Collembola altered the fungal-mediated channelling via top-down processes, which may optimize nutrient transfer to plants at the cost of increased bacterial competition [55], while earthworms accelerated bacteria-based nutrient mineralization by alleviating resource limitation and stress [56]. In the bean rhizosphere, effects of collembola and earthworms on microbial

community characteristics were independent of each other. By contrast—in the rhizosphere of wheat—the effects of collembola and earthworms on microbial community characteristics depended on each other. In the presence of both decomposers, the fungi/bacteria ratio increased synergistically. This synergistic shift towards fungal dominance suggests that collembola and earthworm activities complemented each other. Presumably, collembola grazing suppressed bacteria or stimulated fungal growth, while earthworms—through burrowing and mixing of litter and mineral soil—further promoted fungal proliferation [53,57]. This is supported by the observed increase in the fungi/bacteria ratio in the bean rhizosphere in the presence of both decomposers, indicating that each independently promotes fungal dominance, albeit likely via different mechanisms.

Generally, mixed cropping reduced the sat/mono PLFA ratio in bulk soil of beans, whereas it increased this ratio in bulk soil of wheat. By contrast—and consistent with patterns observed in the rhizosphere—earthworms decreased the sat/mono ratio in wheat bulk soil, presumably reflecting enhanced nutrient mobilisation and reduced microbial stress [58]. Notably, earthworms lowered the Gram⁺/Gram[−] bacteria ratio in wheat bulk soil, indicating a shift towards Gram[−] dominance, likely driven by increased substrate availability resulting from the mixing of litter and mineral soil in earthworm casts [39].

Critically, our results revealed distinct microbial responses in the wheat rhizosphere to intercropping and fauna interactions that were previously underexplored. Mixed cropping with beans consistently increased the fungi/bacteria ratio in the wheat rhizosphere, indicating a shift towards fungal-dominated communities under interspecific competition. This aligns with the increased sat/mono ratio, suggesting nutritional stress in wheat rhizosphere microbes when competing with beans [59]. Notably, the combined presence of earthworms and collembola further increased the fungi/bacteria ratio in the wheat rhizosphere. This implies that both taxa jointly intensified fungal dominance potentially via complementary processes, with collembola grazing stimulating fungal growth and earthworms improving soil structure for fungi [20]. Furthermore, our results indicated that the effect of beans on microorganisms in the wheat rhizosphere varies with bean genotype. Mixed cropping with bean genotype S-62 significantly reduced the Gram⁺/Gram[−] ratio, indicating enrichment of Gram[−] bacteria with high nutrient acquisition capacity. This aligns with the resource-acquisitive strategy of bean genotype S-62, which likely is associated with high root exudation favouring fast-growing Gram[−] bacteria [47,60]. Conversely, in wheat bulk soil, mixed cropping with bean genotype S-69 increased the Gram⁺/Gram[−] ratio, suggesting enrichment of stress-tolerant Gram⁺ bacteria. This likely reflects the low investment of bean genotype S-69 into root exudates, selecting for oligotrophic microbes adapted to nutrient-limited conditions [41,49].

Our results confirm unique rhizosphere and bulk soil microbial responses that were fundamentally shaped by crop identity and further modulated by decomposer fauna. Rhizosphere soil consistently exhibited elevated fungal dominance than bulk soil across all treatments. These findings are consistent with root exudation promoting saprotrophic fungi [39]. Notably, collembola amplified this rhizosphere effect in particular in beans, likely due to bean-specific root metabolites enhancing fungal–collembola interactions [57]. Furthermore, the sat/mono PLFA ratio was higher in bulk soil than in rhizosphere soil, indicating greater nutritional stress distant to roots. Earthworms mitigated this stress in particular in bulk soil, as indicated by the reduced sat/mono ratio, presumably due to enhanced nutrient mobilisation and mixing of litter and soil in casts. Conversely, collembola increased the sat/mono ratio in the bean rhizosphere, suggesting grazing-induced microbial stress. Generally, the animal effects on PLFA indicators varied with root vicinity, with earthworms predominantly affecting bulk soil microorganisms and collembola more strongly affecting microorganisms in the rhizosphere, elevating fungal dominance and increasing microbial stress [39,53].

Mixed cropping with wheat generally reduced the Gram⁺/Gram[−] bacteria ratio in bean treatment soil samples, indicating a shift towards Gram[−] bacteria—typically r-strategists with high growth capacity [44,60]. This shift closely corresponded with increased microbial biomass, enhanced basal respiration and reduced qCO₂, suggesting greater substrate availability in mixed cropping systems, likely driven by complementary root exudate profiles [61]. The reduced metabolic quotient further suggests more efficient carbon use due to mixed cropping [62].

Collembola and earthworms affected microbial biomass and activity in bulk soil in a context-dependent way, reflecting shifts in microbial functioning and carbon use efficiency. Collembola consistently increased basal respiration in the bulk soils of bean and wheat and concurrently increased the microbial metabolic quotient—suggesting that they generally enhanced microbial metabolic activity while reducing microbial carbon use efficiency. Presumably, this reflects increased microbial community turnover and increased energy costs due to grazing [6]. Earthworms alone neither affected microbial biomass nor respiration in bulk soil of bean treatments but interacted with collembola in affecting these parameters. The combined presence of earthworms and collembola increased microbial biomass and basal respiration, supporting our second hypothesis. This synergy again points to complementary functional roles of collembola and earthworms with collembola stimulating

microbial activity via grazing and earthworms enhancing microbial resource availability through mixing of litter and soil and forming favourable habitat conditions for microorganisms [63,64].

The $\delta^{13}\text{C}$ values of microbial PLFAs provided key insights into the incorporation of litter carbon into different microbial groups and their modification by animal decomposers. Collembola significantly reduced the $\delta^{13}\text{C}$ values of Gram⁻ bacteria within bean bulk soil. Given that Gram⁻ bacteria typically use labile, recent plant-derived carbon [65], this reduction suggests that collembola grazing redirects bacterial carbon use from litter to root exudates. Conversely, earthworms increased $\delta^{13}\text{C}$ values of Gram⁻ bacteria, indicating a shift towards the use of carbon from labelled maize litter [65]. In the combined treatment with both decomposers, $\delta^{13}\text{C}$ values of Gram⁺ bacteria and fungi were reduced in bean bulk soil, indicating that in combination their carbon use shifted from plant litter to root-derived resources, potentially including fine root tissue [53]. This aligns with the finding that collembola increased the specific length of bean fine roots, which likely increased the flux of plant-derived carbon into the soil. Therefore, the increase in Gram⁻ bacteria in the combined treatment with collembola and earthworms likely resulted from the combination of collembola-induced increase in specific fine root length and earthworm-induced increase in the availability of litter resources in soil [64]. In wheat bulk soil, the presence of earthworms reduced $\delta^{13}\text{C}$ values of Gram⁺ bacteria; however, they increased wheat root mass density, indicating that this enhanced the availability of plant-derived carbon to Gram⁺ bacteria [66]. Overall, the results suggest that collembola stimulate the use of root-derived carbon by Gram⁻ bacteria, whereas earthworms promote the use of litter carbon by Gram⁺ positive bacteria and fungi [64].

The results clearly indicated that the abundance/biomass and the incorporation of litter carbon of both decomposer animals studied varied significantly with plant genotype, thereby showing feedback loops between plant genotypes and decomposer animals [67]. In treatments with bean genotype S-69, earthworm biomass was lower than in treatments with bean genotype S-62, whereas earthworm $\delta^{13}\text{C}$ values were (slightly) higher in the latter. Bean genotype S-69 was associated with distinct rhizosphere characteristics, with a higher Gram⁺/Gram⁻ bacteria ratio and sat/mono ratio, indicating microbial stress [68]. Furthermore, the higher root mass density, thicker fine roots and potentially high secondary metabolite production, often associated with a resource-conservative strategy of plants such as bean genotype S-69, may have reduced earthworm nutrition and forced them to more intensively feed on maize litter [69]. The mitigation of earthworm biomass loss by collembola may have been due to increased fungal/bacterial ratio and associated enhanced fungal degradation of phytotoxic compounds in the bean rhizosphere [70]. This is corroborated by lower $\delta^{13}\text{C}$ values in fungal PLFAs in presence of collembola (and earthworms), pointing to a shift towards the use of root-derived rather than litter-derived carbon compounds [71]. Notably, intercropping amplified bean genotype effects, as indicated by the peak in collembola abundance in mixed cropping systems with bean genotype S-69.

The divergent $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of collembola in the two bean genotype treatments may point to a fungal-driven decoupling of the acquisition of C and N by collembola. Fungi preferentially metabolise soluble root exudates (e.g., carboxylates) because these require less energy to process than complex polymers [72]. The significantly higher $\delta^{15}\text{N}$ values of collembola in soil of bean genotype S-69 compared to that of bean genotype S-62 point to plant genotype-specific nitrogen routing to collembola. In soil of bean genotype S-69, the fungi/bacteria PLFA ratio was higher than in that of bean genotype S-62, indicating a more fungi-based nitrogen cycling. Low $\delta^{15}\text{N}$ values of collembola coincided with high fungal assimilation of litter-derived nitrogen, positioning fungi as the main nitrogen vector for litter nitrogen [27]. The conservative root architecture of bean genotype S-69 and associated low availability of labile root-derived carbon likely favoured fungi and stimulated the capture and translocation of litter nitrogen by fungi and thereby by collembola. Therefore, collembola may benefit from fungal-mediated nitrogen flux between rhizosphere and detritusphere—a mechanism intensified by conservative root traits.

It is important to acknowledge the limitations of our microcosm-based approach. The 6-month incubation, while sufficient to capture early-stage plant-soil interactions and decomposer colonisation, may not reflect long-term dynamics in field soils. Microcosm conditions (e.g., homogenised soil, controlled temperature and humidity, absence of external colonisers and vertical migration constraints) simplify the complexity of real agroecosystems. Earthworm and collembola behaviour, including vertical migration, trophic interactions with predators and spatial heterogeneity in soil, differ between confined and unconfined soils. Furthermore, our findings are based on a single soil type and climatic context, and the observed genotype, soil fauna and cropping system interactions may vary with soil edaphic properties, landscape position and management history. Extrapolation of the documented mechanisms to field conditions and breeding applications therefore requires cautious interpretation and targeted field validation across multiple environments and growing seasons. Furthermore, we acknowledge the inherent limitations of PLFA-based inference in the present study. PLFA ratios (sat/mono, cy/pre, Gram⁺/Gram⁻) are indirect proxies for microbial physiological state and resource availability, and their interpretation is context-dependent. For example, while the sat/mono ratio has been widely used as an indicator of nutritional or

environmental stress in microorganisms, it can also vary with community composition and soil moisture [73,74]. Accordingly, PLFA-based interpretations in this study should be considered as working hypotheses to be validated by complementary approaches such as metagenomics, metatranscriptomics or enzyme assays.

5. Conclusions

The results document that faba bean genotypes form distinct belowground networks based on their contrasting root architecture. This determines rhizosphere microbial communities and decomposer animal activity. The resource-acquisitive bean genotype S-62 favours bacteria-mediated residue decomposition, whereas the resource-conservative bean genotype S-69 supports fungal-based pathways and fungi–collembola interactions. Soil animal decomposers, such as earthworms and collembola, facilitate rhizosphere processes and feedbacks to plants in a complementary way, with collembola stimulating the growth of fine roots and accelerating root-derived carbon flux, favouring the fungal channel and inducing microbial stress through grazing. Earthworms, in contrast, enhance the decomposition of plant residues by physical soil engineering and mixing of litter and soil (bioturbation), thereby stimulating nutrient mobilisation. Overall, collembola and earthworms mitigate genotype-specific constraints and reinforce fungal pathways, increase microbial carbon efficiency and enhance carbon–nitrogen coupling in mixed cropping systems. Genotype-specific responses to decomposer activity underpin the differential performance in monoculture versus mixed systems, with bean genotype S-69 exhibiting greater sensitivity to fauna-induced changes due to its resource-conservative root strategy. These findings reveal a hierarchical plant–soil feedback framework where the root phenotype orchestrates decomposer-mediated resource processing. Within this framework, root architecture acts as the primary determinant of the microbial channel (bacterial vs. fungal) engaged by decomposer fauna, which in turn governs the pathway through which carbon and nitrogen are processed and transferred across the rhizosphere–detritusphere continuum. This mechanistic insight offers a clear target for future research: root architectural traits that modulate rhizosphere carbon–nitrogen coupling and decomposer feedback loops hold potential for enhancing nutrient cycling and yield stability in legume–cereal intercrops, provided that the identified mechanisms are validated under field conditions with diverse genotype panels.

Supplementary Materials

The additional data and information can be downloaded at: <https://media.sciltp.com/articles/others/2608030928549867/SE-26010155-SM.pdf>. Figure S1: Specific root length of bean fine roots as affected by collembolans, earthworms and mixed cropping with wheat; means with 95% confidence intervals, means sharing the same letter do not differ significantly (Tukey's post hoc pairwise comparison, $p < 0.05$); Figure S2: Interactive effects of plant type (wheat monoculture, mixed cropping of wheat and bean genotype S-62 or S-69) and collembola on wheat fine root diameter. Means with 95% confidence intervals, means sharing the same letter do not differ significantly (Tukey's post hoc pairwise comparison, $p < 0.05$); Figure S3: Interactive effects of bean genotype, mixed cropping with wheat and presence of collembola on the change in earthworm biomass during the experiment. Means with 95% confidence intervals; means sharing the same letter do not differ significantly (Tukey's post hoc pairwise comparison, $p < 0.05$); Table S1: Biomass changes of earthworms, numbers of collembola, and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of collembola and earthworms in plant treatments (bean genotype S-62, bean genotype S-69, wheat, mixture of wheat and S-62, mixture of wheat and S-69) (a), and animal treatments (with and without collembola or earthworms) (b); lower.CL and upper.CL represent the lower and upper bounds of the confidence intervals of the means; capital letters indicate significant differences of respective variables between plant (a) and animal treatments (b); means sharing the same letter do not differ significantly, Tukey's post hoc pairwise comparison, $p < 0.05$.

Author Contributions

Z.-Y.W.: Conceptualization, Funding acquisition, Methodology, Investigation, Writing—original draft, Writing—review & editing; S.L.: Writing—original draft, Formal analysis; J.-Z.L.: Formal analysis; A.E.: Formal analysis; L.-B.F.: Conceptualization; W.-D.C.: Conceptualization; P.-L.W.: Methodology, Formal analysis, Writing—review & editing; S.S.: Conceptualization, Funding acquisition, Methodology, Supervision, Project administration, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

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Informed Consent Statement

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare the following financial interests/personal relationships which may be considered potential competing interests: Zhi-Yuan Wang reports financial support was provided by the China Scholarship Council. The other authors declare that they have no competing interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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