



## Crop rotation phase has a greater impact on soil biology than crop rotation diversity

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### ABSTRACT

The effect of plant diversity on the belowground soil food web remains poorly understood. In this study the soil microbial community structure and biomass, and the abundance of microfauna, mesofauna, and macrofauna were assessed at three levels of crop rotation diversity: A Simple rotation (2 plant species), a Moderate rotation (4 plant species), and a Diverse rotation (10 plant species). Soils subjected to more diverse crop rotations did not differ in their microbial community structure, were lower in soil total C, and exhibited a smaller microbial biomass, but a higher crop yield. The mean abundance of Collembola and mites exhibited a trend of Simple > Moderate > Diverse. These observations may be associated with higher levels of disturbance in soils of more diverse rotations due to more frequent tillage operations to establish a greater diversity of crops. The lack of a significant positive effect of crop rotation diversity on soil biology was observed despite the field experiment being established three to four years prior to these measurements. We did observe effects due to the phase of the crop rotation. Within the Simple rotation, we found a significant effect of crop rotation phase on collembolan and mite abundances, and within the Diverse rotation on earthworm biomass. These observations suggest that the crop rotation phase, and perhaps the identity of the individual plants used in a crop rotation, affect soil biology more than the diversity of the crop rotation *per se*.

### 1. Introduction

Biodiversity is critical to the delivery of global food security, and therefore the provision of healthy diets for all in the 21st century (Dannenberg et al., 2024). It is well established that agricultural intensification leads to decreases in associated biodiversity (Hooper et al., 2005). However, ecological intensification and biological diversification of farming systems have been proposed as methods to increase ecosystem service delivery, improve the resilience of food production systems (Tilman et al., 2006; Lin, 2011; Bommarco et al., 2013), decrease agrochemical input use, improve soil health, and reduce the environmental damage ensuing from modern agriculture (such as greenhouse gas emissions and biodiversity loss) (Kremen et al., 2012).

Soils provide a habitat to 59 % of species that inhabit Earth (Anthony et al., 2023). However, the impacts of human activities, such as agricultural land use, on belowground soil biology remain understudied (Geisen et al., 2019) and observed relationships between aboveground and belowground biodiversity are not consistent (Bardgett and Wardle, 2010; Sabais et al., 2011).

Farming systems can be diversified by increasing plant diversity temporally (e.g., crop rotations) or spatially (e.g., intercropping, establishment of field margins, hedgerows, and other landscape features) (Kremen et al., 2012). Diversification at the field scale can be realised by growing a combination of different crops by means of intercropping or undersowing, and/or growing different genetic varieties of the same crop (Kremen et al., 2012). There is considerable

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evidence that plant species can shape the soil microbial community composition due to the influence of crop residues (Veen et al., 2019) and rhizodeposits (Nannipieri et al., 2023). This evidence leads to our first hypothesis; that arable crop rotation diversity alters soil microbial community composition.

Several authors report differences in properties of soils under crop rotations, compared to continuous monocultures. These differences include increased total C content of the soil (Lange et al., 2015), increased microbial biomass C (McDaniel et al., 2014), increases in certain microbial communities (Tiemann et al., 2015), and increased soil faunal diversity and biomass (Tresch et al., 2019). The primary mechanism explaining the greater belowground biodiversity and higher soil organic matter found in more diverse cropping systems is the production of different qualities of plant-derived organic substrates (Dufour, 2025). These substrates include aboveground inputs in the form of plant residues (Shu et al., 2022) and belowground inputs in the form of rhizodeposits (Nannipieri et al., 2023) and root litter (Liu et al., 2023). This evidence leads to our second hypothesis, that soil microbial biomass, soil C, and soil N will increase with crop rotation diversity.

These organic inputs create a larger and more biochemically heterogeneous resource base that reduces interspecific competition and feeds into a greater number of trophic niches, resulting in a differently structured soil food web (Wardle, 2006; Armbrecht et al., 2004). Greater diversity of belowground microbial community composition, in turn, has been associated with agro-ecosystem multifunctionality, including increases in plant diversity, decomposition rate, and retention and cycling of nutrients (Wagg et al., 2014; Wagg et al., 2019). Differences in the quantity of resources provided by different plant species has been identified as a mechanism for greater soil biota abundance (Salamon et al., 2011; Wissuwa et al., 2012). Diversification of arable systems by increasing the number of plant species has also been linked to a higher diversity (Simpson's evenness) of nematodes (De Deyn et al., 2004). This evidence leads to our third hypothesis, that greater microbial biomass and more diverse litter inputs will lead to greater populations of both microbivorous and detritivorous soil fauna, as reflected in the populations of nematodes, microarthropods and earthworms.

De Deyn et al. (2004) noted that it can be the plant identity rather than diversity or biomass that mainly affects belowground species diversity. The importance of plant identity rather than plant diversity has been noted in numerous studies, including on nematodes (Kostenko et al., 2015; Viketoft et al., 2009; Wardle et al., 2003), mesofauna (Beugnon et al., 2019; Salomon et al., 2011; Wissuwa et al., 2012), and earthworms (Gastine et al., 2003). For example, leguminous plants are often considered to provide higher-quality resources that positively affect the soil faunal groups studied (Spehn et al., 2000). This evidence leads to our fourth hypothesis, that differences between crop rotation phases will be most pronounced in more diverse crop rotations, because it is likely that the plant currently growing may exert some influence on the soil biological community and therefore it is more likely to observe differences in soil biological communities between plots growing different plants than plots growing the same plants.

In this study, the link between plant diversity and soil biodiversity was investigated in an arable cropping system by comparing the soil biological community in soils samples taken from a field plot experiment which contained crop rotations with different degrees of diversity: A Simple rotation (2 plant species), a Moderate rotation (4 plant species), and a Diverse rotation (10 plant species). Soil samples were analysed for total C and N, pH, and soil microbial community. In addition, the abundance and community composition of microarthropods (mites and Collembola), nematodes, and earthworms were quantified. The experiment was designed such that each phase of the 4-phase crop rotations were represented each year of the experiment in a space-for-time substitution. This enabled us to differentiate the impact of crop rotation diversity and the impact of individual plants that exist at each crop rotation phase.

## 2. Methods

### 2.1. Description of the field site and experimental design

The field experiment was established in 2013 at the Crop Research Unit, University of Reading, Sonning, UK ( $51^{\circ}28'50.8''N$   $0^{\circ}54'07.3''W$ ) in a free draining sandy/silty loam containing on average 5.6 % clay, 50.7 % silt, and 43.7 % sand (Degani, 2019), overlaying coarse red-brown sand of the Sonning series (Jarvis, 1968). The Soil Survey of England and Wales classification of the Sonning series has been correlated and reclassified using the World Reference Base (2006, Tier 1 Version) as a Chromic Endoskeletal Luvisol. Establishment of the field site succeeded many years of grass ley, one season of winter barley (2011–2012) and one season of winter wheat (2012–2013). The experiment was laid out in a split-plot randomized complete block design, where a block containing all three rotations (Simple, Moderate, and Diverse; Table 1) was replicated four times. Each rotation treatment comprised four 12 m  $\times$  10 m subplots, representing the four different phases (i.e., years) of the rotation. The design of the experiment relies on a space-for-time substitution, so that each phase in the crop rotation is represented by one of the four subplots in the rotation at any one time. Each subplot was divided into five 1.9 m wide strips with enough space in between to allow for crop management and access to sampling and measurement instrumentation. Crop yield was measured at the end of each cropping season using a plot-scale combine harvester from the middle three strips of each subplot. See [Supplementary Information](#) Section S3 for maps with plot designation for each year and [Supplementary Information](#) Section S4 for a full description of all agronomic operations.

Nitrogen fertilisation was performed at 50 % of the rate recommended by the Agriculture and Horticulture Development Board (AHDB, 2019) to reflect a low input system and maximise the likelihood of observing diversity-driven differences between the treatments. This was  $50 \text{ kg N ha}^{-1} + 50 \text{ kg SO}_3 \text{ ha}^{-1}$ , applied as ammonium nitrate (34.5 % N) and ammonium sulphate nitrate (26 % N, 37 % SO<sub>3</sub>). Fungicide was applied at 50 % recommended rate, and herbicide was applied at 100 % recommended rate, except for the Diverse plots, which were not treated with a second herbicide dose in phases 1 and 3 to encourage establishment of the legume understorey. The Diverse plots also had an additional power harrowing prior to cover crop drilling and ploughing to 20–25 cm prior to spring crop drilling. Therefore, the Diverse plots were subject to a greater level of soil disturbance than the Simple or Moderate plots.

Sample collections and measurements were carried out in selected plots during June 2016 (for soil characterisation and soil microbial community assessment) and all plots during June 2017 (for soil characterisation and soil fauna survey). All 48 subplots of the experiment representing all three diversity levels and all four crop phases were sampled in June 2017. Nematodes were collected to represent microfauna, Collembola and mites to represent mesofauna, and earthworms to represent macrofauna. In 2016 only the 12 plots where the crop was winter wheat at phase 3 of the rotation were sampled for soil characterisation (C, N, pH) and soil microbial community assessment

**Table 1**

Sequence of crops in each rotation (Simple, Moderate and Diverse) in the field experiment.

	Simple	Moderate	Diverse
Phase 1	Winter wheat	Winter wheat	Winter wheat under-sown with legume mixture
Phase 2	Winter wheat	Oilseed rape	Oilseed rape
Phase 3	Winter wheat	Winter wheat	Winter wheat under-sown with legume mixture
Phase 4	Oilseed rape	Winter beans	Brassica winter cover crop followed by spring beans

(Table S1). In the Simple rotation these plots were previously cropped with two years of wheat. In the Moderate rotation these plots were previously cropped with a year of oilseed rape (OSR) following a year of wheat. In the Diverse rotation these plots were previously cropped with a year of spring beans (after a brassica winter cover crop) following wheat under-sown with a legume mixture. The combination of the 12 plots sampled in June 2016 and the 48 plots sampled in June 2017 resulted in a total of 60 samples collected in total.

## 2.2. Description of the soil characterisation methods

Soils were sampled from the middle three strips of selected subplots in June 2016 and all plots in June 2017. We took five 15 cm deep cores in a 'W' layout and homogenised these into one composite sample per plot. The soil samples were sieved to 2 mm and air-dried. Subsamples of 10 g each were shaken in 25 ml Ultrapure ( $> 18.2 \Omega\text{W.cm}$ ) water for 15 min and the pH was measured using a pH electrode. For measurement of total C and N, subsamples were ball-milled (Fritsch Pulverisette 4) and analysed by Dumas dry combustion (Flash 2000, Thermo Fisher Scientific, Cambridge, U.K.).

## 2.3. Description of the soil microbial community structure and biomass measurement

Cores of 15 cm depth were used to collect 5 soil samples per plot from selected plots in a 'W' layout across the three middle strips of each subplot in July 2016. We used a gouge auger and homogenised these into one composite sample per subplot for phospholipid fatty acid (PLFA) analysis. Soils were stored in a cool box during field sampling and subsequently transferred to a cold room and stored at 4 °C prior to sieving to 4 mm, freezing, and freeze-drying. Microbial community structure and biomass were assessed using PLFA profiles following the methods described in Struijk et al. (2023).

## 2.4. Description of the soil fauna survey

Soils were sampled from all plots in June 2017 for nematodes using a gouge auger. Five 30 mm diameter soil cores were collected from the middle three strips of each subplot combined in one composite sample. Duplicate subsamples were then prepared per plot for extraction of nematodes using a modified version of the Baermann funnel method (Baermann, 1917), as described in Supplementary Methods S1.

A 10 cm deep core of 9.8 cm diameter ( $754 \text{ cm}^3$ ) was collected from each plot to collect microarthropods (Collembola and mites) in June 2017. Each core was then placed upside down and extracted for three days under a hot lamp in Tüllgren funnels, allowing microarthropods to drop through a 2 mm mesh into collection receptacles containing 70 % ethanol. Collembola specimens were identified by x10 stereo microscope to the orders *Poduromorpha*, *Entomobryomorpha* and *Sympyleona*, and mite specimens were identified to the orders/suborders *Prostigmata*, *Mesostigmata* and *Oribatida* (which included Astigmatid mites).

A 20 cm × 20 cm × 20 cm soil pit was excavated from each plot and transported to the lab, where it was hand sorted for earthworms in June 2017. Juveniles were distinguished from adults based on the absence of a saddle and then adults (and some juveniles) were identified to species level, following Sherlock (2012). The biomass of each species was recorded. Five litres of mustard solution ( $6 \text{ g L}^{-1}$  Coleman's mustard powder) was poured into each soil pit immediately after excavation and observed to retrieve deep-burrowing anecic earthworms, but none were retrieved from any of the plots sampled.

## 2.5. Description of the data analyses

Statistical analyses were performed in R version 4.1.3 (R Core Team, 2022) using RStudio version 2023.03.0.386 (Posit Team, 2023). Soil

faunal abundance and biomass were analysed by analysis of variance (ANOVA) with a nested treatment structure (Diversity/(Scrop + Mcrop + Dcrop)) and a nested blocking structure (block/mainplot/subplot). The Diversity factor indicates whether a main plot is in the Simple, Moderate or Diverse rotation. The nested factors indicate which of the four crop phases within Diversity level each subplot was in: one of the S (imple), M(oderate), or D(iverse) crop phases. Assumptions of the ANOVAs were assessed by inspecting q-q (normality) and fitted values (variance homogeneity) plots and data transformed where necessary as indicated in the statistical output tables. Pearson correlations were performed to investigate relationships between different variables.

PLFA data (expressed in nmol per gram dry soil) were converted into proportions and analysed by nonmetric multidimensional scaling (NMDS) ordination and subsequent permutational analysis of variance (PERMANOVA). The fungal:bacterial (F:B) ratio was calculated based on the classification of PLFAs specified in Struijk et al. (2023) to provide an indication of the presence of these microbial groups, although we recognise the shortcomings of the F:B calculation from PLFA profiles (Strickland and Rousk, 2010). ANOVAs (with experimental blocking structure) were performed on the biomass of all fatty acids as well as F:B, G+ :G-, actinomycetes, and total PLFA biomass. Homoscedasticity was evaluated with a Levene test of the data set. The normal distribution of the residuals was evaluated with a Shapiro-Wilk test of the residuals of the ANOVA.

## 3. Results

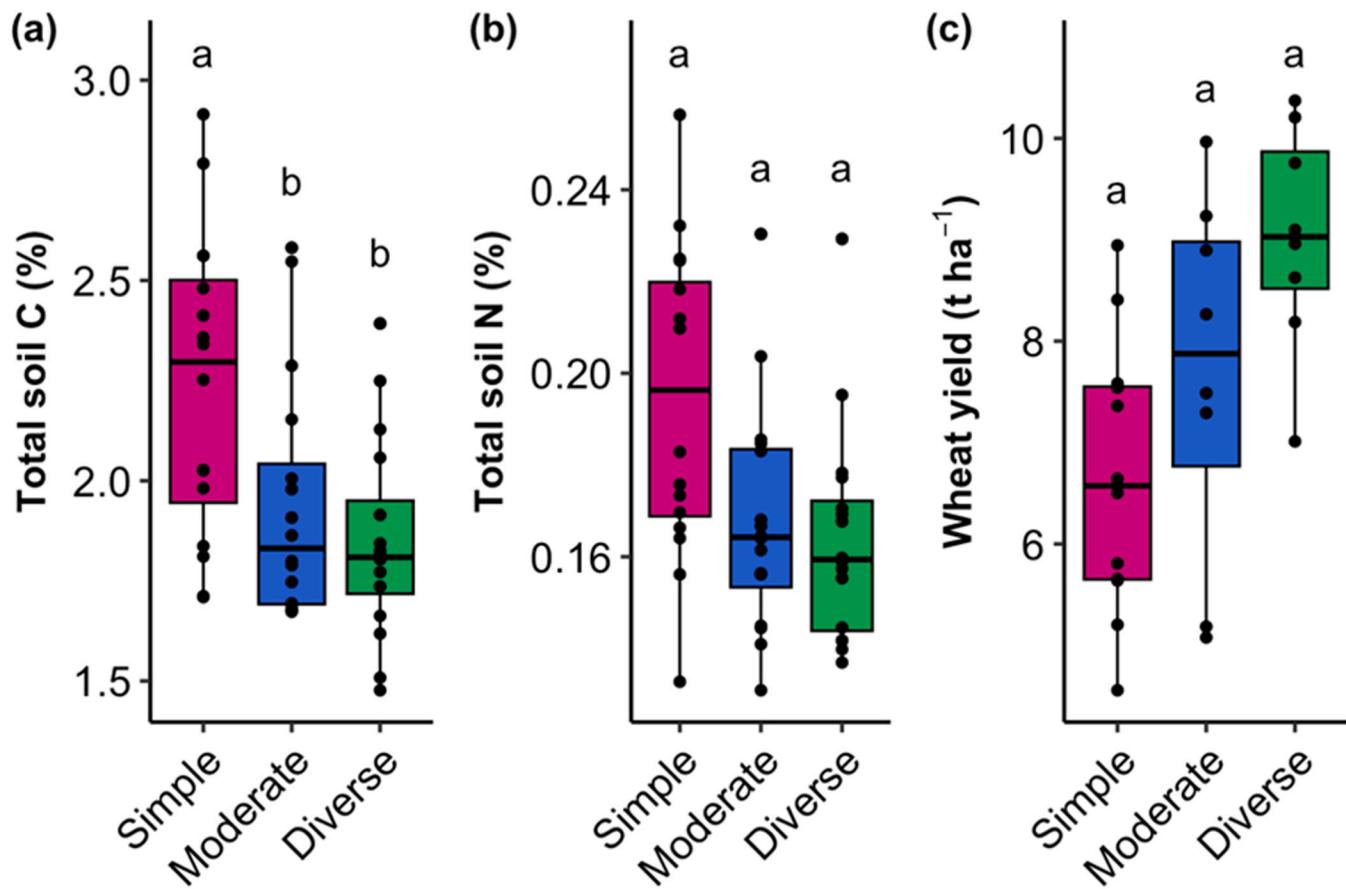
### 3.1. Effects of crop rotation diversity on crop yield, soil biology, and soil chemistry

The crop rotations of varying degrees of diversity (Simple, Moderate, Diverse) mostly affected the chemical characteristics of soils. Total soil C was significantly higher in the Simple rotation (Fig. 1a). The same pattern was observed in the total soil N data (Fig. 1b), but this was not statistically significant (Table 2). While wheat yield followed the opposite pattern, being lowest in the Simple rotation and highest in the Diverse rotation, there were no significant differences in wheat yield between rotations (Fig. 1c).

While the mean abundance of some mite and collembolan taxa also follow the pattern Simple > Moderate > Diverse (Fig. 2), crop rotational diversity was not a significant predictor of nematode, mite, collembolan or earthworm abundances (Table 2). We encountered mostly Collembola of the order *Entomobryomorpha*, followed by *Poduromorpha*. The collembolan order *Sympyleona* was absent from all soil cores. For nematodes, bacterial feeders were the most dominant trophic group in all crop rotations, followed by plant parasites and a small proportion of predatory species (Figure S3). We identified two adult earthworm species in the plots, *Aporrectodea rosea* and *Octolasion cyaneum*, juveniles of *Allolobophora chlorotica*, and numerous other unidentifiable juvenile specimens. All identified adult earthworms were soil-dwelling endogeic species. Adult earthworms were rare and only appeared in the Moderate rotation. Earthworm abundance correlated positively with plant parasitic nematodes ( $r = 0.45$ ,  $p = 0.02$ ) and negatively with bacterivorous nematodes ( $r = -0.50$ ,  $p < 0.01$ ) but there was no significant effect of crop rotation diversity on earthworm abundance or biomass (Figure S4).

The soil microbial community structures observed in the wheat plots at phase 3 of the crop rotation in June 2016 were similar across all rotations (treatment  $R^2 = 0.151$ ,  $p = 0.78$ ; PERMANOVA) (Fig. 3a). The total PLFA biomass, as well as the F:B ratio, were highest in the wheat plots of the Simple rotation, followed by the Moderate and then the Diverse rotation (Figs. 3b and 3c), but these differences were not statistically significant (PLFA biomass:  $F = 2.426_{2,8}$ ,  $p = 0.150$ ; F:B ratio:  $F = 0.573_{2,8}$ ,  $p = 0.586$ ) (Table S2).

Total soil C content in the 2016 soil samples (Figure S1a) was strongly and positively correlated with PLFA biomass, fungal biomass, bacterial biomass, actinomycetes, G+ biomass and G- biomass



**Fig. 1.** Total soil C (a), total soil N (b), and wheat yield (c) by crop rotation in the 2016–2017 growing season. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual datapoints, occasionally overlapping. Different letters indicate significant difference ( $p < 0.05$ ; post-hoc Tukey HSD). Total soil C and N data and wheat yield data from the 2015–2016 growing season are included in the Supplementary Information in Fig. S1 and Fig. S2.

**Table 2**

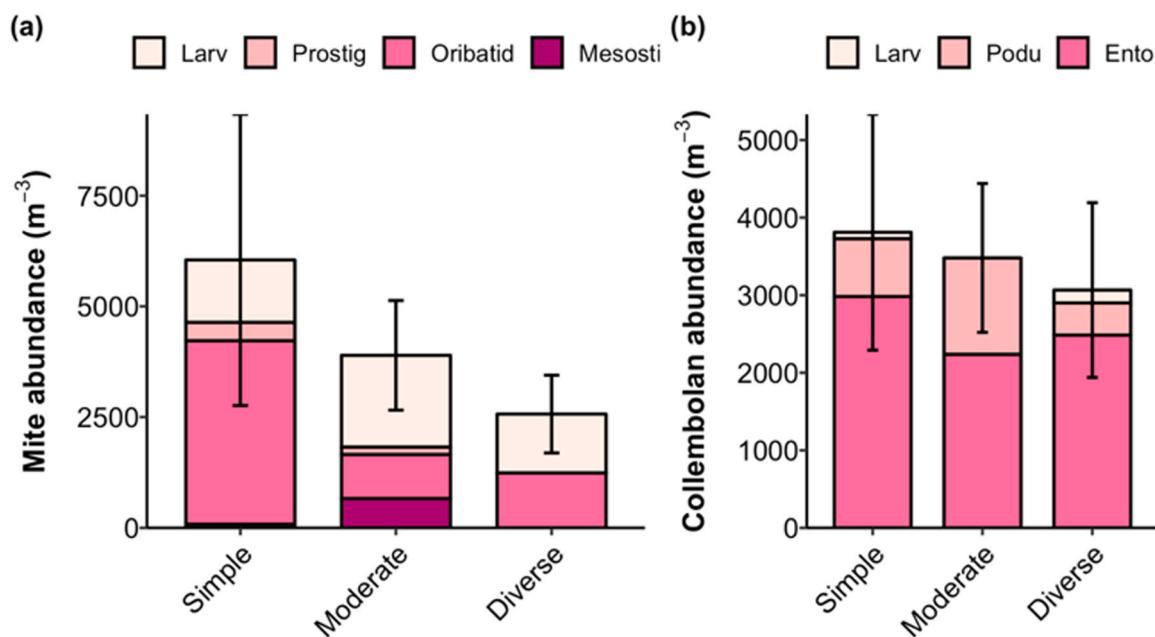
Statistical output from ANOVAs with nested treatment structure: Diversity/(Scrop + Mcrop + Dcrop), where Diversity refers to the overall effect of different crop rotations of varying crop rotation diversities, and Scrop, Mcrop and Dcrop refer to the effect of crop phases within the Simple, Moderate, and Diverse rotations, respectively. Soil fauna were sampled in 2017. 'Abundance' is abbreviated as 'abun.'. Data transformed by square root are indicated by \*, and data transformed by Ordered Quantile (ORQ) normalization transformation are indicated by \*\*. Significance is indicated as  $p < 0.05$  and  $p > 0.05$ .

Response variable	Diversity/		(Scrop +		Mcrop +		Dcrop)	
	F	p	F	p	F	p	F	p
Collembola abun.*	0.126 <sub>2,6</sub>	0.884	3.399 <sub>3,27</sub>	<b>0.032</b>	0.015 <sub>3,27</sub>	0.997	0.837 <sub>3,27</sub>	0.485
<i>Entomobryomorpha</i> *	0.072 <sub>2,6</sub>	0.932	4.153 <sub>3,27</sub>	<b>0.015</b>	0.205 <sub>3,27</sub>	0.892	1.464 <sub>3,27</sub>	0.247
Mite abundance*	0.337 <sub>2,6</sub>	0.727	5.452 <sub>3,27</sub>	<b>0.005</b>	0.745 <sub>3,27</sub>	0.535	0.918 <sub>3,27</sub>	0.445
Nematode abun.	0.268 <sub>2,2</sub>	0.788	1.502 <sub>3,11</sub>	0.268	0.033 <sub>3,11</sub>	0.991	2.214 <sub>3,11</sub>	0.144
Bacterial	0.634 <sub>2,2</sub>	0.612	0.611 <sub>3,10</sub>	0.623	0.489 <sub>3,10</sub>	0.698	0.633 <sub>3,10</sub>	0.610
Plant	0.677 <sub>2,2</sub>	0.596	0.377 <sub>3,10</sub>	0.772	0.255 <sub>3,10</sub>	0.856	0.549 <sub>3,10</sub>	0.660
Predator*	4.174 <sub>2,2</sub>	0.193	0.430 <sub>3,10</sub>	0.736	2.659 <sub>3,10</sub>	0.105	0.997 <sub>3,10</sub>	0.434
Earthworm abun.*	0.702 <sub>2,6</sub>	0.532	0.601 <sub>3,27</sub>	0.620	1.354 <sub>3,27</sub>	0.278	3.445 <sub>3,27</sub>	<b>0.031</b>
Earthworm biomass**	0.760 <sub>2,6</sub>	0.508	1.431 <sub>3,27</sub>	0.255	2.037 <sub>3,27</sub>	0.132	4.623 <sub>3,27</sub>	<b>0.009</b>
Total soil C 2016	4.519 <sub>2,6</sub>	0.062	0.272 <sub>3,27</sub>	0.845	0.467 <sub>3,27</sub>	0.708	0.473 <sub>3,27</sub>	0.704
Total soil N 2016	5.020 <sub>2,6</sub>	0.052	0.208 <sub>3,27</sub>	0.890	0.644 <sub>3,27</sub>	0.594	0.419 <sub>3,27</sub>	0.741
Soil C:N 2016**	0.170 <sub>2,6</sub>	0.847	1.082 <sub>3,27</sub>	0.373	0.897 <sub>3,27</sub>	0.455	0.893 <sub>3,27</sub>	0.457
Total soil C 2017	6.381 <sub>2,6</sub>	<b>0.033</b>	2.716 <sub>3,27</sub>	0.064	1.763 <sub>3,27</sub>	0.178	0.389 <sub>3,27</sub>	0.762
Total soil N 2017	2.832 <sub>2,6</sub>	0.136	0.820 <sub>3,27</sub>	0.494	1.687 <sub>3,27</sub>	0.193	0.232 <sub>3,27</sub>	0.873
Soil C:N 2017**	0.952 <sub>2,6</sub>	0.437	2.063 <sub>3,27</sub>	0.129	0.306 <sub>3,27</sub>	0.821	0.480 <sub>3,27</sub>	0.699
pH 2016	27.95 <sub>2,6</sub>	< 0.01	0.632 <sub>3,27</sub>	0.601	0.681 <sub>3,27</sub>	0.571	0.043 <sub>3,27</sub>	0.988
Wheat yield 2016	4.810 <sub>2,6</sub>	0.057	2.474 <sub>2,12</sub>	0.126	1.138 <sub>1,12</sub>	0.307	0.001 <sub>1,12</sub>	0.979
Wheat yield 2017	3.282 <sub>2,6</sub>	0.109	1.064 <sub>2,12</sub>	0.375	0.014 <sub>1,12</sub>	0.907	0.644 <sub>1,12</sub>	0.438

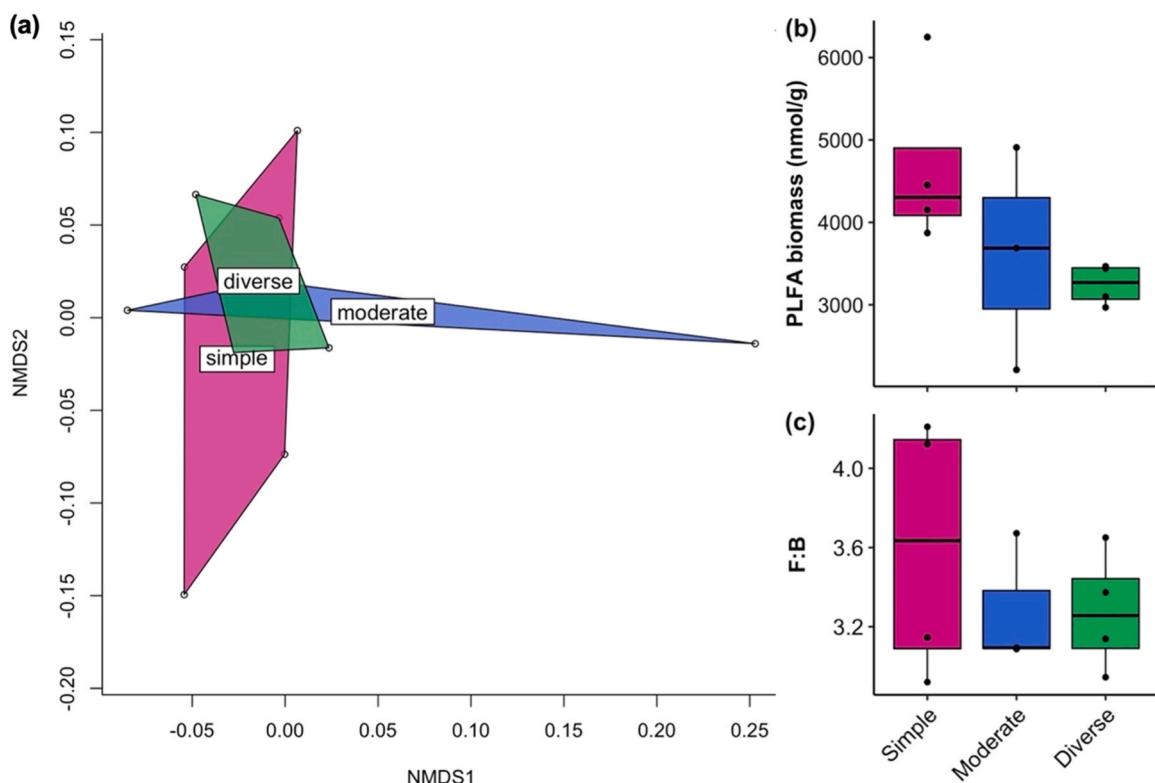
( $p < 0.05$ ,  $r > 0.60$ ; Table S3). Total soil N (Figure S1b) only correlated significantly ( $p < 0.05$ ) with bacterial biomass ( $r = 0.60$ ) and actinomycetes ( $r = 0.67$ ) (Table S3). Soil pH (Figure S1c) did not exhibit noteworthy correlations with the variables obtained via PLFA analysis (Table S3).

### 3.2. Effects of crop rotation phase on crop yield, soil biology, and soil chemistry

The phase of the crop rotation resulted in more significant differences between soil faunal abundances than overall crop rotation



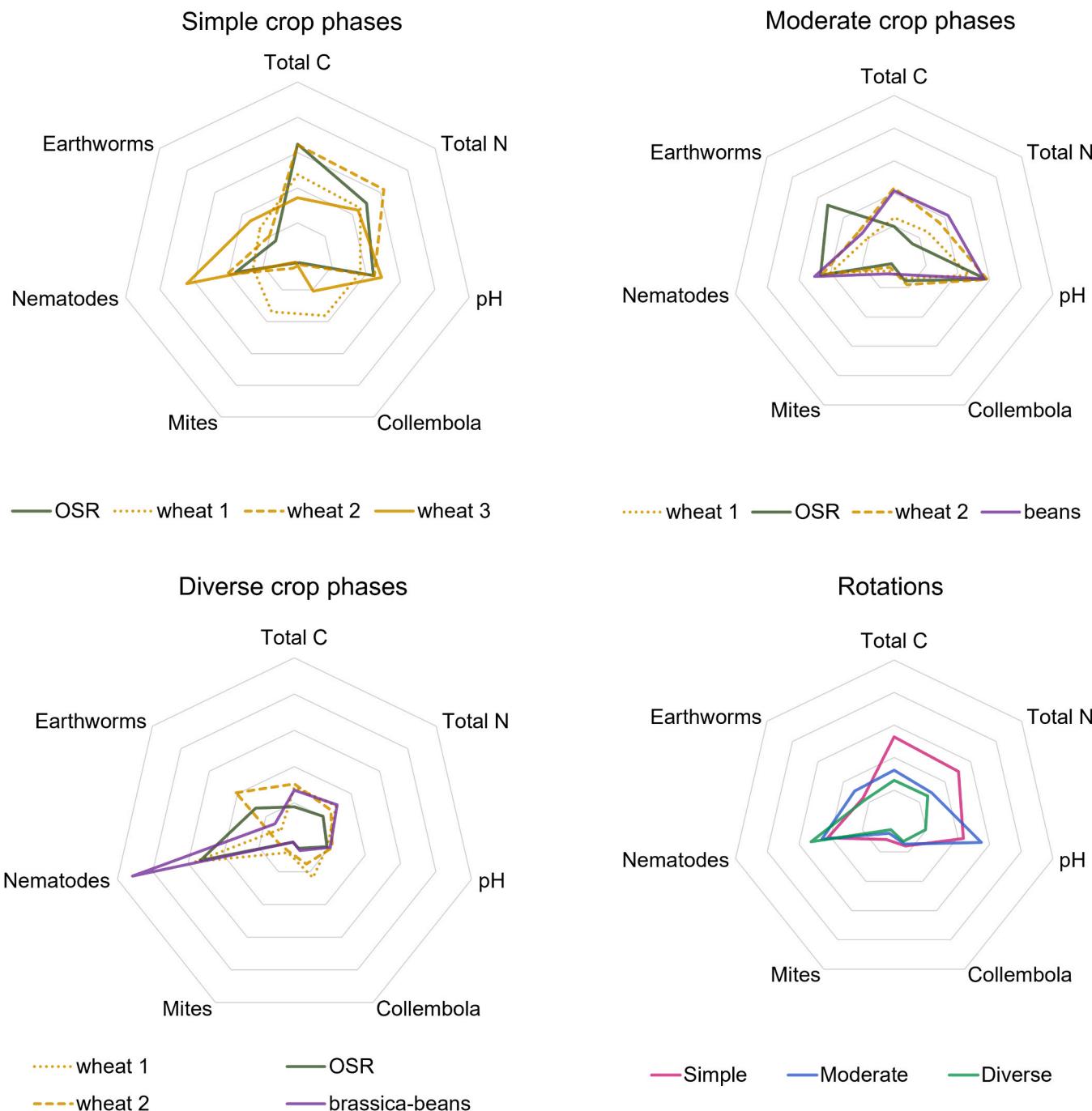
**Fig. 2.** Mean mite (a) and collembolan (b) abundance per rotation. Error bars represent standard error of the mean of total abundance ( $n = 16$ ). Abbreviations: Larv = larvae, Prostig = Prostigmata, Oribatid = Oribatida, Mesostig = Mesostigmata, Podu = Poduromorpha, Ento = Entomobryomorpha.



**Fig. 3.** (a) Nonmetric multidimensional scaling (NMDS) ordination of relative abundances of identified fatty acids (based on Bray-Curtis dissimilarity matrix; stress = 0.042). Each data point represents a PLFA profile in a replicate plot of the rotations. Data points that are closer to each other represent more similar microbial community structures. Boxplots per rotation of (b) the total PLFA biomass based on identified fatty acids and (c) the fungal:bacterial ratio. Lower and upper hinges correspond to the 25th and 75th percentiles; black dots represent individual datapoints, occasionally overlapping ( $n = 4$ ).

diversity (Table 2). In the Simple rotation, collembolan and mite abundances were significantly higher in the first wheat crop phase than in the OSR crop phase (Fig. 4; Figure S5). In the Moderate rotation, we found no significant crop rotation phase effects. In the Diverse rotation, earthworm abundance and biomass were significantly affected by the

crop rotation phase (Fig. 4; Table 2). Earthworm biomass was significantly higher in the second wheat phase (phase 3) than in the spring beans phase (phase 4). The spring beans (phase 4) was subjected to additional cultivations to establish an over-winter brassica cover crop which was terminated and incorporated prior to establishing the spring



**Fig. 4.** Radar plots showing the relative effects of crop rotations ( $n = 12$ ) and crop phases ( $n = 4$ ) within rotations (2016–2017 growing season) on soil faunal abundances and soil chemical parameters. OSR = Oilseed Rape. Data were min-max normalised so that each variable was expressed on a scale of 0–1. Data were collected in the 2016–2017 growing season, except pH which was collected in the 2015–2016 growing season. Greater distance from the centre of the plot corresponds to greater values, with concentric polygons increasing with steps of 0.2.

beans. Total soil C, total soil N, and wheat yield were not significantly different between any of the crop phases within the different rotations (Table 2) and instead were more influenced by crop rotation diversity (see Section 3.1).

#### 4. Discussion

##### 4.1. Hypothesis 1: soil microbial community structures will differ between the three rotations

We detected no significant differences in the soil microbial

community structures in the wheat plots between the Simple, Moderate or Diverse crop rotations (Fig. 3a). We made this observation despite the field experiment being established three years prior to sampling for PLFA analysis. This finding could be due to a number of reasons: (1) A more diverse mixture of plants aboveground does not produce a more diverse mixture of substrates belowground. [El Moujahid et al. \(2017\)](#) demonstrated that increasing plant diversity increased the diversity of a pool of extractable soil organic acids, fatty acids and phenolics. However, as noted by [Hooper et al. \(2000\)](#), one plant species can create the same diversity of litter qualities and chemical substrates as a mixture of plant species, so it is the diversity of substrates rather than species that

matters. Several papers investigating the impact of plant diversity on soil microbial processes apply mixtures of artificial chemicals to mimic plant root exudate diversity (Steinauer et al., 2016; Afzal et al., 2024; Kawasaki et al., 2021). However, a review of the literature by Wardle (2006) indicates that the effect of plant diversity on soil biology is inconsistent. (2) The soil microbial community could be primarily composed of generalist species in terms of habitat or diet. Generalist species would not be affected by the creation of more niches because they are equally as adapted to one resource as they are to the other (Armbrecht et al., 2004). However, Dhungana et al. (2023) demonstrated that some plants are able to secrete specific compounds which select for a specific rhizosphere community. It might be the case that analysis of the rhizosphere soil rather than the bulk soil may have yielded clear plant-induced differences. (3) PLFA analysis may not have been able to capture the changes in the microbial community taking place in this ecosystem. Although PLFA provides results that are broadly comparable to 16S rRNA gene metabarcoding (Orwin et al., 2018), the cell walls of microbial species that respond to greater resource diversity in the Diverse rotation may contain the same fatty acids as those present in less diverse environments.

#### 4.2. Hypothesis 2: soil microbial biomass, soil C, and soil N will increase with crop rotation diversity

Soil microbial biomass was highest in the wheat plots of the Simple rotation and lowest in the Diverse rotation, although this difference was not statistically significant. A similar pattern was observed in the soil C and N data. This finding contrasts with previous studies on crop diversification and soil C and N levels (Lange et al., 2015; Spohn et al., 2023; Lange et al., 2023). However, most studies have focused on grassland systems, rather than arable crop rotations. Nevertheless, in a meta-analysis on the impact of crop diversity on soil properties, increases in soil microbial biomass C, and total soil C and N were found in systems with a polyculture of crops compared to monocultural systems (McDaniel et al., 2014). Shu et al. (2022) showed that mixing crop residues increased microbial biomass C. It may be that in our study the lower microbial biomass and soil C content in the Diverse rotation resulted from greater soil disturbance due to more passes of a seed drill to establish additional understorey or cover crops as well as cash crops, increasing aggregate turnover, and increasing decomposition of soil organic matter (Six et al., 2000). Low soil C and soil microbial biomass levels in the Diverse rotation could also be related to differences in priming due to inter-species effects on the rhizosphere priming effect (Pausch et al., 2013), and subsequently greater mineralisation rates in these soils. Our previous work showed that the N mineralisation rate in the Diverse plots was greater than in the Moderate and Simple rotations (Degani et al., 2019). However, Shu et al. (2022) found that mixing crop residues resulted in no additional priming beyond that expected by applying the residues of individual plants.

#### 4.3. Hypothesis 3: greater microbial biomass and more diverse litter inputs will lead to greater populations of both microbivorous and detritivorous soil fauna

The abundance of none of the soil faunal groups sampled in this experiment was significantly influenced by plant diversity of the crop rotation. This result could be related to the relatively high level of spatial and temporal variance often observed when quantifying soil biological communities (Caruso and Bardgett, 2021; Ettema and Wardle, 2002). However, plant diversity has previously been shown to have a greater influence on the soil food web than CO<sub>2</sub> fertilisation or enhanced N deposition (Eisenhauer et al., 2013). Microarthropods (Collembola and mites) did exhibit a clear pattern with higher mean population abundance in the order Simple > Moderate > Diverse rotation. Because crop rotation diversity was negatively associated with microbial biomass and soil C and N, there may be less available substrate for soil fauna (Potapov

et al., 2019). Alternatively, the greater soil disturbance from drilling additional intercrops and cover crops in the Diverse rotation may have had a direct impact on the soil food web. Lower microarthropod abundance has previously been observed in organically managed soils compared to conventionally managed soils due to disturbance from tillage activities replacing herbicide applications (Mazzoncini et al., 2010) and reduced tillage operations have been associated with greater microarthropod abundance (Liu et al., 2024b). Alternatively, microarthropods may have been more abundant in the Simple rotation because they graze on microbes and these soils contained more C, a greater microbial biomass, and nematode populations, and therefore provided greater food resources (Beare et al., 1997; Potapov et al., 2019). However, we observed a negative correlation between nematode abundance and mite abundance which may reflect lower predation pressure on nematodes when mites were more disturbed in the Diverse rotation (Figure S5).

For mites, it was mostly the *Oribatida* that were of higher abundance in the Simple rotation (Fig. 2). The abundances of the other mite sub-orders were similar in all three rotations. *Oribatida* mites are known to be food generalists, occupying three to four trophic levels, as determined by stable isotope studies (Schneider et al., 2004), so they may be able to better adapt to a lower diversity of resources available in the Simple rotation compared to other faunal groups that might occupy more specialist niches. Indeed, it has been demonstrated that the identity and diversity of litter has a relatively minor impact on oribatid mite communities in a temperate forest ecosystem (Bluhm et al., 2019). Since oribatid mite and collembolan species are susceptible to physical disturbance (Maraun et al., 2003), it is likely that the additional cultivations in the Diverse crop rotation reduced the population abundance. However, plots in the Diverse rotation may have also harboured more insects and natural predators which may have increased predation of soil fauna (Heinen et al., 2024).

Crop rotation diversity did not significantly influence populations of earthworms or nematodes, although a slightly higher average nematode abundance could be observed in plots of the Diverse rotation (Figure S4). Since nematodes tend to reside near roots (Ingham et al., 1985), this observation could be related to more numerous and more diverse rooting systems in the Diverse rotation. Earthworms feed on organic matter present in the soil, effectively partnering with soil microbial communities to mineralise soil organic matter (Medina-Sauza et al., 2019). Earthworm biomass and abundance tends to increase with greater inputs of organic matter (Deibert and Utter, 1994; Fraser et al., 1996; Sizmur et al., 2017). Considering the lower soil C and microbial biomass present in the Diverse rotation soils, it is perhaps surprising that there is no corresponding drop in earthworm abundance. The earthworms identified in this experiment were all endogeic, occupying the soil including the root zone (Capowiez et al., 2024), and nematodes are also known to reside closely to the root zone of plants (Ingham et al., 1985). Microarthropods tend to inhabit more shallow soil layers (top ca. 5 cm) than earthworms or nematodes (Sharma and Parvez, 2017). Therefore, even minor levels of soil disturbance or drying of the topsoil layer in the summer may have affected microarthropod populations (Meyer et al., 2021; Betancur-Corredor et al., 2022), while endogeic earthworms at slightly deeper levels and nematodes closer to the root zone are comparatively less affected and may recover more easily (Postma-Blaauw et al., 2012).

#### 4.4. Hypothesis 4: differences between crop rotation phases will be most pronounced in more diverse crop rotations

Although we found no statistically significant effects of crop rotation diversity on soil chemical properties or biological communities, different phases of the same crop rotation revealed some significant effects on soil fauna (Table 2). However, contrary to Hypothesis 4, more significant effects were observed in the Simple rotation than the Moderate or Diverse rotation. We observed a significantly higher abundance

of mites and Collembola in the first wheat plots of the Simple rotation, compared to the other phases in the rotation. Collembola in particular are susceptible to disturbances, including tillage (Liu et al., 2024a), organic amendments (Pommeresche et al., 2017), and pesticides (Joimel et al., 2022). However, Gergocs et al. (2022) found that crop identity (Wheat or Maize) had a greater influence on microarthropod assemblages than fertilisation regime. The crop-phase effect that we observed was most significant for the collembolan order *Entomobryomorpha*, which are epigean (Lima et al., 2021), and therefore most likely affected by changes to crop residues. Microarthropods require sufficient microhabitats and heterogeneity in the top layer of the soil, which may have been more abundant after OSR (Nielsen et al., 2010) and provided a greater contrast in the Simple rotation than the Moderate or Diverse rotation. Therefore, habitat heterogeneity, may have made the first wheat crop phase (i.e., directly after OSR) more favourable for microarthropods.

Earthworm biomass (but not abundance) was significantly lower in bean plots than second wheat plots in the Diverse rotation. This could be due the brassica cover crop directly preceding the spring beans since brassica residues are not a preferred food choice for earthworms (Valckx et al., 2011) and the additional tillage operation to incorporate the cover crop residues may have reduced the earthworm populations (Briones and Schmidt, 2017) and counteracted any positive influence of substrate addition on earthworm abundance (Sizmur et al., 2017).

Generally, differences in scales of the processes that influence aboveground and belowground systems make it difficult to distinguish different mechanisms from each other (Hooper et al., 2000). Scales of soil food web processes differ (1) spatially (Scharroba et al., 2012), as species reside at different depths and in different pore spaces; (2) temporally (Hedde et al., 2024), as species have different life cycles and respond differently to changes in temperature, moisture and other abiotic conditions; and (3) functionally (Potapov, 2022), as species each fulfil different roles in a community or ecosystem.

#### 4.5. Limitations of the study

While the experimental treatments were established several years before measurements were made, these measurements of the soil food web were only made at a single time point during the summer months and this leads to a limitation of our study since it is known that soil biological communities are highly temporally dynamic. Another limitation is that the diversity of the plants included within each crop rotation cannot be disentangled from the physical disturbance associated with their establishment and this hampers a mechanistic understanding of the interactions between plant diversity and soil biology. Lastly, the taxonomic resolution of our surveys were relatively coarse (e.g., use of PLFA to distinguish major microbial groups and identification of Collembola and mites to orders/suborders). It is possible that shifts in the abundance of individual species were masked by this resolution.

#### 5. Conclusions

Although we hypothesised that crop rotation diversity would increase soil C, soil microbial biomass, shift microbial community composition, and increase soil fauna abundance and biomass, we found no statistically significant influence of crop rotation diversity on any of these parameters. In fact, we observed an, albeit largely not statistically significant, negative impact of crop rotation diversity on each of these parameters. For example, we observed greater total PLFA biomass, higher soil C and N content, and greater mean mite and collembolan abundances in the Simple rotation soils. It is likely that the additional tillage operations required to establish cover crops and intercrops in the diverse rotation created physical disturbances that mineralised soil organic matter and negatively impacted soil fauna.

Statistically significant differences were observed between the different phases of the crop rotation within the Simple and Diverse

rotation. Crop rotation phase significantly affected mite and collembolan abundances in the Simple rotation. A higher abundance of mites and Collembola were observed in the first wheat plots of the Simple rotation, possibly due to greater habitat heterogeneity provided by the preceding OSR crop. Crop rotation phase significantly affected earthworm abundance and biomass in the Diverse rotation. A lower earthworm biomass was observed in spring bean plots of the Diverse rotation, possibly due to the additional disturbance caused by a preceding cover crop establishment, termination, and incorporation. We therefore conclude that crop rotation phase and soil disturbance have greater impact on soil biology than crop rotation diversity.

#### Author contributions

HJ designed the Liberation field experiment. TS, ED and MS designed the soil biodiversity experiment presented in this paper. MS, ED, EB and ST performed field work and data collection with help from FA, TS, and SL. MS analysed the data with support from TS and SC. MS and TS prepared the manuscript with critical review by all authors. TS, SM, ED and MS secured funding. TS supervised the project and AW and SM co-supervised the project.

#### CRedit authorship contribution statement

**Samuel G. Leigh:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Erika Degani:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Sion Thomas:** Writing – review & editing, Investigation, Data curation. **Emma Bowen:** Writing – review & editing, Investigation, Data curation. **Tom Sizmur:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Suzanne J. Clark:** Writing – review & editing, Formal analysis. **Marijke Struijk:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Simon R. Mortimer:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Frank Ashwood:** Writing – review & editing, Methodology, Investigation. **Andrew P. Whitmore:** Writing – review & editing, Supervision, Investigation, 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.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.110023.

## Data availability

The data associated with this manuscript is freely available online at <https://doi.org/10.17632/jkpr2d6zfm.1>

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