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Earthworms are sustained in a 116-year rye monoculture with ploughing by provision of soil organic carbon from farmyard manure

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A B S T R A C T

In arable fields, earthworm abundance declines with the level of intensification, due to soil disturbance and reduced food resources. In this context, continuous cropping of monocultures and removed plant residues are drivers for a decline in earthworm populations. Therefore, the earthworm communities of the 'Eternal rye', a 116-year field trial, were investigated. The Eternal rye was instigated in 1906 Vienna, Austria, mainly to compare the application of mineral fertiliser, farmyard manure (FYM) and no fertiliser application (unfertilised). A two-factorial design with factor fertiliser comprised of FYM, mineral fertiliser and unfertilised and factor crop rotation with levels crop rotation of winter rye (*Secale cereale* L.) – spring barley (*Hordeum vulgare* L.) – bare fallow) *versus* continuous cropping of winter rye. All plots were ploughed (25-30 cm) at the same time, after the application of FYM and before sowing of rye in late October. Earthworms were investigated for continuous rye and rye in crop rotation in April 2020-2022 by hand sorting. Results show that only fertilisation treatments impacted earthworm communities with highest total abundances under FYM (120 ± 92.9 m^{-2}) followed by mineral fertiliser (27.6 ± 21.4 m^{-2}) and unfertilised (9.55 ± 9.27 m^{-2}). The most abundant species was *Allolobophora chlorotica* under FYM and mineral fertiliser, followed by small numbers of *Aporrectodea caliginosa*, *Aporrectodea rosea* and *Lumbricus terrestris*. Interestingly, *L. terrestris* was observed only under FYM (2.69 ± 7.81 m^{-2}). Similar to earthworm parameters, soil organic carbon, NO$_3^-$-N and leaf area index were highest for FYM compared to mineral and unfertilised within and across cropping systems, while soil decomposition rate was highest for FYM only in continuous rye. The application of FYM compensated the effect of conventional management practices, such as monoculture and ploughing on earthworms and enhanced numbers of endogeic earthworm and *L. terrestris* compared to mineral and unfertilised treatments.

1. Introduction

Earthworms are affected by field management practices such as crop rotations, availability of food sources, fertilisation regimes and soil tillage [1–4]. Especially, numbers of deep burrowing earthworms, such as *Lumbricus terrestris* (L., 1758), tend to decline under a ploughing regime, because *L. terrestris* depend on undisturbed soil structure, feed on and incorporate plant residues from the soil surface [1,5]. Earthworms can also be affected by the fertilisation regime. Several authors [2,6,7] found that application of farmyard manure (FYM) increased abundance of earthworms compared to mineral fertiliser or unfertilised

treatments, but did not affect earthworm community compositions.

Farmyard manure is an important fertiliser and nitrogen source (N) in Austria as 23% of the farms are organic and 78% of them keep livestock with straw beddings or similar natural materials [8]. Generally, N is an essential plant nutrient, improves plant growth and N application increases N availability and N uptake by plants [9]. However for FYM, mineralisation and N uptake is additionally enhanced through soil fauna diversity compared to mineral fertiliser [10–12]. Application of FYM also increases carbon (C) sequestration, microbial activity and plant growth by adding organic C and feeding soil organisms [13,14]. As a consequence of C sequestration, microbial activity drives litter

decomposition and mineralisation *via* interactions with soil structural development and soil moisture [15–17]. In addition, earthworms can form and stabilise soil aggregates, increase water holding capacity and improve microbial biomass through their burrowing and casting activities [18–20]. Interactions of earthworms and the soil microbiome then affect litter decomposition and mineralisation [16,17,21], but field trials are scarce and relatively short-lived (e.g., Athmann et al. [17]).

Long-term trials such as the 'Eternal Rye' from Austria are of particular value due to the rarity of field experiments exceeding 100 years. Only a small number of such trials exist globally, providing essential insights into the long-term effects of agricultural practices [22, 23]. While previous studies have examined the impact of fertilisation, crop rotation on crop yield, even energy efficiency and soil fertility, soil biological parameters have often been overlooked despite their crucial role in soil functioning [23–26].

Due to a lack of soil biological data for long-term trials, we investigated the impact of field management on the Eternal rye, a 116-year-old fertilisation experiment with continuous cropping (monoculture) of winter rye (*Secale cereale* L.) *vs*. a crop rotation of winter rye, spring barley (*Hordeum vulgare* L.) and bare fallow on earthworm communities, crop production and soil parameters. The Eternal rye was instigated in 1906 to evaluate the application of FYM *versus* mineral fertiliser *versus* unfertilised treatment in crop rotation or monoculture. To determine the long-term impacts of field management intensification on the earthworm community (i.e., within the Eternal rye), the following objectives were investigated: Measurement of i) soil biological parameters such as earthworm community composition, abundance, biomass and litter decomposition, ii) soil parameters such as nitrogen content, organic carbon and moisture, and iii) plant performance such as biomass production, leaf area index and soil cover. It was hypothesised that FYM would increase earthworm abundance, litter decomposition, soil organic carbon and plant growth. We further hypothesised that earthworm, plant, and soil parameters, such as litter decomposition, soil nitrogen content, soil organic carbon and soil moisture, would not be negatively impacted by monoculture.

2. Materials and methods

2.1. Site conditions, experimental design and field management

In 1906, the Eternal rye was established at the Experimental Farm of the BOKU University, in Gross-Enzersdorf east of Vienna, Austria (48°11′ N,16°33′ E; 153 m a.s.l.). The field plots have Chernozem soil [27] with a silty loam texture and a pH_{CaCl_2} of 7.6. The site is located in the north-western part of the Pannonian basin with a long-term mean annual temperature of 11.21 °C, and a mean annual precipitation of 560 mm (Supplementary Fig. S1). The experiment was set-up with two factors: i) crop rotation and ii) fertiliser, and conducted in this manner over 116-years. Factor crop rotation consisted of two levels: rye in monoculture and rye in crop rotation. Factor fertiliser consisted of three levels unfertilised, mineral fertilised and FYM application. The mineral fertiliser application is utilised in two equal parts in mid-March and mid-April and contains in total 117 kg N ha^{-1} (calcium ammonium nitrate); 100 kg P$_2$O$_5$ ha^{-1} (triple superphosphate) and 150 kg K$_2$O ha^{-1} (potassium chloride) and FYM comprised of 20 t fresh weight ha^{-1} cattle FYM (110–125 kg N ha^{-1}, 90–110 kg P$_2$O$_5$ ha^{-1}, 140–160 kg K$_2$O ha^{-1}) and is applied in September before ploughing (25–30 cm depth); straw is removed after harvest in July; stubbles and roots remained at the plots. Collected straw was historically used for livestock, and this practice has been maintained. In the current study, only earthworm and additional soil and plant parameters of rye plots from crop rotation and monoculture were compared, while barley and bare fallow plots were not sampled. Across the study years, both rye treatments were sown in mid-October with an Austrian variety 'Tschermaks veredelter Marchfelder', at a seed density of 180 kg ha^{-1} and a row distance of 12.5 cm. The field trial covers a total area of 4000 m^2 divided into four equal sub-plots (20 × 13 m) for treatment crop rotation and three fertiliser treatments forming four pseudo-replicates of 10 × 6.5 m.

2.2. Earthworm parameters

Earthworms were sampled in mid-April 2020, 2021 and 2022 by excavation of soil monoliths using a spade (20 × 20 × 30 cm, L x W x D) and hand-sorting (40 min block^{-1}), with four subsamples pseudo-replicate^{-1}. Hand-sorting without usage of a vermifuge such as mustard suspension was applied, due to sensitivity of silty soil to soil tillage, which can block infiltration of any liquid [28,29]. Earthworms were counted and biomass taken by life stage (juvenile; adult), main ecological groups (viz: epigeic; endogeic; anecic; epi-anecic; based on Bouché and Bottinelli et al. [30,31]) and species level [32]. In detail, *Aporrectodea caliginosa* (Savingy, 1826), *A. rosea* (Savingy, 1826), *Allolobophora chlorotica* (Savingy, 1826) were assigned to endogeic and *L. terrestris* was assigned to epi-anecic. Earthworm cocoons were also collected each April using a soil auger of 750 cm^3 volume to a depth of 10 cm. Soil was then wet sieved through mesh sizes of 2.5 and 1 mm and number of cocoons were recorded. Earthworm diversity was analysed through the total species richness and the Shannon diversity index [33,34].

2.3. Soil parameter

Soil mineral nitrogen, as nitrate-nitrogen (NO$_3^-$-N) was analysed in March 2020, 2021 and 2022, before the first application of mineral fertiliser, with three pooled subsamples pseudo-replicate^{-1} to a depth of 30 cm. The soil mineral nitrogen content was determined photometrically (FIASTAR 5000, FOSS GmbH, Hamburg, Germany), after extraction with 0.0125 M CaCl$_2$ in a soil extraction ratio of 1:4 (w/v) for 1 h, using an overhead shaker [35]. The water content was obtained gravimetrically by drying soil samples at 105 °C for 24 h.

Soil organic carbon (SOC) was analysed on soil samples from 0 to 30 cm depth, similar to soil mineral nitrogen samples, in March 2020 and March 2021. Samples were air-dried and ground (Planetary Ball Mill PM 400; RETSCH GmbH, Haan, Germany). Organic carbon was analysed via dry combustion (soliTOC; Elementar Analysesyteme GmbH, Langensebold, Germany) with a temperature of 400 °C according to DIN 19539:2016-12 [36]. In addition, topsoil moisture was measured weekly for six weeks from March to mid-April in 2020, 2021 and 2022 with three subsamples pseudo-replicate^{-1} at a depth of 0–7 cm (WET-Sensor, Delta-T Devices Ltd, Cambridge, UK).

Litter stabilisation and decomposition in soil was determined using the tea bag index [37]. Therefore, one tea bag of Lipton green tea (EAN: 87 22700 05552 5) and one Lipton rooibos tea (EAN: 87 22700 18843 8) per pseudo-replicate were buried (8 cm depth) during three periods from i) January to April, ii) February to May and iii) March to June in 2020, 2021 and 2022. Before burying the tea bags, the initial mass of each (including mesh bag, string and label) was taken, labels were marked and after 90 days they were excavated and dried at 60 °C for 48 h. Tea bags were then freed from attached soil, opened and mass of the remaining tea (excluding mesh bag, string and label) was recorded with a precision balance (accuracy: 0.0001 g). Data were processed with the provided data submission sheet for non-woven bags to computed stabilisation rate (*S*) and decomposition rate (*k*) [38].

2.4. Crop parameters

Annually, crop biomass of 4 × 0.25 m^2 pseudo-replicate^{-1} was cut by mid-April (BBCH 31-39) in 2020, 2021 and 2022, dried at 105 °C for 24 h and weighed. Before cutting, leaf area index (LAI) and photographs for soil cover analyses were taken. For LAI, a LAI ceptometer (LP-80 AccuPAR, METER Group, Munich, Germany) was used with four subsamples per plot. For each subsample, a reference measure was taken to determine the total photosynthetically active radiation above plant canopy and four measurements of the plant leaves 2 cm aboveground

were taken. For the soil cover, four photographs per plot were taken from 1 m aboveground and were analysed for percentage covering of the soil surface by living plants (plant_cover) and plant residues (residue_cover) with the web application 'Soil Cover' [39] according to the protocol of Riegler-Nurscher et al. [40].

2.5. Data analysis

Data were analysed with two-way linear mixed-models (2-way LMM) in R 4.5.1 [41]. Fixed factors were cropping system (2 levels: monoculture; crop rotation), fertilisation (3 levels: FYM; mineral fertiliser; unfertilised) and as random effects year (3 levels: 2020; 2021; 2022) and plot to account for the pseudo-replicates within each plot. The full model for all parameters was cropping system (CS) + fertiliser (FT) + CS: FT + (Year: Plot), except for the tea bag index where the model was CS + FT + CS: FT + (Year: Period: Plot), with colons indicating interaction terms and random effects in parentheses [42]. Function 'lmer' ('*lme4*' package; [43]) was used for LMM with compound symmetry and residual maximum likelihood for estimation. Analyses of variance (function 'Anova') was applied with Wald-type F-tests and the Satterthwaite's method for denominator degrees of freedom and type III hypotheses. A post-hoc test function 'emmeans' with adjust 'tukey' (package '*emmeans*'; [44]) was applied in pairwise mean comparisons ($P < 0.05$) for factor combinations. For assumptions of normal distribution of residuals and homogeneity of the variance, QQ-plots and residuals against fitted values were checked visually. Parameters which failed the assumptions such as soil mineral nitrogen were log transformed or square root transformed such as: plant biomass, topsoil moisture, k and all earthworm parameters. All data provided are mean values and standard deviation (mean ± SD).

3. Results

3.1. Earthworm parameters

Earthworm total abundance, biomass, juvenile abundance and biomass and number of cocoons were unaffected by cropping system (Table 1; Figs. 1 and 3). Only abundance and biomass of endogeic earthworms were affected by the cropping system, with abundance and biomass being 1.5- and 1.4-times higher respectively, in crop rotation than in monoculture (Table 1; Fig. 2). However, earthworm total abundance and biomass, juvenile abundance and biomass and number of cocoons were significantly affected by fertilisation. They did not differ between mineral and unfertilised and were 5.5-, 5.8-, 7.3-, 6.1- and 2.9-times higher in FYM than the average of mineral and unfertilised, respectively (Table 1; Figs. 1–3). In total, four earthworm species were found. The percentage of adults and juveniles was 50.4% and 49.6%, respectively across all sampled treatments, with no significant differences observed in any treatment. In addition, species richness was 2.57-times higher, the Shannon diversity index 13.3-times higher in FYM compared to the average of mineral and unfertilised (Table 1) and showed additionally that *L. terrestris* was only found under FYM. Overall, numbers of earthworms (mean over 3 years) in FYM were 41.7 ± 49.5 *A. chlorotica* m^{-2}, 1.6 ± 7.2 *A. caliginosa* m^{-2}, 0.4 ± 3.2 *A. rosea* m^{-2} and 2.7 ± 7.8 *L. terrestris* m^{-2} (Fig. 4). By comparison only 13.3 ± 21.8 *A. chlorotica* m^{-2}, 0.8 ± 4.4 *A. caliginosa* m^{-2} and 0.8 ± 4.4 *A. rosea* m^{-2} were detected in mineral and 5.0 ± 10.6 *A. chlorotica* m^{-2} and 0.3 ± 2.6 *A. caliginosa* m^{-2} in unfertilised (Fig. 4).

3.2. Soil parameters

NO$_3^-$-N content from 0 to 30 cm depth followed a gradient of FYM ≥ mineral ≥ unfertilised and was only significant for fertilisation (Table 1; Fig. 5), whereas SOC showed an interaction of cropping system and fertilisation (Table 1; Fig. 6). On average, NO$_3^-$-N content under FYM was 2.7-times higher than under mineral fertilisation and 3.9-times

Table 1
ANOVA results (2-way LMM) of earthworm parameters, decomposition rate (k), stabilisation rate (S), topsoil moisture, soil mineral nitrogen, soil organic carbon, plant biomass, leaf area index, soil cover by living plants, plant residues and bare soil with fixed effects cropping system (CS; crop rotation; monoculture) and fertilisation (FT; farmyard manure; mineral fertiliser; unfertilised) of the Eternal rye in Austria from April (2020, 2021 and 2022). Degrees of freedom CS = 1; FT = 2; CS × FT = 2.

Parameter	F-value			P-value		
	CS	FT	CS × FT	CS	FT	CS × FT
Total earthworm abundance	0.790	29.0	0.862	0.377	<0.001	0.427
Total earthworm biomass	0.184	23.5	0.522	0.667	<0.001	0.596
Juvenile abundance	0.396	47.9	1.58	0.531	<0.001	0.213
Juvenile biomass	1.39	28.3	0.666	0.243	<0.001	0.517
Endogeic abundance	5.88	28.3	2.9	0.018	<0.001	0.059
Endogeic biomass	4.32	18.7	2.3	0.041	<0.001	0.102
Number of cocoons	0.063	6.11	0.866	0.803	0.004	0.426
Shannon diversity index	1.27	5.91	0.433	0.286	0.003	0.784
Species richness	2.68	9.90	0.818	0.073	<0.001	0.517
Litter stabilisation rate (S)	0.025	0.156	0.005	0.876	0.857	0.995
Litter decomposition rate (k)	13.2	8.67	2.23	<0.001	<0.001	0.12
Topsoil moisture	0.028	0.144	0.001	0.868	0.867	0.999
Soil NO$_3^-$-N	0.196	6.34	0.876	0.673	0.033	0.464
Soil organic carbon	21.9	87.4	5.59	0.003	<0.001	0.043
Crop biomass	0.056	2.50	0.134	0.818	0.124	0.876
Crop leaf area index	2.38	28.3	1.54	0.149	<0.001	0.255
Crop cover	0.001	41.3	0.808	0.983	<0.001	0.469
Residue cover	0.241	7.66	0.297	0.632	0.007	0.749
Bare soil cover	0.071	31.3	1.12	0.794	<0.001	0.357

higher than in unfertilised plots. SOC was highest for FYM in monoculture followed by FYM in crop rotation and mineral fertiliser in monoculture (−12.5%), while mineral fertiliser in crop rotation and unfertilised showed lowest SOC (−33.2%) in comparison to FYM.

Topsoil moisture was not affected by fertilisation or cropping system (Table 1; Supplementary Fig. 2). However, the tea bag index showed that only k was affected by cropping system and fertilisation, while S was totally unaffected by any treatment (Table 1; Fig. 7). Decomposition rate k, was 41.1 % higher for FYM in crop rotation than for the remaining treatments.

3.3. Rye parameters

Plant biomass and LAI were not affected by cropping system but ranked among fertilisation treatments as follows: FYM ≥ mineral ≥ unfertilised and FYM > mineral > unfertilised, respectively (Table 1; Fig. 8). Plant biomass was 3.7-times higher for FYM than unfertilised. The LAI for FYM was higher than for mineral (1.4-times) and for unfertilised (5.1-times) and that of mineral was 3.6-times higher than unfertilised. In addition, soil surfaces covered by living plants were 99.1 and 153.9% higher and by plant residues were 54.7 and 65.6% lower for mineral and FYM compared to unfertilised, respectively (Table 1; Fig. 9A and B). By contrast, proportion of bare soil was 45.9 and 72.6% higher for unfertilised than for mineral and for FYM, respectively (Table 1; Fig. 9C).

4. Discussion

4.1. Cropping system has a minor impact

We consider the Eternal rye as a case study for an intensified crop production with or without livestock and unfertilised as control. As hypothesised, 116-years of monoculture had no effect on the total and
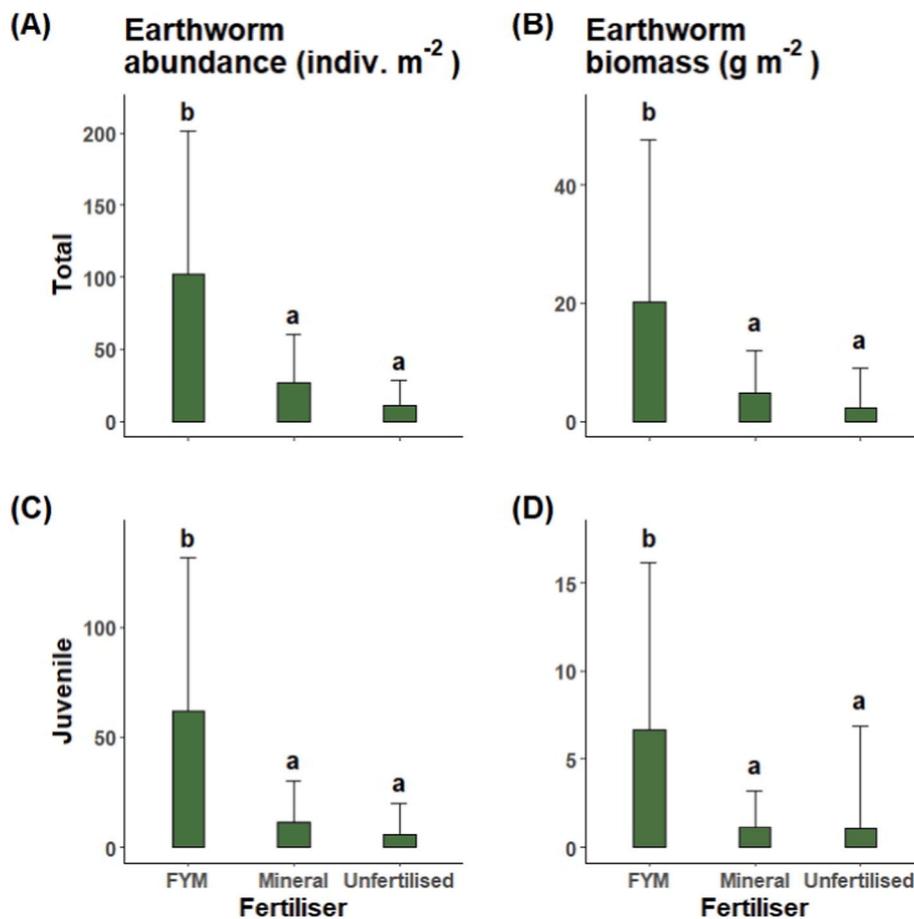
Fig. 1. Overall mean of total earthworm abundance (A), total biomass (B), juvenile abundance (C) and juvenile biomass (D) with fixed effects fertilisation (farmyard manure (FYM); mineral fertiliser; unfertilised) and across cropping system (crop rotation; monoculture) of the Eternal rye in Austria from April (2020, 2021 and 2022). Treatments having no letter in common are significantly different by pairwise comparison (2-way LMM, Tukey; $P < 0.05$). Mean + SD.

juvenile earthworm abundance and biomass, except for endogeic earthworms. Biomass and abundance of endogeic earthworms were higher for crop rotation than monoculture under FYM and mineral fertiliser, but higher for monoculture compared to crop rotation in the unfertilised plots. Endogeic earthworms are usually sensitive to bare fallow, which causes a decline in their numbers [4,45,46]. It is important to note that the crop rotation comprising winter rye, spring barley and bare fallow had a historical background and is not considered representative for any sustainable crop rotation, as a whole year of bare fallow is now either economically or ecologically preferable [47]. However, Euteneuer et al. [4,45] found less endogeic earthworms for bare fallow compared to cover crops after only three to eight months in field trials, but the crop rotation of the Eternal rye includes bare fallow and showed higher endogeic earthworm abundance than winter rye in monoculture. Torppa and Taylor [48] reported similar results in a long-term crop rotation trial in Sweden. Their applied crop rotation comprised wheat (*Triticum aestivum* L.), peas (*Pisum sativum* subsp. *arvense* (L.), A. and G.), oilseed rape (*Brassica napus* L.) and they found 58% more endogeic earthworms than in a simplified crop rotation with only wheat and barley. The amplitude of the diverse crop rotation is higher than that of the Eternal rye, likely due to the use of legumes and brassica crops. Legumes and brassicas are, due to their low C:N ratio, a favourable food for earthworms [4,45,46]. However, as the simple grain crop rotation of the Eternal rye has higher endogeic earthworm numbers than monoculture, it emphasises overall that earthworms need diverse food sources [4,49].

Against our second hypothesis, the decomposition rate k and SOC were also affected by the cropping system, while k was higher for crop rotation than for monoculture and SOC by comparison showed higher values for monoculture, especially for FYM and mineral fertilised plots. We can only consider that the crop rotation has impacted soil microbial and micro and mesofauna activity, and therefore k, as indicated by Sae-Tun et al. [15]. These authors reported that dissolved organic carbon can be enhanced by using crop rotations, and this could have affected k in the Eternal rye. However, the main driver for SOC in monoculture may have been the continuous higher C input in the form of straw stubble and root biomass of winter rye, compared to lower C inputs from bare fallow and spring barley in the crop rotation. In addition, due to the decreased k in monoculture, the turnover rate of C could have also been decreased and led to a higher SOC [15,17,50].

4.2. Fertilisation is the main driver

Overall, as hypothesised, fertilisation was the main driver for earthworm, plant and soil parameters and before the first mineral fertiliser application in mid-March, the soil mineral nitrogen content followed the order of FYM > mineral > unfertilised. This was caused by the earlier FYM application in September of the previous year. It has been demonstrated by many studies that long-term FYM application stimulates soil biological activity, microbial mineralisation of organic matter and improves nutrient supply during periods of slower plant growth in winter [51–53]. Consequently, soil mineral nitrogen was available in early spring and promoted plant growth, LAI and soil cover for FYM compared to mineral and unfertilised [54]. By contrast, first application of mineral fertiliser was in mid-March, four weeks before plant sampling took place. Mineral fertiliser becomes instantly plant-available once
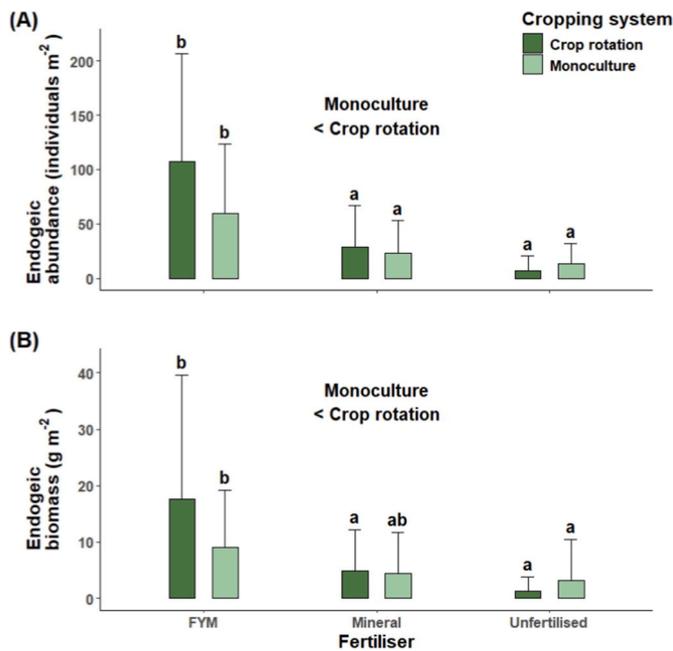
Fig. 2. Overall mean of abundance (A) and biomass (B) of endogeic earthworms with fixed effects cropping system (crop rotation; monoculture) and fertilisation (farmyard manure (FYM); mineral fertiliser; unfertilised) of the Eternal rye in Austria from April (2020, 2021 and 2022). Fertilisation treatments within crop rotation treatments having no letter in common are significantly different by pairwise comparison (2-way LMM, Tukey; $P < 0.05$). Mean, +SD.
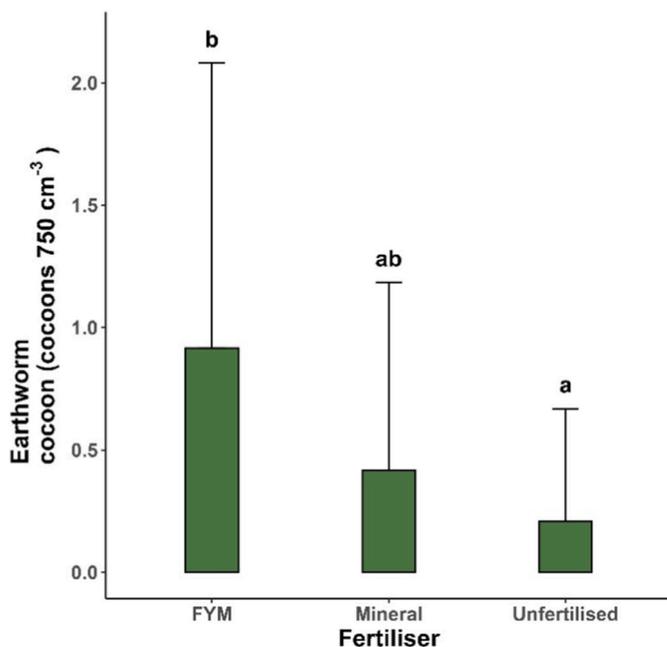

Fig. 3. Overall mean of total earthworm cocoon abundance from soil depth 0-10 cm with fixed effects fertilisation (farmyard manure (FYM); mineral fertiliser; unfertilised) and across cropping system (crop rotation; monoculture) of the Eternal rye in Austria from April (2020, 2021 and 2022). Treatments having no letter in common are significantly different by pairwise comparison (2-way LMM, Tukey; $P < 0.05$). Mean, +SD.

Fig. 4. Mean relative species abundance of the Eternal rye in Austria from April (2020, 2021 and 2022). Species displayed are *Lumbricus terrestris* (Lter), *Aporrectodea caliginosa* (Acal), *Aporrectodea rosea* (Aros) and *Allolobophora chlorotica* (Achl) with fertilisation (farmyard manure (FYM); mineral fertiliser; unfertilised) for cropping system (crop rotation; monoculture).

Fig. 5. Overall mean of the soil nitrogen content of the first 30 cm soil depth with fixed effects fertilisation (farmyard manure (FYM); mineral fertiliser; unfertilised) and across cropping system (crop rotation; monoculture) of the Eternal rye in Austria from March pre-fertilisation (2020, 2021 and 2022). Treatments having no letter in common are significantly different by pairwise comparison (2-way LMM, Tukey; $P < 0.05$). Mean, +SD.

dissolved in the soil solution, but weather conditions were not favourable for plant uptake [55]. In detail, precipitation of zero mm in 2020 and only single rain events of 11.6 and 11.1 mm by the April 13, 2021
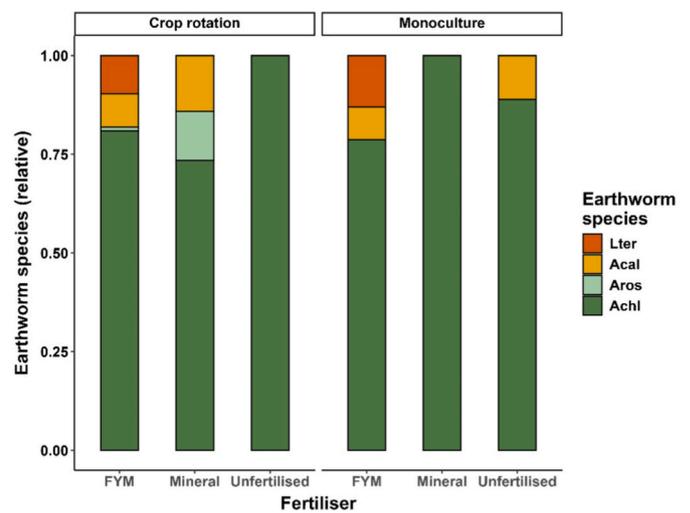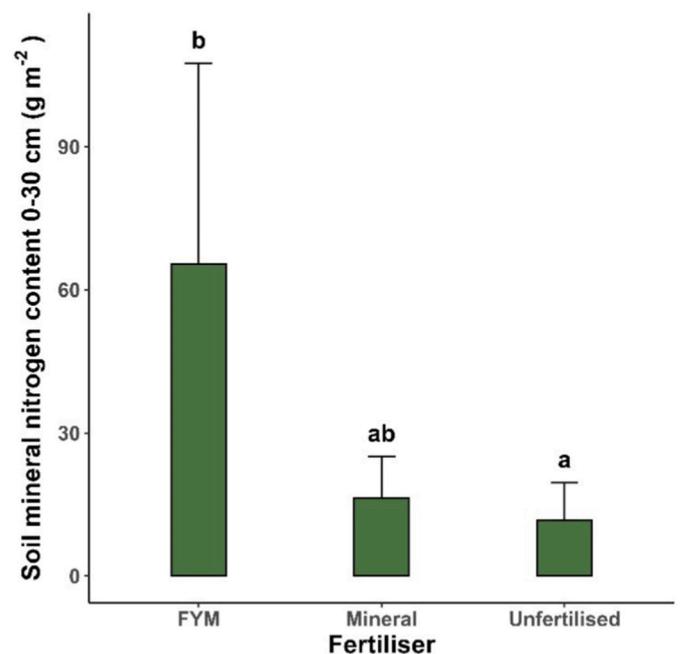
and March 31, 2022, respectively, during the critical period from March to April, were insufficient. Otherwise, precipitation was only rare, with short-lived showers of no more than 3 mm per day. The lack of precipitation may have impeded plant growth for mineral and given FYM a head start into the growing season [56,57]. In addition, topsoil moisture was mainly affected by the low amount of precipitation and not by any treatment as hypothesised [4].

Soil biological processes such as k can be enhanced for FYM by microbial activity and diversity compared to mineral fertilisation. In two long-term trials in Germany (established in 1878 and in 1963), Langer
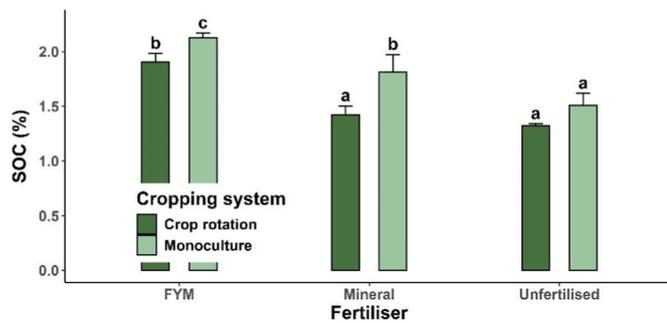
Fig. 6. Overall mean of soil organic carbon (SOC) with fixed effects cropping system (crop rotation; monoculture) and fertilisation (farmyard manure (FYM); mineral fertiliser; unfertilised) of the Eternal rye from March (2020 and 2021). Treatments having no letter in common are significantly different by pairwise comparison (2-way LMM, Tukey; $P < 0.05$). Mean, +SD.

Fig. 7. Overall mean of stabilisation rate (S; A) and decomposition rate (k; B) of the tea bag index with fixed effects cropping system (crop rotation; monoculture) and fertilisation (farmyard manure (FYM); mineral fertiliser; unfertilised) and across tea bag inoculation periods of the Eternal rye in Austria. Tea bags were inoculated for 90-day-periods from January to April, February to May and March to June in 2020, 2021 and 2022. Treatments having no letter in common are significantly different by pairwise comparison (2-way LMM, Tukey; $P < 0.05$). Mean, +SD.
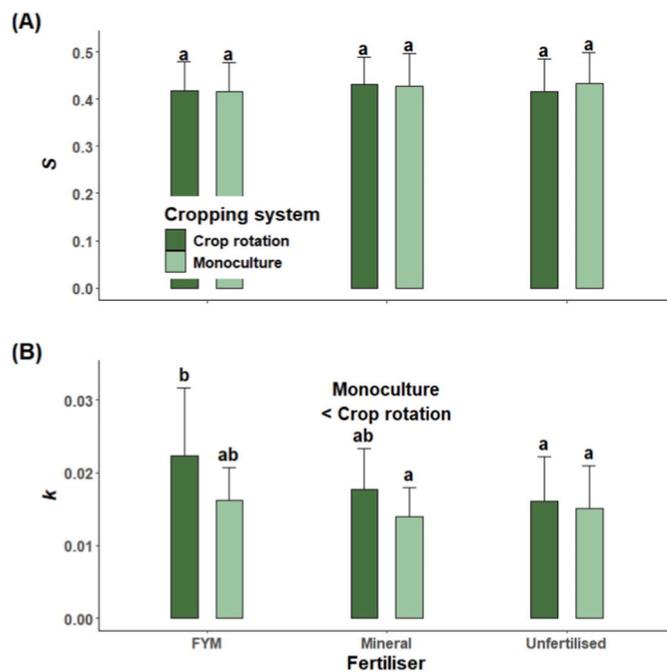
and Klimanek and Stark et al. [58,59] demonstrated that soil microbial communities differed significantly across management regimes. These authors showed that FYM application caused richer and more evenly distributed microbial populations than mineral or unfertilised. This enhanced microbial diversity and activity for FYM can explain the observed increased rate of k, which exceeded those measured in mineral and unfertilised treatments within crop rotation and monoculture. In addition, SOC concentrations were highest for FYM followed by mineral and unfertilised. Abdalla et al. [60] found similar results in a long-term fertilisation experiment at Dikopshof in Germany (established in 1904), where application of 20 t FYM ha^{-1} year^{-1} increased topsoil SOC by up to 58% compared to an unfertilised control. The authors attributed this increase to a favourable carbon balance, with organic inputs that exceeded losses through mineralisation.
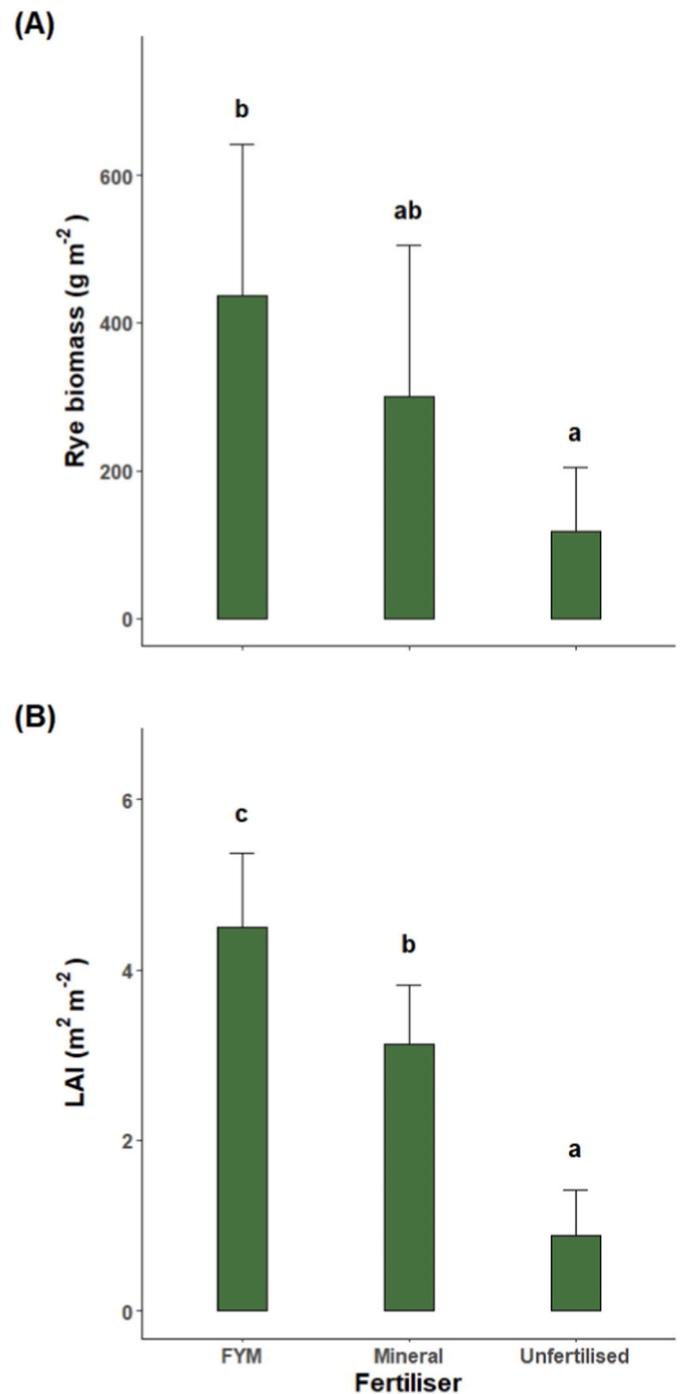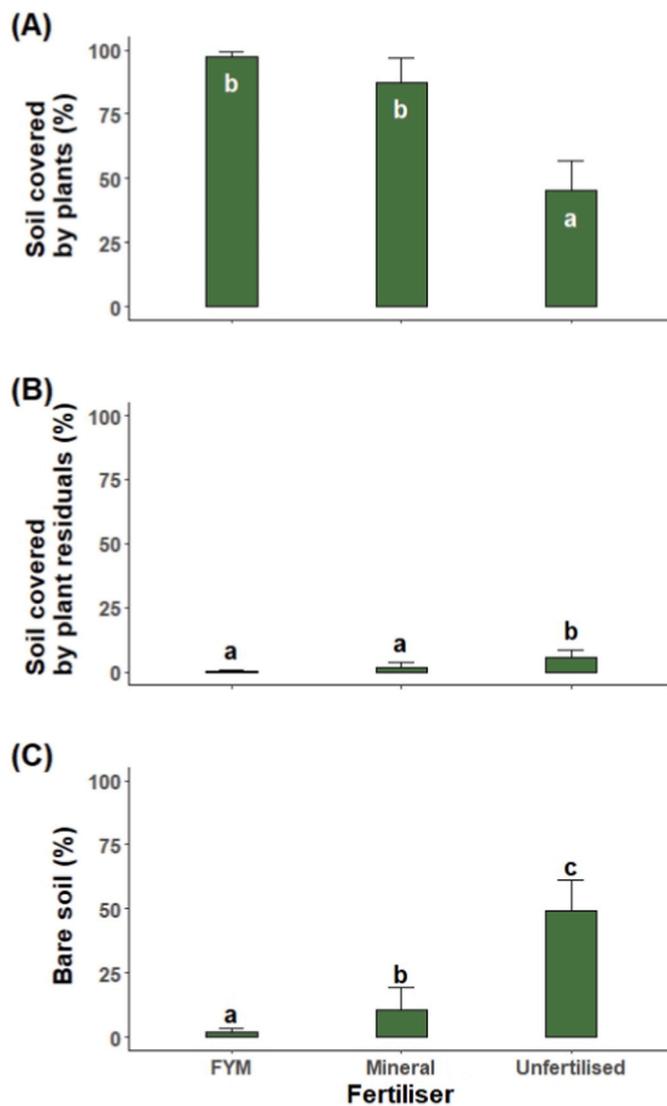

Fig. 8. Overall mean of rye biomass (A) and leaf area index (LAI; B) with fixed effects fertilisation (farmyard manure (FYM); mineral fertiliser; unfertilised) and across cropping system (crop rotation; monoculture) of the Eternal rye in Austria from April (2020, 2021 and 2022). Fertilisation treatments having no letter in common are significantly different by pairwise comparison (2-way LMM, Tukey; $P < 0.05$). Mean, +SD.

Earthworm parameters were also affected by fertilisation and FYM led to the highest levels of total biomass and abundance, higher species richness and a higher Shannon diversity index, in addition to juveniles, endogeics and cocoons. These findings are supported by Leroy et al. [2, 61], who found that FYM and cattle slurry with crop residues incorporated to a depth of 20 cm led to higher total earthworm abundance compared to crop residues only, and composts from vegetable, fruit and garden waste. The authors concluded that the higher availability of organic matter and nitrogen in FYM and cattle slurry with crop residues,

Fig. 9. Overall mean of soil covered by plants (A), plant residues (B) and uncovered soil with fixed effects fertilisation (FYM = farmyard manure; mineral fertiliser; unfertilised) and across cropping system (crop rotation; monoculture) of the Eternal rye in Austria from April (2020, 2021 and 2022). Fertiliser treatments having no letter in common are significantly different by pairwise comparison (2-way LMM, Tukey; $P < 0.05$). Mean, +SD.

improved nutrient availability and led to a higher abundance of earthworms and cocoons. Slurry can serve as an alternative to FYM, but differences between cattle and pig slurry were detected in a 33-year grassland experiment by Murchie et al. [62]. In this experiment, cattle slurry increased earthworm abundance, whereas pig slurry had little effect compared to mineral fertiliser. However, Moinard et al. [63] showed that usage of anaerobic digestate and cattle slurry caused short-term mortality of earthworms, while after two years of repeated applications earthworm abundance increased relative to mineral fertiliser treatments. Moinard et al. [63] showed that up to 2% of adult earthworms died within a few hours of surface application and concluded that mortality depended on ammonia concentration and therefore on the high pH of slurry and other unidentified reasons. Also, farmers frequently report finding dead earthworms on the soil surface after the application of slurry, especially under wet soil condition or rain events shortly after the application. These findings and observations therefore show that the effects of liquid organic fertilisers such as slurry on earthworm parameters should be further investigated, because their effects are not uniformly positive and depend on type of input including

their pre-treatments, type of application, soil condition and time since application [62–64]. Additionally, Murchie et al. [62] found some changes in earthworm community structure as *L. rubellus* (H., 1843) were associated with cattle slurry, *Aporrectodea spp.* With mineral fertiliser, while no particular species were associated with pig slurry. However, the current study found that species richness was higher for FYM than mineral and unfertilised and *L. terrestris* was only found in FYM, similar to a long-term trial of Edwards and Lofty [65] at Rothamsted (UK). These authors attributed the presence of *L. terrestris* to a higher SOC content, but also emphasized the important role of food supply through organic material. While Leroy et al. [2,61] reported increased total earthworm biomass and abundance, they did not detect changes in earthworm community composition under FYM. Taken together, the enhanced food supply, improved soil conditions and increased SOC content explain the elevated abundance of *L. terrestris* for FYM.

5. Conclusion

Our results confirm earlier findings, that the application of organic inputs, such as FYM, can enhance earthworm species richness and abundance. The application of FYM demonstrably increased earthworm abundance, earthworm biomass, cocoons and altered species composition, including the presence of *L. terrestris*. Our findings show that an augmented food supply by the application of FYM can improve the habitat conditions for *L. terrestris*, even after 116-years of intensified plant production under monoculture, removal of plant residue and ploughing.

CRediT authorship contribution statement

Laura Sturm: Writing – original draft, Visualization, Investigation. **Helmut Wagentristl:** Resources, Project administration. **Reinhard W. Neugschwandtner:** Writing – original draft, Validation. **Caroline Huber:** Investigation. **Kevin R. Butt:** Writing – original draft, Methodology, Conceptualization. **Johann G. Zaller:** Writing – original draft, Validation. **Pia Euteneuer:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Has patent pending to. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2026.103815.

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