

Contribution of *Lumbricus terrestris* enhancement to soil fertility and maize and wheat yields in soil tillage systems

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ABSTRACT

Earthworms provide important ecosystem services such as plant yield increase and soil structure improvement. Soil tillage systems affect earthworm abundance, e.g., that of *Lumbricus terrestris*, the burrows of which can increase water infiltration. We tested the impact and mechanisms of additional *L. terrestris* on plant growth and soil physical parameters in Austria. At two sites, 14 individuals m⁻² were inoculated under different soil tillage systems into large enclosures of 7.5 m², to compare enhanced versus ambient numbers of *L. terrestris* for maize (*Zea mays*) followed by wheat (*Triticum aestivum*). Tested soil tillage systems were plough (CON, 30 cm depth), cultivator (RED; 15 cm depth) and no soil tillage (NT; 0 cm depth). The success rate of inoculation was similar between the soil tillage systems at 33–44%. Number of middens were increased for enhancement than control and was highest for NT than RED and CON. At level enhancement, grain yield and N grain yield of maize were increased by 10; 13% and 17; 23% respectively for CON and RED, while wheat was unaffected, due to insufficient precipitation. At flowering of maize, topsoil moisture at site 2 was 18% and 17% higher for enhancement than control for CON and RED, while NT and wheat were unaffected. Soil aggregate stability was 2.7–2.9 times more stable at level enhancement than control for CON and RED for maize, respectively. It was shown that earthworms contribute to plant growth and soil fertility within a relatively short period. Increased numbers of earthworms had the highest effect on plants and soil parameters for CON and RED, which are usually considered to have a weaker soil structure than NT. Earthworms can therefore facilitate the conversion from CON and RED to NT for climate change adaptation and can improve soil fertility.

1. Introduction

Ecosystem services play an important role in agroecosystems and have shifted from being a benefit to a necessity for climate change mitigation (Barrios, 2007; Bardgett and van der Putten, 2014; Helfenstein and Kienast, 2014). Earthworms provide important ecosystem services such as plant yield increase and improvement of soil structure (Blouin et al., 2013), but their impacts under field conditions are still elusive (van Groenigen et al., 2015; Euteneuer et al., 2024). Earthworms can facilitate processes such as soil aggregate formation and stabilisation, change in pore size distribution, and thus impact soil water fluxes (Six et al., 2004; Reck et al., 2018; Schneider et al., 2018). Earthworms actively assist soil structural development through macropore creation

(Pelosi et al., 2017) and their activities (burrowing and cast formation) can increase water infiltration (Shipitalo et al., 2004), help prevent soil erosion and alter topsoil moisture content due to enhanced hydrological effectiveness (Capowiez et al., 2014; Lipiec et al., 2015; Schneider et al., 2018). Epi-anecic (deep burrowing) earthworms such as *Lumbricus terrestris* (Linnaeus, 1758) have the greatest effects on mass water flow through macropores (Shipitalo and Butt, 1999; van Schaik et al., 2014), but for interactions with the soil-matrix, geophagous, endogeic (shallow working) species such as *Aporrectodea caliginosa* (Savigny, 1826) may be more important as they have greater abundance in most agroecosystems (van Schaik et al., 2014; Euteneuer et al., 2020; Butt et al., 2022). But earthworms and soil aggregate stability are sensitive to soil tillage and both can be reduced under conventional approaches (Euteneuer et al.,

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2024; Simon et al., 2025). Conversely, conservation tillage, i.e., reduced, minimized or no mechanical soil disturbance, is known to enhance earthworm abundance and diversity, formation of soil aggregates and soil water storage (Bodner et al., 2015; Dekemati et al., 2019; Euteneuer et al., 2024). Soil structure and its interactions with earthworms are important components in conservation agriculture (Brussaard et al., 2007; Lehmann et al., 2017; Bodner et al., 2023).

Earthworm effects on plant production have shown that their presence in agroecosystems leads to an average 25 % increase in crop yield and a 23 % increase in aboveground biomass (van Groenigen et al., 2015). Similar was seen in a global study by Fonte et al. (2023), who estimated the average earthworm contribution to grain yield of major cereal crops was 6.5 %, with higher contributions of 7.4 % for Europe, due to greater earthworm abundance. Interactions of plants and earthworms have been studied many times and have shown that earthworms contribute to plant growth, due to improvement of plant nutrition and/or plant health (Wurst and Jones, 2003; Wurst, 2010; Bityutskii et al., 2012; Athmann et al., 2017). A further meta-analysis relating to earthworm casts, showed that by comparison with bulk soil, casts are many times more fertile (van Groenigen et al., 2019), but overall effects on plant growth depended on complex interactions between earthworm species traits and specific soil properties.

As shown by Fonte et al. (2023), earthworm densities can make a positive difference to grain yield, but the authors also stated that their results might be an overestimate, because most studies in their meta-analysis used mesocosms in greenhouses or fields and added or excluded earthworms at unrealistic densities. However, direct manipulation of earthworm communities in agricultural soils is possible and has been undertaken by numerous authors, e.g., Eriksen-Hamel and Whalen (2007) found significantly higher soil mineral nitrogen and microbial biomass nitrogen in increased earthworm treatment plots (2.9 m²) dominated by *A. caliginosa* (50–130 individuals m⁻²) compared to zero earthworm in the control. Long-term monitoring by Nuutinen et al. (2017) of introduced *L. terrestris* to a boreal, clay soil, showed that this did not affect overall field macroporosity, but did influence enchytraeid existence. *L. terrestris* has been used at high density (56 individuals m⁻²) in a mesocosm field experiment of 0.071 m², when heavy rain events reduced plant biomass in the control with no earthworms (Andriuzzi et al., 2015). In addition, Fonte et al. (2023); argued that some of the short-term experiments used fail to capture the complete range of ecosystem services provided by earthworms. To overcome some of the shortfalls mentioned by Fonte et al. (2023), we tested the impacts of earthworms on soil structure and plant growth under different soil tillage systems by using large enclosures of 7.5 m², to compare enhanced versus ambient numbers of *L. terrestris* over two years, at two sites in Austria.

Although not a given, earthworms, particularly *L. terrestris*, have been found to have positive results with respect to soil structure and plant growth when used and established in a variety of agroecosystems. The aim of this study was to assess effects and mechanism of earthworm (*L. terrestris*) enhancement on selected soil and plant parameters within conventional and reduced tillage systems in an already existing cropping sequence of maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) cultivation under genuine field conditions rather than controlled greenhouse trials. *L. terrestris* was selected, due to its positive impact on water infiltration, soil aggregate stabilisation, water-holding capacity, were easy to obtain and has a relatively low abundance compared to endogeic earthworms (Andriuzzi et al., 2015; Hallam and Hodson, 2020; Simon et al., 2025). We discarded the idea to inoculate endogeic earthworms such as *Aporrectodea caliginosa*, because we calculated that it would have required twice as many individuals of the ambient abundance of endogeic earthworms (a total of 5040 individuals) to increase the enhanced treatment at a similar level as for *L. terrestris* (details provided in Sections 2.1 and 3.1) (Butt et al., 1997; Simon et al., 2025).

Earthworm activity depends highly on soil moisture content (Lowe and Butt, 2005) and the Pannonian basin in north-east Austria, where

our field trial was located, receives an average of 560 mm of precipitation per year (Euteneuer et al., 2020; Euteneuer and Butt, 2025). Earthworm activity might be restricted under this environment, but to the best of our knowledge no earthworm inoculation trials have tested ecosystem services of *L. terrestris* under such conditions in a field experiment. It was not our intention to advocate earthworm inoculation within tillage systems as this might not be sustainable, and be quite costly for farmers, but to evaluate known ecosystem services of *L. terrestris* such as increased aggregate stability, N uptake by plants and the overall effects on plant productions in enclosures closer to field conditions rather than small mesocosm trials. Specific objectives were to: i) measure *L. terrestris* parameters, ii) record interactions with tillage systems as key drivers of soil structure, soil moisture and crop growth in water-limited environments. It was hypothesized that in plots where *L. terrestris* numbers were artificially enhanced, earthworm would mediate soil structure improvements and promote soil fertility and that both crops would respond with higher grain yield and N uptake as seen with studies of van Groenigen et al., (2015);(2019), Fonte et al. (2023) and Euteneuer et al. (2024).

2. Materials and methods

2.1. Study sites and experimental set up

This research was undertaken at two long-term tillage experiments in Hollabrunn (site 1; in 2019–2020; 48°34'N, 16°5'E) and in Raasdorf (site 2; in 2020–2021; 48°14'N, 16°33'E), in north-east Austria. The distance between the sites is approximately 53 km, both sites have similar climatic characteristics and similar soil; a loamy silt chernozem (WRB, 2014) (Table 1) (GeoSphere Austria, 2022). Climate data for site 1 were provided by GeoSphere Austria (2022) and for site 2 by a meteorological station on site (Adcon A733, OTT Hydromet GmbH, Kempton, Germany) (Supplementary figure 1).

The experimental setup for both sites consisted of three soil tillage treatments in a randomised block design, with three blocks at site 1 and four blocks at site 2. The tillage treatments included conventional (plough; 25 cm depth), reduced (cultivator; 10 cm depth) and no-till (0 cm depth). The plots at site 1 were 6 × 60 m and at site 2 were 24 × 40 m. The cropping sequence at both sites was winter wheat

Table 1

Overview of the two experimental sites, soil parameters and cropping conditions in Lower Austria, Austria, for soil tillage treatment no-till (NT), reduced (RED) and conventional (CON).

Experimental details	Site 1		Site 2	
Instigated in	2006		1996	
Bulk density (NT/RED/CON)	1.51/1.27/1.27 ^a		1.15/1.01/1.06 ^b	
Clay/Silt/Sand (g kg ⁻¹)	210/550/240 ^a		217/570/213 ^b	
pH _{CaCl2}	7.5 ^c		7.6 ^d	
Total organic carbon (g kg ⁻¹)	26.5 ^c		23.5 ^d	
Mean temperature (°C)	10.15		11.21	
Mean of precipitation (mm)	517		560	
Previous crop (year)	Winter wheat (2018)		Winter wheat (2019)	
	Maize	Wheat	Maize	Wheat
Study year	1	2	1	2
Crop variety	DKC 3629	Emilio	Waxy P 9074E	Arnold
Seeding rate (m ⁻²)	7.5	350	7.5	320
Row spacing (cm)	75	10	75	10
Seeding date	11th Apr 2019	11th Nov 2019	17th Apr 2020	15th Nov 2020
Harvest date	23rd Oct 2019	27th Jul 2020	5th Oct 2020	12th Jul 2021
Nitrogen (kg N ha ⁻¹)	130	130	130	130

^aWeninger et al., (2019); ^bLiebhard et al., (2022); ^cRosner et al., (2018);

^dNeugschwandtner et al., (2020)

(previous crop), maize (study year 1) followed by winter wheat (study year 2) (Table 1).

Purchased *L. terrestris* (14 adult individuals m^{-2}) (Fisherman's Partner, Gross-Enzersdorf, Austria) were released on the soil surface in May 2019 (site 1; mean individual mass 4 ± 0.21 g) or 2020 (site 2; mean individuals mass 3.87 ± 0.46 g) into $7.5 m^2$ enclosures in maize. The enclosures for the enhancement treatment had dimensions of $3 \times 2.5 \times 0.2 \times 0.2$ m (length, width, depth, height) and consisted of heavy-duty plastic sheeting and a wooden frame (Fig. 1), similar to Grigoropoulou and Butt (2010). The control treatment, contained the ambient earthworm community, had no fence and was only marked with corner pegs on the soil surface (3×2.5 m). For soil tillage and seeding of winter wheat in November 2019 and 2020, enclosures were removed and not reinstalled and plots for both treatments were marked on the soil surface.

2.2. Midden monitoring

To encourage midden formation at both sites, wheat straw ($178 g m^{-2}$) was spread across the enhancement and control area for maize in May and June 2019 and 2020, and for wheat in October and February 2020 and 2021, with counts conducted in November 2019, 2020, and March 2020, 2021, at site 1 and 2, respectively. Since each burrow typically has one midden, but occasionally two exits, we accounted for potential overestimations, by checking for occupancy of ten randomly selected middens using mustard suspension (Stroud et al., 2016) to determine occupancy rates, and the final midden count (m^{-2}) was corrected accordingly (Simon et al., 2025). Collected *L. terrestris* were rinsed of mustard, blotted dry, life stage (immature; adult) and biomass was recorded. The inoculation success was determined by subtraction of corrected numbers of middens in control from middens in the enhanced enclosure.

2.3. Soil data

Soil samples for soil aggregate stability were taken with two subsamples per plot in November 2019 or 2020 and March 2020 or 2021 at site 1 and 2, respectively, air-dried and sieved to 2–5 mm as previously described by Euteneuer et al. (2024). In brief, the two sub-samples per plot were taken from the bulk soil and pooled. Then aggregate analyses

were repeated five time per plot with each three soil aggregates per analysis. For measurement, the smartphone application MOULDER (formerly SLAKES) (Fajardo et al., 2016; Fajardo and McBratney, 2023) was used following the analytical protocol of Flynn et al. (2020) with an iPhone 7 (Apple Inc., Cupertino, California, USA). MOULDER analyses the dispersion of the soil aggregate submerged in deionised water over 10 min and compares it to a dry references image taken at the start of the measurement. The result is presented as a max. slaking α -coefficient, which is the maximum prediction of dispersion fitted to a Gompertz function (Gompertz, 1825). Details of the max. slaking α -coefficient, the Slaking index and the method, are described by Fajardo et al. (2016).

Topsoil moisture (0–7 cm depth) was measured on a weekly basis with 5 subsamples per plot only at site 2 from May to November 2020 and March to June 2021 (WET-Sensor, Delta-T Devices Ltd, Cambridge, UK). Soil mineral nitrate was sampled at site 2 after the harvest of maize and wheat in November 2020 and July 2021 at soil depth of 0–30 cm. Soil nitrate ($g NO_3 m^{-2}$) was extracted with 0.0125 M $CaCl_2$ in a ratio of 1:4 (w/v) for 1 h using an overhead shaker (ÖNORM L, 1091, 2020) and analysed photometrically (FIASSTAR 5000, FOSS GmbH, Hamburg, Germany).

2.4. Plant data

To determine grain yield, the whole plot was harvested for maize and $2 m^2$ of each plot were harvested for wheat by cutting plants at 4 cm above the soil surface. All ears per plot were counted for ear density per m^2 . Maize ears were first pre-dried at room temperature for 10 days and then, as for wheat ears, dried at $105^\circ C$ for 24 h to determine dry matter content. After drying, ears were counted and maize seeds were separated by hand from the cob or wheat ears were processed with a laboratory thresher (LD 180, Wintersteiger AG, Ried, Austria) to measure grain yield ($g m^{-2}$). Total mass of grains was taken of five medium sized maize ears or of 20 wheat ears per plot to determine ear grain yield ($g ear^{-1}$). Thousand kernel mass (TKM; g) of a randomly selected subsample of each plot were recorded. For residue dry matter, again a randomly selected subsample of 5 maize plants $plot^{-1}$ without seeds were chopped and then as for wheat residues, dried at $105^\circ C$ for 24 h to determine dry matter content. For wheat residue, dry matter of the whole $2 m^2$ was used. Nitrogen for N grain yield and N residue yield was determined by the Dumas combustion method (vario MACRO cube CNS; Elementar

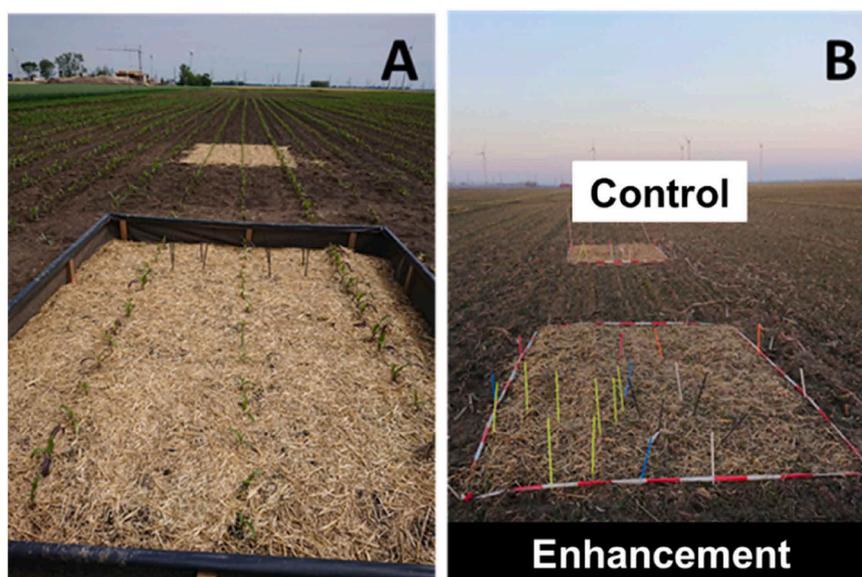


Fig. 1. Earthworm plots with enclosures (enhancement with additional 14 *Lumbricus terrestris* m^{-2}) and ambient earthworm community (control) at site 2 in May 2020 with maize (A) and March 2021 with winter wheat (B). Enclosures were not reinstalled after being removed for soil tillage and seeding of winter wheat in November 2020. All plots received a mulch layer of wheat straw to enable the building of middens by *L. terrestris*.

Analysesysteme GmbH, Germany) (Winkler et al., 2000). Nitrogen was analysed after grinding and sieving (<1 mm) by inductively coupled plasma-optical emission spectrophotometry (iCap 7000 Series ICP-OES; Thermo Fisher Scientific, Waltham, USA) after nitric acid digestion of the material. Concentration of N was multiplied by grain yield and residue dry matter and divided by 100 accordingly to calculate N grain yield (g N m^{-2}) and N residue yield (g N m^{-2}), respectively.

To measure the influence of earthworms on water stress mitigation and enhanced N uptake, total C and N, bulk ^{13}C and ^{15}N isotope analysis were performed by elemental isotope ratio mass spectrometry. All grain samples were dried (40 °C for 72 h), finely ground and accurately weighed (3–5 mg) into 9×5 mm tin cups. Samples were analysed using a Thermo Flash 2000 Organic Elemental analyser, linked to a Thermo Delta V Advantage automated isotope ratio mass spectrometer (Thermo, Bremen, Germany). A full complement of internal and external standards was run with the samples to calculate isotopic ratios and %C and %N values. The isotope ratios were expressed as parts per thousand or δ deviation from the internationally recognised standards Vienna Pee Dee Belemnite and ambient air.

2.5. Data analyses

Data were analysed with linear mixed models (LMM) over both sites except for topsoil moisture where data was only available for site 2. In the overall model, crop was considered as a repeated factor, with tillage and enhancement as fixed and site, block, plot and enclosure (2 levels; enclosure; control) as random effects. The experimental design with the earthworm enclosures is a split-plot design with soil tillage the main-plot and enclosures the split-plot factor, because enclosures and control were situated within the main plot (Fig. 1) (Neugschwandtner et al., 2022). The main-plot error is therefore included by using enclosures as a random effect (Piepho et al., 2003). In detail, a three-way LMM (3-way LMM) were used for parameters grain yield, grain yield ear^{-1} , ear density, residues dry matter, TKM, N grain yield, N residue yield, soil nitrate, aggregate stability, plant $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analysed with fixed factors enhancement (2 levels; enhanced versus control), soil tillage (3 levels; conventional; reduced; no-till) and crop (2 levels; maize; wheat) (Piepho et al., 2004). The major differences between maize and wheat were considered through nesting fixed factors enhancement and soil tillage within crops (Euteneuer et al., 2022). Measurements took place only once per year for wheat and in the following year for wheat and therefore autocorrelation was considered as similar between the samplings with a compound symmetry structure by using site \times year, which in this case also corresponded to an autoregressive model of order 1. In addition, as topsoil moisture was measured weekly, we used the mean topsoil moisture for the month of flowering and applied a similar approach as described before. The only difference was that site was excluded as a random effect, as topsoil moisture was only measured at site 2, similar to soil nitrate.

Two-way LMM (2-way LMM) was used for monitoring *L. terrestris* parameters such as inoculation success rate, occupancy rate, proportion of adult and immature *L. terrestris* and their biomasses with fixed effect soil tillage and site (2 levels; site 1; site 2) and random effect block. For normality and homogeneity of occupancy rate, proportion of adult and immature *L. terrestris* and their biomasses, number of middens, grain yield, TKM, N grain yield, $\delta^{15}\text{N}$, topsoil moisture and soil nitrate were square root transformed, while grain yield ear^{-1} , ear density and $\delta^{13}\text{C}$ were log transformed. Normal distribution of residuals was inspected in QQ-plots and homogeneity of the variance was determined by plotting residuals against fitted values.

Linear MM was performed with 'lmer' ('lme4' package) (Bates, 2015) in R (R Core Team, 2025) using the residual maximum likelihood method. Function 'anova' (with type III hypotheses) was used for ANOVA with Wald-type *F*-tests and Satterthwaite's method to obtain denominator degrees of freedom. For pairwise comparisons of factor combinations (Tukey; $P < 0.05$), function 'emmeans' of package

'emmeans' (Lenth, 2022) and to create figures, function 'ggplot' of package 'ggplot2' (Wickham, 2016) were used.

Principal component analysis (PCA) was performed with function 'prcomp' (package 'factoextra') (Kassambara and Mundt, 2020) and based on centred and scaled data of grain yield, TKM, residue dry matter, middens, topsoil moisture and soil aggregate stability only for site 2, because not all parameters were available for both sites. Biplots were plotted with function 'fviz_pca_biplot' (package 'FactoMineR') (Le et al., 2008) and include scores and loading vectors for the first and second principal components.

3. Results

3.1. Environmental conditions

Mean temperatures for maize and wheat at both sites were similar with 17.7 °C (maize: May to October) and 9.6 °C (wheat: November to July) and in the range of the long-term averages at the two sites (Supplementary figure 1). Rainfall during the same period at site 1 was 17 mm below the long-term average precipitation and at site 2 was 103 mm above the average for maize. In total, maize at site 1 received 119 mm less precipitation from May to October than site 2 and wheat at site 1 had 110 mm more precipitation from November to July than site 2. From March to May, wheat received 38 mm and 33 mm less precipitation during the growing period than the long-term averages at site 1 and 2, respectively, while site 1 received 27 mm less than site 2.

The number of possible days for earthworm activity (at temperatures between 5–25 °C) were 182 and 180 for maize and 166 and 148 for wheat, at site 1 and 2, respectively. Topsoil moisture was measured on a weekly basis at site 2 and number of weeks with a soil moisture content > 15 % were 15–18 for maize and 7–10 for wheat depending on the soil tillage system (Supplementary figure 2).

3.2. Monitoring of *Lumbricus terrestris*

Occupancy rate of middens of *L. terrestris* showed differences between site and tillage systems, where site 1 had 14–29 % more occupied middens than site 2 and no-till had a 27–40 % higher occupancy rate than conventional (Table 2). Occupancy rate of adult *L. terrestris* was 47 % higher at site 1 than at site 2 but was similar between the tillage systems (Table 2). Conversely, the rate of immature *L. terrestris* at site 2 was twice as high as at site 1 and was affected by soil tillage (Table 2). At site 2, no-till had the lowest abundance of immature *L. terrestris*, while none were expelled in reduced tillage at site 1 (Table 2). Only soil tillage had an impact on biomass of *L. terrestris*. Adults showed similar size across sites and were the largest in reduced tillage within site 2 and immatures were the smallest for no-till at both sites (Table 2). In addition, number of middens (corrected by occupancy rate) was affected by soil tillage (conventional = reduced < no-till; at both sites) where enhancement had more middens after inoculation (conventional 2.5, 4.5; reduced 2.5, 2.7; no-till 1.3, 1.5 times for maize and wheat, respectively) than control (Fig. 2). However, the inoculation success rate was similar between the soil tillage systems with 44.3 ± 42.7 % for no-till, 41.7 ± 21.2 % for reduced and 33.7 ± 17.6 % for conventional ($F_{\text{site}} = 2.7$, $P = 0.125$; $F_{\text{tillage}} = 1.7$, $P = 0.234$; $F_{\text{site} \times \text{Tillage}} = 1.16$, $P = 0.353$).

3.3. Interactions of crops, *Lumbricus terrestris* and soil tillage

For a better understanding and overview, we separated the results for effects of *L. terrestris* and soil tillage, since, except for TKM and topsoil moisture, the 3-way interaction of crop, soil tillage and enhancement of *L. terrestris* were not significant (Table 3). Parameters affected by crop \times enhancement such as grain yield ear^{-1} and N residue yield were not affected by crop \times tillage (Table 3). Vice versa parameters such as ear density, $\delta^{13}\text{C}$ and soil nitrate were only affected by crop \times tillage, while

Table 2

ANOVA results of occupancy rate (relative) of adult and immature burrows of *Lumbricus terrestris* and their biomass (g individuals⁻¹) at level enhancement with fixed factor tillage and site in November 2019 or 2020 for site 1 or 2, respectively. Upper-case letters: Sites having no letter in common are significantly different by pairwise comparison; Lower-case letters: Tillage treatments across sites having no letter in common are significantly different by pairwise comparison (2-way LMM, Tukey; $P < 0.05$). Tillage (T): degrees of freedom (df) = 2; Site (S): df = 1; S × T: df = 1. Standard error (SE), N = 3 or 4.

Parameter	F-value			Site	Mean ± SE					
	T	S	S × T		Conventional		Reduced		No-till	
Occupancy rate	6.73 **	8.84 *	0.677	1 B	0.66 ± 0.093	b	0.799 ± 0.102	ab	0.927 ± 0.14	a
				2 A	0.421 ± 0.063	b	0.513 ± 0.069	ab	0.822 ± 0.088	a
Adult rate	1.98	24.8 ***	0.121	1 B	0.755 ± 0.069	a	0.893 ± 0.069	a	0.845 ± 0.088	a
				2 A	0.519 ± 0.058	a	0.63 ± 0.058	a	0.543 ± 0.058	a
Immature rate	9.53 **	9.39 **	0.139	1 A	2.71 ± 0.221	a	0	b	1.3 ± 0.13	b
				2 B	2.59 ± 0.163	a	2.8 ± 0.178	a	1.18 ± 0.11	b
Adult biomass	4.58 *	0.006	0.175	1 A	3.61 ± 0.294	ab	4.07 ± 0.313	a	3.31 ± 0.361	b
				2 A	3.42 ± 0.242	ab	4.2 ± 0.628	a	3.32 ± 0.238	b
Immature biomass	184 ***	1.89	0.064	1 A	2.4	a	-	-	1.14 ± 0.17	b
				2 A	2.6 ± 0.361	a	2.6 ± 0.36	a	1.18 ± 0.167	b

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

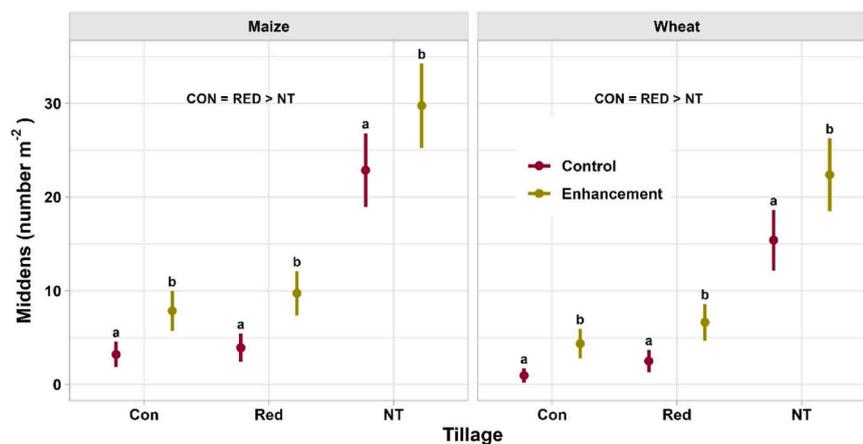


Fig. 2. Number of middens corrected by occupancy rate for maize and wheat for soil tillage systems conventional (Con), reduced tillage (Red) and no-till (NT) at two enhancement levels of *Lumbricus terrestris* (Control; Enhancement) at both study sites. Treatments having no letter in common are significantly different by pairwise comparison, for enhancement levels within crops (three-way linear mixed model, Tukey). Displayed are mean values and standard error.

grain yield, residue dry matter, N grain yield, $\delta^{15}\text{N}$ and aggregate stability were affected by crop × enhancement and crop × tillage (Table 3).

3.3.1. Effect of adding *Lumbricus terrestris*

First, we present the effect of the enhancement of *L. terrestris* on yield components and then of plant quality parameters. In detail, grain yield of maize was higher for enhancement than control, for conventional by 10 % and for reduced tillage by 13 % (Fig. 3A). Similar trends were seen for maize grain yield ear⁻¹ with higher values for enhancement than control for conventional (153 ± 13.7; 140 ± 12.6) and reduced tillage (159 ± 14.2; 140 ± 12.5), respectively. In addition, residue dry matter of maize was higher by 16 % for enhancement than control across soil tillage systems and TKM was also increased by 8 % and 9 %, for reduced and conventional tillage, respectively (Figures 3BC). Whereas subsequent wheat was not affected by inoculated *L. terrestris* for any of these parameters (Figures 3ABC).

Nitrogen grain yield, N residue yield and $\delta^{15}\text{N}$ showed differences for maize, but not wheat (Table 3; Fig. 4 ABC). Nitrogen grain yield of maize was higher for enhancement than control for conventional and for reduced tillage by 17 % and 23 %, respectively (Fig. 4A), whereas no differences between enhancement and control were found for no-till. But this was different for N residue yield of maize, when enhancement was increased for all soil tillage systems by 17 % (Fig. 4B). In addition, plant $\delta^{15}\text{N}$ was only higher for conventional in enhancement by 25 % compared to control (Fig. 4C).

Soil aggregates were affected by additional *L. terrestris* in maize, but not in wheat (Fig. 5). Aggregates in maize were 2.7 and 2.9 times more stable for enhancement than control for conventional and reduced tillage, respectively, but not for no-till (Fig. 5). Topsoil moisture for maize at flowering was higher for enhancement than control for conventional by 18 % and for reduced tillage by 17 % but was similar for no-till and wheat was not affected by enhancement at all (Fig. 6).

3.3.2. Effect of soil tillage

In this section we focus on significant interactions of crop × tillage from Table 3. In detail, grain yield of maize was not affected by soil tillage, but were increased for wheat at conventional (25 %) than reduced tillage, while no-till was similar to conventional and reduced tillage (Table 3; Fig. 3A). Residue dry matter of maize was also affected by tillage with higher values by 37 % for reduced tillage than no-till, when conventional did not differ from reduced tillage and no-till. (Fig. 3B). For wheat conventional was higher by 29 % than reduced tillage and did not differ from no-till (Fig. 3B). Similar was seen for ear density for wheat, when soil tillage followed the order of conventional > reduced = no-till (525 ± 37; 452 ± 31.9; 450 ± 32.1 ears m⁻², respectively), but was similar between all treatments for maize (7.6 ± 0.5 ears m⁻²).

The differences caused by soil tillage treatments for N uptake were seen in wheat for N grain yield and $\delta^{15}\text{N}$ (Table 3), while maize was unaffected for any parameter (Fig. 4 AC). N grain yield of wheat for

Table 3

ANOVA results of grain yield, ear grain yield, residue dry matter, TKM, ear density, N grain yield, N residue yield, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, aggregate stability, topsoil moisture at flowering and soil nitrate with fixed factor crop (C; maize, wheat), tillage (T; conventional, reduced, no-till) and enhancement level (E; control, enhancement) for both sites. Crops having no letter in common are significantly different by pairwise comparison (3-way LMM, Tukey; $P < 0.05$). Further details are given in Figs. 3-6. Degrees of freedom: C = 1; C \times T = 4; C \times E = 2; C \times T \times E = 4. Standard error (SE), $N = 4$.

Parameter	C	C \times T	C \times E	C \times T \times E	Crop	Mean \pm SE
Grain yield (g m ⁻²)	8.34	7.13 **	10.23 **	0.448	Maize	1167 \pm 157 a
					Wheat	508 \pm 103 a
Ear grain yield (g ear ⁻¹)	2259 **	2.12	8.99 ***	1.27	Maize	152 \pm 12.6 b
					Wheat	1.23 \pm 0.102a
Residue dry matter (g m ⁻²)	3.62	7.5 **	13.5 ***	0.654	Maize	591 \pm 59.5 a
					Wheat	539 \pm 59.4 a
TKM (g)	225 **	1.94	19.1 ***	6.54 **	Maize	260 \pm 15.5 b
					Wheat	35.1 \pm 5.55 a
Ear density (ears m ⁻²)	2194 **	10.1 ***	0.979	0.729	Maize	7.67 \pm 0.529 a
					Wheat	474 \pm 32.7 b
N grain yield (g N m ⁻²)	0.002	3.96 *	13.3 ***	0.633	Maize	14.3 \pm 1.47 a
					Wheat	11.8 \pm 1.61 a
N residue yield (g N m ⁻²)	4.01	1.79	9.07 **	0.238	Maize	5.15 \pm 1.3 a
					Wheat	1.2 \pm 1.68 a
$\delta^{15}\text{N}$ (‰)	13.2 *	5.99 **	7.63 **	1.52	Maize	2.37 \pm 0.251 a
					Wheat	2.55 \pm 0.26 b
$\delta^{13}\text{C}$ (‰)	280 **	3.79 *	0.771	0.534	Maize	-12.7 \pm 0.372 a
					Wheat	-26.6 \pm 0.781 b
Aggregate stability (max. slaking α -coefficient)	2.01	15.7 ***	39.3 ***	1.96	Maize	1.82 \pm 0.294a
					Wheat	1.67 \pm 0.294 a
Topsoil moisture (%)	0.387	11.7 **	14.5 **	7.43 **	Maize	17.5 \pm 0.713 a
					Wheat	16.3 \pm 0.688 a
Soil nitrate (g NO ₃ m ⁻²)	9.60 **	7.52 **	0.823	0.787	Maize	6.17 \pm 1.83 a
					Wheat	14.2 \pm 2.78 b

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

conventional was 26 % higher than reduced tillage and similar to no-till (Fig. 4A). In addition, plant $\delta^{15}\text{N}$ of wheat for conventional was higher than reduced tillage by 49 % and no-till by 61 % (Fig. 4C), while for $\delta^{13}\text{C}$ (‰) of wheat no-till (-27.1 \pm 0.814) was decreased compared to conventional (-26.2 \pm 0.782) and similar to reduced tillage (-26.4 \pm 0.79) (Table 3). In addition, soil nitrate (0–30 cm) at harvest was affected by soil tillage for maize and wheat and followed the order of conventional \geq reduced \geq no-till and conventional = reduced \geq no-till, respectively (Supplementary figure 3).

Soil aggregates were affected by soil tillage and were 2–2.5 times more stable for no-till than conventional and reduced tillage for maize and 1.7–2.9 times for no-till and reduced tillage than conventional for wheat (Fig. 5). In addition, topsoil moisture at flowering of maize was similar between the treatments and by 28 % higher for no-till than conventional and reduced tillage for wheat (Fig. 6).

3.4. Principal component analysis

Principal component analysis showed that the first dimension (Dim1) for maize and wheat comprised soil parameters, middens and $\delta^{13}\text{C}$ and the second dimension (Dim 2) held plant parameters grain yield and residue dry matter. Highly correlating plant parameters for grain yield and residue dry matter such as grain yield ear⁻¹, ear density, N grain yield and N residue yield were excluded after the first PCA (data not shown). $\delta^{15}\text{N}$ was also excluded, because it had only minor contributions for Dim1 and Dim2 and was always associated with the third dimension. For both PCAs eigenvalues were only above 1 for Dim1 and Dim2 and explained 55; 59 % and 20 % of the variation, respectively (Fig. 7). For maize, $\delta^{13}\text{C}$ (r 0.356), topsoil moisture (r 0.439) and number of middens (r 0.462) were positively correlated with Dim1, while soil aggregate stability (r -0.416) was negatively correlated. Maize parameters grain yield (r 0.480) and residue dry matter (r 0.739) were positively correlated with Dim2. In summary, grain yield and $\delta^{13}\text{C}$ were associated between reduced, conventional for enhancement and both enhancement levels of no-till and negatively correlated with a weak soil aggregate stability, which was positioned at control for conventional tillage. In addition, residue dry matter showed no relationship with soil

aggregate stability and $\delta^{13}\text{C}$. For wheat, grain yield was negatively correlated with $\delta^{13}\text{C}$ and a weak aggregate stability, while the latter parameters were associated with reduced and conventional. Both PCAs associated soil moisture and middens with no-till, while conventional and reduced were at the opposite ends of the spectrum. Within soil tillage systems for maize, control and enhancement were clearly separated from each other but showed overlaps for wheat.

4. Discussion

4.1. Inoculation success

This study examined the effects of *L. terrestris* inoculation on plant growth and soil physical properties in different soil tillage systems at two sites. Overall, the inoculation was successful at both sites and showed that 33.7–44.3 % of the introduced *L. terrestris* settled in the enclosures. Inoculation of *L. terrestris* has been used before in different studies, but was not always successful. Grigoropoulou and Butt (2010) for example, failed to increase *L. terrestris* abundance in a deciduous tree trial with an existing high ambient abundance of 34.2 \pm 2.48 *L. terrestris* m⁻². Adding 30 and 60 *L. terrestris* m⁻² neither increased abundance nor numbers of middens (overall: 30 \pm 0.77 m⁻²). However, the authors also provided traps to monitor migration of *L. terrestris*, due to population density, food availability and life stage of individuals. Results showed that numbers of captured *L. terrestris* were not affected by food availability (100 or 300 g dry matter leaf litter m⁻²), but by inoculation intensity. The authors stated that, numbers of *L. terrestris* captured from inoculation plots were 7–12-times higher than that from ambient population. In addition, in the second year Grigoropoulou and Butt (2010) added purchased, and field-collected *L. terrestris* from nearby and tagged them. It was seen that local, field-collected *L. terrestris* were more likely to settle and to adapt to environmental conditions which they were already used to than purchased individuals from an unknown origin. The authors concluded that a large proportion of purchased *L. terrestris* were unable or unwilling to settle or to create a new burrow, due to the high population density. For our study we used only purchased *L. terrestris* and found a similar pattern to Grigoropoulou and Butt

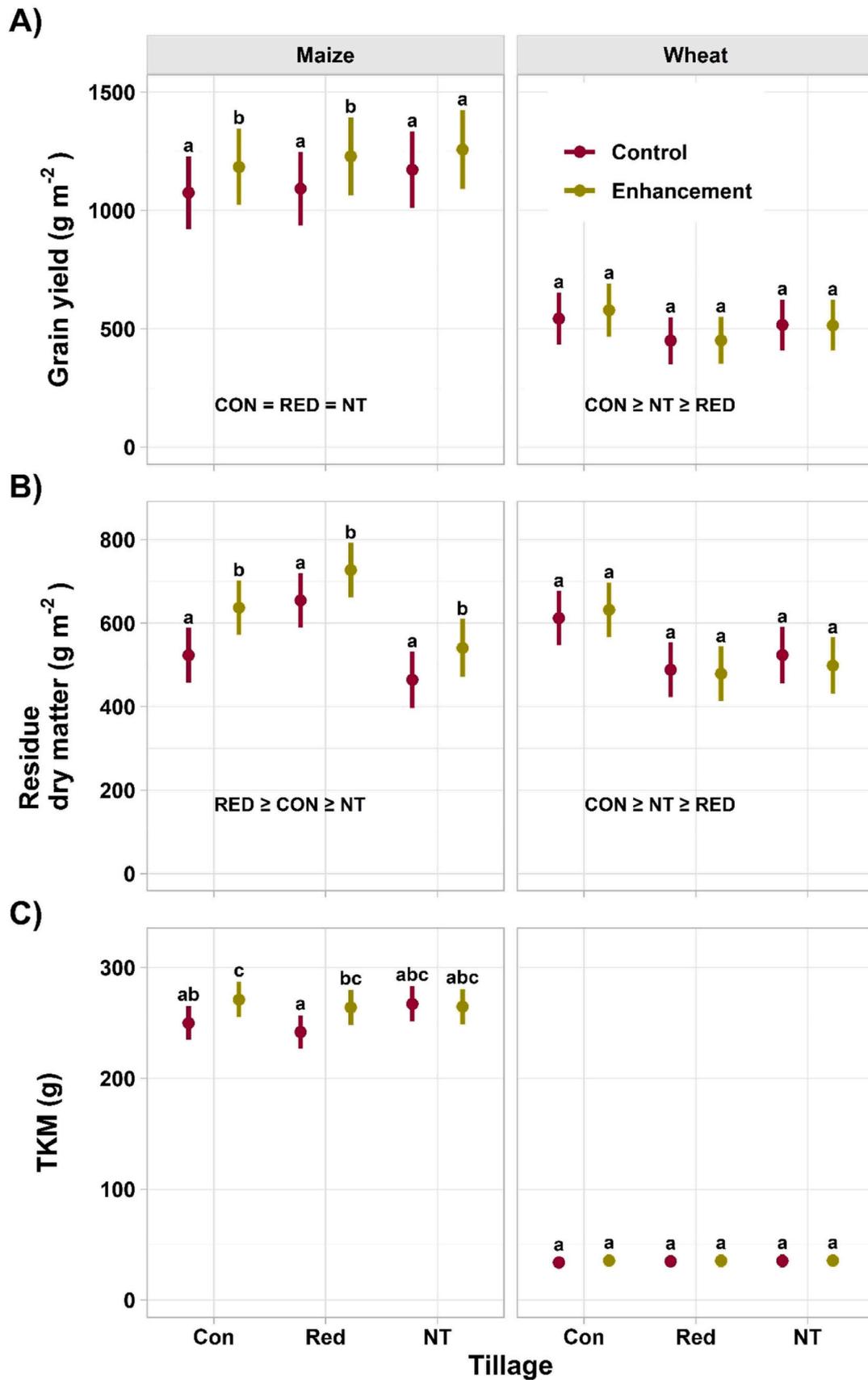


Fig. 3. Grain yield (A), residue dry matter (B) and thousand kernel mass (C; TKM) of maize and wheat for soil tillage systems conventional (Con), reduced tillage (Red) and no-till (NT) at two enhancement levels of *Lumbricus terrestris* (Control; Enhancement) at both study sites. Treatments having no letter in common are significantly different by pairwise comparison, for enhancement levels within crops, except for TKM where they differ for enhancement × tillage within crops (three-way linear mixed model, Tukey). Displayed are mean values and standard error.

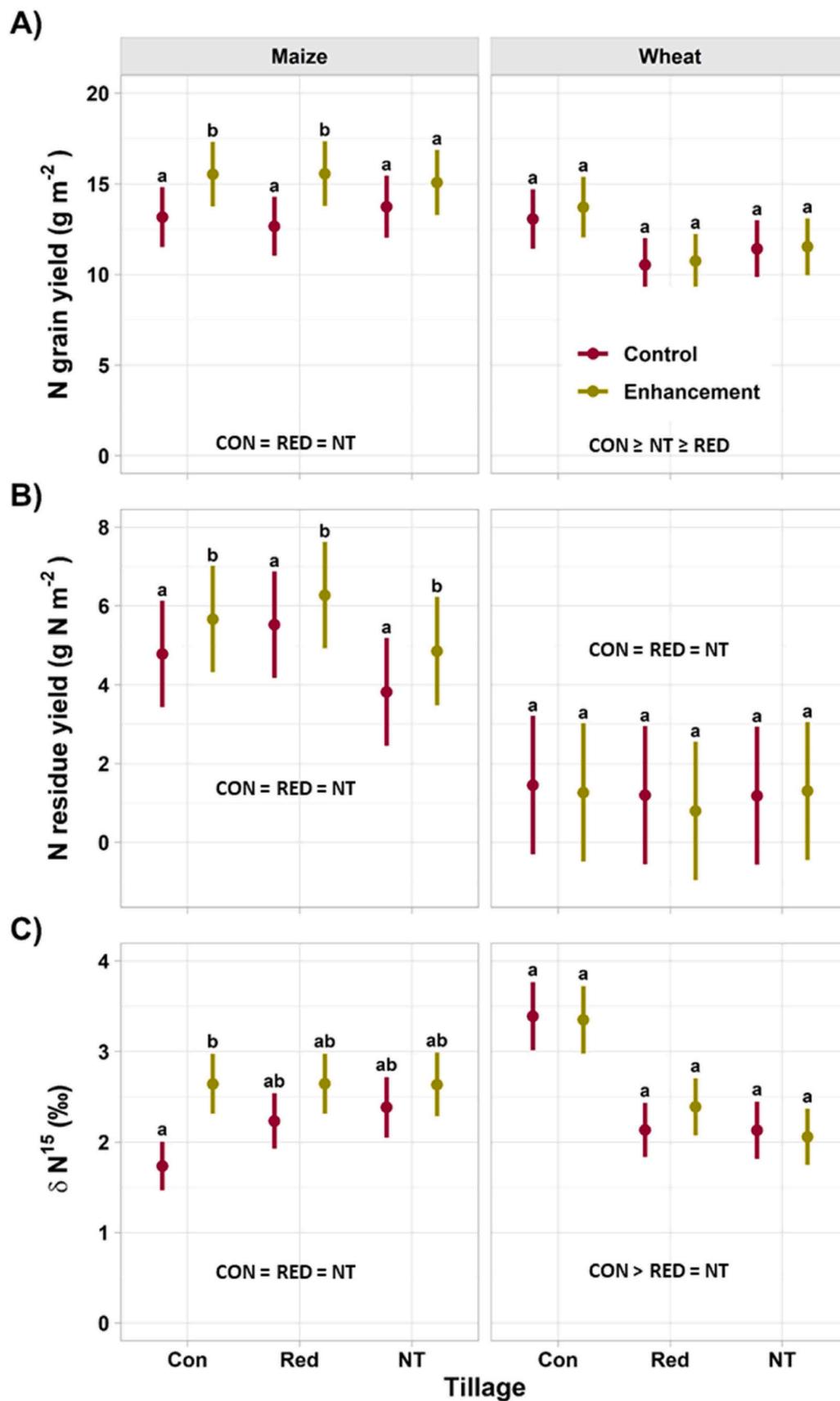


Fig. 4. Nitrogen (N) grain yield (A), N residues yield (B) and $\delta^{15}\text{N}$ (C) of maize and wheat for soil tillage systems conventional (Con), reduced tillage (Red) and no-till (NT) at two earthworm enhancement levels (Control; Enhancement) at both study sites. Treatments having no letter in common are significantly different by pairwise comparison, for enhancement levels within crops (three-way linear mixed model, Tukey). Displayed are mean values and standard error.

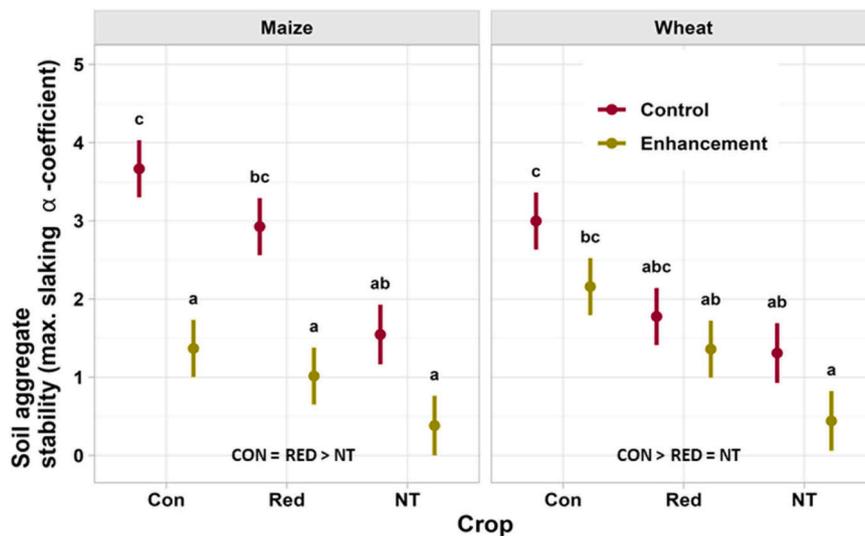


Fig. 5. Soil aggregate stability for maize and wheat for soil tillage systems conventional (Con), reduced tillage (Red) and no-till (NT) at two enhancement levels (Control; Enhancement) at both study sites. Treatments having no letter in common are significantly different by pairwise comparison, for enhancement levels and soil tillage systems (three-way linear mixed model, Tukey). Displayed are mean values and standard error. Note: a low max. slaking α -coefficient represents a high aggregate stability.

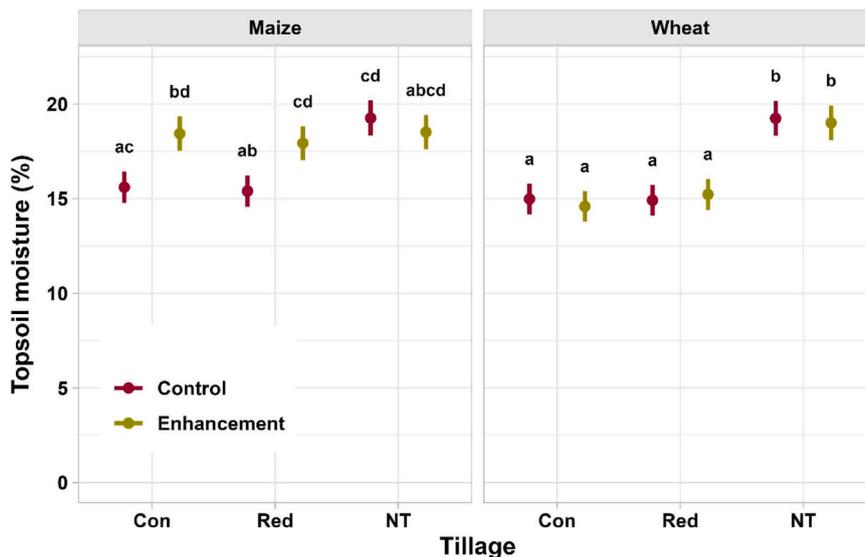


Fig. 6. Mean topsoil moisture over flowering of Maize (July 2020) and Wheat (May 2021) for soil tillage systems conventional (Con), reduced tillage (Red) and no-till (NT) at two enhancement levels of *Lumbricus terrestris* (Control; Enhancement) at site 2. Treatments having no letter in common are significantly different by pairwise comparison, for enhancement levels within crops (three-way linear mixed model, Tukey). Displayed are mean values and standard errors.

(2010), when 60–70 % of *L. terrestris* failed to settle. We had no traps installed and did not find any dead individuals the following days, so can only consider that some of the inoculated *L. terrestris* were preyed upon, even though we inoculated the earthworms shortly before sunset and protected them until it was dark. In addition, Shuster et al. (2003) observed that inoculation success of *L. terrestris* depended on soil tillage intensity and was higher for reduced tillage (ridge till) than more intensive chisel tillage, due to less soil disturbance and resource availability such as plant residues. This was not observed in our study, as we provided the same amount of straw in all plots. However, once settled, numbers of middens did not differ between maize in the first year and the following wheat crop, even when soil tillage was applied for reduced and conventional treatments between the harvest of maize and seeding of winter wheat in November. It is possible that *L. terrestris* retreated to deeper soil layers at the time of soil tillage application, due to colder temperatures and therefore survived soil tillage treatments and rebuilt

their middens by March (Nuutinen and Butt, 2009). Overall, midden abundance was highest for no-till followed by reduced and conventional tillage. These negative effects on earthworm abundance of soil tillage has been seen in many previous studies (Briones and Schmidt, 2017; Euteneuer and Butt, 2025; Simon et al., 2025). In their meta-analysis, Briones and Schmidt (2017) found that adult earthworms were slightly, but not significantly more sensitive to soil tillage intensity than immature earthworms and related this to their body size. We have not seen this effect in our study as adult *L. terrestris* were not affected by the soil tillage system, because soil tillage took place after the sampling. However, biomass of immature *L. terrestris* were higher for reduced tillage than no-till at both sites and site 2, respectively. An explanation might be that food accessibility was higher at reduced tillage compared to no-till as the amount of straw applied was similar between the treatments, but we cannot be certain as no-till already had a mulching layer from previous crops (wheat residues from 2019 and maize residues from 2020).

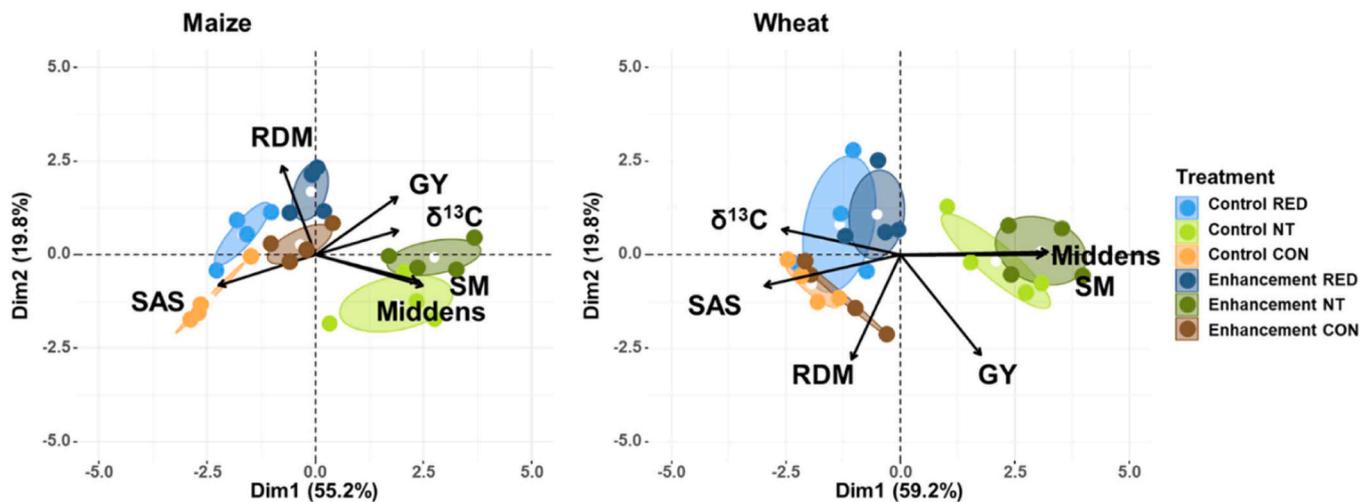


Fig. 7. Biplot of principal component analyses for maize and wheat at site 2 with vectors grain yield (GY), residue dry matter (RDM), $\delta^{13}\text{C}$, soil aggregate stability (SAS; max. slaking α -coefficient), topsoil moisture (SM) and number of middens (Middens) with treatments soil tillage systems conventional (Con), reduced (Red), no-till (NT) and enhancement level (control versus enhancement). First dimension of principal component (Dim1), second dimension of principal component (Dim2). Note: a high max. slaking α -coefficient represents a low aggregate stability.

In addition, immature individuals from reduced tillage could also have been older than immatures found for no-till.

4.2. Inoculation affected soil physical properties

Topsoil moisture was only measured at site 2 and enhancement treatments of maize for conventional and reduced tillage showed higher topsoil moisture than control, while no-till showed the highest topsoil moisture in control for both crops. In addition, soil aggregates were more stable in enhancement than control for conventional and reduced tillage for maize. These results can be partially explained by the findings of Hallam and Hodson (2020), Hodson et al. (2023) and Euteneuer et al. (2024). Euteneuer et al. (2024) reported, that water stable aggregates increased with number of earthworms for bulk soil in conventional and reduced tillage, while aggregate stability in a 5 cm radius of *L. terrestris* burrows (burrow-midden-complex) only increased for conventional. Similar was also stated by Schrader and Zhang (1997) and Spurgeon et al. (2013), who reported that aggregate stability of soils with weaker structure was increased mostly by earthworm activity. In addition, a laboratory study of Hallam and Hodson (2020) found that soil aggregate stability and water holding capacity were increased by endogeic earthworms such as *Allolobophora chlorotica* (Savigny, 1826). Earthworms actively assist in soil structure development through soil aggregate formation and macropore creation (Six et al., 2004; Elmholt et al., 2008; Pelosi et al., 2017) and this increases water infiltration and alters topsoil moisture content due to an increased hydrological effectiveness (Spurgeon et al., 2013; Andriuzzi et al., 2015; Lipiec et al., 2015; Schneider et al., 2018). In addition, Hodson et al. (2023) found that soil moisture was higher with earthworms than without for a watered treatment, but was similar within a drought treatment. This supports our findings that topsoil moisture was increased by enhancement for maize in conventional and reduced tillage. For wheat, the enhancement showed no effect during flowering, due to the dry weather conditions. Precipitation was below the long-term average and wheat was under water stress as seen from the results of $\delta^{13}\text{C}$. PCA showed that topsoil moisture had a large effect on grain yield of maize and wheat. While for wheat, the impact on grain yield was clearly related to low precipitation and expressed in low values of $\delta^{13}\text{C}$. For maize, $\delta^{13}\text{C}$ was correlated positively with grain yield, because it received higher precipitation than wheat. In addition, maize as a C_4 plant showed isotopic discrimination on water supply, which was opposite in direction to wheat as a C_3 plant. Sufficient water supply is essential for plant growth and N uptake

(Dercon et al., 2006; Wang et al., 2017; Monteleone et al., 2022), and our results (of grain yield, residue dry matter, TKM and N grain yield) for conventional and reduced tillage corresponded with topsoil moisture at flowering.

4.3. Earthworms affected plant growth and N yield of maize

Long-term temperature and precipitation at both sites are similar, but annual weather conditions were different. Maize at site 2 received 119 mm more rain than at site 1, while in spring wheat at site 1 and 2 received 38 mm and 33 mm less precipitation than the long-term averages, respectively. Annual weather conditions and selected crops (maize; wheat) affected grain yield immensely and justified our approach to use site as a random factor and to nest fixed factors soil tillage and enhancement level in crops to evaluate the overall effects of additional *L. terrestris* rather than environmental effects or crop species (Piepho et al., 2003, 2004; Euteneuer et al., 2022).

Maize grain yield in control was 2.7 t ha^{-1} higher than the average grain yield of Moitzi et al. (2021) at site 2, but only 0.6 t ha^{-1} higher than the highest grain yield between 2003 and 2016. Maize grain yield, TKM, grain yield ear^{-1} and residue dry matter were higher in enhancement for conventional and reduced tillage. These maize parameters can be affected by ear density (Csathó et al., 2025), but ear density was similar for both enhancement levels, so it is more likely that maize parameters were affected by water supply, due to higher topsoil moisture for enhancement than control throughout the growth period. According to Monteleone et al. (2022), maize has the highest vulnerability to water stress during flowering (85 %) and less than 40 % during yield formation for loamy soils. Therefore, the higher topsoil moisture could have increased the grain yield of maize, as topsoil moisture at flowering was higher in enhancement than control for conventional and reduced, but not for no-till. Wittwer et al. (2023) demonstrated that maize grain yield was reduced by drought by 34 %, but no difference between soil tillage systems was observed. Also Moitzi et al. (2021) found no differences between soil tillage treatments at our second study site over a six year comparison of maize grain yield. In our study, grain yield, N grain yield, residue dry matter and N residue yield were also not or minor affected by soil tillage systems, but were increase for enhancement compared to control for conventional and reduced tillage. However, while grain yield and N grain yield for no-till were not affected by enhancement, residue dry matter and N residue yield showed higher values for enhancement than control. We cannot exclude nitrogen

deficits at flowering for no-till (La Menza et al., 2025), because we have no data, but soil nitrate at harvest were highest for no-till. Similar was seen by Pecci Canisares et al. (2021) when no-till showed higher N content in soil and in aboveground biomass compared to plough. The authors concluded that higher N stock and N mineralisation (0–20 cm depth) after 48 years of no-till were responsible for the higher N aboveground yield. Pecci Canisares et al. (2021) related the lower mineralisation rate for plough to an immobilisation period, due to higher C:N ratio of incorporated plant residues. In our study, mineralisation processes induced by earthworms (van Groenigen et al., 2015; van Groenigen et al., 2019) could explain the higher residue dry matter and N grain yield for enhancement than control for maize and the overall similar results between the soil tillage treatments. In this case, conventional and reduced tillage at enhancement had the same level of mineralisation as no-till. In addition, N uptake of plants depends on water availability according to Dercon et al. (2006) and Wang et al. (2017). Both can explain the higher plant $\delta^{15}\text{N}$ for enhancement over control for conventional with maize. This is supported by previous field studies, when leaf N content increased, due to lower plant heat stress and *vice versa* by water stress (Rodriguez et al., 2006; Song et al., 2016; Cao et al., 2019). In addition, Blouin et al. (2005) found a higher chlorophyll content of rice in watered mesocosms with earthworms than without earthworms, while Hodson et al. (2023) also observed a higher chlorophyll content with earthworms than without, but only before a 15-day period of drought. After the drought period this effect was no longer detectable. Similar plant \times soil N \times soil moisture \times earthworm interactions were reported by Eriksen-Hamel and Whalen (2007), who found no effect for the second year of their field trial under dry weather conditions, but a higher N content and plant biomass with enhanced earthworm numbers for soybean in the first year. While van Groenigen et al. (2015) related such effects of earthworms on plant growth and N content to a higher availability of soil N, Hodson et al. (2023) were unable to show this correlation in a pot experiment, but concluded that enhanced earthworms increased plant growth, due to changes in the soil microbiota community rather than nutrient availability.

Overall, earthworms had the most impact on maize parameters for conventional and reduced tillage, but less in no-till. We can rule out any kind of fertilisation effect of dead earthworm, because according to Schmidt (1999) N content of earthworms is 10.1 % of their dry body mass. This would lead to a maximum of 0.06 g N individual⁻¹ by a mean fresh body mass of 4 g individual⁻¹ and an estimated dry body mass proportion of 15.4 % according to Petersen and Luxton (1982). In our study, 56 % of *L. terrestris* have failed to settle in no-till, 59 % in reduced and 67 % in conventional tillage. Assuming that these individuals died during the first study year, maize would have received an additional application of 0.488 g N m⁻² for no-till, 0.514 g N m⁻² for reduced and 0.584 g N m⁻² for conventional tillage, but the differences of 0.07 and 0.096 g N m⁻² between the treatments cannot explain the higher N yield for reduced and conventional for enhancement. Additionally, soil nitrate content in November after maize at site 2 was similar for enhancement and control. Earthworm cast is considered nutrient rich, but needs to be relatively fresh, due to leaching of water-soluble nutrients within 48 h or mineralisation of C and N within 3–30 days and after 45–90 days cast nutrient levels decrease towards bulk soil (Zangerlé et al., 2016). This, and the plant uptake of maize, can explain the similar soil nitrate content between enhancement and control in November in our study. However, it is estimated that earthworms can contribute up to 1.8–10.5 g N m⁻² year⁻¹ depending on their population size and community structure (Whalen and Parmelee, 2000; Abail and Whalen, 2021). Related to the community structure, Euteneuer et al. (2024) reported an increase of earthworm abundance below middens at site 2 in November in study year 1. The authors found that total earthworm abundance was 3-times higher in the burrow-midden-complex than in bulk soil with mainly endogeic species such as *Aporrectodea caliginosa*, *A. rosea* (Savigny, 1826) and *A. chlorotica*. In conclusion, total number of earthworms could have increased with numbers of middens. Therefore,

differences in N yield of maize through our earthworm enhancement are possible and could have contributed to plant available N as reported in van Groenigen et al. (2015) and/or through soil microorganisms according to Hodson et al. (2023).

4.4. Earthworm effects on wheat were impeded by dry weather conditions

Earthworms are most active during autumn and spring in temperate regions, when soil temperature is 5–25 °C and soil moisture is sufficient (optimum at 20–40 % or 25 %; Diop et al., 2023; Lowe and Butt, 2005). But at both sites, wheat had a reduced period of earthworm activity according to environmental conditions prone to earthworm activity (7–10 weeks), while this period was 15–18 weeks for maize, alongside increased topsoil moisture for earthworm enhancement during flowering and grain filling (conventional and reduced tillage). Topsoil moisture for wheat was not increased during flowering and not sufficient to affect wheat growth, plant $\delta^{13}\text{C}$ accumulation or N uptake. Koppensteiner et al. (2022) and Satorre and Slafer (1999) showed, that wheat yield is substantially affected by N supply and environmental conditions during certain development stages such as flowering and grain filling. However, wheat yield (4.4–5.2 t ha⁻¹) was within the range of average yield levels measured in the region (Neugschwandtner et al., 2015). Prior to our study, Neugschwandtner et al. (2015) investigated wheat production at site 2 and observed that in drier years with below average precipitation, no-till had higher grain yield than conventional, while in years with average or higher precipitation, conventional was increased compared to no-till. In our earthworm inoculation study, wheat received only 66 % (site 1) and 75 % (site 2) of the average precipitation from March to May. We saw no effect of soil tillage on grain yield similar to Wittwer et al. (2023) in a drought experiment. These authors showed yield losses between rain-fed and drought, but not between conventional and no-till. Thus, average differences in topsoil moisture (16–24 %) did not affect grain yield of wheat. This was also seen by Waibel et al. (2024), who also found no differences in grain yield among the soil tillage treatments, but a higher aboveground biomass and grain N content for plough than reduced tillage and no-till. Waibel et al. (Waibel et al., 2024) related this to drought conditions during spring (April–June) and higher growth performance due to root characteristics, such as increased root area, root volume and number of root tips under ploughing than no-till. In addition, a higher proportion of fine roots increased water and N uptake for ploughing than no-till, due to early growth promotion through lower soil penetration resistance for ploughing. However, due to low precipitation, earthworm activity and cast production could have been reduced accordingly, as 16 % of soil moisture is already at the lower range for earthworm activity (Whalen et al., 2004; Lowe and Butt, 2005; Diop et al., 2023). In consequence, nutrient mobilisation through the action of earthworms and earthworm enhanced N uptake of plants could have also been restricted, due to insufficient supply of water as results of plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ suggested.

Overall, our field trial showed that increased earthworm (*L. terrestris*) numbers can provide major contributions to soil health and plant production, when soil structure is improved by earthworms as in enhancement conventional and reduced soil tillage systems. Simon et al. (2025) showed that soil structure and soil health parameters such as soil aggregate stability, bulk soil density, dissolved organic carbon and the earthworm communities are impaired for conventional and reduced tillage at site 2. Our hypothesis that in plots with enhanced numbers of *L. terrestris*, earthworm would mediate soil structure improvements and promote soil fertility can be confirmed for conventional and reduced tillage, but not for no-till, which can conserve physical and biological soil quality (Sae-Tun et al., 2022). It was seen that even topsoil moisture can be increased over weeks by the enhancement of *L. terrestris* and this has positively affected crop production and quality of maize, but only when precipitation was sufficient.

These beneficial effects of *L. terrestris* suggest the possibility to facilitate the transfer from conventional farming to no-till farming, to

shorten the time of conversion and to mitigate any negative effects during conversion such as soil drainage, yield reduction or financial risks (Soane et al., 2012; Heller et al., 2024). Once numbers of *L. terrestris* were enhanced, positive effects were seen within six months. In this context further experiments could focus on how to rapidly increase earthworm numbers and especially anecic, epi-anecic and endogeic earthworms which sustainably increase water infiltration, water holding capacity and aggregate stability (Andriuzzi et al., 2015; Lipiec et al., 2015; Hallam and Hodson, 2020). Management practices such as cover cropping and no-till systems can support earthworm communities (Euteneuer et al., 2020; Simon et al., 2025), but may be slow to take effect (Nuutinen et al., 2017). However, there is a need to assist farmers with a more rapid transition to no-till farming and earthworm can certainly support this.

5. Conclusion on ecosystem services of *L. terrestris*

The aim of this study was not to justify earthworm inoculation as this might be considered unsustainable, but rather to test mechanisms of interaction between agricultural strategies such as soil tillage and ecosystem services provided by *L. terrestris* under field conditions. We demonstrated that *L. terrestris* can relatively rapidly contribute to increased topsoil moisture, soil aggregate stability, N uptake by plants and plant biomass production. Overall, topsoil moisture was increased for enhancement over control, and it was confirmed that earthworms depend on precipitation and sufficient soil moisture to show their full capacity of ecosystem service provision. In drier years, as under wheat, the number of earthworms had no effect on plant production, but under moister conditions and especially under conventional and reduced soil tillage, higher earthworm numbers increased maize yield, plant N uptake and improved soil structure. Our findings serve as a baseline for any interdisciplinary investigations in drier areas, to support farmers in climate-adapted agriculture, and show the contribution that earthworm can make to soil fertility.

CRedit authorship contribution statement

P. Euteneuer: Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **H. Wagentristsl:** Resources, Writing – original draft. **R. Hood-Nowotny:** Investigation, Writing – original draft. **R.W. Neuschwandtner:** Investigation, Writing – original draft. **G. Bodner:** Investigation, Writing – original draft. **A. Hofer:** Investigation. **M. Windisch:** Investigation. **E. Weiler:** Investigation. **S. Widy:** Investigation. **K.R. Butt:** Conceptualization, Methodology, Supervision, Writing – original draft.

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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.still.2026.107087.

Data availability

Data will be made available on request.

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