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Research paper

Impact of tillage practices and soil texture on soil health and earthworms in the Pannonian region: A comparative study from Austria and Hungary

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A B S T R A C T

Soil tillage has major impacts on physical, chemical and biological soil parameters. Two long-term soil tillage experiments in North-East Austria (AT) and Central-North Hungary (HU), both located in the Pannonian region, were studied in 2020 and in 2021. The three soil tillage systems comprised mouldboard ploughing (P), shallow cultivation (C), and no-till (NT) in a completely randomised block design with four blocks. Major differences between AT and HU concerned clay content and pH value (clay: 20 %; 36 % and pH: 7.7; 4.5, respectively). This affected most parameters such as dissolved organic carbon, soil aggregate stability, bulk density, earthworm abundance, and biomass and Shannon index. These parameters decreased with soil tillage intensification at both sites. In addition, for all mentioned parameters, C and P were similar in AT, while in HU this was the case for NT and C. Additionally, epi-anecic *Lumbricus terrestris* was only found in AT, while endogeic *Aporrectodea georgii* was only present in HU. Arbuscular mycorrhizal root colonization was not responsive to different tillage practices when sampled in August in AT. In conclusion, reduced soil tillage such as C and NT can show similar affects towards soil health, but site-specific properties such as soil texture need to be considered for a final evaluation.

1. Introduction

Soil tillage is an important management practice in agriculture with a high impact on the soil physical (Dekemati et al., 2019; Euteneuer et al., 2024), biological (Dekemati et al., 2020, 2021; Euteneuer and Butt, 2025), and chemical properties (Neugschwandtner et al., 2020; Weidhuner et al., 2021). Soil tillage is practised for the incorporation of plant residues, seedbed preparation, weed and pest control (Gajri et al., 2002; Mairhofer et al., 2019), but there are several disadvantages of soil tillage depending on the management practices. Ploughing (P) can cause intensive disturbances by inverting the top 30 cm of soil (Obour et al., 2017), and the resulting bare soil surface (Jug et al., 2019) is exposed to wind erosion, decreased soil aggregate stability (SAS) (Klik and Rosner, 2020), and loss of soil biodiversity (Briones and Schmidt, 2017). As opposed to ploughing, conservation agriculture is based on complete omission (no-till) or reduced tillage practices (Busari et al., 2015). No-till (NT) soils are more resilient to abiotic impacts and usually have higher water retention (Liebhard et al., 2022), soil carbon storage

Abbreviations: AMF, arbuscular mycorrhizal fungi; AT, Austria; BD, bulk density; DOC, dissolved organic carbon; EEG, easily extractable glomalin; HU, Hungary; NMDS, Non–metric multidimensional scaling; NT, no–till; P, ploughing; PCA, Principal component analysis; SAS, soil aggregate stability; C, shallow cultivation; SOC, soil organic carbon; SPR, soil penetration resistance.

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(Rosinger et al., 2022; Sae-Tun et al., 2022), more stable soil aggregates (Sae-Tun et al., 2022) and earthworm abundance (Dekemati et al., 2019; Euteneuer et al., 2024). Moreover, shallow cultivation (C) represents a form of conservation agriculture and is considered to have an intermediate impact on soil physics and biodiversity compared to NT and P (Klik and Rosner, 2020; Liebhard et al., 2022; Sae-Tun et al., 2022).

Soil organisms, such as earthworms and arbuscular mycorrhiza fungi (AMF) are also affected by soil tillage (Jansa et al., 2002; Säle et al., 2015; Briones and Schmidt, 2017; Rosner et al., 2018). Earthworms and AMF are important for agricultural soil functionality, especially in the development of soil structure (Jongmans et al., 2003), soil porosity (Pérès et al., 2010), and changes in soil organic carbon (SOC) (Sae-Tun et al., 2022). AMF contribute to soil carbon sequestration and soil aggregation via the release of a soil glycoprotein initially termed 'glomalin' during AMF hyphae turnover (Wright and Upadhyaya, 1998; Rillig et al., 2001; Driver et al., 2005; Thomopoulos et al., 2023). Glomalin or glomalin-related soil protein (Irving et al., 2021) contains >85 % sugars and thus provides a strong connection between mineral and organic soil particles (Gunina and Kuzyakov, 2015). Furthermore, it enhances carbon sequestration due to its recalcitrant property (Singh et al., 2022). In addition, dissolved organic carbon (DOC) comprises photosynthetically-derived carbon such as mucilage and exudates of organic compounds (Kalbitz et al., 2000) and can be metabolized by soil bacteria and fungi into more complex carbon compounds and lead to higher soil aggregate stabilization (SAS) (van Groenigen et al., 2010; Sokol et al., 2019; Sae-Tun et al., 2022). In addition, earthworms also have an important role in SAS through casting and burrowing (Lehmann et al., 2017; Euteneuer et al., 2024). Hence, SAS governs ecosystem services such as SOC sequestration (Six et al., 2000, 2004; Klik and Rosner, 2020), prevents soil erosion (Klik and Rosner, 2020), allows gas and water fluxes (Amézketa, 1999; Finn et al., 2017) and thus supports soil fertility (Blanchart et al., 2009; Arai et al., 2018). High-intensity soil tillage affects SAS negatively, while soil biological activity has a positive effect (Lehmann et al., 2017; Sae-Tun et al., 2022).

The current study aimed to investigate the effects of tillage (NT; C; P) on parameters under similar climatic conditions with calcaric or endocalcic Chernozem soil with respect to i) physical (soil penetration resistance; bulk density; SAS); ii) chemical (pH, DOC, glomalin), and iii) biological properties (earthworm abundance, biomass, community composition; AMF root colonization rates) in two long-term tillage experiments in Austria and Hungary. It was hypothesized that the effects of soil tillage intensity on soil properties and biological communities would vary between Chernozems form Austria and Hungary mostly due to significant differences in soil pH and texture. Specifically, we hypothesized that regional differences in soil properties would interact with tillage practices to produce distinctive impacts on soil health, with these effects being more pronounced in clay-rich, acidic soils in Hungary compared to the calcareous soils in Austria. The interaction between tillage practices and site-specific conditions (e.g., soil pH and texture) is expected to influence soil physical properties, nutrient availability, and biological parameters differently across the Pannonian region.

2. Materials and methods

2.1. Study sites and years

The experiment in Austria (AT) was instigated in 1996 at the Experimental farm of the University of Natural Resources and Life Sciences, Vienna, in Raasdorf (48°14′N, 16°33′E; 153 m a.s.l.). The experimental field site is located to the east of Vienna (Lower Austria) on the edge of the Marchfeld plain in the north-western part of the Pannonian Basin. The silt loam soil is classified as a calcaric Chernozem of alluvial origin (WRB, 2014). The experiment is conducted as a complete random block design with four replications of plot size 24 × 40 m and different tillage systems, P (30 cm depth), C (10 cm depth) and NT (0 cm). The cropping sequence in AT from 2018 to 2021 was sorghum (*Sorghum bicolor* Mönch), winter wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) (study year 2020), winter wheat (study year 2021).

The experiment in Hungary (HU) is located at the Józsefmajor Experimental and Training farm of the Hungarian University of Agricultural and Life Sciences (47°41′N, 19°36′E; 110 m a.s.l.), in Pest County, Central Hungary. The long-term tillage experiment was set up in 2002 with three tillage systems, P (30 cm depth), C (18 cm depth) and NT (0 cm). The clay loam soil is classified as an endocalcic Chernozem (WRB, 2014). The experiment is arranged in a complete random block design with four replicates and plot size of 13 × 180 m. The cropping sequence between 2018 and 2021 in HU was soybean (*Glycine* max Merr.), winter wheat, winter oat (*Avena sativa* L.) (study year 2020), sunflower (*Helianthus annuus* L.) (study year 2021).

Both sites had a similar continental climate Dfb (1961–1990) in the Köppen-Geiger climate classification (Kottek et al., 2006) with a shift to Csa (1991–2020) for AT and Dfa for HU and an upcoming transition for HU to Csa (2041–2071; Beck et al., 2023). The long-term mean annual temperature for AT is 11.2 °C and 10.3 °C for HU, and the long-term annual precipitation is 560 mm for both countries. For details of soil properties, weather data and time of sampling, see Table 1.

2.2. Soil chemical analyses

Soil samples for SOC measurements in HU were taken from depths of 0–10 and 25–30 cm, from four random locations within each plot in autumn 2020. The SOC content of the HU samples was determined by wet oxidation using the Walkley (1947) method. SOC data and soil texture of AT were taken from Liebhard et al. (2022) derived from 2020 in the same plots as the current study. SOC was sampled at 0–10 and 0–30 cm and analysed according to Austrian Standards with dry combustion (ÖNORM L 1080, 1999) and hence, results are not directly comparable with SOC from HU. Therefore, parameters were not statistically analysed but are provided to complete site soil parameters.

For pH_{CaCl_2} measurement, the 0.01 mol L^{-1} $CaCl_2$ extracts were performed in the ratio 1:10 w v^{-1} (4 g of fresh soil (≤ 5 mm) 40 ml^{-1} of extract) (Houba et al., 2000). After 2 h of shaking, the pH value was measured. The suspension was subsequently centrifuged at 5000g for 5 min. The content of DOC was measured using SKALAR SAN^{PLUS} SYSTEM (Netherlands) and was recalculated on soil dry mass obtained from mass difference after 105 °C for 24 h. Glomalin-related soil protein or easily extractable glomalin (EEG) was extracted after Wright and Upadhyaya

Table 1
Site parameters of soil tillage trials in Austria and Hungary with treatments no-till (NT), cultivator (C) and plough (P). Current precipitation and average temperature during growth period in 2020 and 2021, soil organic carbon (SOC) and sampling times of physical soil parameters such as soil penetration resistance (SPR), bulk density (BD), soil aggregate stability (SAS); soil chemical parameters such as pH, dissolved organic carbon (DOC), easy extractable glomalin (EEG) and soil biological parameters such as earthworm sampling and plant roots colonised by arbuscular mycorrhiza fungi (AMF). Note that SOC in AT is dry combustion and in HU it is based on wet oxidation.

	Austria		Hungary	
Parameter	2020	2021	2020	2021
Sand/clay/silt (%)	27/20/53		37/36/27	
SOC (10 cm) NT/C/P	2.7/2.5/2.3		2.3/2.1/1.8	
SOC (30 cm) NT/C/P	2.5/2.4/2.3		1.7/1.9/1.7	
Current precipitation (mm)	496	268	359	302
SPR	May	May	Mar	Mar
BD	May	May	Mar	Mar
SAS	Nov	May	Mar	May
pH_{CaCl_2}	Nov	Jul	Sep	Jul
EEG	Nov	—	—	Sep
DOC	Nov	—	—	Sep
SOC	Jun	—	Sep	—
Earthworm	May & Oct	Mar	Mar & Sep	Mar & Nov
AMF	Aug	—	—	—

(1998) with 20 mmol L^{-1} sodium citrate at pH_{CaCl_2} 7.0 for 30 min at 121 °C. The air-dried soil (≤ 2 mm) $solution^{-1}$ ratio was 1:8. After extraction, samples were centrifuged at 5000*g* for 5 min. The EEG content was measured colorimetrically using an extract of dye brilliant blue reagent and Bovine serum albumin as a standard (Protein assay kit II, Bio-Rad, USA). For the analysis, 10 μl of the extract was dispensed onto a microplate, followed by the addition of 200 μl of dye reagent (diluted at a 1:4 ratio). Subsequently, the microplate was horizontally shaken at 593 rpm for 30 s and then measured after a 5-minute stabilization period at a wavelength of 590 nm (Spark, Tecan Ltd., Zurich, Switzerland).

2.3. Soil physical analyses

SAS was measured with the smartphone application MOULDER Version 2.0 (formerly: SLAKES) (Fajardo et al., 2016; Fajardo and McBratney, 2019) and according to the analytical protocol established by Flynn et al. (2020) and utilising an iPhone 8 (Apple Inc. Cupertino, California, USA) in AT and Huawei P30 lite MAR-LX 1A (Huawei Technologies Co., Ltd., Bantian, Longgang, Shenzhen, China) in HU. In summary, air-dried soil samples were sieved to a particle size range of 2–5 mm, with three soil aggregates per sample and five subsamples per plot analysed (Euteneuer et al., 2024). The initial step in the protocol involves capturing a reference photo of the dry soil aggregates positioned in a dry Petri dish against a high-contrast background. Subsequently, the aggregates are transferred to a Petri dish containing deionized water, ensuring that they are placed in the same orientation as in the reference image. The application then measures the expansion of the dispersed soil area, starting from the reference image and determining the final area after a 10-minute interval. Upon completion of the ten-minute period, the α-coefficient is displayed on the screen. The α-coefficient represents the maximum predicted dispersion of a soil aggregate and is derived from the Slaking index, which is fitted to the Gompertz function (Gompertz, 1833). For comprehensive details regarding the α-coefficient, the Slaking index, and the methodology, refer to Fajardo et al. (2016). A lower α-coefficient indicates more stable soil, with zero being the most stable value. For comparison of MOULDER to more established methods, such as Cornell Rainfall Simulator (Moebius-Clune et al., 2016), wet sieve procedure (Kemper and Rosenau, 1986; Nimmo and Perkins, 2002) or water stable aggregate mean weight diameter (Franzluebbers et al., 2000) related to earthworm processed soil or soil health, refer to Euteneuer et al. (2024) and Rieke et al. (2022).

Soil bulk density (BD) samples were taken in the topsoil (0–5 cm) with an undisturbed soil sampler, using 100 cm^3 rings in four subplots. Soil penetration resistance (SPR) was measured at both sites by an electronic penetrometer (Penetrologger, Royal Eijkelkamp, Giesbeck, Netherlands). The measurements were carried out at 20 random points $plot^{-1}$. SPR values were measured by 1 cm, 1 N accuracy, between 0 and 40 cm, with a penetration speed of 2 cm s^{-1}, with a 1 cm^2 cone.

2.4. Soil biological analyses

Earthworms were sampled by hand-sorting 25 × 25 × 30 cm soil blocks, in four randomly chosen places of each treatment at both sites (ISO 23611-1, 2018). Parameters measured were earthworm abundance (individuals m^{-2}), earthworm biomass (g m^{-2}), juvenile:adult ratio, juvenile:adult biomass ratio, average biomass of endogeic adult earthworm (g $individual^{-1}$) and species composition according to Christian and Zicsi (1999) and Csuzdi and Zicsi (2003) for AT and HU, respectively. In addition to hand-sorting, a common practice is the use of a vermifuge, such as a mustard suspension (5 g L^{-1} mustard powder), allyl isothiocyanate or formaldehyde (formalin) to expel earthworms from soil, particularly deep burrowing earthworms such as *Lumbricus terrestris* (Linnaeus, 1758) (Butt and Grigoropoulou, 2010). The disadvantage of a vermifuge for silty soil such as in AT is the increased infiltration rate (1–2 h; Euteneuer unpublished). Silty Chernozem soil is highly sensitive to soil tillage, which can block infiltration of any liquid (Weninger et al., 2019) and was therefore not applicable. As an alternative for an expellant, we counted middens of *L. terrestris.* To determine the middens, wheat straw (178 g m^{-2}) was added onto an area of 7 m^2 in May and June 2020 to allow *L. terrestris* to build middens (Euteneuer et al., 2024). These middens were then counted in November 2020. Normally, there is one *L. terrestris* burrow under each midden, but sometimes a burrow can have two openings to the soil surface, which can appear as two individual middens and could lead to an overestimation of *L. terrestris* abundance (Butt and Grigoropoulou, 2010; Grigoropoulou and Butt, 2010). To verify our approach, we checked ten randomly selected middens $plot^{-1}$ for occupancy using mustard suspension (Stroud et al., 2016). Earthworms were taken to the laboratory and biomass were recorded (g $individual^{-1}$), but not added to the earthworms from hand-sorting. The proportion of *L. terrestris* (total; adult; juvenile) expelled by mustard suspension from the middens (occupancy rate), was calculated by number of middens occupied divided by ten middens $plot^{-1}$. Subsequently, recorded middens m^{-2} were corrected by total occupancy rate of middens.

For AMF assessment, 5 randomly chosen plants $plot^{-1}$ were sampled in late August 2020, but only at the AT site due to COVID-19 travelling restrictions. Roots were stained according to the method of Vierheilig et al. (1998). Briefly, maize roots were cleared in 10 % potassium hydroxide (KOH) for 6 min at 90 °C and then roots were stained in 5 % ink-vinegar solution for 6 min at 90 °C. The stained roots were then stored in 30 % ethanol at 4 °C. For each sample, 30 root pieces were mounted on a microscope slide and AMF colonization was determined according to the modified method of Trouvelot et al. (1986) using the INOQ calculator Advanced (Mercy, 2017). Parameters measured were mycorrhizal frequency (F %), mycorrhizal intensity (M %), arbuscule abundance (A %), vesicle abundance (V %) and hyphal abundance (H %). Details of sampling time at both sites are provided in Table 1.

2.5. Statistical analyses

Two-way linear mixed model (2-way LMM) with the fixed effect site (2 levels; AT, HU) and tillage (3 levels; P, C, NT) were used to analyse parameters BD, SAS, SPR, pH, DOC, EEG, earthworm abundance, earthworm biomass, juvenile:adult ratio, juvenile:adult biomass ratio, average biomass of endogeic adult earthworms and Shannon index. Shannon index was obtained from package '*vegan*' (Oksanen et al., 2020) function 'diversity'. Middens were only found in AT and parameters occupancy rate, numbers of middens, proportion of adult and immature *L. terrestris* and their biomasses were analysed with fixed effect tillage and random effect replicate using a one-way LMM (1-way LMM).

For 2-way LMM replicates, years (2 levels; 2020, 2021), sites and sampling dates (according to Table 1) were set random. All LMMs applied function 'lmer' ('*lme4*' package; Bates, 2015) in RStudio 6.1.524 (Posit team, 2023) using R 4.3.1 (R Core Team, 2023) with compound symmetry as a variance-covariance structure for repeated measurements and fitted for residual maximum likelihood (REML) method was used. Compound symmetry was selected as both long-term trials have existed for >20 years and variance in data are considered as momentary effects due to weather conditions as soil tillage systems are believed to have reached some form of stabilization after two decades. Function 'Anova' was applied for the analyses of variance with Wald-type *F*-tests and the Satterthwaite's method for denominator degrees of freedom and type III hypotheses. Tukey post-hoc test with function 'emmeans' (package '*emmeans*'; Lenth, 2022) was applied in multiple mean comparisons ($P < 0.05$) for factor combinations. All data provided are mean values and standard deviation (mean ± SD). Residual distributions were checked visually by frequency of residuals and homogeneity of the variance by residuals against fitted values per model. Parameters that did not meet these assumptions, such as EEG, average biomass of earthworm, occupancy rate, number of middens, proportion and biomass of adult and immature *L. terrestris*, were square root transformed. Total earthworm biomass, juvenile:adult ratio, juvenile:adult biomass ratio were log transformed.

Non-metric multidimensional scaling (NMDS) was applied for ordination of rank orders for soil parameters (Table 1) and mean earthworm species abundance for both sites (Paliy and Shankar, 2016). For NMDS, package '*vegan*' and function 'metaMDS' with Bray-Curtis distances was used and was solved with k = 2 and a stress score of 0.141 after an interaction of 20 tries (Kenkel and Orloci, 1986; Clarke, 1993). Plotting of NMDS was done with package '*ggplot2*' (Wickham, 2016) and 'score' function to extract the results of vector fitting by function 'envfit' (package '*vegan*') with scaling 'species' for earthworm species and 'site' for soil and site parameters.

A Principal Component Analysis (PCA) with AT data was performed on the soil parameters, earthworm data from October 2020 and AMF data from August 2020 to depict the interplay and the contribution of the selected variables. For PCA analysis and visualization the packages '*FactoMineR*' (Le et al., 2008) and '*factoextra*' (Kassambara and Mundt, 2020) were used.

3. Results

3.1. Soil chemistry and physics

The results of the 2-way LMM showed interaction of site × soil tillage for pH (Table 2), with a higher pH in AT than HU, and within HU highest for P, whereas pH did not differ between tillage treatments in AT (Fig. 1A). The EEG was only affected by site and had a 25 % lower concentration in AT than HU (Fig. 1B). In addition, DOC differed between sites and was 2.7 times higher in AT than HU and decreased with soil tillage intensity at both sites (AT: NT > C = P > HU: NT = C > P) (Fig. 1C). Soil physical parameters SAS, BD and SPR showed an interaction of site × soil tillage (Table 2). Aggregate stability was 2.25 times greater for NT in AT than C and P, whereas in HU C ≥ NT were 1.7 times more stable than P (Fig. 1D). Bulk density followed a similar pattern and decreased with soil tillage intensity and was 1.2 times higher in HU than AT (Fig. 1E). Two-way LMM of SPR showed that resistance across depths was lowest in NT and P in HU, followed by P ≤ C in AT and ≤ C in HU and ≤ NT in AT (Fig. 1F).

Table 2
ANOVA results of chemical, physical and biological soil parameters (2-way LMM) with fixed factors site (S; Austria, Hungary) and tillage (T; no-till, cultivation, plough). Easily extractable glomalin (EEG), dissolved organic carbon (DOC), soil aggregate stability (SAS), bulk density (BD), soil penetration resistance (SPR) and earthworm parameters. Degrees of freedom: S = 1, *T* = 2, S × T = 2, *N* = 4.

	F-value			*P*-value		
Parameter	S	T	S × T	S	T	S × T
pH_{CaCl_2}	3544	1.61	13.06	<0.001	0.218	<0.001
EEG	59.9	3.24	0.423	<0.001	0.054	0.66
DOC	267	31.2	5.08	<0.001	<0.001	0.013
SAS	0.053	17.9	5.12	0.819	<0.001	0.013
BD	159	18.7	9.89	<0.001	<0.001	<0.001
SPR	0.694	5.79	15.0	0.406	0.004	<0.001
Earthworm abundance	4.22	16.7	8.51	0.046	<0.001	<0.001
Earthworm biomass	14.6	7.64	30.5	0.001	<0.001	<0.001
Adult biomass	2.66	0.22	9.38	0.108	0.801	<0.001
Juvenile:adult ratio	3.57	9.05	6.04	0.065	<0.001	0.004
Juvenile:adult biomass ratio	17.3	6.25	3.53	<0.001	0.004	0.036
Shannon diversity	4.38	3.48	5.05	0.043	0.045	0.013

3.2. Soil biology and biodiversity

All earthworm parameters were affected by site × soil tillage, except for adult biomass which was only affected by site (Table 2). In detail, earthworm abundance and biomass were 1.6–1.9 or 4–6 times higher for NT in AT or HU, respectively, and decreased with soil tillage intensity and was lowest in P in HU, while in AT, P was similar to C (Fig. 2A, B). Overall, NT in AT showed the greatest juvenile:adult ratio compared to the remaining treatments (Fig. 2C), but the juvenile:adult biomass ratio for C, P and NT in HU showed similar results to NT in AT (Fig. 2D). In addition, average biomass of an adult earthworm was similar within sites but higher for C in AT than P in HU (Fig. 2F). The Shannon diversity index was highest in NT and C in HU and NT in AT, followed by C and P in AT and lowest in P in HU (Fig. 2E). The earthworm community at the two sites consisted mainly of endogeic earthworms dominated by *Aporrectodea caliginosa* (Savigny, 1826), *A. rosea* (Savigny, 1826), plus *Allolobophora chlorotica* (Savigny, 1826). Differences were found, as *Aporrectodea georgii* (Michaelsen, 1890) was present in HU, but absent in AT, while *L. terrestris* was found in AT, but not in HU (Fig. 3). In addition, the presence of *L. terrestris* at both sites were also assessed by counting middens and corrected by the occupancy rate of middens. No middens were found in HU and the number of middens in AT were 10–12 times higher in NT than C and P (Table 3). Similar was seen with the occupancy rate, which was 30–40 % higher in NT than C and P (Table 3). In addition, the proportion of adults did not differ between the tillage system, but smaller and more immature *L. terrestris* were expelled in NT than C, while biomass of adult was similar between the tillage systems (Table 3). The presence and absence of the earthworm species mainly affected the outcome of the NMDS (Fig. 4A). Thus, a clear separation of soil tillage systems P and NT and sites was observed by vectors EEG (R^2 = 0.467), SAS (R^2 = 0.294), BD (R^2 = 0.539), clay and sand content (R^2 = 0.459) at the HU site (Fig. 4B).

The AMF parameters F % ($F_{1,2}$ = 0.375, P = 0.690), M % ($F_{1,2}$ = 1.066, P = 0.354) and A % ($F_{1,2}$ = 2.312, P = 0.113) were not affected by soil management. AMF frequency reached between 98 and 99 % (Table 4). AMF intensities ranged between 29 and 31 %. Arbuscule abundance was in the range of 26 to 29 %. Vesicle abundance ($F_{1,2}$ = 5.378, P < 0.01) and hyphal abundance ($F_{1,2}$ = 7.036, P < 0.01) were significantly affected by soil management. Vesicle abundance was lowest in NT (5 %) and highest in the C treatment (9 %). For hyphal abundance, the lowest values (5 %) were seen in the C treatment and highest (9 %) in the NT treatment.

Results of the PCA that depict the interplay between AMF root colonization parameters, earthworm parameters and soil properties are provided in Fig. 5. Parameters identified in a first PCA (data not shown) as less important, such as DOC, soil penetration resistance from 0 to 10 cm, mean soil penetration resistance from 0 to 40 cm and soil pH were excluded from the data analysis. The PCA identified 3 principal components explaining 68.9 % of the total variance. Juvenile earthworm mass (0.920), juvenile earthworm abundance (0.825) followed by the soil parameters bulk density (0.734) and soil organic carbon 0–10 cm (0.637) correlated to a great extent to PC1 explaining 30.2 % of the variance (Fig. 5). Furthermore, soil aggregate stability was negatively correlated (−0.639) with PC1. The second PC explained 22.8 % of the variance. The most highly correlated variables were mycorrhizal intensity (M %) (0.874) and arbuscule abundance (A %) (0.822). Additional variables correlated with PC2 were SOC 0–30 cm (0.634) and vesicle abundance (V %) (0.592). The third PC explained 16.0 % of the variance and was excluded from the PCA plot to increase readability. Adult earthworm biomass (0.766) and adult earthworm abundance (0.739) correlated positively with PC3 and hyphal abundance (−0.583), and glomalin (−0.613) correlated negatively with PC3.

Additionally, individual data points of the respective treatments were included in the PCA biplot (Fig. 5). C and P samples were completely separated from the NT samples. This separation mainly depended on parameters such as BD, SAS and SOC and earthworms and

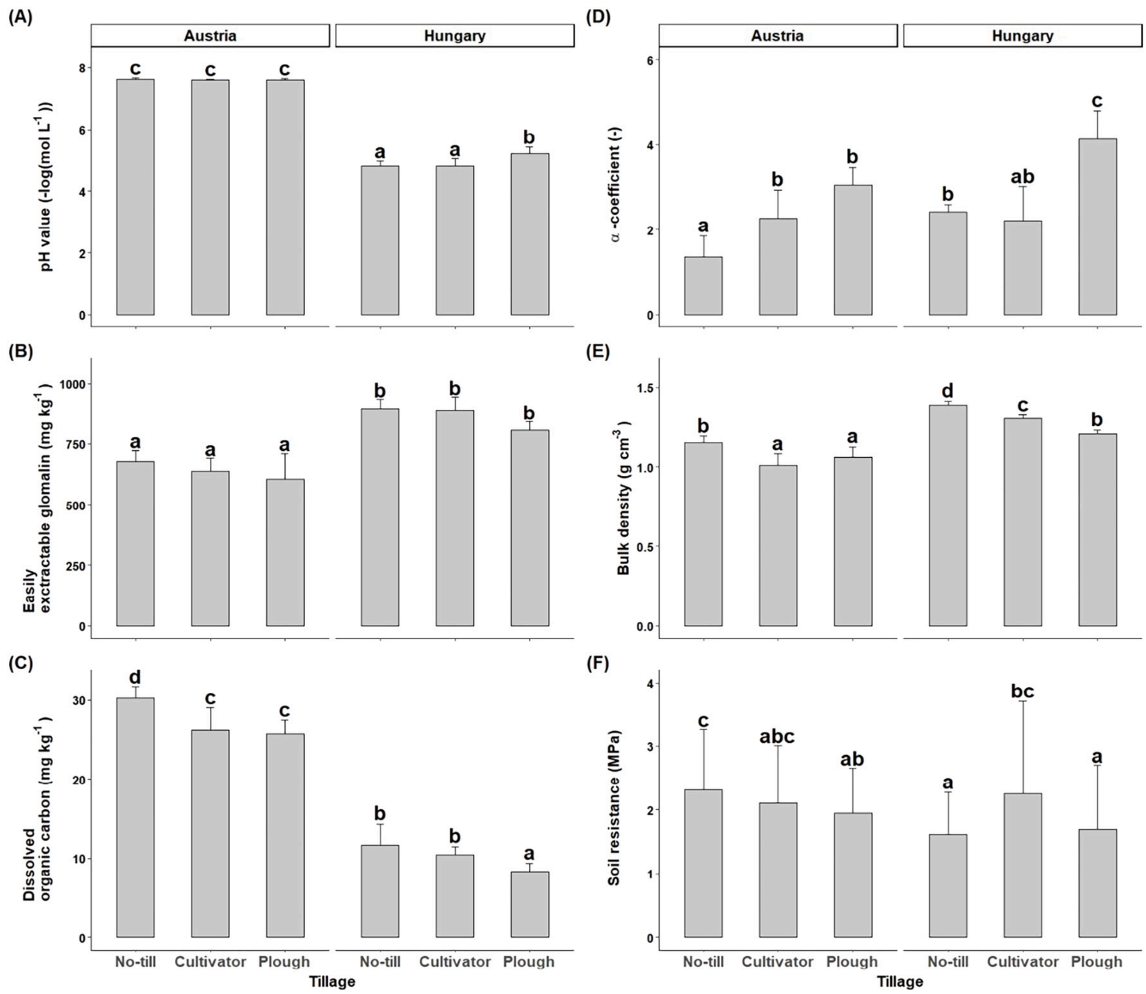

Fig. 1. Soil chemical parameters pH_{CaCl_2} (A), Easily extractable glomalin (B), dissolved organic carbon (C), and soil physical parameters soil aggregate stability (α-coefficient; D), bulk density (E) and mean soil penetrations resistance of 0–40 cm (F) in Austria or Hungary of three tillage treatments in 2020 and 2021. Tillage treatments having no letter in common are significantly different by pairwise comparison (2-way LMM, Tukey; $P < 0.05$). Mean + SD, $N = 4$. Note: More stable soil aggregates have a lower α-coefficient.

was associated with NT. PCA (Fig. 5) showed that PC1 accounted for 35.9 % of variance and was associated with earthworm parameters, EEG, SOC and soil physical parameters such as BD and SAS. Parameters of AMF were linked to PC2 and explained 25.4 % of variance. Soil tillage system ellipses overlapped slightly in P and C and all AMF parameters, except hyphal abundance, were related to C. Hyphal abundance was not linked to any soil tillage system and negatively correlated to remaining AMF parameters. By contrast, all earthworm parameters were clearly associated with NT, while SAS decreased with soil tillage intensity and was negatively correlated to earthworm parameters. AMF and earthworm data appeared to be independent at the AT site.

4. Discussion

4.1. Soil chemistry and physics

Overall, results of soil chemistry and physics were highly affected by soil tillage systems and differences for soil pH and clay or silt content. The calcareous loess parent material in AT was relevant for the high pH value (Bolan et al., 2023) and was not affected by soil tillage treatments as seen in Neugschwandtner et al. (2022) and mainly affected EEG and DOC. EEG was higher in HU than AT and clearly benefited from a lower pH, as seen by Singh et al. (2016), due to a reduced nutrient availability, which can affect AMF growth negatively (Rillig et al., 2001; Wright and Upadhyaya, 1996). The greater DOC values in AT than HU were also pH-related and decreased with soil tillage intensity at both sites. Sae-Tun et al. (2022) showed that DOC decreased with soil tillage intensification in the order of NT > C > P in a long-term soil tillage trial in the same area of AT, with a similar silty loam Chernozem and soil pH as in the current study. Soil pH can determine the release of DOC, as the availability of dissolved organic substances in soils increases with increasing pH (Kalbitz et al., 2000; Jones and Willett, 2006) and explains the higher concentrations of DOC in AT (pH 7.7) than HU (pH 4.5). In addition, the clay loam texture in HU (36 %; compared with 20 % clay content in AT) may have caused a stronger adsorption of DOC to clay minerals, as suggested by Ussiri and Johnson (2004) and Saidy et al. (2015).

For soil physical parameters, SAS decreased with soil tillage intensification at both sites. While Flynn et al. (2020) found a clear separation of SAS between soil tillage systems in clayey soils, aggregates in HU showed similar results for NT and C. But when Bagnall and Morgan (2021) compared tillage systems for soils with 27–38 % clay content, they found no differences for SAS between NT and chisel-ploughing (20–25 cm depth), which can be considered as less destructive than P, but with higher intensity than C. Moreover, Bagnall and Morgan (2021) stated that SAS is independent of clay content, but that clayey soils need a higher content of organic carbon to show similar SAS than soils with lower clay content. In addition, Schrader and Zhang (1997) found that soil texture is a predictor for SAS and that soils which are more sensitive

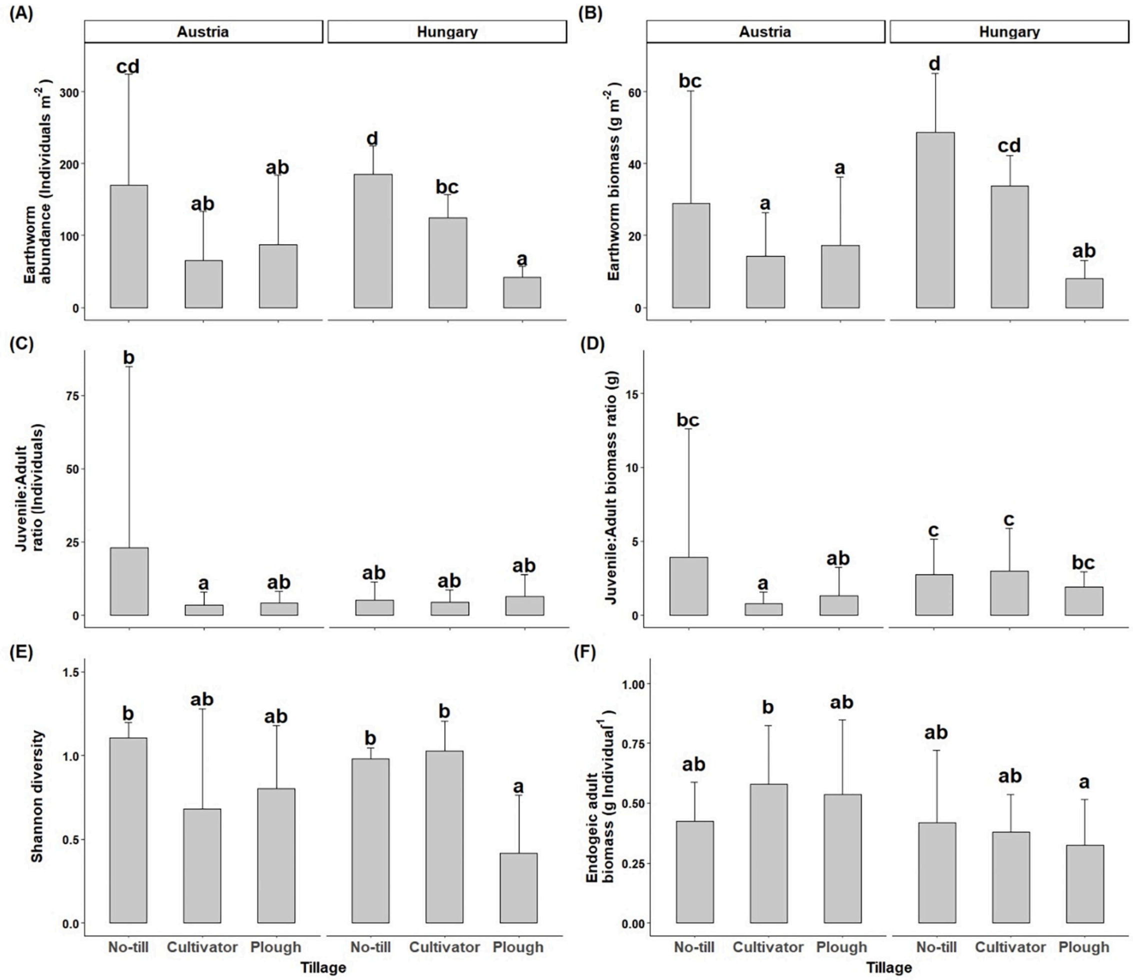

Fig. 2. Earthworm parameters total abundance (A), biomass (B), juvenile:adult ratio (C), juvenile:adult biomass ratio (D), Shannon diversity index (E), and average biomass of endogeic adult earthworm (F) in Austria or Hungary of three tillage treatments in 2020 and 2021. Tillage treatments having no letter in common are significantly different by pairwise comparison, except for adult biomass (F) which was only affected by site (2-way LMM, Tukey; $P < 0.05$). Mean + SD, $N = 4$.

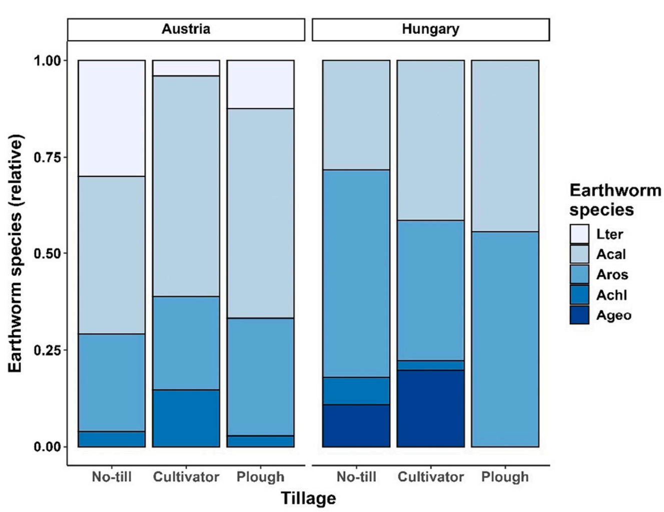

Fig. 3. Earthworm community composition of *Aporrectodea caliginosa* (Acal), *A. rosea* (Aros), *A. georgii* (Ageo), *Allolobophora chlorotica* (Achl) and *Lumbricus terrestris* (Lter) in Austria or Hungary of three tillage systems in 2020 and 2021 assessed by hand-sorting. Mean, $N = 4$.

Table 3
ANOVA results of rate of occupied burrows of *Lumbricus terrestris* (occupancy rate), number of middens corrected by the occupancy rate (middens), rate of adult and immature *L. terrestris* burrow occupancy and biomass with fixed factor tillage in Austria (2020). Tillage treatments having no letter in common are significantly different by pairwise comparison (1-way LMM, Tukey; $P < 0.05$). Mean ± SD, $N = 4$, degrees of freedom = 2.

Parameter	No-till	Cultivator	Plough	*F*-value	*P*-value
Occupancy rate	0.825 ± 0.126 ^{b}	0.525 ± 0.171 ^{a}	0.425 ± 0.096 ^{a}	14.3	0.005
Middens	28.7 ± 7.23 ^{b}	2.85 ± 2.76 ^{a}	2.28 ± 1.42 ^{a}	56.5	>0.001
Adult rate	0.325 ± 0.222 ^{a}	0.4 ± 0.082 ^{a}	0.275 ± 0.096 ^{a}	1.28	0.344
Immature rate	0.5 ± 0.141 ^{b}	0.125 ± 0.126 ^{a}	0.15 ± 0.1 ^{ab}	7.63	0.011
Biomass adult	3.33 ± 0.463 ^{a}	4.22 ± 0.708 ^{a}	3.43 ± 0.468 ^{a}	2.94	0.104
Biomass immature	1.18 ± 0.167 ^{a}	2.74 ± 0.366 ^{b}	2.6 ± 0.358 ^{b}	215	>0.001

to physical disturbance, such as soils with high silt content (Weninger et al., 2019), show the highest effect on SAS through earthworm casting, while clayey soils have an inverse effect. These results are in line with current findings, when data from AT showed a gradient in SAS from NT

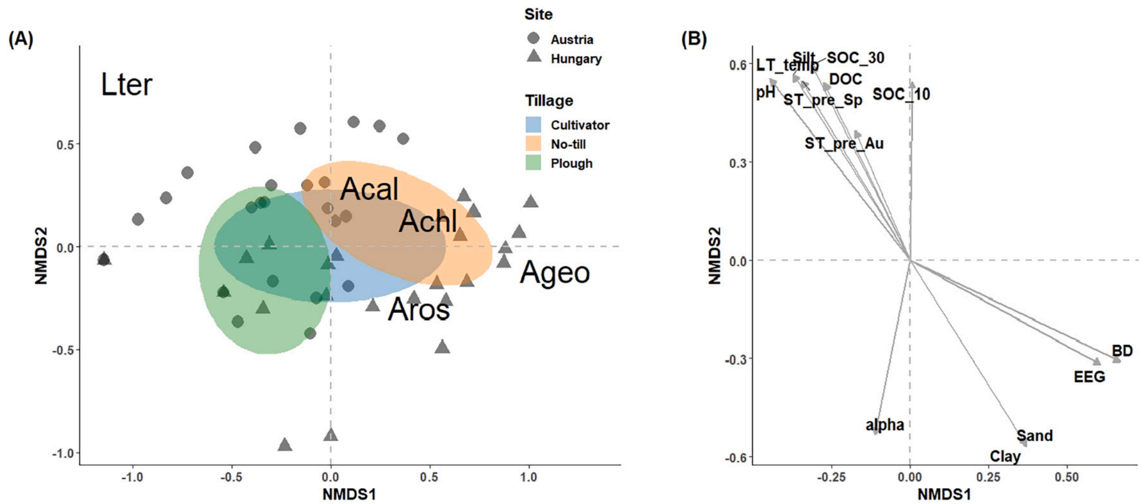

Fig. 4. Non-metric multidimensional scaling (NMDS) of earthworm species (A) in Austria and Hungary under three soil tillage treatments from 2020 to 2021. Earthworm species found were *Lumbricus terrestris* (Lter), *Aporrectodea caliginosa* (Acal), *A. rosea* (Aros), *A. georgii* (Ageo) and *Allolobophora chlorotica* (Achl). Site parameters of NMDS (B) include soil aggregate stability (alpha), bulk density (BD), soil organic matter from 0 to 10 cm and 0–30 cm (SOC_10, SOC_30 cm; respectively), dissolved organic carbon (DOC), soil texture (Sand, Clay, Silt), pH, easily extractable glomalin (EEG), long-term temperature and precipitation (LT_temp, LT_pre; respectively) and annual precipitation (ST_pre).

Table 4
Arbuscular mycorrhiza colonization parameters for maize roots under three tillage treatments at the Austrian site (2020). Tillage treatments having no letter in common are significantly different by pairwise comparison (2-way LMM, Tukey; $P < 0.05$). Mean, +SD, $N = 4$. F (% mycorrhizal frequency), M (% mycorrhizal intensity), A (% arbuscule abundance), V (% vesicle abundance), H (% hyphal abundance) in the root system.

Tillage	F (%)		M (%)		A (%)		V (%)		H (%)	
No-till	98	± 5 a	31	± 3 a	27	± 5 a	5	± 3 a	9	± 4 b
Cultivator	99	± 4 a	31	± 4 a	29	± 5 a	9	± 7 b	5	± 4 a
Plough	99	± 2 a	29	± 4 a	26	± 6 a	6	± 4 ab	7	± 5 ab

> C = P and with similar results of Euteneuer et al. (2024) from the same area in AT. In addition, BD was increased by P ≥ C > NT, while BD was higher in HU than AT. Klik and Rosner (2020) showed similar with BD increased with reduced soil tillage independently of soil texture within 0–20 cm soil depth in three long-term soil tillage trials in AT. However, SPR differed between soil tillage systems, where NT in AT was higher than P, but NT in HU was similar to P. These results in AT concur with previous publications (e.g., Dekemati et al., 2019).

4.2. Earthworm community and AMF

Earthworm communities at both sites were affected by soil tillage systems, showed highest abundance and biomass in NT and decreased with intensity, similar to previous studies of Dekemati et al. (2019) at the HU site. This finding is supported by a meta-analysis of Briones and Schmidt (2017), they reported that anecic and larger-sized species such as *L. terrestris* benefit more from reduced soil tillage than endogeic and smaller-sized earthworms such as *A. chlorotica* and *A. caliginosa*. In the current study, *A. caliginosa* was less sensitive to soil tillage compared to *L. terrestris*, but also increased with reduced soil tillage intensity, but not as much as *L. terrestris* in AT, when 12-times more middens were counted in NT than P (Briones and Schmidt, 2017; Capowiez et al., 2009; Euteneuer et al., 2024). Overall, endogeic earthworms were found in all soil tillage systems except for *A. chlorotica* for P in HU, therefore the Shannon index was higher in NT at both sites and lower for P in HU. Interestingly, *A. georgii* and *L. terrestris* were found mostly in reduced tillage

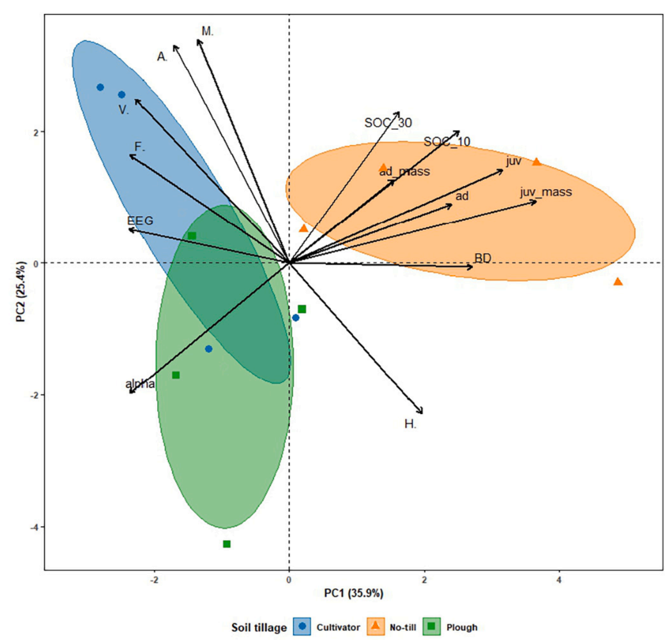

Fig. 5. Principal Component Analysis (PCA) biplot depicting the interplay between arbuscular mycorrhizal root colonization parameters, earthworm parameters, and soil properties at the Austrian site (2020). Ellipses are confidence ellipses based on the 0.95 level. F (mycorrhizal frequency), M (mycorrhizal intensity), A (arbuscule abundance), V (vesicle abundance), H (hyphal abundance) in the root system, BD (bulk density), alpha (soil aggregate stability), EEG (easily extractable glomalin), SOC_10 (soil organic carbon from 0 to 10 cm), SOC_30 (soil organic carbon from 0 to 30 cm), ad (adult earthworm abundance (individuals m^{-2}), ad_mass (adult earthworm biomass (g m^{-2}), jv (juvenile earthworm abundance (individuals m^{-2}), juv_mass (juvenile earthworm biomass (g m^{-2}).

and seem to have the same demands related to undisturbed soils, but *A. georgii* was absence in AT, while *L. terrestris* was missing in HU. Similar to Briones and Schmidt (2017), we believe that ecological groups or body size are not sufficient to explain the presence or absence in certain soil tillage systems or site. Currently, ecological groups and functional groups are undergoing some revisions and clarifications

(Bottinelli et al., 2020; Bottinelli and Capowiez, 2021; Capowiez et al., 2024). For example, anecic for *L. terrestris* is used by Briones and Schmidt (2017) and Butt et al. (2022) or epi-anecic by Bouché (1972), Bottinelli and Capowiez (2021), and Capowiez et al. (2024). In addition, Capowiez et al. (2024) categorised *L. terrestris* to functional group burrower in relation to burrowing, feeding and casting activities close to the surface or at the surface. *A. caliginosa* is also determined as epi-anecic with functional group burrower by Capowiez et al. (2024) and for ecological group as endogeic by Bouché (1972), Bottinelli and Capowiez (2021) and Capowiez et al. (2024). Perhaps neither ecological groups nor functional groups are sufficient to explain the sensitivity of certain earthworm species to soil tillage systems, but additional information is needed, such as preferred burrowing mode (ingestion or cavity expansion; Arrázola-Vásquez et al., 2022), habitat requirements, survival strategies, soil properties and site history (Briones and Schmidt, 2017) to evaluate the sensitivity, presence or absence of certain earthworm species. As the climatic data at both sites are similar, the NMDS result suggested that differences in earthworm community composition are mainly related to soil properties pH and soil texture, but leaving out the other mentioned factors such as burrowing mode, survival and site history. The pH and clay data dominated the NMDS analysis, but according to Misirlioğlu et al. (2016), *L. terrestris* can be found in soils with low pH values (3.5–3.7) and Edwards and Lofty (1975) emphasized that the abundance of the same earthworm species enhanced as the acidic pH values (pH 3.7) increased to neutral (pH 7.0), suggesting that earthworms favour neutral pH ranges to acidic soils. The high clay content in HU might explain why *A. georgii* was only detected at HU site, which is in line with Csuzdi and Zicsi (2003), who reported that *A. georgii* can be found in moist clayey soils in southern Europe. In addition, Zicsi (1994) found *A. georgii* only 8 km from the AT site, in a pasture in the Lobau (an alluvial forest in Donau-Auen National Park) with mostly loamy sand Fluvisol (WRB, 2014; BFW, 2023). The moisture content of the soil might therefore be more important than the clay content, but cannot be addressed in this study as unknown factors may have caused the absence/presence of *A. georgii*.

Apart from earthworms, AMF are an important indicator for soil health, therefore the parameters of AMF colonization and EEG were selected to monitor AMF development. AMF colonization rates did not differ between the treatments at the sampling date in August in AT, at the corn filling stage. As obligate biotrophs, AMF development is strongly dependent on an available host plant and fluctuates over the growing season (Abbott and Robson, 1985; Kabir et al., 1997). In a field study with maize, hyphal density and density of metabolic active hyphae were the lowest in spring before maize was sown and increased thereafter at the 12–14 leaf stage, peaked at the silking stage and decreased again following root senescence (Kabir et al., 1997). In addition, differences between soil management practices also depended on the sampling time. Treatments with P showed diminished AMF parameters compared to C and NT only at the silking stage. In the current study, root samples were taken at the corn filling stage where differences were probably no longer evident. Furthermore, EEG can be used as a proxy for arbuscules and hyphal lengths of AMF, as it correlates closely with phospholipid fatty acids 16:1ω5 (Thomopoulos et al., 2023). EEG did not follow the expectation that soil tillage should influence EEG values. However, by tendency, EEG was lower in the P treatment than in the C and NT treatments as seen by Thomopoulos et al. (2023). The same tendency was seen in HU when the magnitude of the EEG values was in general higher than in AT. In another study investigating the link between EEG and AM hyphal biomass in different soil aggregate classes, Helgason et al. (2010) could find only a correlation between EEG and AMF biomass in the P treatment, but not in the NT treatment, indicating a potential threshold from where AMF ceased to affect aggregate stability. Although EEG is proposed as a useful proxy for assessing AMF biomass and its potential impact on SAS, its association with soil tillage practices may be complex and dependent on site-specific factors. Holátko et al. (2021) recently reported that any correlation between AMF and EEG may be indirect or coincidental, due to unknown interplays of the soil food web, turnover rates of glomalin or differences in glomalin productions rates of AMF species.

To date, studies linking AMF and earthworm data are scarce. In a combined principal components analysis, AMF and earthworm parameters at the AT site were not correlated. Earthworm parameters were clearly linked to NT, while AMF parameters were not affected by tillage. As discussed above, results for earthworm parameters are supported by many previous studies, but AMF parameters were also expected to increase in NT and decrease with soil tillage intensity (Kabir et al., 1997; Rosner et al., 2018; Thomopoulos et al., 2023). Non-responsiveness of AMF data might be linked to the sampling date, or a site-specific physiological maximum for root colonization.

Earthworm activity, in addition to AMF hyphal activity/degradation, contributes to SAS (Six et al., 2004; Helgason et al., 2010; Euteneuer et al., 2024). In a recent review, it was highlighted that earthworms ingest AMF and that AMF propagules stay active in the digestive system resulting in the dispersal of AMF propagules and enhancement of root colonization (Pelosi et al., 2024). However, grazing and burrowing activity can simultaneously result in a decrease in hyphal length and disruption of hyphal networks (Meng et al., 2022; Pelosi et al., 2024). As the contributions of AMF and earthworms to SAS follow very distinct pathways, it remains to be determined if their contributions can be linked and may provide synergistic effects. Investigating these potential links may require consideration of the behaviour and niche preferences of different earthworm species, specific soil and site factors, and the overall history of soil management.

4.3. Future perspectives

As a causal relationship between AMF and earthworm activity remains elusive, further studies on different soils and at varying scales could provide more insight. For example, investigating aggregate size classes, i.e., moving from bulk soil to a finer scale, could offer a clearer understanding of potential synergistic effects (Helgason et al., 2010). In addition, microcosm experiments using a range of field soils may serve as the preferred test system in such cases. To enhance clarity, future research should aim to define the nature of the AMF-earthworm relationship, address specific knowledge gaps and guide investigations at different scales and soil types.

Soil tillage showed different impacts on soil and earthworm parameters related to soil properties rather than soil tillage treatments. The higher silt content in AT made the soil particularly more sensitive to soil tillage than in HU. Even reduced tillage showed a similar impact on DOC, SAS, BD, SPR and earthworms compared with conventional tillage, so our hypothesis cannot be fully accepted or rejected. While NT improved soil health parameters such as DOC, SAS and earthworms, reduced soil management depended on soil texture. Further research and reviews on various topics might usefully consider soil texture, when comparing soil tillage systems.

5. Conclusion

The current study showed that within the same soil type, site specific soil properties such as pH and soil texture have a high impact on soil chemistry and the earthworm community. In addition, other parameters such as DOC, earthworm abundance and biomass, and soil physical properties were clearly affected by soil tillage. Nevertheless, the extent of the influence of the soil tillage system depended on soil properties. For most parameters, it was seen that C showed some intermediate position between P and NT in HU, while in AT, C was rather similar to P. Hence, soil tillage, and especially reduced tillage such as C, needs site-specific evaluation with respect to soil health.

CRediT authorship contribution statement

Barbara Simon: Writing – original draft, Methodology, Investigation, Conceptualization. **Igor Dekemati:** Writing – original draft, Methodology, Data curation. **Hanaa T.M. Ibrahim:** Writing – original draft, Investigation. **Maxwell M. Modiba:** Writing – original draft, Investigation. **Márta Birkás:** Validation, Resources, Methodology, Conceptualization. **János Grósz:** Writing – original draft, Investigation. **Martin Kulhanek:** Writing – original draft, Methodology, Investigation. **Reinhard W. Neugschwandtner:** Writing – original draft, Resources, Project administration, Conceptualization. **Anna Hofer:** Investigation. **Viola Wagner:** Investigation. **Marion Windisch:** Investigation. **Karin Hage-Ahmed:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Kevin R. Butt:** Writing – original draft, Methodology, Investigation. **Pia Euteneuer:** Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

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Data availability

Data will be made available on request.

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