Plant Soil
https://doi.org/10.1007/s11104-026-08743-w

RESEARCH ARTICLE

Leaf litter distribution as a key driver of earthworms in a mature temperate agroforestry system

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Received: 13 March 2026 / Accepted: 6 June 2026

Abstract

Background and aims Alley cropping agroforestry, which is the integration of tree strips into arable land, is known to enhance soil biodiversity as compared to open cropland. Earthworms, a key group of soil fauna, are strongly promoted through trees in agroforestry. Although tree leaf litter is often assumed to play an integral role in the promotion of earthworms, experimental data is lacking. This study aims to shed light on the effect of tree leaf litter on earthworm communities in agroforestry.

Methods Our study was conducted in a 17-year-old poplar alley cropping system in Germany. Earthworms were collected using chemical extraction along point transects at multiple distances from the tree strip into the windward and leeward section of its adjacent crop alleys. Additionally, leaf litter was manually collected from the soil surface and soil samples for general soil properties were taken at 0—30 cm soil depth.

Results Overall distribution of earthworms mirrored the spatially heterogeneous distribution of tree leaf litter with greater population density and biomass in the tree strip and in the leeward crop alley as compared to the windward crop alley. However, earthworm distribution patterns were species-specific. While anecic and epigeic earthworms were positively associated with tree leaf litter, endogeic earthworms showed no observable response.

Conclusion Our results confirm tree leaf litter inputs as a major driver of earthworm communities in mature agroforestry systems. Furthermore, species-specific responses of earthworms to tree leaf litter are likely attributed to distinct feeding behavior and habitat requirements.

Keywords Temperate agroforestry · Alley cropping · Earthworms · Soil fauna · Leaf litter distribution · Spatial heterogeneity

Responsible Editor: Erik J. Joner.

Lukas Beule and Anna Vaupel shared senior authorship.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-026-08743-w.

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Introduction

Silvoarable agroforestry, the establishment of woody perennials on arable land, is a land-use practice that can provide multiple environmental benefits over open croplands. Alley cropping systems that alternate strips of trees with alleys of crops are gaining

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Published online: 20 June 2026

increasing popularity as the width of the crop alleys can be matched to the working widths of the agricultural machinery, which facilitates crop management and enhances scalability. Benefits of alley cropping cover the effective reduction of wind speeds and subsequently the risk of soil erosion (van Ramshorst et al. 2022) as well as the promotion of biodiversity (Kletty et al. 2023) including its beneficial ecosystem functions such as natural pest control (Staton et al. 2019; Matevski et al. 2024) and pollination services (Varah et al. 2013, 2020). Furthermore, there is evidence that alley cropping systems can effectively reduce leaching of nutrients through a "safety-net" of deep-rooting trees (Allen et al. 2004; Wang et al. 2011) taking up excess nutrients that are leached below the main rooting zone of the crops. In addition to improved nutrient cycling, alley cropping systems have shown to sequester significant amounts of soil organic C (SOC) (e.g. Mayer et al. 2022), making them not only a land-use practice for the adaption to climate change but also for its mitigation. As the ecosystem functions provided by alley cropping systems are manifold, several authors labeled them as a multifunctional land-use practice. In 2023, Veldkamp et al. validated this claim by publishing a comprehensive field-based study showing that multifunctionality of alley cropping outperforms open cropland. Their work demonstrated that temperate alley cropping enhances soil biological activity by improving habitat conditions, which is congruent with the vast majority of studies that carried out assessments on soil organisms in these systems. The promotion of soil organisms through alley cropping ranges from soil microorganisms like bacteria and fungi (Bainard et al. 2011; Beule et al. 2020; Beule and Karlovsky 2021; D'Hervilly et al. 2020) to soil macrofauna like earthworms (Cardinael et al. 2019; D'Hervilly et al. 2022; Vaupel et al. 2023). Based on their morphology and behavior, earthworms can be classified into ecological groups of which three generalized main groups have become widely established: anecic, endogeic, and epigeic earthworms (Bottinelli et al. 2020; Bouché 1972). Anecic species live in predominantly vertical, deep, and permanent burrows that extend from the soil surface to mineral soil horizons and mainly feed on aboveground organic material such as litter which they pull into their burrows. Endogeic species primarily inhabit the topsoil, where they create rather horizontal, non-permanent burrows, ingesting large amounts of mineral soil, whereas epigeic species dwell in and feed on aboveground litter.

In order to capture the spatial heterogeneity introduced by the establishment of tree strips on arable land, spatial sampling designs within alley cropping systems often require sampling along transects spanning from the tree into the crop component of the system (i.e. transect sampling; see Minarsch et al. 2025). The few studies that assessed earthworm communities along point transects in alley cropping systems revealed that the promotion of earthworms through agroforestry is mainly restricted to the tree strips and the extension into the crop alleys is limited to short distances and differs among earthworm species (Cardinael et al. 2019; D'Hervilly et al. 2022; Price and Gordon 1998; Vaupel et al. 2023). Aside from tree strips offering an undisturbed habitat in croplands (Cardinael et al. 2019), tree leaf litter input has been assumed to be a promoting factor for the abundance and diversity of soil organisms in agroforestry systems (Beule et al. 2022; Marsden et al. 2020). For instance, in 2023, we found a gradual decline in the population density of endogeic earthworms with increasing distance from the trees into the crop alleys of an alley cropping system (Vaupel et al. 2023). We attributed the promotion of endogeic earthworms in close vicinity to the trees to the exponential decline of tree leaf litter with increasing distance from the trees, as reported by Oelbermann et al. (2004). It is self-evident that the spatial distribution of tree leaf litter within alley cropping systems not just depends on the distance to the trees but also on the orientation of the tree strips to the wind direction during litter fall. Indeed, Swieter et al. (2019, 2022) showed that the amount of leaf litter deposited in crop alleys is larger at the leeward than the windward side. Despite these differences in litter deposition, assessments of soil organisms in temperate alley cropping systems i) rarely consider or determine the spatial distribution of tree leaf litter or ii) differentiate between the windward and leeward section of the crop alleys. To our knowledge, no study attempted to assess the influence of the spatially heterogeneous distribution of tree leaf litter within temperate agroforestry systems on key representatives of the soil fauna: earthworms.

Here, we investigated earthworm populations, soil properties, and the amount of leaf litter in a tree strip as well as the windward and leeward section of the bordering crop alleys of a mature temperate alley cropping system in which poplar leaf litter was deposited for almost two decades. As tree leaf litter can serve as habitat and food source for earthworms and is unevenly distributed within alley cropping systems, we hypothesized that i) overall earthworm population density corresponds to the spatially heterogenous distribution of tree leaf litter. However, since different earthworm species have different habitat requirements and are dependent on different food sources, we expected ii) species-specific promotion of earthworms through tree leaf litter.

Materials & methods

Study site and sampling design

To test the impact of the windward and leeward section of the crop alleys (hereafter referred to as windward and leeward crop alley, respectively) of a temperate agroforestry system on earthworm communities, we selected a mature poplar-based alley cropping system near Dornburg, Germany (51°00′40″ N, 11°38′46″ E; m.a.m.s.l.: 289; mean annual air temperature: 10.3 °C; mean annual precipitation: 595.6 mm (Jena climate station (ID: 2444); German Meteorological Service n.d.)). The alley cropping system was established in 2007 on a Calcaric Phaeozem soil by manually planting 12-m wide strips of poplar trees (clone Max 1: *Populus nigra* × *P. maximowiczii*) that were alternated by 48-m wide crop alleys. Tree strips were planted in North–South orientation perpendicular to the main wind direction of 218.8° (west-to-east), as determined by Callejas-Rodelas et al. (2026). Since their establishment, the tree strips did not receive fertilizer and plant protection products, were not tilled, and were harvested for biomass production in January 2015. The crop alleys were managed conventionally (i.e. application of mineral fertilizer and plant protection products, and soil management). During our year of sampling (2024), winter wheat was cultivated at the study site (pre crops were winter oilseed rape (2023), winter barley (2022), and winter wheat (2021)). Last soil management prior to earthworm sampling was conducted on October 7, 2023, followed by seeding of winter wheat on October 11, 2023.

To capture spatial heterogeneity across the windward and leeward side within the agroforestry system, we established eight point transects spanning 18 m distance from the trees into the crop alleys on both the windward and leeward side (transect sampling; Fig. 1). The transects were established orthogonal to the orientation of the tree strips and samples were collected at different sampling locations across the gradient from the windward to the leeward side. We sampled in the crop alley at the windward side at 1, 3, 6, and 18 m distance to the trees, in the center of the tree strip, and at 1, 3, 6, and 18 m distance to the trees in the crop alley at the leeward side (Fig. 1). In total, we collected samples at 72 sampling locations (9 sampling positions per transect (i.e. 1, 3, 6, and 18 m windward crop alley, tree strip, and 1, 3, 6, and 18 m leeward crop alley) × 8 transects = 72 sampling locations). At each sampling location, we determined general soil properties, collected earthworms and leaf litter. Sampling for soil properties and earthworms was performed in April 2024, while leaf litter was collected in December 2024. Therefore, the agroforestry system was 17 years old during our sampling and the first and last harvest of the poplar trees was nine years ago, allowing for long-term deposition of tree leaf litter.

General soil properties

Samples for general soil properties were collected at 0–30 cm soil depth using a stainless-steel auger (Ø 3.5 cm). At each sampling location, we collected three soil cores that were thoroughly homogenized in a sterile polyethylene bag in the field and an aliquot of approximately 300 g of fresh soil was transported to the laboratory. Upon arrival at the laboratory, soil samples were air-dried at room temperature, sieved to < 2 mm, and plant material was thoroughly removed. Soil pH was determined from 10 g sieved soil in deionized H_2O at a ratio of 1:2.5 (soil/water (w/v)). Soil organic C (SOC) and total N were determined following acid fumigation to remove inorganic C from the samples prior to measurement. For this, sieved soil samples were finely ground using a swing mill and fumigated with 12 M HCl in a desiccator as described by Harris et al. (2001). Following acid fumigation, samples were dried at 60 °C and

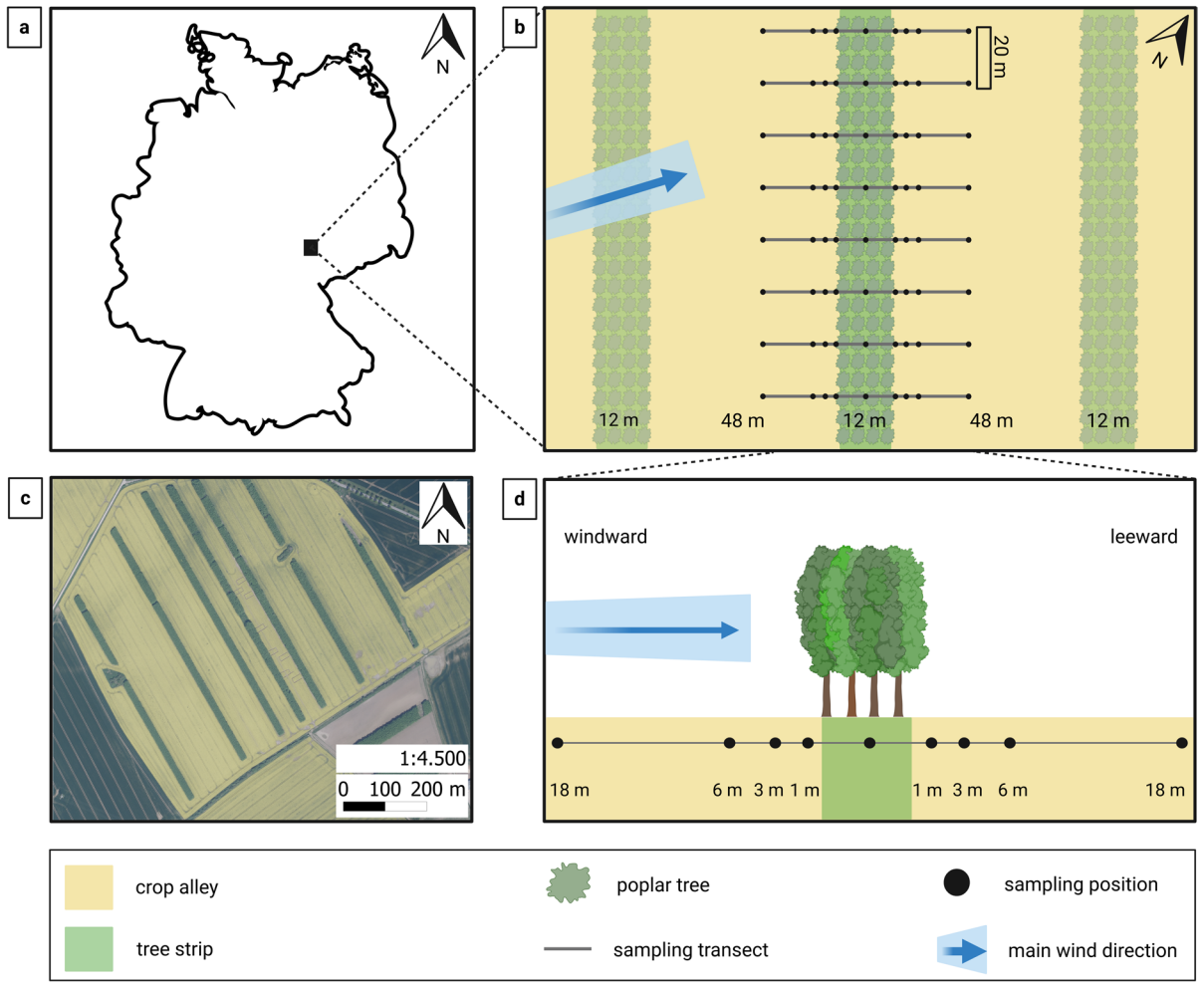

Fig. 1 Study site location and study design. Location of the study site near Dornburg, Thuringia, Germany (**a**). Schematic illustration of the locations of the eight point transects and the nine sampling positions per transect including the measured main wind direction of 218.8° (Callejas-Rodelas et al. 2026) (**b**). Orthophoto of the study site with a scale of 1:4.500 (© GDI-Th, edited in QGIS 3.44.0) (**c**). Schematic illustration of the distances between the sampling positions within each point transect (**d**) (created in https://BioRender.com)

concentrations of SOC and total N were determined using a CN elemental analyzer (Vario EL Cube, Elementar, Germany).

Earthworm communities

Earthworms were collected using chemical extraction as per Vaupel et al. (2023). Briefly, 5 L of a freshly prepared 0.01% (w/v) allyl isothiocyanate (AITC) solution were poured onto an area of 0.25 m^2 limited by a 50×50 cm open metal frame to guide infiltration. In order to improve spotting of surfacing earthworms, plant material was carefully removed prior to the application of the AITC solution. Following the application of the AITC solution, the soil surface was continuously monitored for 30 min and surfacing earthworms were collected, washed with tap water and subsequently stored in tap water at 5 °C in the dark until morphological identification in the laboratory. Earthworms were counted, weighed (fresh biomass including gut content), and identified on species level based on their morphological and behavioral traits as per Krück (2018) and released alive within 48 h after sampling. Individuals that

were not identifiable based on their morphology and behavior were subjected to molecular identification using Sanger sequencing of the COI region as described by Vaupel et al. (2022). In total, seven species of earthworms were found during the study and were classified into the three ecological groups originally introduced by Bouché (1972) as follows: anecic (*Lumbricus terrestris*), endogeic (*Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea rosea*, and *Octolasion tyrtaeum*) and epigeic (*Lumbricus castaneus* and *Lumbricus rubellus*). Additionally, developmental stage (juvenile or adult) of each individual earthworm was determined. To compare the whole windward and leeward crop alleys and the tree strip, sampling positions within the crop alleys were weighted by their areal coverages (i.e. area-weighted average) as previously done by Beule et al. (2019), Shao et al. (2023), and Veldkamp et al. (2023) in temperate agroforestry systems. The area-weighted average ($\bar{x}_{weighted}$) for each windward and leeward sampling transect was calculated using the following equation

$$\bar{x}_{weighted} = \frac{\sum_{i=1}^{n} X_i w_i}{\sum_{i=1}^{n} w_i}$$

where X_i represents the value of the respective variable at the sampling position i and w_i is the corresponding weighting factor as assigned below. Weighting factors of the windward and leeward crop alley (1/2 width of the 48-m wide crop alleys: 24 m), were determined as follows: the sampling position at 1 m distance to the tree strip was assigned a weighting factor of 2, 3 m distance of 2.5, 6 m distance of 7.5, and 18 m distance of 12.

Tree leaf litter distribution

Tree leaf litter was collected on 10 December 2024, following complete leaf abscission. Leaf litter was collected after earthworm sampling to avoid interfering with earthworm population density or biomass. At each sampling location, litter was manually collected on a surface area of one square meter (50 × 200 cm; with the 200 cm side parallel to the North–South orientation of the tree strip). Fresh litter was dried at 60 °C for 48 h and the dry litter mass was determined.

Statistical analyses

To analyze differences in soil pH, leaf litter biomass, SOC and total N among the nine different sampling positions, we used linear mixed-effect (LME) models with sampling position as fixed term and sampling transect (eight different sampling transects) as random term utilizing the 'nlme' package (v. 3.1–168) in R. If the addition of a random term did not improve the model fit, generalized least squares fitted (GLS) models were used with sampling position as explanatory variable using the 'nlme' package. All models were fitted by maximizing the restricted log-likelihood and visually inspected for normal distribution of the residues and homogeneity of variances. In cases of heteroscedasticity, a 'VarIdent' variance structure was fitted using sampling position (i.e. tree strip and four different distances in the crop alley into each sampling direction) and/or sampling direction (i.e. windward and leeward side of the crop alley) as variance covariates. If adding a variance structure did not resolve heteroscedasticity, data was log(x + 1) (leaf litter biomass) transformed to meet the criteria. Model selection was performed based on the Akaike information criterion (AIC) and all models were visually inspected for linearity and independence. To analyze the effect of sampling direction, a subset of the data without data points from the tree strip was analyzed as described above but with sampling distance, sampling direction, and their interaction as fixed term.

Differences in ecological count data (earthworm population densities) between different sampling positions were analyzed using generalized linear mixed models (GLMMs) utilizing the 'glmmTMB' R-package (v. 1.1.13). Negative binomial distribution (Nbinom1) was found to be the most fitting distribution for all ecological groups of earthworms and zero inflation was accounted for using ziformula = ~ 1, whenever present. Model selection was performed based on the AIC and likelihood ratio tests. All final models were visually inspected using QQ-Plots of residuals as well as checked for overdispersion and homoscedasticity using the 'performance' R-package (v.0.15.2). The influence of sampling direction was analyzed using a subset of the data without data points from the tree strip. For this, GLMMs were used as described above but with sampling distance, sampling direction, and their interaction as fixed term.

For all models that returned statistically significant differences among groups using ANOVA, subsequent Tukey's honestly significant difference test was performed. Relationships between the amount of tree leaf litter and earthworm population density were assessed using Pearson Correlation. Pearson Correlation was calculated using data from individual sampling locations. For all statistical tests, statistical significance was considered at $p < 0.05$. All statistical analyses were performed in R (version 4.3.3).

Results

General soil properties

General soil properties at 0–30 cm soil depth showed only minor differences between the tree strip and crop alleys of the 17-years-old agroforestry system (Supplementary Table 1 in Online Resource 1). Soil pH ranged from 6.5 ± 0.1 to 6.9 ± 0.4, with slight differences among individual sampling locations without a discernible effect between windward and leeward crop alley or distance to the tree strip. Soil organic carbon (SOC) content was greatest in the tree strip (16.9 ± 1.3 g kg^{-1}), but values barely differed between windward and leeward crop alleys ranging from 14.7 ± 0.7 (6 m leeward) and 16.0 ± 0.7 (1 m windward). Total soil N did not differ among sampling positions, while the C/N ratio ranged from 8.5 ± 0.3 to 8.9 ± 0.4 with slightly greater values in the leeward than in the windward crop alley ($p > 0.05$, Supplementary Table 1 in Online Resource 1).

Earthworm communities

Earthworm population density

In total, 3,716 individuals belonging to seven different earthworm species were collected (anecic: *Lumbricus terrestris*; endogeic: *Allolobophora chlorotica, Aporrectodea caliginosa, Aporrectodea rosea, Octolasion tyrtaeum*; epigeic: *Lumbricus castaneus*, *Lumbricus rubellus*). Across all sampling locations, our study site was numerically dominated by *L. castaneus* (1,576 individuals, 42.4%) and *L. terrestris* (992 individuals, 26.7%), followed by *A. rosea* (608 individuals, 16.4%), *A. caliginosa* (368 individuals, 9.9%), *L. rubellus* (72 individuals, 1.9%), *O. tyrtaeum* (60 individuals, 1.6%), and *A. chlorotica* (40 individuals, 1.1%).

Within the agroforestry system, total earthworm population density peaked in the tree strip (169.5 ± 65.0 individuals m^{-2}). Leeward, population density ranged between 24.0 ± 16.1 and 93.5 ± 27.3 individuals m^{-2}, whereas it varied between 5.0 ± 8.2 and 12.0 ± 14.0 individuals m^{-2} in the windward crop alley (Fig. 2a). Differences between the tree strip and the windward crop alley (area-weighted average: 9.5 ± 4.3 individuals m^{-2}) were particularly large, with almost 18 times more earthworms underneath the trees than the crops ($p < 0.001$) (Supplementary Table 2 in Online Resource 1). In contrast, only three times more earthworms were found in the tree strip than in the leeward crop alley (area-weighted average: 51.2 ± 9.9 individuals m^{-2}) ($p < 0.05$). Furthermore, earthworm population density was greater at 1 m, 3 m and 6 m into the leeward crop alley than at every sampling position of the windward crop alley.

The examination of developmental stages revealed that the majority of earthworms were juvenile (59.85%) (Fig. 2b, Supplementary Table 2 in Online Resource 1). Juvenile earthworms showed a similar trend as all earthworms with greatest population density in the tree strip (127.0 ± 45.0 individuals m^{-2}), counting almost 24 times more individuals than in the windward crop alley (area-weighted average: 5.3 ± 2.3 individuals m^{-2}; $p < 0.0001$). The leeward crop alley harbored close to five times more juvenile individuals (area-weighted average: 26.0 ± 5.4 individuals m^{-2}) than its windward counterpart. A slightly different spatial distribution pattern was observed for adult earthworm population densities (Fig. 2c). As for juvenile earthworms, the windward crop alley (area-weighted average: 4.2 ± 2.1 individuals m^{-2}) harbored less adults than the tree strip (42.5 ± 21.7 individuals m^{-2}) ($p < 0.0001$). Contrary to juveniles, up to 6 m into the leeward crop alley, adults were promoted to a similar degree than in the tree strip ($p > 0.05$). Therefore, we did not observe an abrupt decline in population density from the tree strip into the leeward crop alley as found for juveniles (cf. Figure 2 b, c).

Spatial distribution patterns within the agroforestry system differed strongly among ecological groups of earthworms. Anecic *L. terrestris* was the most recovered species at each sampling position in the windward crop alley (Fig. 2d). Although the population density of *L. terrestris* peaked in the tree strip (28.0 ± 18.1 individuals m^{-2}, 87.5% juveniles), statistically significant differences among sampling positions were scarce (Fig. 2d), except for a lower population density at 3 m windward compared to the tree strip ($p = 0.020$) and 3 m leeward ($p = 0.032$).

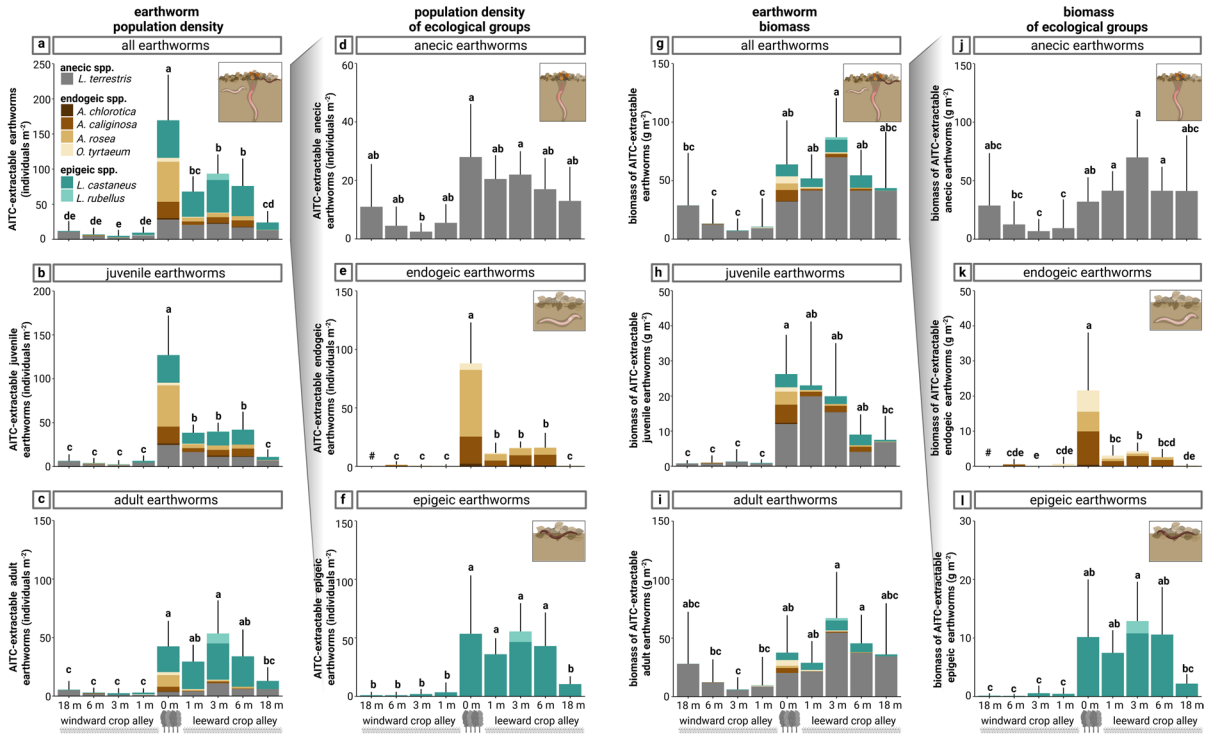

Fig. 2 Earthworm population density and biomass of different species and developmental stages. Mean earthworm population density (individuals m^{-2}), and biomass (fresh weight g m^{-2} including gut content) with standard deviation ($n = 8$) for all (**a, g**), juvenile (**b, h**), and adult (**c, i**) allyl isothiocyanate (AITC)-extractable earthworms as well as different ecological groups (**d, e, f, j, k, l**) are shown. Different colors represent different earthworm species (anecic: *Lumbricus terrestris*; endogeic: *Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea rosea*, and *Octolasion tyrtaeum*; epigeic: *Lumbricus castaneus* and *Lumbricus rubellus*). Different letters indicate statistically significant differences ($p \leq 0.05$) among the nine sampling positions (i.e. 1, 3, 6, and 18 m windward crop alley, tree strip, and 1, 3, 6, and 18 m leeward crop alley) (created in https://BioRender.com)

Endogeic earthworms were strongly promoted by the tree strip (88.0 ± 35.0 individuals m^{-2}) (Fig. 2e, Supplementary Table 2, 3 in Online Resource 1), counting 156 times more endogeics than in the windward and 11 times more than in the leeward crop alley ($p < 0.0001$). The tree strip was numerically dominated by *A. rosea* (57.0 ± 40.0 individuals m^{-2}) of which 82.5% were juveniles (Fig. 2e). Population densities at 1 m, 3 m, and 6 m distance from the trees were greater in the leeward than windward crop alley ($p < 0.05$). Furthermore, endogeic earthworms were absent at 18 m windward. Overall, the prevalence of endogeic species in the windward crop alley was low. This trend was observed for all endogeic species despite small fluctuations in their population density; no species-specific distribution patterns were observed.

Epigeic earthworms were promoted by the tree strip (53.50 ± 49.87 individuals m^{-2}) but unlike endogeics, also similarly abundant in the leeward crop alley up to 6 m distance. Leeward, population densities ranged between 36.0 ± 13.7 and 55.5 ± 24.1 individuals m^{-2}, which was markedly greater than in the windward crop alley ($p < 0.01$) (Fig. 2f). Among epigeic earthworms, *L. castaneus* occurred at every sampling position (57.6% adults), whereas *L. rubellus* was only found sporadically at 3 m into the leeward crop alley (94.4% adults) (Supplementary Table 3 in Online Resource 1).

Earthworm biomass

Due to the strong correlation between earthworm biomass and population density (Supplementary Fig. 1 in Online Resource 1), results for biomass mirrored those for population density (cf. Figure 2).

Total biomass in the tree strip (63.8 ± 37.5 g m^{-2}) (Fig. 2g, Supplementary Table 2 in Online Resource 1) was 22% ($p > 0.05$) greater than in the leeward crop alley

and approx. 215% greater than in the windward crop alley if considering the area-weighted average (with statistically significant differences of $p<0.01$ up to 6 m). A gradual decrease in juvenile biomass was observed from the tree strip (26.2±11.2 g m^{-2}) up to 18 m distance into the leeward crop alley (7.5±6.8 g m^{-2}) (Fig. 2h). Area-weighted biomass of juveniles in the leeward crop alley (10.6±2.2 g m^{-2}) was approx. 11 times greater than windward, whereas biomass in the windward crop alley did not differ among sampling positions. Among adults, biomass barely differed between the tree strip and leeward crop alley (Fig. 2i).

An examination of ecological groups revealed that the anecic species *L. terrestris* had the greatest biomass at all sampling positions (Fig. 2j). At distances up to 6 m into the crop alleys, biomass was greater in the leeward than the windward side ($p<0.01$). Biomass of endogeic species in the tree strip (21.6±16.5 g m^{-2}) exceeded those of the area-weighted windward (0.2±0.2 g m^{-2}; $p<0.0001$) and leeward crop alleys (1.6±0.5 g m^{-2}; $p<0.01$) (Fig. 2k, Supplementary Table 2 in Online Resource 1). Biomass of epigeic earthworms was greatest in the tree strip (10.2±9.8 g m^{-2}) as well as up to 6 m in the leeward crop alleys ranging between 7.5±3.9 g m^{-2} and 12.9±6.7 g m^{-2} (Fig. 2l).

Impact of tree leaf litter distribution on earthworms

Poplar dry leaf litter biomass gradually declined with increasing distance to the tree strip into both the windward and leeward crop alley (Fig. 3a). With

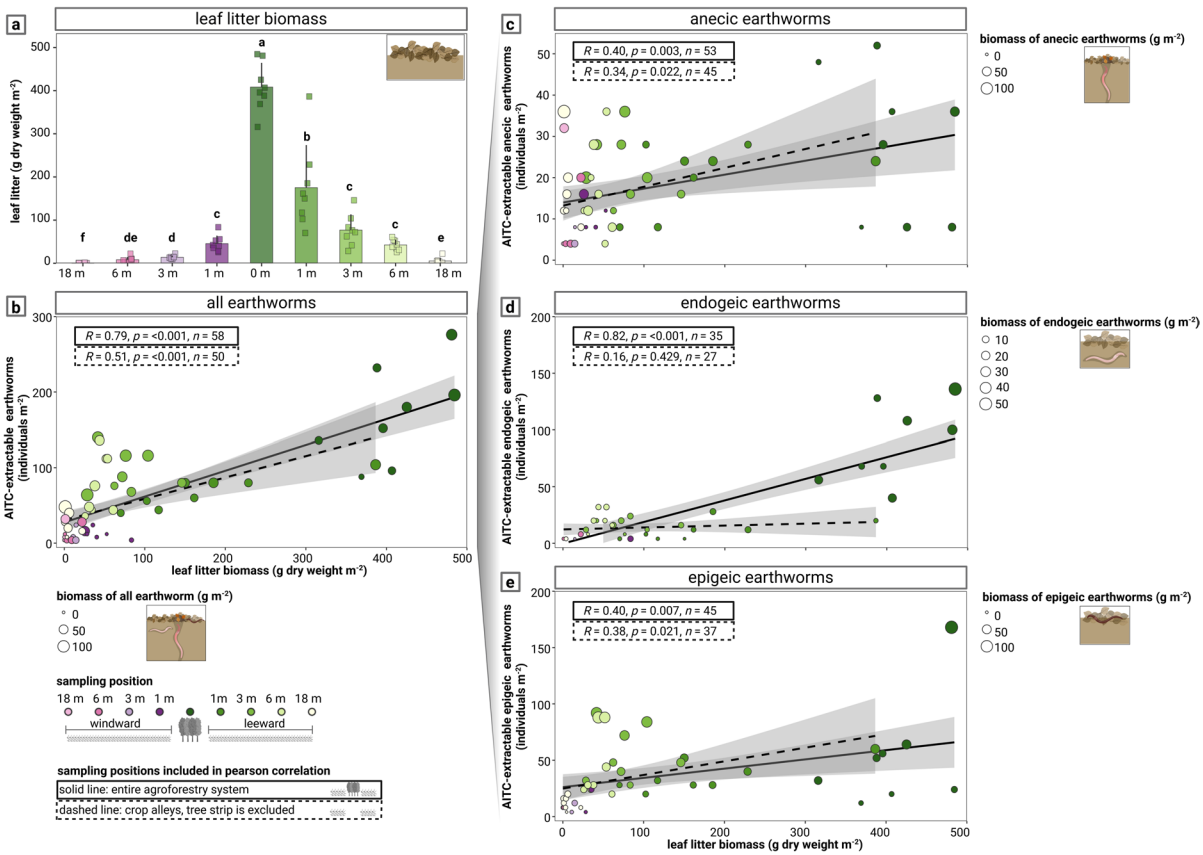

Fig. 3 Leaf litter biomass and correlations with earthworm population density. Mean leaf litter biomass (g dry weight m^{-2}) with standard deviation ($n=8$) is shown. Different letters indicate statistically significant differences ($p\leq0.05$) among the nine sampling positions (i.e. 1, 3, 6, and 18 m windward crop alley, tree strip, and 1, 3, 6, and 18 m leeward crop alley). Squares represent individual sampling locations (**a**). Correlation of mean leaf litter biomass with population density of all allyl isothiocyanate (AITC)-extractable earthworms (**b**) and different ecological groups (**c, d, e**) are indicated. Pearson Correlation coefficient *R* calculated using data from individual sampling locations, *p*-values and sample sizes are given for the entire agroforestry system (solid line) as well as crop alleys only (dashed line). Size of dots represents earthworm biomass (fresh weight g m^{-2} including gut content) at individual sampling locations (created in https://BioRender.com)

408.4 ± 56.1 g m^{-2} leaf litter biomass, the tree strip accumulated 10.6 times more leaf litter than the leeward crop alley (area-weighted average: 38.5 ± 6.9 g m^{-2}) and 51.7 times more than the windward crop alley (area-weighted average: 7.9 ± 1.8 g m^{-2}).

Leaf litter biomass and total earthworm population density showed a strong positive correlation (Fig. 3b). The correlation's strength slightly decreased if the tree strip was excluded from analysis to account for other confounding factors such as the absence of soil management under the trees. Likewise, population densities of epigeic and anecic earthworms positively correlated with the amount of leaf litter, both across all sampling positions as well as only in the crop alley (i.e. exclusion of the tree strip) (Fig. 3c, e). For endogeic earthworms, a strong positive relationship between population density and leaf litter was found across all sampling locations; however, if the tree strip was excluded from the analysis, no relationship was detected (Fig. 3d). Nevertheless, distance to the trees ($p < 0.0001$) as well as the effect of windward versus leeward crop alleys ($p < 0.0001$) remained statistically significant for endogeic earthworms if the tree strip was excluded from the model (Supplementary Table 4 in Online Resource 1).

Discussion

Our study was able to demonstrate for the first time that earthworm population density and biomass in alley cropping systems are not just affected by the distance to the trees but also show distinct responses to the leeward and windward side of the crop alley. Population density and biomass closely resembled the spatially heterogenous distribution of poplar leaf litter, which was characterized by the accumulation of litter in the tree strip and a steady decrease into the crop alleys with the leeward crop alley harboring significantly larger amounts of leaf litter than the windward crop alley. Specifically, our findings suggest that the accumulation of leaf litter on the soil surface promotes epigeic and anecic but not endogeic earthworms, which can be explained by differences in their lifestyle, especially feeding behavior.

Spatially distinct accumulation patterns of tree leaf litter in windward and leeward crop alleys

The main wind direction determined the spatial distribution pattern of tree leaf litter with greater leaf litter accumulation in the leeward than in the windward crop alley. Consistent with previous observations (Oelbermann et al. 2004; Swieter et al. 2022), leaf litter declined exponentially with increasing distance to the tree strip in both crop alleys (Fig. 3a). Previous investigations that studied poplar leaf litter distribution in alley cropping systems used litter traps (Schmidt et al. 2021; Swieter et al. 2022; Thevathasan and Gordon 1997). Assuming that leaf litter is not substantially redistributed by wind in December, we decided to collect leaf litter from the soil surface, thereby representing the amount of leaf litter available to earthworms at a given sampling position.

The considerable accumulation of leaf litter in the tree strip and the exponential decline into the crop alleys was not reflected in the assessed soil properties at 0–30 cm soil depth, except for a slightly elevated SOC content underneath the trees. Other soil chemical properties that can be affected by litter input (i.e., pH, total N, and C/N ratio) remained unaffected (Supplementary Table 1 in Online Resource 1). The elevated SOC content in the tree strip is congruent with previous results obtained for temperate alley cropping systems (Mayer et al. 2022) as well as our study site in particular (Schmidt et al. 2021). In line with previous research, C inputs through tree litter and rhizodeposition likely contributed to the accumulation of SOC under the trees.

Tree leaf litter distribution impacts earthworm communities

In this study, we chemically extracted earthworms from the soil using AITC. Generally, chemical extraction has proven to be an effective method for the expulsion of anecic and epigeic species (Bouché and Gardner 1984; Zaborski 2003); however, endogeic species are likely to be underestimated (Bartlett et al. 2006; Gutiérrez-López et al. 2016; Pelosi et al. 2009). Although combining AITC extraction with hand sorting (as stated in ISO 23611–1 2018) can increase efficiency, it is, however, highly labor-intensive, therefore hardly implementable in large-scale studies, and destructive to the topsoil (Čoja et al. 2008; Pelosi et al.

2009). For this reason, it was not applicable in our study. In agreement with previous studies, total earthworm population density and biomass were greater in the tree strip than in the crop alleys (Cardinael et al. 2019; Vaupel et al. 2023). As previously hypothesized by others, the absence of soil management as well as the accumulation of tree litter likely accounted for the promotion of earthworms under the trees (Cardinael et al. 2019; Vaupel et al. 2023). As shown above, tree leaf litter accumulated unevenly within our agroforestry system – not just between the tree strip and the crop alleys but also within the crop alleys depending on the distance from and orientation to the trees (i.e. leeward and windward side of crop alley).

Species-specific responses of earthworms to tree leaf litter distribution

Ecological groups showed varying spatial distribution patterns that were in the majority of cases linked to leaf litter accumulation. Among ecological groups, litter-dwelling earthworms (i.e. epigeics), particularly the abundant *L. castaneus* (Fig. 2f), showed the strongest response to leaf litter and were promoted up to a distance of 6 m from the tree strip leeward (Fig. 3d, e, f), confirming our hypothesis of species-specific promotion of earthworms through leaf litter. Presumably, the leeward crop alley provides a suitable habitat for epigeic earthworms due to the presence of sufficient amounts of leaf litter in combination with favorable microclimatic conditions (Jacobs et al. 2022). Their great mobility, in turn, enables them to potentially utilize this habitat (Mathieu et al. 2010).

Population density and biomass of anecic *L. terrestris* in the tree strip and leeward crop alley notably exceeded that of the windward crop alley (Fig. 2d, j). These results partly agree with D'Hervilly et al. (2020) and Vaupel et al. (2023), who observed a promotion of anecic earthworms in the tree strips in contrast to the crop alleys but did not differentiate between windward and leeward sections of the crop alley in their sampling design. Surprisingly, the promotion of anecics in the tree strip in our study was mainly driven by juveniles. We propose that the abundant root system of the densely planted poplar trees in our mature agroforestry system could have impeded the formation of vertical burrows of adult *L. terrestris*, whereas juveniles with a lifestyle resembling epigeics likely found a suitable habitat (Supplementary Table 2, 3 in Online Resource 1). This may also explain the comparatively low *L. terrestris* biomass in the tree strip and at 1 m leeward, as adults with great biomass were scarce and juveniles predominated. The tree strip, as a habitat not directly affect by agricultural management, may have promoted reproduction and served as a refuge for juveniles. In contrast to epigeic species, anecic earthworms do not depend on the constant availability of surface litter as a habitat, hence differences between the windward and leeward crop alley are probably attributable to leaf litter as an additional food source or favorable microclimatic conditions.

Unlike anecic and epigeic earthworms, endogeic species primarily feed on mineral soil as well as soil organic matter that underwent greater microbial decomposition and, hence, may utilize rather recalcitrant carbon sources (Ferlian et al. 2014; Potapov et al. 2022). Instead of mainly feeding on aboveground litter, endogeic earthworms in the tree strip may access a substantial amount of belowground litter from trees and understory vegetation as food source (e.g. rhizodeposits and root litter) (D'Hervilly et al. 2020). The belowground inputs under the trees, combined with the omission of soil management, likely led to the strong increase in both juvenile and adult endogeic earthworms in the tree strip. Nevertheless, the potential underestimation of individuals resulting from AITC extraction should be considered. Aboveground leaf litter presumably played a negligible role as a food source for endogeics as increased leaf litter biomass was not associated with greater population density in the crop alleys (Fig. 3d).

Still, the incorporation of aboveground leaf litter into the soil of the crop alleys through soil management will make it available for endogeic species, whereas belowground tree root litter inputs in the crop alley are limited, as only a small amount of tree roots was found in the topsoil of the crop alley at our study site (Schmidt et al. 2021). In an earlier study at a different study site, we observed a gradual decline in endogeic earthworm abundance with increasing distance to the tree strip and proposed that tree leaf litter exerts a positive influence on endogeics in the crop alley (Vaupel et al. 2023). While no gradual decline in endogeic earthworm population density was observed in the present study, we still found a considerable increase of endogeic earthworms up

to 6 m into the leeward crop alley as compared to its windward counterpart that we cannot attribute to the availability of aboveground leaf litter (Fig. 2e, 3d). Therefore, future studies should investigate effects of tree leaf litter on earthworm communities after incorporation through soil management as well as litter preferences of earthworms in agroforestry systems featuring multiple tree species. Furthermore, environmental factors (e.g. microclimatic conditions), temporal migration patterns, as well as their combination should also be investigated, as they likely accounted for the observed increase in endogeic earthworms in the leeward section of the crop alley.

Conclusion

The spatially heterogeneous tree leaf litter distribution within the alley cropping system strongly influenced earthworm communities. Here, we provide initial evidence that total earthworm population density and biomass aligned with aboveground tree leaf litter accumulation. This was reflected in a strong promotion of earthworm communities within the tree strip and distinct differences between the windward and leeward section of crop alleys, with a substantial elevation on the leeward but only few earthworms on the windward crop alley. These findings strongly suggest that the exposure to either the windward or leeward crop alley is a decisive influencing factor for earthworm population density, in addition to the distance to the trees. However, the magnitude of influence of aboveground tree leaf accumulation on earthworm communities varied among ecological groups and developmental stage (i.e. juvenile and adult). We observed species-specific variations with anecic and epigeic earthworms benefiting from aboveground leaf litter input, whereas no influence on endogeic species was apparent, most likely due to their distinct habitat requirements and feeding behavior. Altogether, our results underline the overall promoting effects of aboveground tree leaf litter in agroforestry systems on earthworm communities. Future research should investigate the effect of tree leaf litter incorporation into the soil and include confounding variables such as environmental factors and temporal migration patterns of earthworms.

Author contributions AV and LB contributed to the conception and design of the study. AV, LB, MA, OE and VMT performed the field work. AV, LB, OE and VMT performed the laboratory work. AV and VMT performed the data analysis. VMT wrote the first draft of the manuscript. AV, LB, MA, NH and OE critically revised the manuscript. All authors read and approved the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. VMT was supported by funds of the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH) based on a decision of the Parliament of the Federal Republic of Germany via Agency for Renewable Resources (FNR) under the funding program "Sustainable Renewable Resources" (project MODEMA, grant number 2222NR061H). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability Raw data analyzed during the current study is available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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