Responses of earthworm communities to crop residue management after inoculation of the earthworm Lumbricus terrestris (Linnaeus, 1758)

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Abstract

Earthworms are important for soil functioning in arable cropping systems and earthworm species differ in their response to soil tillage and crop residue management. *Lumbricus terrestris* (Linnaeus, 1758) are rare in intensively tilled arable fields. In two parallel field trials with either non-inversion (NIT) or conventional tillage (CT), we investigated the feasibility of inoculating *L. terrestris* under different crop residue management (amounts and placement). Simultaneously, we monitored the response of the existing earthworm communities to *L. terrestris* inoculation and to crop residue treatments in terms of earthworm density, species diversity and composition, ecological groups and functional diversity. *L. terrestris* densities were not affected by residue management. We were not able to infer effects of the inoculation on the existing earthworm communities since *L. terrestris* also colonized non-inoculated plots. In NIT and two years after trial establishment, the overall native earthworm density was 1.4 and 1.6 times higher, and the epigeic density 2.5 times higher, in treatments with highest residue application (S_{100}) compared to 25% (S_{25}) or no (S_0) crop residues, respectively. Residue management did not affect earthworm species composition, nor the functional trait diversity and composition, except for an increase of the community weighted means of bifide typhlosolis in S_0 compared to S_{100}. In CT, however, crop residues did have a strong effect on species composition, ecological groups and functional traits. Without crop residues (S_0), epigeic density was respectively 20 and 30% lower than with crop residues placed on the soil surface (S_{100}) or incorporated (I_{100}). Community composition was clearly affected by crop residues. Trait diversity was 2.6 to 3 times larger when crop residues were provided, irrespective of placement. Crop residues in CT also resulted in heavier earthworms and in a shift in the community towards species with a thicker epidermis and cuticle, a feather typhlosolis shape, and a higher average cocoon production rate. We conclude

that earthworm communities under conventional tillage respond more strongly to the amount of crop residue than to its placement. Under non-inversion tillage, crop residue amounts affected earthworm communities, but to a smaller degree than under conventional tillage.

Key-words: arable field, tillage, crop residue availability, trait-based approach, community weighted mean, Rao's quadratic entropy

1. Introduction

Earthworms contribute crucially to soil processes, including in arable cropping systems (Edwards, 2004) and have been classified into ecological groups (Bouché, 1977) to infer effects on soil functioning. Endogeic species burrow horizontally in deeper soil layers and are geophagous, feeding on soil organic matter. Epigeic species inhabit the topsoil without much burrowing and anecic species dig deep permanent burrows with important effects on continuous burrow formation and water infiltration (Keith and Robinson, 2012). Both epigeics and anecics are saprophagous and feed on plant litter on the soil surface (Curry and Schmidt, 2007). Earthworm communities in arable fields are dominated by endogeics (e.g., Crittenden et al., 2014; Frazão et al., 2017), whereas epigeics and anecics usually occur at low densities, if at all. This may result in an underperformance of earthworm-mediated soil functions that are central for soil quality (Andriuzzi et al., 2015; Postma-Blaauw et al., 2006). The scarcity of epigeics and anecics in arable fields is thought to be the result of intensive conventional tillage (Chan, 2001): direct negative effects are exposure to predation and destruction of permanent burrows of deep-burrowing anecics, and indirect effects are related to crop residue incorporation into the soil profile. Residue incorporation is negative for epigeics and anecics (Frazão et al., 2019), but positive for endogeics, by increasing the soil organic matter in the deeper layers of the soil profile. Farmers are keen on having anecics inhabiting their arable soils, due to their contribution to soil structure formation and water infiltration (Andriuzzi et al., 2015; Bertrand et al., 2015). Previous studies have reported the effects of the anecic *Lumbricus terrestris* (Linnaeus, 1758) on soil porosity and other soil fauna (enchytraeids, nematodes and other earthworms) seventeen years after inoculation (Nuutinen et al., 2017).

Community response to disturbance has traditionally been analysed through taxonomic approaches, focussing on species richness and composition (Feld et al., 2009), and in case of earthworms also through broad ecological groups. However, additional information on the functional ecology of communities may reflect important patterns of community assembly and species coexistence (Mouchet et al., 2010), which can be better predictors of ecosystem function than taxonomic indicators (Gagic et al., 2015). In this respect, Ricotta and Moretti (2011) argued that community weighted means (CWM) (Garnier et al., 2004) and Rao's quadratic entropy (RaoQ) (Botta-Dukát, 2005) represent two complementary aspects of functional composition and diversity of communities, i.e. the mean and the diversity of functional traits within a given species assemblage, respectively. Inoculating *L. terrestris* in combination with improved conditions conducive to its survival, as well as stimulating epigeics through the accessibility of crop residues on the soil surface could be an alternative to amend functional diversity of earthworm communities in arable fields.

In the present study, we investigated the response of earthworm communities to crop residue amount and placement in the soil profile, in arable fields under different tillage practices: conventional mouldboard ploughing (hereafter "CT") and non-inversion tillage (hereafter "NIT"). Our objectives were: (i) to evaluate the feasibility of inoculating *L. terrestris* under different crop residue management in the two tillage systems; (ii) to assess how local earthworm communities (density, diversity, species composition, ecological groups, and functional diversity) are affected by crop residue management and inoculation of *L. terrestris*. In any trait-based approach, one of the critical aspects is trait selection. Here, we chose traits that are expected to respond to food availability and position in the soil, i.e. body weight, number of cocoons, time to maturity, reproductive strategy, typhlosolis shape, and tegument (cuticle and epidermis) thickness.

We hypothesised that i) the inoculation of *L. terrestris* would be more successful where crop residues were provided on the soil surface, particularly concurring with less intensive soil disturbance typical of the NIT trial; ii) crop residue management and the inoculation of *L. terrestris* would affect the earthworm community composition, with epigeics benefitting from crop residue availability on the soil surface, but being subject to competition with *L. terrestris* where inoculated; and endogeics being facilitated by the inoculation of *L. terrestris*; and iii) the availability of crop residue on the soil surface would favour trait diversity, as well as heavier earthworms with larger reproductive output, faster developmental time, with a less complex typhlosolis shape and thinner tegument.

2. Materials and Methods

2.1 Study site

In the summer of 2013, two parallel field trials were installed at the PPO Westmaas research farm of Wageningen University and Research, located in the southwest of The Netherlands. The trials were situated in two adjacent arable fields that differed in tillage practices since 2009: CT and NIT. The CT field was mouldboard ploughed annually and the NIT field was loosened without soil inversion, either with a paragrubber (2009-2012 and 2014-2015) or with a spading machine (2013). Previous sampling indicated that both fields lacked *L. terrestris* (Frazão et al., unpublished results). The soil type is a Haplic Fluvisol (WRB, 2006), developed in calcareous marine deposits with a sandy clay loam texture (49% sand, 24% clay) and a pH of 7.9 in the top 30 cm. Daily average temperature was 10.8 °C and annual precipitation was 883 mm over the experimental period (Royal Netherlands Meteorological Institute, 2016). The crop rotation of both fields was as follows: winter wheat in 2013, followed by radish (*Raphanus sativus* subsp. *oleiferus*) as cover crop, sugar beet in 2014 and winter barley in 2015. Both fields received similar mineral fertilization and synthetic crop protection; no animal manure was used throughout the experimental period.

2.2 Experimental design

In August 2013, 24 plots (6x6 m) were established in the two neighbouring tillage fields, arranged in a split-plot design with two factors and replicated in four blocks. Within each block, the main plots were randomly assigned to the factor *L. terrestris* inoculation (two levels: "+", with inoculation and "–", without inoculation), and subplots were randomly assigned to the factor crop residue application (three levels that differed per trial). In the CT field the factor crop residue application comprised three levels: (i) no crop residues (hereafter "S_0"), (ii) incorporation of crop residues (hereafter "I_{100}"), and (iii) soil surface applied residues (hereafter "S_{100}"). In the NIT field, the factor crop residue application comprised the levels (i) no residues (hereafter "S_0"), (ii) 25% of crop residues placed on the soil surface (hereafter "S_{25}"), and (iii) 100% of crop residues placed on the soil surface (hereafter "S_{100}") (Fig. 1A). Inherent to the tillage regimes, crop residue treatments under study were not exactly the same for the NIT and CT systems, as it was impossible to test an incorporated crop residue treatment under non-inversion tillage.

The crop residue amounts used in S_{100} (CT and NIT trials) and I_{100} were the same and were applied annually in both trials. We kept the crop residue types as similar as possible across the years, depending on availability. In 2013 a mixture of winter wheat stubble and radish

(*Raphanus sativus* subsp. *oleiferus*) was applied, as those were the crops grown in both fields. In 2014 a mixture of winter wheat straw and radish was applied after the removal of sugar beet residues, which was the crop harvested at the time, and in 2015 only winter barley stubbles were applied. Grain crop residues were applied at a rate of 4.7 t ha^{-1} and radish at a rate of 1.1 t ha^{-1} (DW) in the treatments S_{100} and I_{100} of both trials.

In October 2013, seven weeks before Fall tillage, (sub)adults of *L. terrestris* (Starfood, Barneveld, The Netherlands) were inoculated in the "+" plots of both fields at a density of 20 ind. m^{-2}. For a week prior to inoculation, the individuals were acclimatized in tempex boxes with a compost substrate provided by Starfood, at 6 °C in a climate chamber. Each individual was carefully checked and the ones not appearing healthy and vigorous were discarded. In each of the "+" plots, a 3x3 grid with 2 m spacing (Fig. 1B) was established and around each of the intersects four holes were dug to 40 cm deep, and 20 individuals of *L. terrestris* were placed in each hole. Soil pits were moistened before and after introducing earthworms, and refilled with moistened soil. The order of the plots to be inoculated was *a priori* randomized. To prevent predation by birds, flags and cannon sounds were used and upon observing mole activity, mole traps were placed in the fields.

2.3 Data collection

2.3.1 <u>Earthworm sampling</u>

Earthworms were sampled in Spring (May) and Fall (September) 2014 and in Fall (October) 2015 in the CT and NIT trials. During the first two sampling events three soil monoliths of 30x30x20 (lxbxd) cm were collected in each plot, whereas in the last sampling event, only two monoliths were taken per plot, due to logistical constraints. After digging a monolith, 2.5 l of allyl isothiocyanate (AITC) solution (1 ml AITC dispersed in 20 ml 2-propanol added to 10 l of water and mixed thoroughly) was applied to the pit, to expel deep burrowing earthworms. Andriuzzi et al. (2017) have demonstrated that this is a suitable earthworm sampling method for all earthworm ecological groups in arable systems. Individuals expelled by AITC were rinsed and collected alive for further laboratory work. Monoliths were stored separately in plastic bags for transportation and storage in the lab at 2 °C until hand-sorting.

2.3.2 Earthworm sample processing and body weight measurements

Earthworm samples were hand-sorted in the laboratory and individuals were kept alive in pots with moist paper tissue at 16 ˚C for 48 h to void the guts. After voiding of the guts, live body weight and developmental stage (juvenile, subadult or adult) were recorded individually for the Spring 2014 samples. Specimens were then killed in 70% alcohol and identified to species immediately. For the hand-sorted individuals collected in Fall 2014 and 2015, some adjustments were made to reduce sample processing time. Therefore, (part of) the individuals were stored in 70% alcohol immediately after voiding of the guts. In those cases, the dead body weight was measured after placing the specimens in water for 10 minutes, to allow body rehydration. As in Spring 2014, individuals were weighed, assigned to their developmental stage and identified to species. To correct for differences in the method of body weight measurement among different samplings, 20 individuals sampled in Spring 2014 (live body weights ranging from 0.1 to 1.6 g) were re-weighed after being stored for two years in alcohol. A linear regression (Equation 1, adjusted $R^2 = 0.90$; $p = 1.318 \times 10^{-10}$) was computed between the rehydrated alcohol-conserved body weight of 2016 ($BW_{ethanol}$ in Equation 1) and the live body weight of 2014 (BW_{live} in Equation 1).

$$BW_{\text{live}} = BW_{\text{ethanol}} \times 1.05663 + 0.03372 \qquad \text{Equation 1}$$

The regression coefficients in Equation 1 were used as a correction factor to express all body weight values per g live weight. For the purpose of this study, only (sub)adult individuals were used, given that trait values for juveniles are lacking and might differ from adult trait values. Adult individuals were identified using Sims and Gerard (1999) and juveniles using (Stöp-Bowitz, 1969), and complete individuals, as well as heads, were considered for identification. Body weight was measured for intact individuals only excluding some 12% of the sampled specimens.

2.4 Functional traits

We assessed seven functional traits (five continuous and two categorical) (Table 1) that were expected to respond to resource availability: body weight in grams (measured per individual, corrected for different weighing methods at different sampling occasions – see equation 1 – and averaged for each species over the study duration), average number of cocoons produced per year, reproductive strategy, typhlosolis shape, average time to maturity in weeks (Hedde et al., 2012a), and cuticle and epidermis thickness in µm (Briones and Álvarez-Otero, 2018). Body weight was used as an indicator for the condition of the individuals and relates to the energetic investment in growth; reproductive strategy and number of cocoons relate to the investment in reproduction, thereby reflecting the potential for population recovery after disturbance; typhlosolis shape relates to the nutrient uptake efficiency (Pelosi et al., 2013); time to maturity reflects the investment in individual development, and often represents a trade-off with reproductive investment (Stearns, 1976); finally, tegument thickness (cuticle and epidermis) reflects the burrowing ability of the species (Briones and Álvarez-Otero, 2018).

2.5 Data analysis

2.5.1 Taxonomic and ecological group approaches

Earthworm species densities and ecological group densities (epigeic and endogeic) of subsamples were averaged per plot for each sampling period and expressed as number of individuals per meter square. Shannon diversity index was computed per plot, as a measure of species diversity (richness and relative abundance).

2.5.2 Trait-based approach

Functional diversity was assessed by community weighted means (CWM) and Rao's quadratic entropy (RaoQ). CWM was calculated for each trait, as the mean of trait values for each species in the community, weighted by the relative abundance of the species associated with that value (Lavorel et al., 2008). RaoQ was calculated for the complete set of traits as the dissimilarity between pairs of species within each plot, weighted by the product of the relative abundance of both species (Leps et al., 2006).

2.5.3 Statistical analysis

The taxonomic, ecological group and trait data were analysed using univariate and multivariate statistics. NIT and CT trials were considered separate datasets, to avoid statistical pseudoreplication (Hurlbert, 1984), since the sample size of each tillage system was only one. As we were interested in the effects of inoculation of *L. terrestris*, we excluded this species from the analyses. The univariate approach consisted of mixed linear models using crop residue application and inoculation treatments as fixed factors. The structure of the split-plot design was incorporated in each model by nesting the crop residue application within the inoculation factor in the random factors. Several response variables were modelled for each sampling season: (sub)adult earthworm density, Shannon diversity index, epigeic and endogeic densities, CWM for each trait, and RaoQ for all traits combined. If overall linear mixed models were statistically significant at $p < 0.05$, pairwise comparisons were computed. P-value adjustments to avoid inflation type I errors were considered necessary when the interaction between the fixed effects was significant due to the large number of pairwise comparisons. In those cases, *post-hoc* adjustments (Tukey HSD) were used. Overall models' distribution and variance assumptions were inspected visually, and if needed, a variance structure was used to avoid heteroscedasticity (Zuur et al., 2009).

The multivariate approach consisted of testing the centroid "location" (Anderson, 2001) and the "dispersion" (Anderson, 2006) of the community's species composition. An analogy towards the CWM was made with a multivariate test of the "CWM composition". The centroid "location" analysis is a non-parametric version of a multivariate ANOVA, whereas the "dispersion" analysis tests the homogeneity of multivariate dispersions (Anderson, 2006). Both analyses are based on dissimilarity matrices. For the species composition analysis, a Bray-Curtis dissimilarity matrix was used, after square root transformation of the earthworm density data. For the CWM composition analysis a Gower dissimilarity matrix was used, allowing the combination of categorical and continuous variables. If the centroid location analysis was significant, a nonmetric multidimensional scaling (NMDS) was plotted to visualize the results. As in the univariate analysis, crop residue application and *L. terrestris* inoculation were used as explanatory variables, and the split-plot design structure was incorporated in a permutation scheme that considered our nested design.

We present the Fall 2015 results in the main text of this article. As most univariate and multivariate tests of Spring and Fall 2014 appeared as not significant, these are presented in the Supplementary materials A (Tables S1 – S9). The raw datasets of all seasons for both experimental trials are available in the Supplementary materials B. All analyses were performed with R 3.3.1 (R CoreTeam, 2014), using packages nlme 3.1–131, lsmeans 2.27-61, FD 1.0-12, ade4 1.7-6 and vegan 2.4-5.

3. Results

3.1 Inoculation of *Lumbricus terrestris*

L. terrestris was found in both experimental trials throughout the sampling seasons (77 individuals in NIT *vs.* 46 in CT, of which 8 and 5 individuals were (sub)adults, respectively), although the patterns were erratic and unrelated to the inoculation and crop residue treatments (Table 2). Furthermore, besides the inoculated (sub)adult individuals, juveniles were also found (Table 2), already in Spring 2014 (just seven months after inoculation). Highest average juvenile density of 9.3 ind.m^{-2} was recorded in Fall 2014 in NIT – S_{25}- and in CT – S_{100}+ (Table 2), while highest average densities of (sub)adults reached 2.8 ind.m^{-2} in NIT – S_{25}+ and 1.9 ind.m^{-2} in CT – I_{100}+, also in Fall 2014. By the end of the study, in Fall 2015, no (sub)adults of *L. terrestris* were found in the CT trial, nor in the non-inoculated plots of the NIT trial. However, irrespective of the crop residue treatments, 1.4 ind.m^{-2} were found in the inoculated plots of the NIT trial. Juveniles were found in higher densities, particularly in the NIT trial, in erratic patterns unrelated to crop residue treatments.

3.2 Earthworm density

In NIT, in Fall 2015, native earthworm (sub)adult density was higher in S_{100} than in S_{25} and S_0 (60 % and 37%, respectively, Table 3), whereas it was not affected by the inoculation of *L. terrestris* nor by the interaction between both factors. In CT, native earthworm (sub)adult density was not affected by *L. terrestris* inoculation, irrespective of residue application (Table 3).

3.3 Species diversity and composition

Besides the inoculated *L. terrestris*, (sub)adult individuals of six other earthworm species were found in the two tillage trials: *Aporrectodea caliginosa* (Savigny, 1826), *Allolobophora chlorotica* (Savigny, 1826), *Aporrectodea rosea* (Savigny, 1826), *Eiseniella tetraedra* (Savigny, 1826), *Lumbricus castaneus* (Savigny, 1826) and *Lumbricus rubellus* (Hoffmeister, 1843). Among them, only one individual of *E. tetraedra* was found in each trial in Spring 2014. *L. castaneus* was not detected during Fall 2014 (both trials), nor in Spring 2014 in the CT trial.

In both trials in Fall 2015, Shannon diversity index was low (≤ 1.0) and was not affected by *L. terrestris* inoculation, irrespective of residue application (Table 3). Furthermore, in NIT, local earthworm community composition was not affected by *L. terrestris* inoculation, irrespective of residue application, whereas in CT, earthworm community composition showed differences in terms of centroid location in the multivariate dimensional space, both with respect to the crop residue application and to *L. terrestris* inoculation (Table 4, Fig. 2). The community composition showed a separation between the surface-applied (S_{100}) and the incorporated (I_{100}) crop residue treatments *vs.* the treatment where no crop residues (S_0) were provided. The separation between *L. terrestris* inoculation treatments was less clear (Fig. 2), concurring with the p-value of 0.042, which although significant was rather high.

3.4 Ecological groups' distribution

The NIT trial, in Fall 2015 showed a pronounced effect of surface availability of crop residues on earthworm ecological groups (Table 3). Epigeics' density was about 2.5 times higher in S_{100} than in the other treatments. Endogeics also increased significantly with crop residue availability on the soil surface, although the effect was less pronounced, and the patterns were more erratic. Endogeics were about 40% more abundant in S_{100} than in S_{25}, but were not significantly different from S_0 (Table 3). The inoculation of *L. terrestris* did not affect earthworms in terms of ecological groups (Table 3).

In the CT trial only epigeics responded to the crop residue treatments in Fall 2015 (Table 3). Epigeic density in S_0 treatment was 20 and 30% lower than when residues were applied on the soil surface (S_{100}) or incorporated into the soil (I_{100}), respectively. No significant differences in density of epigeics were found between S_{100} and I_{100} (Table 3). Similarly to the findings in the NIT trial, the inoculation of *L. terrestris* did not affect earthworms in terms of ecological groups (Table 3).

3.5 Trait composition and diversity

In the NIT trial, CWM of typhlosolis shape, body weight and epidermis thickness of (sub)adult earthworm species were significantly affected in Fall 2015 by crop residue availability on the soil surface (Table 5). In S_{100}, the proportion of species with a bifide typhlosolis was significantly smaller (-15%) compared to absence of crop residues, whereas I_{100} did not differ from other treatments (Table 5). Neither body weight nor epidermis thickness, although significant in the overall linear models, showed significant pairwise differences among any of the three crop residue treatments.

In the CT trial in Fall 2015, the CWM body weight, number of cocoons, typhlosolis shape, and cuticle and epidermis thickness were affected by the crop residue application. The distribution of reproductive strategies was modified by the inoculation of *L. terrestris,* and the time to maturity by the interaction of both factors (Table 6). CWM of (sub)adult earthworms' body weight was larger in S_{100} and I_{100} than in the S_0 (16% and 9%, respectively). CWM of the number of cocoons was 22% higher in S_{100} than in I_{100}, which was, in turn, 40% higher than in S_0. The proportion of species with a bifide typhlosolis was 52% and 23% higher in S_0 than in S_{100} and I_{100}, respectively. CWM of cuticle thickness was 33% larger in S_{100} than in I_{100}, and in turn, it was 57% larger in I_{100} than in S_0. Epidermis thickness was 4% larger in S_{100} and 3% larger in I_{100} than in S_0. Inoculation of *L. terrestris* increased biparental reproduction in the local earthworm community by 6%. Finally, an interactive effect between crop residue and inoculation of *L.* terrestris was found for the CWM of time to maturity: it was 11% higher in S_0+ treatments than in I_{100}+, and between 11 to 13% higher in I_{100}- and S_0- than in S_{100}-.

Multivariate analyses showed no significant patterns in CWM composition for NIT in Fall 2015, but in CT, plots with crop residues (S_{100} and I_{100}) were separated from plots without (S_0) (Table 7, Fig. 3). Although significant, trait composition as affected by the inoculation of *L. terrestris* (Table 7) did not show such a clear separation between plots where *L. terrestris* had been inoculated or not (Fig. 3).

Regarding trait diversity in Fall 2015, RaoQ was 2.6 and 3.0 times higher in S_{100} and I_{100} than when no crop residues (S_0) were provided in CT, while not different in NIT (Table 8).

4. Discussion

4.1 Attainment of *L. terrestris* inoculation in arable fields

Particularly from a farmer's perspective, *L. terrestris* was successfully inoculated in both experiments, since this species has established and reproduced in both fields. However the success rate depended on tillage regime. The NIT trial provided better conditions for establishment of this species, considering that 1.7 times as many individuals were found compared to the CT trial. Additionally, more reproduction took place in the NIT trial, as 1.7 times more juveniles were found compared to the CT trial. Our ratio of *L. terrestris* individuals collected between the CT and the NIT trials is much smaller than that of Nuutinen et al. (2011), who found an average of 0.6 ind. m^{-2} and 4.3 ind. m^{-2} in conventional tillage and no-till systems, respectively. However, in their study, the time span between *L. terrestris* inoculation and sampling was 13 years. Surprisingly, in our study, *L. terrestris* was also found in non-inoculated plots, sometimes even at higher densities than in plots that had been inoculated. We could not enclose the experimental plots with physical barriers, which would have, most likely, minimized the colonization of non-inoculated plots by *L. terrestris*. The existence of physical barriers would have hampered the use of agricultural machinery, which would not be feasible under conventional agricultural practices. Instead, we maximised the distances between inoculated *vs.* non-inoculated plots (between 21 and 30 m; Fig 1A) to prevent colonization of non-inoculated plots, but unfortunately this appeared not to be sufficient. Although Mather and Christensen (1988) quantified the length of the surface movement of individuals of *L. terrestris* at 19 m in one night, Eijsackers (2011) reviewed that in grazed grasslands the population's areal expansion varied between 1.5 and 4 m yr^{-1}, and therefore the distances between plots in our experiments were expected to be sufficient to avoid the colonization of non-inoculated plots by *L. terrestris*. However, besides active surface dispersal, passive dispersal by tractor tires (Marinissen and van den Bosch, 1992) may also have promoted the occurrence of *L. terrestris* in non-inoculated plots. In both of the two tillage systems in Spring and Fall 2014, crop residue amount or placement had no effect on *L. terrestris* density, suggesting that *L. terrestris* populations were not necessarily restricted by crop residue availability, in opposition to our first hypothesis. Instead of becoming established where crop residues were not limiting, it is likely that *L. terrestris* have burrowed elsewhere and initiated movement to forage (Butt et al., 2003) in the initial phase of experimentation. On the other hand, by the end of the study (i.e. Fall 2015), distribution patterns of *L. terrestris*, particularly juveniles, seemed to be related to crop residues application, suggesting that the response of this species to crop residue availability takes time. In the NIT trial, densities of juveniles were highest with full crop residue application, as well as in the CT trial, provided that residues were on the soil surface.

Our choice of crop residue for earthworms, both the local communities and the inoculated *L. terrestris* was pragmatic and conformed with common agricultural rotations, i.e., wheat or barley followed by radish as cover crop. Although indoor experiments have shown that earthworms can have good survival rates with those food sources (Al-Maliki and Scullion, 2013; Frazão et al., 2019; Giannopoulos et al., 2010), there is also evidence that earthworms, and in particular *L. terrestris*, show dislike for feeding on species belonging to the Brassicaceae family (Valckx et al., 2011), when subjected to food choice experiments. However, wheat and barley straw applications have been shown to increase *L. terrestris* densities in natural populations (Stroud et al., 2016), while cover cropping with radish has shown no effects on populations of this species (Stroud et al., 2017).

4.2 Crop residue management and earthworm communities

Our results demonstrate that the local community of adult earthworms was affected by crop residue availability and position, both in NIT and CT systems, although crop residue effects were not similar between the tillage types. We were not able to infer effects of the inoculation on the existing earthworm communities since *L. terrestris* colonized non-inoculated plots via active or passive dispersal.

In CT, neither the amount nor the position of crop residues affected (sub)adult total earthworm density or Shannon diversity (Table 3). However, as long as crop residues were applied, either at the surface or incorporated at ploughing depth, epigeics' density was 3.5 to 5 times higher than in absence of residues. A similar response was found for species composition which differed between plots with and without crop residues (Fig. 2). These results suggest that under conventional tillage the application of crop residues, rather than the position in the soil profile, plays a larger role in shaping earthworm communities. These outcomes were unexpected as we hypothesised that epigeics, being known to feed on decaying litter (Bouché, 1977; Curry and Schmidt, 2007), would only profit from crop residues applied on the soil surface. Furthermore, as we anticipated that the most important responses in community composition due to crop residue availability would be found for epigeics, we had expected that when studying species composition in the multivariate space, plots without residue would be more similar to those in which the crop residue was incorporated. Incorporation of crop residues under conventional tillage is often claimed as a reason for the unsuitability of arable fields for epigeics (Kladivko, 2001). Furthermore, in a mesocosm experiment, Frazão et al. (2019) demonstrated that the growth and survival of *L. rubellus* was reduced when crop residues (mixture of wheat straw and radish) were incorporated at 30 cm soil depth.

In the NIT system, crop residue amount had a pronounced effect on earthworm density as well as density of epigeics (Table 3), whereas species composition did not differ among the crop residue treatments, which was rather surprising (Fig. 2). Crop roots that were not removed after harvest may have been a food source to the earthworm populations in the no residue treatments. However, this does not explain the differences in epigeic density among crop residue treatments, unless the duration of our trials was not long enough to pick effects on species composition.

In CT, crop residue stimulated trait diversity (Table 8) and modified the community trait profiles (Table 6). However, in analogy to the ecological group and community composition analyses, the trait based approach indicated that the location of crop residue application (soil surface and incorporated) was trivial, in respect to trait diversity and CWM. The observation in the CT trial that trait diversity (RaoQ) was positively affected by crop residue provision suggests some degree of niche differentiation in those communities. Lower competition for resources as well as higher efficiency in resource utilization have been linked to higher ecosystem function (Mason et al., 2005). Applying crop residues, either on the soil surface or incorporated in the profile, contributed to increased earthworm body weight, and shifted the earthworm community towards species with a thicker epidermis and cuticle, a feather shaped typhlosolis, and species with relative high average rates for cocoon production (Table 6). Moreover, earthworm species that, on average, produce more cocoons and that have a relatively thick cuticle profited even more when crop residues were applied on the surface. However, those effects were always smaller in magnitude than when compared to the no residue treatments (Table 6). These findings suggest that crop residue availability, irrespective of position in the soil profile, promotes earthworms with better burrowing abilities (i.e., larger tegument thickness, see Briones and Álvarez-Otero (2018)), higher recovery from disturbance (i.e., higher reproductive output, measured as average number of cocoons), higher nutrient uptake efficiency (i.e., larger proportion of species with a feathered typhlosolis, see Pelosi et al. (2013)). These characteristics may contribute to a higher performance of the earthworm community (i.e., larger body weight). The suggestion of higher nutrient uptake efficiency by the community is surprising, as we expected that removing and not applying crop residues as a food source would select for species with high nutrient uptake efficiency, i.e. species with a feather shaped typhlosolis. However, typhlosolis morphology is unlikely to be the only trait to determine nutrient uptake efficiency. For example Thakuria et al. (2010) highlighted that earthworm species' gut wall-associated bacterial communities shifted according to food sources provided, although these shifts were more strongly determined by habitat type and ecological group.

In contrast to the CT trial, in the NIT trial crop residue treatments did not affect earthworm trait diversity (Table 8) nor modified the trait profiles, with the exception of typhlosolis shape (Table 5), where patterns were similar to those observed in the CT trial.

Functional responses have been amply studied in plants (e.g., Díaz and Cabido, 2001), while little attention has been given to soil organisms. Nevertheless, earthworm functional response to disturbances has been studied, in relation to tillage intensity (Pelosi et al., 2013; Pelosi et al., 2016), flooding of floodplains (de Lange et al., 2013; Fournier et al., 2012), and soil pollution (Hedde et al., 2012b; Pérès et al., 2011). To our knowledge, this is the first study in the field focussing on earthworm functional responses to crop residue availability and position. Studies that have focused on the relationship between earthworm communities and crop residue availability with more traditional approaches, such as community composition, ecological groups or total density are also rare (but see Eriksen-Hamel et al. (2009)). The latter authors did not find, however, any differences between high *vs.* low crop residues input in earthworm abundance or biomass. Contrary to Pelosi et al. (2013) who obtained dissimilar results with different approaches in studying earthworm community responses to tillage, in our study, analysis of species composition, ecological groups and trait diversity and composition resulted in consistent outcomes in terms of response to crop residue availability and position in NIT and CT systems. Therefore, the additional value of trait-based approaches in assessing the response of earthworms to crop residues management was not fully confirmed with this study. Nevertheless, since functional traits represent explicit links between biology and environment, it remains useful to better understand which traits are affected by crop residues, and in that respect our trait-based approach has added value. In general, in CT, the provision of residues had an effect on several facets of earthworm communities, whereas in NIT, residue quantity had small effects on earthworm communities.

Finally, further research should focus on the hypothesis that increasing earthworm functional diversity, mediated by crop residue application, enhances soil functioning. However, earthworm effects might be less straightforward, as Frazão et al. (2019) found evidence of trade-offs between earthworm-mediated soil porosity and formation of large water-stable macroaggregates related to crop residue placement in the soil profile.

5. Conclusions

Our study clearly illustrates different earthworm community responses to crop residue availability in arable fields under contrasting tillage regimes. The inoculation of *L. terrestris* was successful, but the success was inconsistently related to crop residue management. In contrast, the type of tillage played an important role in terms of the success of inoculations, with less intensive tillage systems providing better conditions for this species than conventional mouldboard ploughing.

The largest differences in earthworm community responses were observed between no residues *vs.* available residues in the CT trial when using the species composition, ecological groups and trait-based approaches, whereas in the NIT trial, only the use of an ecological group approach enabled us to show an effect of crop residue amount on earthworms. Our results suggest that in arable fields earthworms are more affected by the amount of crop residue than by its position in the soil profile.

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Figure captions

Fig. 1. A) Scheme of the experimental design of the CT and NIT trials and list of treatments. B) Details of inoculation scheme within each + plot.

Fig. 2. Nonmetric multidimensional scaling (NMDS) of (sub)adult earthworm communities for the main factor crop residues (panels A) and C)) and main factor inoculation of *L. terrestris* (panels B) and D)) of the non-inversion (NIT, panels A) and B), stress = 0.13) and conventional tillage trials (CT, panels C) and D), stress = 0.16), in Fall 2015. Dissimilarity between species composition was determined through a Bray-Curtis distance matrix and earthworm density was square root transformed. Inoculated *L. terrestris* was excluded from dissimilarity matrices. Polygons in different colours indicate different crop residues (S_{100}: grey, S_{25} / I_{100}: white, S_0: black) and inoculation levels (+: black, –: grey).

Fig. 3. Nonmetric multidimensional scaling (NMDS) of CWM for the main factor crop residues (panels A) and C)) and main factor inoculation of *L. terrestris* (panels B) and D)) of the non-inversion (NIT, panels A) and B), stress = 0.08) and conventional tillage trials (CT, panels C) and D), stress = 0.05), in Fall 2015. Dissimilarity between CWM composition was determined through a Gower distance matrix. Inoculated *L. terrestris* was excluded from dissimilarity matrices. Polygons in different colours indicate different crop residues (S_{100}: grey, S_{25} / I_{100}: white, S_0: black) and inoculation levels (+: black, –: grey).

Figures

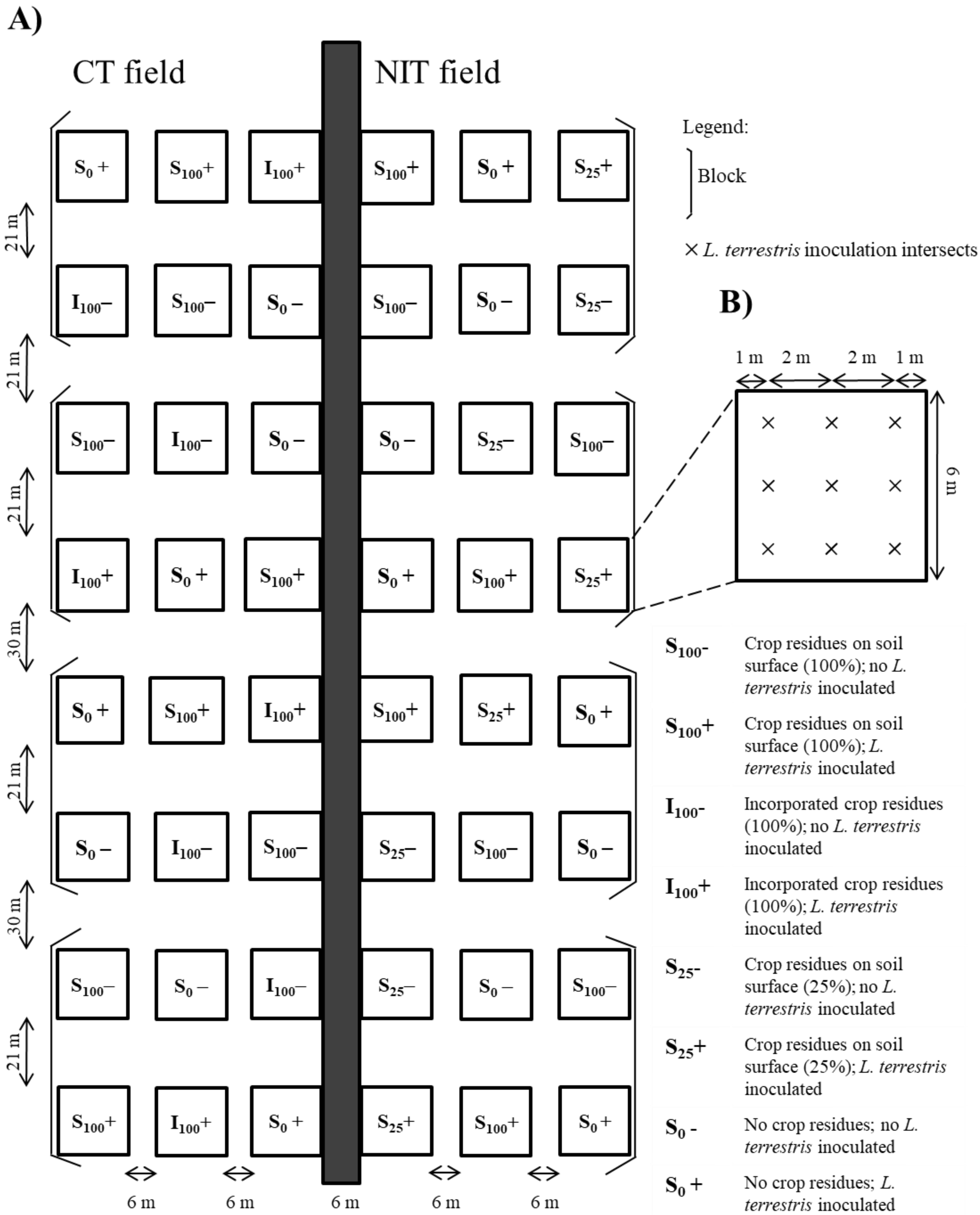
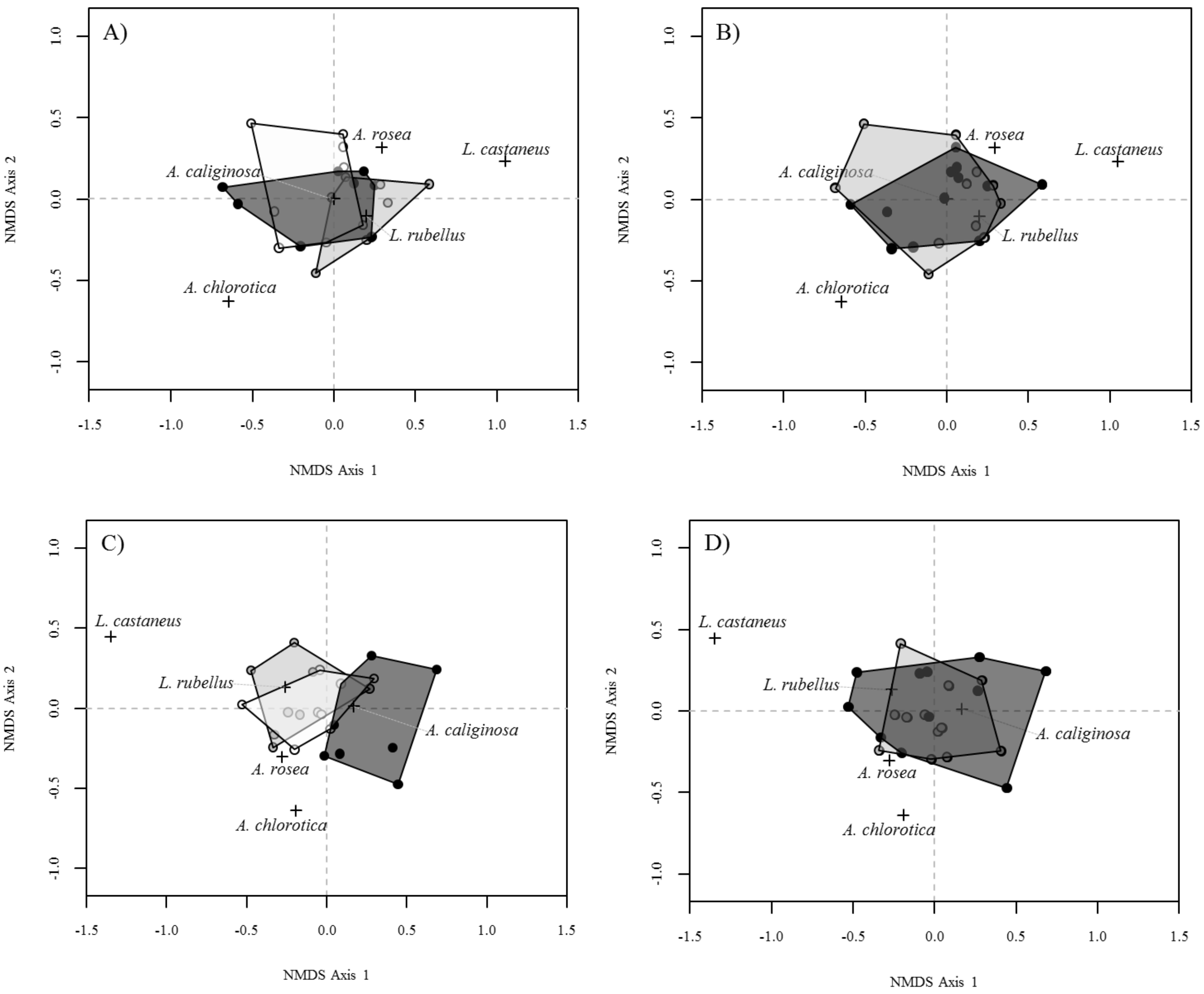

A)
CT field
NIT field
Legend:
Block
× *L. terrestris* inoculation intersects
B)
1 m
2 m
2 m
1 m
6 m
21 m
30 m
6 m
S_{100}- Crop residues on soil surface (100%); no *L. terrestris* inoculated
S_{100}+ Crop residues on soil surface (100%); *L. terrestris* inoculated
I_{100}- Incorporated crop residues (100%); no *L. terrestris* inoculated
I_{100}+ Incorporated crop residues (100%); *L. terrestris* inoculated
S_{25}- Crop residues on soil surface (25%); no *L. terrestris* inoculated
S_{25}+ Crop residues on soil surface (25%); *L. terrestris* inoculated
S_0 - No crop residues; no *L. terrestris* inoculated
S_0 + No crop residues; *L. terrestris* inoculated

Fig. 1



A)
A. rosea
L. castaneus
A. caliginosa
L. rubellus
A. chlorotica
B)
A. rosea
L. castaneus
A. caliginosa
L. rubellus
A. chlorotica
C)
L. castaneus
L. rubellus
A. caliginosa
A. rosea
A. chlorotica
D)
L. castaneus
L. rubellus
A. caliginosa
A. rosea
A. chlorotica
NMDS Axis 1
NMDS Axis 2

663 Fig. 2

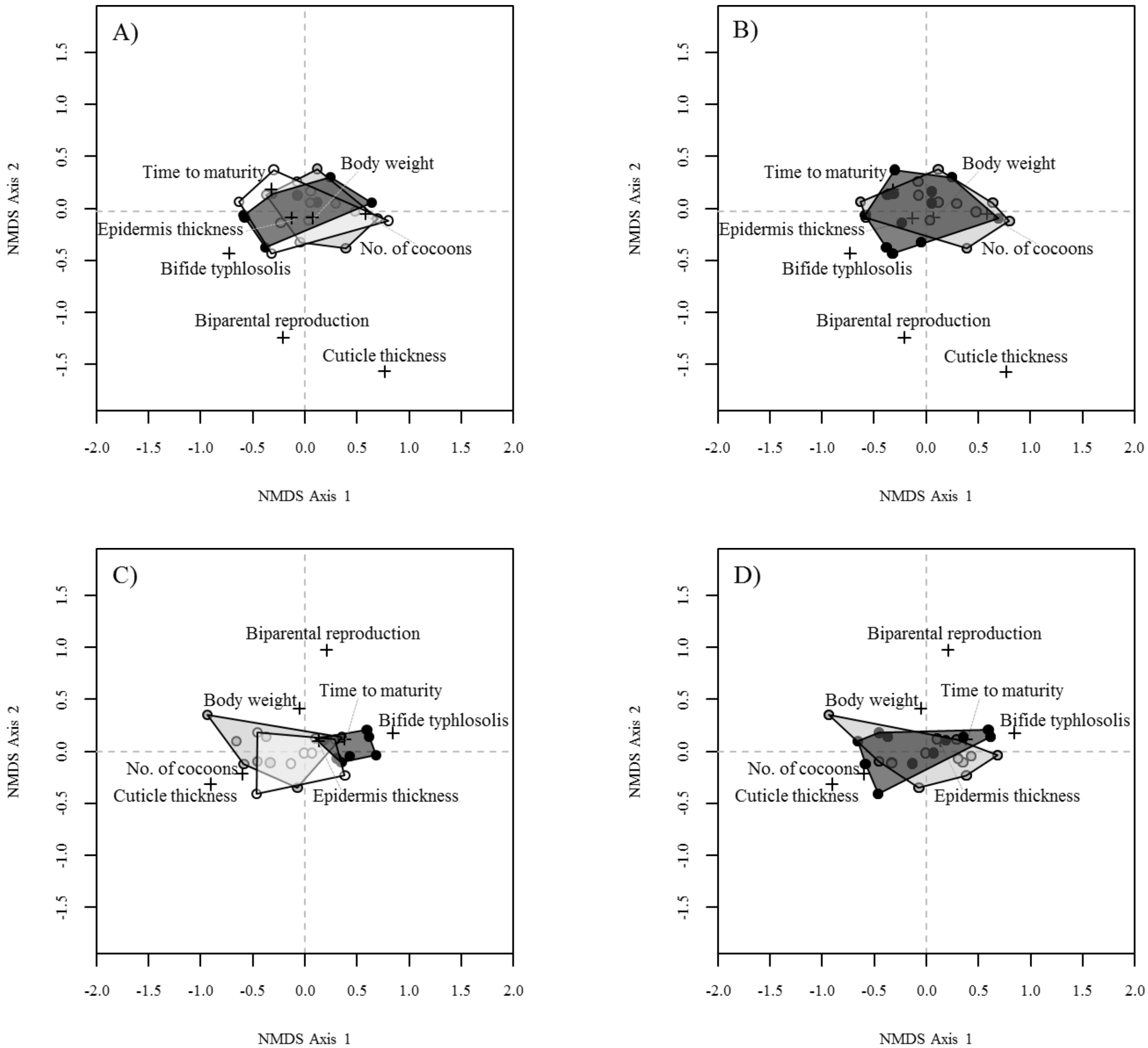

A)
B)
C)
D)
Time to maturity
Body weight
Epidermis thickness
No. of cocoons
Bifide typhlosolis
Biparental reproduction
Cuticle thickness
NMDS Axis 1
NMDS Axis 2

Fig. 3

Tables

Table 1 Literature acquired and measured (body weight) trait values of the species sampled in both trials. Earthworm species are arranged by ecological groups (first three species are endogeics; and last three are epigeics).

Species	**Mean of adult body weight (g)** †	**No. of cocoons (per year)** ‡	**Reproductive strategy** ‡	**Typhlosolis shape** ‡	**Time to maturity (weeks)** ‡	**Cuticle thickness (μm)** §	**Epidermis thickness (μm)** §
A. caliginosa	0.33	27	biparental	bifide	55	0.46	34.19
A. chlorotica	0.22	27	biparental	bifide	36	1.60	27.39
A. rosea	0.18	35	parthenogetic	bifide	55	0.67 #	32.68 #
E. tetraedra	0.08	72	parthenogetic	simple	13	1.74 #	27.27 #
L. castaneus	0.20	65	biparental	feather	24	1.74 #	27.27 #
L. rubellus	0.54	106	biparental	feather	37	3.21	39.42

† measured in this study

‡ Hedde et al. (2012a)

§ Briones and Álvarez-Otero (2018)

Not measured in Briones and Álvarez-Otero (2018). Expert knowledge of Prof. Dr. Maria Briones

Table 2 Mean, standard error (SE) and occurrence in number of plots (Freq) of the density of (sub)adult and juvenile individuals of *L. terrestris* (ind. m^{-2}) in the non-inversion tillage (NIT) and conventional tillage (CT) trials, for each of the sampling times (Spring 2014, Fall 2014 and Fall 2015). For legend of the treatments, see Figure 1.

	NIT trial							**CT trial**					
	Spring 2014		**Fall 2014**		**Fall 2015**			**Spring 2014**		**Fall 2014**		**Fall 2015**	
	Mean (SE)	**Freq**	**Mean (SE)**	**Freq**	**Mean (SE)**	**Freq**		**Mean (SE)**	**Freq**	**Mean (SE)**	**Freq**	**Mean (SE)**	**Freq**
(Sub)adult individuals													
S_{100}-	0.0 (0.0)	0	0.9 (0.9)	1	0.0 (0.0)	0	S_{100}-	0.0 (0.0)	0	0.9 (0.9)	1	0.0 (0.0)	0
S_{100}+	0.0 (0.0)	0	0.09 (0.0)	0	1.4 (1.4)	1	S_{100}+	0.0 (0.0)	0	0.0 (0.0)	0	0.0 (0.0)	0
S_{25}-	0.9 (0.9)	1	0.0 (0.0)	0	0.0 (0.0)	0	I_{100}-	0.0 (0.0)	0	0.0 (0.0)	0	0.0 (0.0)	0
S_{25}+	0.0 (0.0)	0	2.8 (1.5)	2	1.4 (1.4)	1	I_{100}+	0.9 (0.9)	1	1.9 (1.3)	2	0.0 (0.0)	0
S_{0}-	0.0 (0.0)	0	0.0 (0.0)	0	0.0 (0.0)	0	S_{0}-	0.0 (0.0)	0	0.0 (0.0)	0	0.0 (0.0)	0
S_{0}+	0.0 (0.0)	0	0.0 (0.0)	0	1.4 (1.4)	1	S_{0}+	0.0 (0.0)	0	0.0 (0.0)	0	0.0 (0.0)	0
Juvenile individuals													
S_{100}-	1.9 (1.3)	2	1.9 (1.3)	1	8.3 (2.8)	4	S_{100}-	0.9 (0.0)	1	0.9 (0.9)	1	1.4 (1.4)	1
S_{100}+	4.6 (2.1)	3	8.3 (3.1)	3	6.9 (2.9)	4	S_{100}+	0.9 (0.0)	1	9.3 (3.0)	4	4.2 (2.0)	3
S_{25}-	2.8 (2.8)	1	9.3 (4.7)	3	1.4 (1.4)	1	I_{100}-	0.9 (0.0)	1	0.9 (0.9)	1	2.8 (1.8)	2
S_{25}+	0.0 (0.0)	0	7.4 (4.8)	3	0.0 (0.0)	0	I_{100}+	0.0 (0.0)	0	6.5 (3.2)	2	1.4 (1.4)	1

S_0-	1.9 (1.9)	1	3.7 (2.9)	2	4.2 (2.0)	3	**S_0-**	0.0 (0.0)	0	4.6 (2.5)	2	0.0 (0.0)	0
S_0+	0.0 (0.0)	0	8.3 (5.0)	3	0.0 (0.0)	0	**S_0+**	0.0 (0.0)	0	5.6 (2.2)	3	1.4 (1.4)	1

Table 3 Mean and standard error (SE) of earthworm (sub)adult density, density of epigeics and endogeics (ind. m^{-2}) and Shannon diversity index of the non-inversion tillage (NIT) and conventional tillage (CT) trials in Fall 2015. For legend of the treatments, see Figure 1. F-statistics and associated p-value of best fitted linear mixed model of earthworm densities and Shannon diversity index. Capital letters show significant pairwise differences within the main factor Crop residue application and small letters within the main factor *L. terrestris* inoculation.

	NIT trial				**CT trial**			
Treatments	**(Sub)adult density**	**Shannon diversity**	**Epigeics †**	**Endogeics ‡**	**(Sub)adult density**	**Shannon diversity**	**Epigeics †**	**Endogeics ‡**
S_{100}-	109.7 (8.6) Ba	0.9 (0.1)	30.5 (3.6) Ba	79.2 (6.9) Ba	73.6 (11.4)	1.0 (0.1)	29.2 (1.4) Ba	44.4 (10.9)
S_{100}+	97.2 (18.2) Ba	0.7 (0.1)	23.6 (8.9) Ba	73.6 (11.6) Ba	81.9 (11.4)	0.8 (0.2)	31.9 (9.2) Ba	50.0 (9.1)
S_{25}- / I_{100}-	66.7 (22.3) Aa	0.7 (0.2)	15.3 (7.6) Aa	51.4 (17.0) Aa	75.0 (10.3)	0.6 (0.1)	13.9 (3.6) Ba	61.1 (7.5)
S_{25}+ / I_{100}+	62.5 (8.9) Aa	0.8 (0.0)	6.9 (1.4) Aa	55.5 (9.4) Aa	86.1 (7.3)	1.0 (0.1)	29.2 (3.5) Ba	56.9 (9.2)
S_0-	70.8 (15.1) Aa	0.6 (0.1)	13.9 (5.3) Aa	56.9 (11.9) ABa	70.8 (9.2)	0.7 (0.1)	6.9 (2.7) Aa	63.9 (6.6)
S_0+	80.5 (16.1) Aa	0.7 (0.2)	8.3 (4.8) Aa	72.2 (15.2) ABa	56.9 (15.3)	0.3 (0.1)	5.6 (3.9) Aa	51.4 (14.1)

	F	**P**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Crop residues	9.753	***0.003***	1.847	0.200	18.084	***0.0002***	5.800	***0.017***	0.859	0.448	3.616	0.059	58.560	***<0.0001***	0.860	0.448

Inoculation	0.015	0.910	0.035	0.863	2.073	0.246	0.091	0.783	0.038	0.858	0.450	0.550	2.140	0.240	0.212	0.676
Crop residues x inoculation	0.445	0.651	1.456	0.272	0.039	0.962	0.703	0.515	0.690	0.520	3.620	0.059	3.058	0.085	0.422	0.665

† Epigeic species: *Lumbricus castaneus*, *Lumbricus rubellus*

‡ Endogeic species *Aporrectodea caliginosa*, *Allolobophora chlorotica*, *Aporrectodea rosea*

Table 4 F and p-values from non-parametric permutational multivariate analysis of variance (Location) and from multivariate homogeneity of variances (Dispersion) of (sub)adult earthworm community composition for each of the main factors (crop residues and inoculation of *L. terrestris*) and their interaction in the case of Location, of the non-inversion tillage (NIT) and conventional tillage trials (CT), for Fall 2015. Inoculated *L. terrestris* was excluded from distance matrices. Dissimilarity matrix calculated using the Bray-Curtis distance, and densities were square-root transformed.

	NIT trial				**CT trial**			
	Location		**Dispersion**		**Location**		**Dispersion**	
	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Crop residues	1.474	0.082	0.490	0.520	3.555	***0.013***	1.126	0.217
Inoculation	1.064	0.559	0.141	0.778	1.886	***0.042***	2.315	0.223
Crop residues x inoculation	0.335	0.794	-	-	2.095	0.072	-	-

Table 5 Mean and standard error (SE) of community weighted means (CWM) for the trait values in the **non-inversion tillage trial (NIT)**, for Fall 2015. Earthworm community taken into account for the computation excluded inoculated *L. terrestris*. For legend of the treatments, see Figure 1. F-statistics and associated p-value of best fitted linear mixed model of CWM. Both categorical traits only had two trait values, therefore, only one is shown. Capital letters show significant pairwise differences within the main factor Crop residue application and small letters within the main factor *L. terrestris* inoculation.

Treatments	**Body weight (g)**	**No. of cocoons (per year)**	**Reproductive strategy †**	**Typhlosolis shape ‡**	**Time to maturity (weeks)**	**Cuticle thickness (µm)**	**Epidermis thickness (µm)**
S_{100}-	0.37 (0.01) Aa	49.02 (1.55)	0.93 (0.05)	0.72 (0.03) Aa	48.40 (1.55)	1.30 (0.11)	34.87 (0.39) Aa
S_{100}+	0.35 (0.02) Aa	43.00 (4.77)	0.95 (0.03)	0.78 (0.06) Aa	50.37 (1.43)	1.01 (0.17)	34.70 (0.64) Aa
S_{25}-	0.36 (0.02) Aa	42.21 (6.46)	0.95 (0.04)	0.81 (0.08) ABa	50.77 (1.69)	1.04 (0.23)	34.78 (0.54) Aa
S_{25}+	0.33 (0.01) Aa	36.99 (2.48)	0.92 (0.05)	0.88 (0.03) ABa	50.14 (1.35)	0.97 (0.06)	33.71 (0.68) Aa
S_0-	0.36 (0.02) Aa	41.51 (6.05)	0.96 (0.02)	0.82 (0.07) Ba	51.09 (0.90)	1.00 (0.18)	34.82 (0.58) Aa
S_0+	0.33 (0.01) Aa	35.37 (4.59)	0.91 (0.04)	0.90 (0.05) Ba	51.22 (1.20)	0.87 (0.13)	33.83 (0.67) Aa

	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Crop residues	4.310	***0.039***	3.746	0.055	0.044	0.957	4.710	***0.031***	1.444	0.274	1.267	0.317	4.915	***0.028***
Inoculation	1.860	0.266	1.239	0.347	0.801	0.437	1.217	0.351	0.103	0.770	1.321	0.334	0.902	0.412

Crop residues x inoculation	0.553	0.589	0.009	0.991	0.571	0.580	0.035	0.966	0.806	0.469	0.314	0.736	1.638	0.235

† Results presented for the category of biparental reproductive strategy;

‡ Results presented for the category of bifide typhlosolis.

Table 6 Means and standard errors of community weighted means (CWM) for the trait in the **conventional tillage trial (CT)**, for Fall 2015. Earthworm community taken into account for the computation excluded inoculated *L. terrestris*. For legend of the treatments, see Figure 1. F-statistics and associated p-value of best fitted linear mixed model of CWM. Both categorical traits only had two trait values, therefore, only one is shown. Capital letters show significant pairwise differences within the main factor Crop residue application and small letters within the main factor *L. terrestris* inoculation. When only small letters are provided, significant differences refer to the interaction between both treatments.

Treatments	**Body weight (g)**	**No. of cocoons (per year)**	**Reproductive strategy †**	**Typhlosolis shape ‡**	**Time to maturity (weeks)**	**Cuticle thickness (µm)**	**Epidermis thickness (µm)**
S_{100}-	0.40 (0.02) Ba	61.67 (5.21) Ca	0.89 (0.05) Aa	0.57 (0.07) Aa	46.51 (1.46) ab	1.71 (0.19) Ca	35.98 (0.53) Ba
S_{100}+	0.39 (0.01) Ba	55.87 (7.29) Ca	0.97 (0.03) Ab	0.63 (0.10) Aa	47.26 (2.32) abcd	1.50 (0.27) Ca	35.57 (0.34) Ba
I_{100}-	0.36 (0.01) Ba	41.51 (2.27) Ba	0.93 (0.05) Aa	0.82 (0.03) Aa	51.81 (0.53) cd	0.96 (0.08) Ba	35.02 (0.19) Ba
I_{100}+	0.38 (0.01) Ba	54.72 (4.19) Ba	0.90 (0.06) Ab	0.65 (0.06) Aa	47.65 (1.18) ac	1.47 (0.14) Ba	35.37 (0.40) Ba
S_0-	0.33 (0.01) Aa	34.68 (2.38) Aa	0.90 (0.01) Aa	0.91 (0.03) Ba	52.36 (1.04) cd	0.78 (0.11) Aa	34.12 (0.18) Aa
S_0+	0.35 (0.01) Aa	34.24 (4.21) Aa	1.00 (0.00) Ab	0.91 (0.05) Ba	52.72 (0.79) bd	0.75 (0.13) Aa	34.44 (0.45) Aa

	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Crop residues	17.000	***0.0003***	25.566	***<0.00001***	0.579	0.575	53.564	***<0.0001***	16.291	0.0004	13.743	***0.001***	19.060	***0.0002***

Inoculation	1.796	0.273	0.424	0.562	64.751	***<u>0.004</u>***	7.008	0.077	12.328	0.039	0.475	0.540	0.350	0.598
Crop residues x inoculation	0.415	0.670	2.523	0.122	1.466	0.269	2.907	0.093	4.322	***<u>0.039</u>***	2.686	0.109	0.640	0.544

† Results presented for the category of biparental reproductive strategy;

‡ Results presented for the category of bifide typhlosolis.

Table 7 F and p-values from non-parametric permutational multivariate analysis of variance (Location) and from multivariate homogeneity of variances (Dispersion) of CWM for each of the main factors (crop residues and inoculation) and their interaction in the case of Location, of the non-inversion (NIT) and conventional tillage (CT) trials, for Fall 2015. Inoculated *L. terrestris* was excluded from distance matrices. Dissimilarity matrix calculated using the Gower distance.

	NIT trial				**CT trial**			
	Location		**Dispersion**		**Location**		**Dispersion**	
	F	**P**	**F**	**p**	**F**	**p**	**F**	**p**
Crop residues	0.939	0.262	0.0495	0.960	9.690	***0.002***	1.0216	0.177
Inoculation	1.834	0.336	0.0433	0.868	1.306	***0.043***	0.0513	0.834
Crop residues x inoculation	0.085	0.949	-	-	1.779	0.260	-	-

Table 8 Mean and standard error of RaoQ in the non-inversion tillage (NIT) and conventional tillage (CT) trials, for Fall 2015. Earthworm community taken into account for the computation excluded inoculated *L. terrestris*. For legend of the treatments, see Figure 1. F-statistics and associated p-value of best fitted linear mixed model of RaoQ. Capital letters show significant pairwise differences within the main factor Crop residue application and small letters within the main factor *L. terrestris* inoculation.

Treatments	**NIT trial**		**CT trial**	
S_{100}-	0.10 (0.01)		0.12 (0.01) Ba	
S_{100}+	0.07 (0.01)		0.10 (0.02) Ba	
S_{25}- / I_{100}-	0.06 (0.02)		0.07 (0.01) Ba	
S_{25}+ / I_{100}+	0.06 (0.01)		0.11 (0.01) Ba	
S_0-	0.06 (0.02)		0.04 (0.01) Aa	
S_0+	0.05 (0.01)		0.03 (0.02) Aa	
	F	**p**	**F**	**p**
Crop residues	3.731	0.055	17.717	***0.0003***
Inoculation	2.756	0.196	0.138	0.735
Crop residues x inoculation	0.511	0.613	2.792	0.101