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# Soil structure formation and organic matter distribution as affected by earthworm species interactions and crop residue placement

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# Abstract

Earthworms play an important role in soil organic matter (SOM) dynamics and soil structure formation, including soil porosity and aggregate stability. Earthworms feed on organic inputs such as crop residues (CR) which are displaced by mouldboard ploughing. In a 61-day mesocosm experiment, we investigated the effects of CR placement (surface-applied vs. incorporated) and different earthworm species (combinations) on: 1) the survival and biomass of the earthworm species *Lumbricus terrestris*, *L. rubellus*, and *Aporrectodea caliginosa*, representing anecic, epigeic and endogeic ecological groups, respectively; and 2) earthworm-mediated soil structure formation. Earthworms were present either as single species or as species mixtures combining anecics with each of the other groups. Incorporating CR reduced biomass of surface-feeders (*L. terrestris*: -30% of initial body weight vs. -9% when CR were surface-applied; *L. rubellus*: -74% vs. -24%, respectively). *L. rubellus* survival was also lower when CR were incorporated (50%) than when CR were surface-applied (92%). In surface-applied CR treatments, the amount of particulate organic matter (POM) > 250 µm in the soil profile was positively affected by *L. terrestris* in the soil upper 20 cm by 16.5%. A similar but weaker effect was found when CR were incorporated (9% increase). Large water-stable macroaggregates (>2000 µm) increased in the upper 20 cm soil only when CR were surface-applied and *L. terrestris* was present (from 2.7 to 13.1 g $kg^{-1}$). Small water-stable aggregates increased with functional groups interactions at all soil depths, irrespective of the CR placement. Surface-applied CR increased soil porosity at 2.5-10 cm depth. Large water-stable macroaggregate formation by earthworms was hampered through the incorporation of CR, although CR incorporation increased porosity between 2.5 and 30 cm soil depth despite reduced earthworm biomass. Furthermore, small macroaggregate formation was hampered by single species, whereas combining functional groups stimulated their formation. Under field conditions residue incorporation might result in trade-offs between the contribution of surface-feeding earthworms to soil porosity and i) their fitness, as surface-feeding earthworms’ body weight loss was larger than when crop residues were surface-applied; as well as ii) large water-stable macroaggregates formation, as no increase in those was found when CR was incorporated.

# 1. Introduction

Earthworms have long been recognized as soil ecosystem engineers (Jones et al., 1994; Lavelle et al., 1997). Their feeding, burrowing and casting activities strongly impact organic matter distribution and soil structure, thereby modifying soil porosity (Capowiez et al., 2015; Martin, 1982; Pérès et al., 2010), soil aggregate stability (Bossuyt et al., 2006; Hedde et al., 2013), soil organic matter (SOM) dynamics (Pulleman et al., 2003), nutrient availability (van Groenigen et al., 2014), water infiltration (Andriuzzi et al., 2015), soil aeration (Lemtiri et al., 2014) and soil fertility (Syers and Springett, 1984).

Based on their feeding habits and morphological features, Bouché (1977) classified earthworms into three main ecological groups, which reflect their burrowing and feeding habits. He distinguished anecics as detritivores feeding at the soil surface and digging deep vertical permanent burrows, epigeics also as feeding on fresh organic matter at the soil surface, but not commonly associated with burrowing activities, and finally endogeics as geophagous species obtaining their nutrition from organic matter associated to soil mineral particles and being reported to burrow horizontally, creating temporary burrows. In Dutch agricultural soils, the most common species belonging to these groups are, respectively, *Lumbricus terrestris* (Linné, 1758), *Lumbricus rubellus* (Hoffmeister, 1843), although some authors have classified this species as epi-endogeic (Hendrix et al., 1999) or epi-anecic (Briones and Álvarez-Otero, 2018), and *Aporrectodea caliginosa* (Savigny, 1826) (Crittenden et al., 2014; Frazão et al., 2017). *L. terrestris*, although common in pastures, is less common in arable fields, while farmers are very keen on stimulating this species due to its important role in soil structure formation and water infiltration.

In arable fields, management activities have been reported to affect earthworm communities, in particular ploughing, through mechanical soil disturbance and burial of crop residues (Chan, 2001; Crittenden et al., 2014; Ernst and Emmerling, 2009). Soil inversion due to ploughing can destroy anecic earthworm burrows. Re-establishing their burrow system occurs at the high cost of energetic investment of individual earthworm specimens (e.g. Petersen and Luxton (1982) who accounted that during soil modification earthworms respired 74–91% of assimilated carbon). Also, soil tillage, especially soil inversion, displaces crop residues to deeper soil layers, typically to about 20 to 30 cm soil depth in case of mouldboard ploughing. Tillage intensity has been found to negatively affect abundances of anecics and epigeics, but have neutral or positive effects on endogeics (Crittenden et al., 2014; de Oliveira et al., 2012; Ernst and Emmerling, 2009), despite increased exposure to predation risks in the short term (Cuendet, 1983). Thus, earthworm communities in agricultural land are subjected to complex interactions involving factors like crop residue management, changes in microclimate, exposure to predation and burrow destruction. Apart from these human-related factors, complex soil-mediated interactions such as interspecific competition and facilitation can affect their survival and growth (Uvarov, 2009).

Competition or facilitation among earthworm species that share or have contrasting feeding habits has been demonstrated in several studies (Lowe and Butt, 1999; 2002; 2003). These interspecific interactions may have consequences for soil structure formation, e.g., soil porosity (Capowiez et al., 2001) and aggregate stability, and SOM availability in arable agro-ecosystems. Moreover, the distribution of crop residues may affect the feeding behaviour of earthworm species which in turn, is likely to affect their contribution to soil structure formation (Coq et al., 2007). Indeed, several studies have shown that crop residue placement affected the specific contribution of earthworm species to soil porosity (Le Couteulx et al., 2015), SOM dynamics (Giannopoulos et al., 2010; Paul et al., 2012) and aggregate stability (Bossuyt et al., 2006). So far, these studies were restricted to either one or two soil structural features and often focussed on single species effects. Efforts to relate soil porosity, aggregate stability and SOM distribution with earthworm species of the three distinct ecological groups and their interactions, under different crop residue placement in the soil profile have been absent, to the best of our knowledge.

The objectives of this study were two-fold. First, we addressed the effects of applying crop residues on the soil surface *vs.* incorporating them in the soil profile, simulating no-tillage and conventional ploughing, respectively, on the survival and body weight of single earthworm species representing the three ecological groups. Furthermore, we focussed on species mixtures' survival and weight change: anecics were combined with either epigeic or endogeic species. Second, we investigated how crop residue placement and earthworm species (interactions) influenced soil porosity, SOM distribution and aggregate stability.

We hypothesized that incorporation of crop residues would have strong negative effects in single species treatments on surface feeders' (model species: *L. terrestris* and *L. rubellus*), but not on soil feeders' (model species: *A. caliginosa*) body weight and survival. Furthermore, we expected that interspecific competition (expressed in weight loss) would occur in the case of mixtures of species with similar feeding habits (*L. terrestris* combined with *L. rubellus),* whereas facilitation (expressed in weight gain) would take place when contrasting feeding guilds were combined in earthworm species mixtures (*A. caliginosa* with *L. terrestris*).

Finally, we hypothesized that i) when crop residues were surface-applied, *L. terrestris* would cause increased soil porosity, SOM incorporation and stable macroaggregates, aided by endogeic (*A. caliginosa*) and counteracted by epigeic species (*L. rubellus*), and that ii) when crop residues were incorporated soil porosity would be higher, but regardless of the species under focus, and with larger weight loss for surface-feeders, especially *L. rubellus*.

# 2. Materials and methods

## *2.1 Experimental set up*

A mesocosm experiment (61 days) was performed in the greenhouse to compare earthworm effects on SOM, aggregate stability and soil porosity, when providing crop residues either at the soil surface (simulating no-tillage) or incorporated between 20 and 30 cm deep (simulating conventional tillage by mouldboard ploughing). The experimental duration was chosen as a compromise between logistical constraints and expected effects (e.g. Le Couteulx et al. (2015) found earthworm-derived porosity effects after 60 days of experimental time). The earthworm effects considered here focussed on the three ecological groups (anecic, epigeic and endogeic) and interactions between anecics and epi- and endogeics. Each ecological group was represented by one model species only, as financial constraints hampered replicating the experimental set-up to consider more species within each group. Single species earthworm treatments were *Lumbricus terrestris* (LT), *Aporrectodea caliginosa* (AC), and *Lumbricus rubellus* (LR), two-species treatments were *L. terrestris* with *A. caliginosa* (LT+AC) and *L. terrestris* with *L. rubellus* (LT+LR) and an additional earthworm-free control treatment (0) was considered as well (Figure 1). The focus on the interactions between *L. terrestris* and the other two species was triggered by farmers' large interest in the anecics, which mitigate the negative effects of intense rainfall events on e.g., plant growth (Andriuzzi et al., 2015). Crop residues used were a mixture of winter wheat (*Triticum aestivum*) stubble and straw and radish (*Raphanus sativus subsp. oleiferus*), corresponding to commonly used main and cover crops in the Netherlands. Stubble, straw and radish were chopped roughly to 2 cm and provided to each mesocosm in the following amounts: 4.7 g, 14.2 g, 5.1 g, respectively, corresponding to 0.4 t ha$^{-1}$, 1.3 and 0.5 t ha$^{-1}$. The experiment was set up in a completely randomized block design with four replicates. Each experimental unit (mesocosm) had a total height of 49.5 cm and a diameter of 19 cm. Four PVC rings with heights of 12, 20, 10, and 7.5 cm (Figure 1) were mounted on top of each other using duct-tape. Each column was closed at the bottom. In order to prevent earthworms from escaping two parallel 1 cm wide strips of velcro were glued on the inside of the column, a few cm below the top (Lubbers and van Groenigen, 2013). Additionally, each column was covered with a cotton cloth allowing gas exchange, and attached with a rubber band. Calcareous marine loam soil (de Bakker and Schelling, 1966) was collected from a conventionally tilled arable field of the Westmaas experimental farm of Wageningen University and Research, located in the southwest of The Netherlands. Soil (36.9 g OM kg$^{-1}$, pH of 7.9 and a texture of 48 % sand and 25 % clay) was collected to a depth of 20 cm, sieved through a 4-mm screen, air-dried at 25°C and thoroughly mixed to guarantee homogeneity. Nine days prior to the inoculation of earthworms, each column was packed with 12.5 kg air-dried soil at a bulk density of 1.20 g cm$^{-3}$ resulting in a total depth of 37.5 cm. Each ring was filled independently ensuring the same bulk density throughout the whole column. The upper ring did not contain soil, but only the crop residues in surface-applied treatments (Figure 1). Crop residues were either incorporated in the profile between 20 and 30 cm deep, by mixing them thoroughly with the soil prior to filling that PVC ring or applied on the soil surface after the complete column was filled. Gravimetric soil moisture was brought to 234 g kg $^{-1}$ of soil, corresponding to 65% of water-filled pore space (WFPS) and was adjusted gravimetrically once a week to maintain the soil moisture constant by applying tap water at the soil surface. All columns were incubated at a constant temperature of 15.5°C and a light cycle of 15hrs light/9 hrs dark.

Three to four weeks prior to the inoculation of earthworms, (sub)adult individuals of *L. terrestris* were commercially obtained from Starfood (Barneveld, The Netherlands), whereas adults of *A. caliginosa* and *L. rubellus* were sampled in parks in the vicinity of Wageningen University and Research Centre. Earthworms were kept in plastic containers at 2 °C with the same soil used as in the experiment and were fed with alder leaves. Two days prior to the inoculation of earthworms in each block, individuals of each species were placed in clean plastic pots at 16 °C with moist kitchen paper to allow them to void their guts and their initial body weights were recorded to 0.1 g accurately. Treatments with *L. terrestris* (LT, LT+AC and LT+LR) received three individuals of *L. terrestris* with total weight of about 15g, treatments with *L. rubellus* (LR and LT+LR) received three individuals of *L. rubellus* with total weight of about 2g and treatments with *A. caliginosa* (AC and LT+AC) received four individuals of *L. rubellus* with total weight of about 1g (Table A1). *A. caliginosa* numbers were based on field data (e.g., Crittenden et al., 2015) and as *L. rubellus* and *L. terrestris* occur usually in lower densities, their experimental density was reduced compared to *A. caliginosa*. However, to ensure that survival rates would be workable, their number could not be lower than three individuals. To avoid earthworms burrowing down along the PVC walls of the mesocosm, they were placed under a 10 cm diameter plastic cup in the centre of the surface area of each column. In the surface-applied crop residue treatments, residues were carefully put aside for the earthworm inoculation, but spread evenly after the individuals had burrowed in the soil.

## *2.2 X-Ray tomography (XRT)*

Sixty-one days after the inoculation of the earthworms, two replicates of the single-species and no species treatments of both crop residue placement treatments were scanned with X-Ray computed tomography. Scans were executed using the v[tome]x m (Phoenix X-ray/General Electric), with a directional X-Ray tube and a tungsten target. The voltage was set to 200 kV with a current of 30 µA with a subsequent power of the Tungsten-target of 60 W. The columns were positioned at 409.022 µm from the target, which corresponds to a voxel size of 230 µm. Because the columns were too tall for a single vertical image, the multi-scan option was selected. Projection images of each experimental unit were taken at 1000 equidistant rotation angles between 0° and 360°. Each image's acquisition time was 333 ms, with a total time of 33 min for each experimental unit. After the scans were completed, the experimental units were harvested destructively to collect earthworms and soil samples for further analysis (see below).

### *2.2.1 Soil porosity*

Images were first transformed into 8-bit format. Greylevel histograms showed two well-separated peaks (one for porosity and one for the soil matrix) and thus images were binarized with the same threshold value. The distribution of porosity with depth was computed for each image as the sum of the areas of all the pores for one image. Total porosity was then calculated for four soil layers (2.5-10, 10-20, 20-30 and 30-35 cm). The upper and lower 2.5 cm were excluded to ensure a clear characterization of the porosity. Since the soil was sieved to 4 mm, the porosity in the images had two origins: burrows and inter-aggregate porosity, the first being dominant. We assumed that the inter-aggregate porosity was similar for all the cores and thus we subtracted the porosity observed in the control cores without earthworms to the porosity for each soil layer.

## *2.3 Destructive sampling*

Surface crop residues and surface casts were carefully removed from each column and oven-dried at 35 °C. Each of the four PCV rings comprising one column were cut horizontally and separated, before the start of the measurements. We double-checked soil moisture contents using a sensor, TRIME PICO 64, IMKO (16 cm long sensor rods) inserted at 0 cm and at 20 cm depth, and bulk density by measuring twice the height and diameter of the soil within each PVC ring, weighing and correcting for the water content. Next, earthworms were carefully removed from the soil, while gently crumbling the soil into aggregates along natural planes of weakness and passing them through a 12 mm mesh, before drying at 35 °C. Earthworms were placed at 16 °C for 48 hrs allowing them to void their guts. Each individual was cleaned, excess water was removed with a tissue, and its body weight was recorded. Representative soil subsamples were taken for i) SOM fractionation and ii) aggregate stability measurements. SOM fractionation was done for each depth layer, i.e. 0-20 cm, 20-30 cm and >30 cm and the surface casts. However, as the amount of cast material was very small, especially in the case of *A. caliginosa* mesocosms, casts were pooled per treatment among blocks. Aggregate stability was measured for 0-20 and 20-30 cm soil layers, and not for casts, as not enough cast material was available after the SOM fractionation.

### 2.4 SOM fractions

Between 80 and 100 g of soil was dispersed with 300 ml of 0.5% solution of NaHMP (5 g $l^{-1}$) in a shaker overnight. In the case of surface casts the complete sample was used, which ranged from 25 to 80 g. The total soil suspension was sieved through three mesh sizes to obtain SOM and mineral soil material of three size fractions: larger than 250 μm (particulate organic matter (POM) plus coarse sand >250 μm: POM > 250), between 53 and 250 μm (POM plus fine sand 53 – 250 μm: POM 53-250) and silt and clay sized soil particles (SOM plus silt and clay <53 μm: SOM < 53). After the three size fractions were dried at 105 °C overnight, loss of ignition (LOI) was used to determine the organic matter content of each size fraction (POM > 250, POM 53-250 and SOM <53).

### 2.5 Aggregate stability

Between 30 to 40 g of soil subsample was used to determine water-stable aggregates (WSA) using the modified wet sieving method of Six et al. (2002), based on Elliott (1986). Three WSA classes of soil aggregates were obtained: large macro-aggregates (WSA > 2000 μm: WSA > 2000), small macro-aggregates (WSA 250 – 2000 μm: WSA 250-2000), micro-aggregates (WSA 53 – 250 μm: WSA 53-250) and the silt and clay fraction (SC <53 μm SC < 53). To obtain these, each soil subsample was placed on a 2 mm sieve and submerged in demi-water and left to slake for five minutes. In the following two minutes, the sieve was moved up and down 50 times to allow water and soil particles to go through the mesh. With the material that had passed through the 2 mm sieve, the same procedure was repeated using sieves of 250 µm and 53 µm. The fractions collected by the sieves were carefully backwashed to pre-weighed aluminium pans, dried overnight at 105 °C and weighed. The suspension smaller than 53 µm was collected in a bucket, its volume was noted down and a subsample of known volume was dried at 105 °C and weighed.

### *2.6 Statistical analysis*

Earthworm biomass (as percentage of the initial body weight) and survival were calculated per column. The single and interactive effects of crop residue placement and presence of other species (i.e. *L. rubellus* or *A. caliginosa*) on the weight change of *L. terrestris* were examined using linear mixed models with a normal distribution, with block as a random factor. Because the variation of *L. terrestris*' survival was very low (only three individuals died during the experiment), it was not possible to compute linear mixed models for *L. terrestris*' survival. For the weight change and survival of *L. rubellus* and *A. caliginosa,* crop residue placement and presence of *L. terrestris* were considered as fixed effects.

The single and interactive effects of *L. terrestris* (present or absent) and other earthworm species (no species, *L. rubellus* and *A. caliginosa*) on SOM size fractions per depth (0-20, 20-30, and >30 cm) and on WSA size classes at 0-20 and 20-30 cm depth were analysed for each crop residue treatment separately, using linear mixed models with a normal distribution, with block as a random factor. For porosity, the fixed effects of the mixed model were slightly different, and corresponded to the (interactive) effects of single earthworm species and soil depth (intervals between 2.5-10, 10-20, 20-0 and 30-35 cm), being analysed separately for each of the crop residue treatments, as well. Porosity was quantified after correcting for inter-aggregate porosity of the earthworm-free treatments and expressed as percentage of the total soil volume, and one-tailed T-tests were computed to check whether mean porosity values were larger than zero ($p < 0.05$). When the overall linear mixed models were statistically significant at the p-level of 0.05, pairwise comparisons were computed refitting the models with the significant (interactive) fixed effects. P-values adjustments to avoid inflation type I errors were only considered necessary when the interaction between the fixed effects was significant due to the large number of pairwise comparisons (15, in the case of aggregate stability SOM and *L. terrestris* weight change or survival; 66, in the case of porosity). In that case, Tukey post-hoc adjustments 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). All analyses were performed with R 3.3.1 (R Core Team, 2014), using packages nlme 3.1–131 and lsmeans 2.27-61.

# 3. Results

## *3.1 Earthworm body weight change and survival*

All earthworm species lost weight during the 61 days of this experiment, but the extent depended on the treatments, i.e. residue placement and species: *L. terrestris* lost on average 30% of the initial weight when residues were incorporated in the profile, and only 9% when surface-applied ($p < 0.0001$), and *L. rubellus* presented a similar, but stronger pattern (74% vs. 24%, $p = 0.003$, Table 1). Body mass of *L. rubellus* was reduced by the presence of *L. terrestris*, irrespective of crop residue placement (-35% when alone vs. -63%, when together with *L. terrestris*, $p = 0.001$, Table 1). Earthworm survival was rather high, particularly for *L. terrestris* (> 90%) and *A. caliginosa* (> 80%). Survival of *L. rubellus* was higher when residues were surface-applied as compared to incorporated into the soil profile (92% vs. 50%, p = 0.039, Table 1). Besides an overall body mass loss of 19-29% during the experiment, *A. caliginosa* body weight or survival did not differ between the treatments (Table 1).

## *3.2 SOM fractions*

When residues were surface-applied, SOM fractions were affected by *L. terrestris* at 0-20 and 20-30 cm depth and by *L. rubellus* at 20-30 cm, whereas neither *A. caliginosa* nor the interaction between both earthworm treatments affected SOM distribution. *L. terrestris* increased POM > 250 at 0 to 20 cm soil depth by 16.5%, from 1.09 (± 0.03) to 1.27 (± 0.06) g $kg^{-1}$ ($p = 0.014$), irrespective of the presence of other species (Table 2), and decreased SOM < 53 at 20 to 30 cm soil depth by 5%, from 34.02 (± 0.62) to 32.32 (± 0.37) g $kg^{-1}$ (overall model $p = 0.005$, Table 2). *L. rubellus*, irrespective of the presence of *L. terrestris*, increased POM 53-250 at 20 to 30 cm soil depth by 26%, from 2.54 (± 0.11) to 3.20 (± 0.17) g $kg^{-1}$ (pairwise $p = 0.010$, Table 2).

When crop residues were incorporated at 20 to 30 cm depth, *L. terrestris* increased POM > 250 in the 0-20 soil layer by 9%, from 0.98 (± 0.01) to 1.07 (± 0.03) g $kg^{-1}$ ($p = 0.043$, Table 3), but the effect was smaller than in the surface-applied residue treatments. At 20-30 cm depth POM > 250 was affected by the overall effect of other species ($p = 0.006$, Table 3), yet, pairwise comparisons within that factor did not show significant effects at the level of $\alpha = 0.05$.

Due to the small amounts of surface casts recovered, those samples had to be pooled across experimental blocks, which made it impossible to test for statistically significant treatment effects. When crop residues were surface-applied, SOM content of casts of all earthworm treatments was consistently higher than when crop residues were incorporated. This was particularly noticeable for the POM > 250 (Table 4). However, the amount of casts produced was consistently higher when crop residues were incorporated than when crop residues were surface-applied, particularly when *L. terrestris* was present (Table 4).

### *3.3 Water stable aggregates*

When residues were surface-applied, both earthworms factors significantly affected aggregate stability at 0 to 20 cm soil depth: when *L. terrestris* was present, irrespective of the presence of the other species, a five times increase in WSA > 2000 was observed (2.71 (± 0.48) vs. 13.08 (± 3.31) g $kg^{-1}$, overall model $p < 0.0001$, Table 5), whereas regardless of the presence of *L. terrestris*, WSA > 2000 increased almost 2.5 times due to *A. caliginosa*, and almost 4.5 times due to *L. rubellus*, (pairwise $p = 0.004$ and $p = 0.016$, respectively, Table 5). Also WSA 250-2000 were strongly affected by earthworm species, but now also by species combinations (overall model $p = 0.002$, Table 5). When only *A. caliginosa* was present, significantly less WSA 250-2000 were found compared to the earthworm-free treatment (54.14 (± 2.06) vs. 67.97 (± 0.67) g $kg^{-1}$, pairwise $p < 0.0001$, Table 5). In contrast, *L. terrestris* almost doubled the amount of WSA 250-2000 when present together with *L. rubellus* (105.18 (± 5.94) vs. 67.97 (± 0.67) g $kg^{-1}$, pairwise $p < 0.001$, Table 5). In combination with *A. caliginosa* this increase was about 60% although not statistically significant different from the earthworm-free control (pairwise $p = 0.068$, Table 5). Regarding the microaggregates, the combination of *L. terrestris* with either *L. rubellus* or *A. caliginosa* resulted in a 10% decrease of the WSA 53-250 between 0 to 20 cm soil depth (pairwise $p = 0.003$ and 0.011, respectively, Table 5 for overall model), and in case of *L. terrestris* combined with *A. caliginosa* a 7% decrease in the 20-30 cm soil layer was also observed (pairwise $p = 0.026$, Table 5). The silt and clay fractions (SC < 53) in the 0 to 20 cm soil layer also decreased. Now, the single species treatments with *A. caliginosa* and *L. rubellus* decreased SC < 53 from 130 to 106 g $kg^{-1}$ (pairwise $p = 0.014$ and 0.003, respectively, Table 5 for overall model). In contrast, at 20 to

30 cm depth, SC < 53 was generally increased due to *L. terrestris*, when present together with either of the other two species, from 119 g kg$^{-1}$ to an average of 158 g kg$^{-1}$ (pairwise p = 0.002 for LT-AC and 0.033 for LT-LR, Table 5).

When residues were incorporated, *L. terrestris* together with *L. rubellus* or *A. caliginosa* increased WSA 250-2000 at 0 to 20 cm depth, from about 65 g kg$^{-1}$ in the control treatment to an average of 100 g kg$^{-1}$ (overall model p < 0.0001, Table 5, pairwise p = 0.004 for LT-AC and 0.049 for LT-LR). In the same soil layer, the combination of *L. terrestris* with *L. rubellus* affected WSA 53-250 in the opposite direction, from about 782 in the earthworm-free treatment to 750 g kg-1 (overall model p < 0.0001, Table 5, pairwise p = 0.006), while single species, namely *A. caliginosa* and *L. terrestris*, resulted in an increase from about 780 to 810 g kg$^{-1}$ (pairwise p = 0.034 and 0.004, respectively, Table 5). None of the (single or mixture) species treatment showed significant shifts in WSA 53-250 compared to earthworm-free control treatments at 20 to 30 cm soil depth, but treatments with *L. rubellus* and *L. terrestris* alone had more WSA 53-250 (ca. 790 g kg$^{-1}$) than mixed-species treatments (720 g kg$^{-1}$) (overall model p = 0.005, pairwise p < 0.05, Table 5). Silt and clay fractions (SC < 53) were generally lower with single species treatments, when compared to earthworm-free control treatments, at 0 to 20 cm soil depth (overall model p < 0.0001, Table 5, pairwise p = 0.001 for LR, p < 0.0001 for AC and LT), whereas at 20 to 30 cm soil depth, only *A. caliginosa* showed a decrease in this fraction compared to the earthworm-free control treatment (pairwise p = 0.010, Table 5).

## *3.4 Soil porosity*

When crop residues were surface-applied, porosity was significantly larger at 2.5 to 10 cm than between 10 and 35 cm soil depth, decreasing from 0.8% of total soil volume to an average of -0.3% (overall model p = 0.006, Table 6, Figure 2A). Porosity in the 2.5 to 10 cm soil layer was the only one that was significantly larger than the earthworm-free control treatments (t = 4.36, p = 0.004). The overall effects of earthworm species and of their interactions with soil depth did not significantly affect soil porosity.

When crop residues were incorporated, porosity was larger in 2.5 to 10, 10 to 20 and 20 to 30 cm, than in the deepest considered layer, between 30 to 35 cm soil depth, decreasing from an average of 1% to 0.3% (overall model p = 0.011, pairwise p < 0.05, Table 6, Figure 2B). Species effects on soil porosity were largest in *L. terrestris* (1.1 ± 0.2%) and larger than in *A. caliginosa* (0.6 ± 0.2%) treatments (overall model p = 0.025, pairwise p < 0.008, Table 6). In all cases of the incorporated crop residues treatments, porosity was significantly larger than the earthworm-free control treatments (p < 0.01).

# 4. Discussion

## *4.1 Response of earthworms to crop residue placement and SOM distribution*

Earthworm survival during the experiment was high, 91% on average, irrespective of crop residue placement, except for LR when residues were incorporated and LT was present (33% survival). Besides, in accordance with our first hypothesis, body weight of surface feeders LR and LT was strongly affected by crop residue placement. Incorporating the residues had stronger negative effects on those species, both in treatments with single species (LT or LR) and when both species were present together (LT+LR). The fact that most earthworms lost weight, particularly in mixtures of surface-feeding species (i.e., *Lumbricus rubellus* and *Lumbricus terrestris*), is consistent with similar studies in literature in which food was limiting as is common in field conditions under arable farming (Giannopoulos et al., 2010; Rizhiya et al., 2007). The fact that *L. rubellus* lost significantly more weight in the presence of *L. terrestris* (-47% and -79% when crop residues were surface-applied and incorporated, respectively, Table 1) than when present alone (-0.4% and -69%, respectively, Table 1) indicates inter-specific competition between both species of the genus *Lumbricus*, as reported earlier by Uvarov (2009). Lowe and Butt (1999) also observed inter-specific competition among both *Lumbricus* species when surface organic matter was limiting. In their study, *L. rubellus* constrained the growth of *L. terrestris*, whereas in our study, it was the presence of *L. terrestris* that had a negative effect on *L. rubellus*. However, it is important to note that Lowe and Butt (1999) started their (three times longer) mesocosm experiments with juvenile individuals. Juveniles of *L. terrestris* and *L. rubellus* are much more similar in size, and the fact that we used (sub)adult individuals could have provided an extra competitive advantage to *L. terrestris* in comparison to *L. rubellus*. It is worthwhile mentioning that despite some dispute in the literature regarding the ecological grouping of *L. rubellus* (e.g. Briones and Álvarez-Otero (2018) considered it an epi-anecic and Hendrix et al. (1999) an epigeic or epi-endogeic) our results indicate negative consequences for *L. rubellus*' survival and body weight when crop residues are incorporated especially so when together with other surface-feeders, in this case with *L. terrestris*. Although those fitness costs of *L. rubellus* do not solve the literature dispute, our results indicate that this species should not be grouped within the endogeics.

Although we expected facilitation effects between *L. terrestris* and *A. caliginosa*, particularly when crop residues were surface-applied, the presence of the former did not show any positive effects on the latter species, nor *vice versa*. It is worthwhile mentioning that our earthworm performance data is limited to body weight and survival, as we did not measure reproductive output during our experiment. Therefore, we cannot know if e.g. more cocoons were produced by *A. caliginosa* in the presence of *L. terrestris*, which could be a facilitation effect. Grubert et al. (2016), in contrast to our results, found a body weight gain of *A.*

*caliginosa* of about 104% in the presence of *L. terrestris*. In temperate arable soils, *A. caliginosa* is the most common earthworm species (Crittenden et al., 2014; Frazão et al., 2017) and it is often assumed that it is stimulated by the incorporation of surface residues by conventional ploughing (Chan, 2001; de Oliveira et al., 2012). Our experimental design aimed at simulating such incorporation of residues, either by manual incorporation or by the activity of *L. terrestris*. However, *A. caliginosa* did not benefit from this, as shown by the similar weight change when this species was subjected alone to experimental conditions or when it was combined with *L. terrestris,* regardless of the crop residue placement (Table 1). Furthermore, irrespective of the presence of *A. caliginosa*, *L. terrestris* incorporated POM > 250 to at least 20 cm soil depth (Tables 2 and 3), and therefore increased the availability of crop residues for *A. caliginosa*. We can only speculate about possible reasons for the lack of benefit of *A. caliginosa* from crop residue incorporation either through tillage or LT, such as the fact that the organic matter could have been possibly too fresh for that species, and/or that the duration of our experiment was too short. On the other hand, it could very well be that the organic matter content (3.7%) of the soil used was sufficiently high, i.e., not limiting, for *A. caliginosa*.

## *4.2 Earthworm effects on soil structure formation*

### *4.2.1 Aggregate stability*

All single earthworm species treatments (LR, AC, and LT) tended to affect WSA similarly, while single species effects were commonly opposite to those of species combinations, irrespective of crop residue placement (Table 5, Figure 3). First, single species always reduced the silt and clay fraction (SC < 53) and increased WSA 53-250 and this effect was most pronounced in under incorporated crop residues for both soil depths (Figure 3B1 and B2), but least pronounced when crop residues were surface-applied and at 20-30 cm depth

(Figure 3A2). Simultaneously, single species treatments never increased macroaggregates (WSA 250-2000 and WSA > 2000) (Figure 3). Second, species combinations always reduced WSA 53-250 (Figure 3). Intriguingly, at 20-30 cm soil depth, this reduction in WSA 53-250 was accompanied particularly by an increase in the silt and clay fraction (SC < 53), irrespective of crop residue placement (Figure 3A2 and B2). However, at 0-20 cm soil depth the decrease in WSA 53-250 coincided with an increase in water-stable macroaggregates, both WSA 250-2000 and WSA > 2000 when crop residues were surface-applied (Figure 3A1), or only WSA 250-2000 when crop residues were incorporated (Figure 3B1). It seems, therefore, that single species treatments have a stabilizing effect at the microaggregate level, whereas combinations of functional groups are more effective in formation and stabilization of  macroaggregates.

The observed patterns may, however, reflect different ecological mechanisms caused by the species combinations applied. We argue the data indicate competition between LT and LR due to food shortage in the surface-applied crop residue treatments, as a result of more individuals within the same feeding guild, i.e. surface-feeders. The food shortage could imply that surface feeders needed to be more active while searching for food which could have resulted in a larger proportion of water-stable macroaggregates, due to larger amounts of ingested soil. This claim is supported by our earthworm performance data (see section 4.1 and Table 1), where competition between both surface feeders was demonstrated, since LR lost more weight when together with LT then when alone. In the case of incorporated crop residues the earthworm performance data did not support facilitation between LT and AC (see section 4.1 and Table 1). However, our data suggests complementarity between those species in terms of soil structure formation, as macroaggregates increased in the presence of LT and AC, at least in the upper 20 cm soil depth.

Our results oppose those found by Bossuyt et al. (2006), Fonte et al. (2007) and Giannopoulos et al. (2010), and, in turn, those studies also showed contrasting results among themselves. Fonte et al. (2007) did not find any effects of earthworms on any aggregate size fraction, whereas Giannopoulos et al. (2010) only found a weak significant increase in water-stable macroaggregates, from 27% to 32%, with *A. caliginosa*, when residues were incorporated. Bossuyt et al. (2006) demonstrated that large water-stable aggregates increased with all earthworm treatments when crop residues were surface-applied and incorporated in the soil. In the case of Fonte et al. (2007), intact soil cores were used, whereas we repacked soil columns. As for Giannopoulos et al. (2010) who also used repacked columns, their soil pre-treatment involved sieving through 8 mm, whereas we used a 4-mm mesh-size. Consequently, in our study, soil structure was “re-set” due to the soil sieving prior to the experiment’s establishment, which could have accounted for the different experimental outcomes. The soil pre-treatment applied by Bossuyt et al. (2006) completely “re-set” initial soil structure, as they sieved their soil through 250 µm. After correcting for the experimental duration, earthworm density and soil volume used, their rate of WSA > 2000 formation was between 3 and 5 times larger than ours in the case of surface-applied residues and between 20 and 70 times larger when residues were incorporated, depending on whether earthworm treatments consisted of single or two species. Caro et al. (2012) demonstrated that increasing intra-specific density increased the mobility of several earthworm species, and therefore their activity. Speculatively, we consider that the results of Bossuyt et al. (2006),who used six earthworms in 500 g of soil (whereas we used a maximum of 0.3 earthworm per 500 g of soil), could also be a product of the unrealistically high earthworm density used.

### *4.2.2 Porosity*

Our experiment revealed that crop residue placement may induce some plasticity in earthworm burrowing behaviour, due to the necessity of earthworms to find food. In a field study in Normandy, Pérès et al. (2010) discussed the possibility that low organic matter availability in maize arable fields would increase the number of burrows made by earthworms as a result of their search for food. Our results are in line with this explanation as we observed an increase of earthworm-mediated soil porosity with soil depth, when crop residues were incorporated in the soil profile (Figure 2B). In contrast, when crop residues were surface-applied, earthworms restricted their burrowing activity up to 10 cm soil depth (Figure 2A). However, it seems that the burrowing plasticity brings a trade-off, as especially *L. rubellus* lost much more weight when crop residues were incorporated (average of 69% body weight loss) than when those were surface-applied (0.4% of body weight loss). To our knowledge, only one study has focused on earthworm burrowing patterns in relation to location of food (Le Couteulx et al., 2015), but it was restricted to endogeic species. It remains therefore difficult to compare our results with current available literature. Furthermore, our findings regarding *A. caliginosa* contrasted those of Le Couteulx et al. (2015), especially when crop residues were surface-applied. In their study, *A. caliginosa* was shown to increase porosity twice as much when food was mixed throughout the soil profile (approximately 0.68% porosity in the upper 10 cm soil depth) than when it was scattered at the soil surface (0.34%). In our study however, porosity made by *A. caliginosa* in the upper 10 cm of soil depth, was approximately 0.79% when residues were incorporated *vs.* 0.93% when residues were surface-applied (data not shown, as it was NS). Although species-mediated porosity was not significant when crop residues were surface-applied, our results suggest that indeed there is an increase of porosity when food is more limiting. Nevertheless, it is worthwhile mentioning that given the fact that the soil used by Le Couteulx et al. (2015) had a much lower organic matter content than ours (2% vs 3.7%), one would have expected a higher porosity with their experimental conditions, which was not the case.

### *4.3 Implications for field conditions*

By incorporating crop residues at ploughing depth, we did not simulate the mouldboard ploughing activity in itself, but one of its consequences, i.e. the displacement of food that would have been available for surface-feeders. In fact, the “real” consequences of ploughing could be even more severe due to the destruction of earthworm burrows and increase in mortality (Chan, 2001), e.g. due to predation. Our results regarding soil structure suggest that large water stable macroaggregates could be reduced through the incorporation of crop residues as compared to surface application. Porosity, however, was stimulated by residue incorporation, at least in single species treatments and within the time frame of 61 days, with the strongest effects for *L. terrestris*. Our data revealed some plasticity in burrowing activities in response to crop residue placement, at least for *L. rubellus*. *A. caliginosa* did not have large effects on soil porosity, stable aggregation or SOM distribution, nor was its population density or biomass affected by crop residue placement. Non-inversion, or minimum tillage practices, by providing crop residues at the soil surface seems to improve the fitness of earthworm species that feed at the soil surface with negligible effects on endogeic species, and contributes to improved soil structure due to an increase of water-stable macroaggregates in the upper 20 cm soil. Furthermore, the combination of anecics (*L. terrestris*) with the other earthworm functional groups also contributes to improving soil structure, due to the increase of large and small macroaggregates.

# 5. Conclusions

We demonstrated that providing crop residues on the soil surface or incorporating them in the soil profile affects earthworm performance, crop residue distribution, soil porosity and aggregate stability. Because of the importance of soil structure maintenance for sustainable land use, and the key role of earthworms belonging to different functional groups in mediating these soil processes, farmers should give careful thought when taking decisions about their crop residue management practices. Those decisions should improve food supply for earthworms belonging to different functional groups.

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# Tables

**Table 1 –** Percentage of body weight change (from the initial body weight) and of survival (mean (SE)) of earthworms used in each of the experimental treatments (crop residue treatments: surface-applied *vs.* incorporated at 20-30 cm soil depth; and earthworm treatments: *L. terrestris* – present (LT) or absent; other species – none, *A. caliginosa* (AC), or *L. rubellus* (LR)), after 61 days. F-statistics and p-values of best fitted linear mixed model of earthworm body weight change (% of initial body weight) and survival. N = 4, but see *.

| **Treatment** | ***L. terrestris* (LT)** | | ***L. rubellus* (LR)** | | ***A. caliginosa* (AC)** | |
|---|---|---|---|---|---|---|
| | **Weight change (%)** | **Survival (%)** | **Weight change (%)** | **Survival (%)** | **Weight change (%)** | **Survival (%)** |
| **Surface applied crop residues** | | | | | | |
| **AC** | - | - | - | - | -18.9 (17.0) | 81.3 (12.0) |
| **LR** | - | - | -0.4 (8.0) | 100 (0.0) | - | - |
| **LT** | -13.9 (12.4) | 91.7 (8.3) | - | - | - | - |
| **LT+AC** | -0.8 (4.9) | 100.0 (0.0) | - | - | -20.8 (9.1) | 100.0 (0.0) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **LT+LR** | -13.4 (2.1) | 100.0 (0.0) | -46.7 (4.3) | 83.4 (9.6) | - | - |
| **Crop residues incorporated at 20-30 cm soil depth** | | | | | | |
| **AC** | - | - | - | - | -28.7 (6.7) | 87.5 (7.2) |
| **LR** | - | - | -68.7 (19.9)* | 66.7 (23.6) | - | - |
| **LT** | -28.2 (3.1) | 100.0 (0.0) | - | - | - | - |
| **LT+AC** | -35.8 (6.7) | 91.7 (8.3) | - | - | -29.3 (4.0) | 93.8 (6.3) |
| **LT+LR** | -27.1 (13.8) | 91.7 (8.3) | -79.0 (15.3) | 33.3 (23.6) | - | - |

| | **Mixed models (F and p-values)** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **F** | **p** | **F**** | **P**** | **F** | **p** | **F** | **p** | **F** | **p** | **F** | **p** |
| **Placement** | 48.27 | ***<0.0001*** | NA | NA | 17.14 | ***0.003*** | 5.81 | ***0.039*** | 1.01 | 0.342 | 0.53 | 0.484 |
| ***L. terrestris*** | - | - | NA | NA | 28.37 | ***0.001*** | 3.85 | 0.081 | 0.01 | 0.920 | 2.67 | 0.137 |
| **Other species** | 0.12 | 0.889 | NA | NA | - | - | - | - | - | - | - | - |
| **Placement x *L. terrestris*** | - | - | NA | NA | 2.04 | 0.191 | 0.23 | 0.641 | 0.004 | 0.951 | 0.67 | 0.435 |
| **Placement x Other species** | 2.52 | 0.114 | NA | NA | - | - | - | - | - | - | - | - |

* In one of the blocks all *L. rubellus* died during the gut voiding-period, thus value refers to n = 3.

** Variation in survival was very low, and therefore statistics are not available (NA).

**Table 2 –** Mean and standard errors of soil organic matter (SOM) size fractions in g $kg^{-1}$ soil (POM > 250 µm, POM 53-250 µm, and SOM < 53 µm) of **surface-applied crop residues** per soil depth (0-20, 20-30 and > 30 cm) after 61 days as affected by different earthworm species and their combinations. No earthworms: 0, *L. terrestris*-LT, *A. caliginosa*-AC, *L. rubellus*-LR. F-statistics and p-values of best fitted linear mixed model of SOM size fractions. Different letters depict pairwise significant differences at $p < 0.05$: capital letters show significant differences within the main factor *L. terrestris*, and small letters within the main factor Other species. N = 4.

| **SOM fraction /earthworm treatment** | **Soil depth** | | |
|---|---|---|---|
| | **0-20 cm** | **20-30 cm** | **>30 cm** |
| **POM > 250 µm** | | | |
| **0** | 1.00 (0.03) Aa | 0.99 (0.05) | 1.03 (0.04) |
| **AC** | 1.08 (0.05) Aa | 1.00 (0.03) | 1.09 (0.06) |
| **LR** | 1.20 (0.05) Aa | 0.99 (0.05) | 1.03 (0.08) |
| **LT** | 1.30 (0.09) Ba | 1.00 (0.04) | 1.02 (0.02) |
| **LT+AC** | 1.32 (0.11) Ba | 1.08 (0.06) | 1.07 (0.06) |
| **LT+LR** | 1.19 (0.10) Ba | 0.96 (0.03) | 1.00 (0.02) |
| **POM 53-250 µm** | | | |
| **0** | 3.11 (0.24) | 2.57 (0.08) Aa | 2.60 (0.14) |
| **AC** | 3.06 (0.09) | 2.75 (0.50) Aab | 2.96 (0.50) |
| **LR** | 2.82 (0.22) | 3.04 (0.27) Ab | 2.72 (0.27) |
| **LT** | 3.12 (0.09) | 2.52 (0.21) Aa | 3.05 (0.21) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **LT+AC** | 2.98 (0.31) | | 2.77 (0.18) Aab | | 2.89 (0.18) | |
| **LT+LR** | 3.52 (0.08) | | 3.36 (0.23) Ab | | 2.70 (0.23) | |
| | | | | | | |
| **SOM < 53 µm** | | | | | | |
| **0** | 33.97 (1.40) | | 32.93 (0.84) Ba | | 32.60 (0.98) | |
| **AC** | 32.09 (0.75) | | 35.11 (1.61) Ba | | 32.02 (0.85) | |
| **LR** | 34.79 (0.64) | | 34.02 (0.44) Ba | | 33.80 (0.69) | |
| **LT** | 32.94 (0.70) | | 32.51 (0.14) Aa | | 34.15 (0.98) | |
| **LT+AC** | 33.82 (1.11) | | 32.84 (0.45) Aa | | 32.11 (1.22) | |
| **LT+LR** | 33.33 (0.74) | | 31.63 (1.01) Aa | | 32.97 (1.35) | |
| | **Mixed models (F and p-values)** | | | | | |
| | **F** | **p** | **F** | **p** | **F** | **p** |
| **POM > 250 µm** | | | | | | |
| ***L. terrestris*** | 7.73 | ***0.014*** | 0.27 | 0.613 | 0.16 | 0.700 |
| **Other species** | 3.45 | 0.059 | 1.03 | 0.380 | 1.36 | 0.287 |
| ***L. terrestris* x Other species** | 2.18 | 0.148 | 0.67 | 0.528 | 0.03 | 0.974 |
| | | | | | | |
| **POM 53-250 µm** | | | | | | |
| ***L. terrestris*** | 1.71 | 0.211 | 0.31 | 0.587 | 1.45 | 0.247 |
| **Other species** | 0.29 | 0.749 | 7.69 | ***0.005*** | 0.29 | 0.754 |
| ***L. terrestris* x Other species** | 2.39 | 0.126 | 0.38 | 0.688 | 0.84 | 0.451 |
| | | | | | | |
| **SOM < 53 µm** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***L. terrestris*** | 0.11 | 0.741 | 10.90 | ***0.005*** | 0.17 | 0.685 |
| **Other species** | 0.71 | 0.508 | 0.42 | 0.663 | 1.84 | 0.192 |
| ***L. terrestris* x Other species** | 1.73 | 0.212 | 1.19 | 0.331 | 1.15 | 0.344 |

**Table 3 –** Mean and standard errors of soil organic matter (SOM) size fractions in g kg$^{-1}$ soil (POM > 250 µm, POM 53-250 µm, and SOM < 53 µm) of **incorporated crop residues** per soil depth (0-20, 20-30 and > 30 cm) after 61 days as affected by different earthworm species and their combinations. No earthworms: 0, *L. terrestris*-LT, *A. caliginosa*-AC, *L. rubellus*-LR. F-statistics and p-values of best fitted linear mixed model of SOM size fractions. Different letters depict pairwise significant differences at $p < 0.05$: capital letters show significant differences within the main factor *L. terrestris*, and small letters within the main factor Other species. N = 4.

| **SOM fraction/earthworm treatment** | **Soil depth** | | |
|---|---|---|---|
| | **0-20 cm** | **20-30 cm** | **>30 cm** |
| **POM > 250 µm** | | | |
| **0** | 1.01 (0.02) Aa | 2.78 (0.13) | 1.18 (0.06) |
| **AC** | 0.99 (0.02) Aa | 2.51 (0.07) | 1.08 (0.07) |
| **LR** | 0.96 (0.03) Aa | 3.13 (0.12) | 1.26 (0.14) |
| **LT** | 1.08 (0.08) Ba | 2.96 (0.40) | 1.12 (0.06) |
| **LT+AC** | 1.05 (0.05) Ba | 2.59 (0.17) | 1.19 (0.04) |
| **LT+LR** | 1.06 (0.07) Ba | 2.79 (0.22) | 1.19 (0.07) |
| | | | |
| **POM 53-250 µm** | | | |
| **0** | 2.69 (0.15) | 2.66 (0.26) | 2.93 (0.17) |
| **AC** | 2.87 (0.25) | 3.27 (0.27) | 2.45 (0.23) |
| **LR** | 2.66 (0.18) | 2.95 (0.27) | 2.91 (0.09) |
| **LT** | 3.02 (0.11) | 2.78 (0.59) | 3.03 (0.14) |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| **LT+AC** | 2.75 (0.17) |  | 3.29 (0.45) |  | 3.02 (0.63) |  |
| **LT+LR** | 2.89 (0.21) |  | 2.96 (0.22) |  | 2.94 (0.23) |  |
|  |  |  |  |  |  |  |
| **SOM < 53 µm** |  |  |  |  |  |  |
| **0** | 34.67 (2.16) |  | 33.88 (0.67) |  | 35.18 (1.14) |  |
| **AC** | 32.95 (0.90) |  | 35.17 (0.92) |  | 35.11 (0.70) |  |
| **LR** | 32.73 (1.31) |  | 33.27 (0.51) |  | 32.35 (1.33) |  |
| **LT** | 33.07 (1.41) |  | 34.04 (1.84) |  | 34.31 (0.84) |  |
| **LT+AC** | 33.22 (1.32) |  | 33.92 (0.51) |  | 33.51 (0.81) |  |
| **LT+LR** | 35.87 (0.76) |  | 34.55 (1.17) |  | 35.71 (1.28) |  |
|  | **Mixed models (F and p-values)** |  |  |  |  |  |
|  | **F** | **p** | **F** | **p** | **F** | **p** |
| **POM > 250 µm** |  |  |  |  |  |  |
| ***L. terrestris*** | 4.92 | ***0.043*** | 0.03 | 0.875 | 0.01 | 0.913 |
| **Other species** | 1.23 | 0.313 | 7.42 | ***0.006*** | 3.43 | 0.060 |
| ***L. terrestris* x Other species** | 0.13 | 0.879 | 0.84 | 0.451 | 1.42 | 0.272 |
|  |  |  |  |  |  |  |
| **POM 53-250 µm** |  |  |  |  |  |  |
| ***L. terrestris*** | 2.44 | 0.139 | 0.01 | 0.923 | 3.91 | 0.067 |
| **Other species** | 0.13 | 0.881 | 1.16 | 0.340 | 1.58 | 0.239 |
| ***L. terrestris* x Other species** | 0.78 | 0.476 | 0.01 | 0.990 | 0.27 | 0.769 |
|  |  |  |  |  |  |  |
| **SOM < 53 µm** |  |  |  |  |  |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***L. terrestris*** | 0.29 | 0.601 | 4.42 | 0.053 | 0.43 | 0.523 |
| **Other species** | 0.40 | 0.678 | 1.15 | 0.343 | 0.21 | 0.810 |
| ***L. terrestris*** x **Other species** | 1.49 | 0.258 | 1.80 | 0.200 | 2.75 | 0.096 |

**Table 4** –SOM fractions (g $kg^{-1}$ cast) and weight of the pooled amount of the surface casts (g) after 61 days, as affected by different earthworm species and their combinations when crop residues were placed at the soil surface or incorporated in the soil profile. No earthworms: 0, *L. terrestris*-LT, *A. caliginosa*-AC, *L. rubellus*-LR. Only means are available because casts were pooled among the four different blocks due to scarcity of cast material.

| **Treatment** | **SOM size fractions** | | | **Weight of casts produced (g)** |
|---|---|---|---|---|
| | **> 250 µm** | **> 53 µm** | **< 53 µm** | |
| **Surface applied crop residues** | | | | |
| **AC** | 3.84 | 3.54 | 36.93 | 45.8 |
| **LR** | 22.24 | 8.52 | 47.58 | 110.7 |
| **LT** | 15.31 | 3.65 | 40.94 | 190.2 |
| **LT+AC** | 14.34 | 4.17 | 36.93 | 135.6 |
| **LT+LR** | 12.71 | 3.41 | 38.39 | 267.0 |
| **Crop residues incorporated at 20-30 cm soil depth** | | | | |
| **AC** | 0.99 | 3.33 | 33.10 | 26.2 |
| **LR** | 1.18 | 2.69 | 32.63 | 35.6 |
| **LT** | 2.27 | 2.57 | 28.17 | 358.5 |
| **LT+AC** | 1.16 | 3.66 | 33.93 | 366.2 |
| **LT+LR** | 1.74 | 2.50 | 33.90 | 456.0 |

**Table 5 –** Mean amounts and standard errors of water-stable aggregate (WSA) size fractions in g kg$^{-1}$ soil (WSA > 2000 µm, WSA 250-2000 µm, WSA 53-250 µm, and silt and clay SC < 53 µm) of surface-applied and incorporated crop residues per soil depth (0-20 and 20-30 cm) after 61 days as affected by different earthworm species and their combinations. No earthworms: 0, *L. terrestris*-LT, *A. caliginosa*-AC, *L. rubellus*-LR. F-statistics and p-values of best fitted linear mixed model of WSA size fractions. Different letters depict pairwise significant differences at $p < 0.05$: capital letters show significant differences within the main factor *L. terrestris*, and small letters within the main factor Other species. When only small letters are provided, significant differences refer to the interaction between both earthworm treatments. N = 4.

| **WSA size class/earthworm treatment** | **Crop residue treatment and soil depth** | | | |
|---|---|---|---|---|
| | **Surface applied crop residues** | | **Incorporated crop residues** | |
| | **0-20 cm** | **20-30 cm** | **0-20 cm** | **20-30 cm** |
| **WSA > 2000 µm (large macroaggregates)** | | | | |
| **0** | 1.18 (0.15) Aa | 1.95 (0.34) | 1.67 (0.32) | 11.17 (1.87) |
| **AC** | 3.35 (0.97) Ab | 0.87 (0.47) | 1.00 (0.27) | 13.27 (1.47) |
| **LR** | 3.60 (0.64) Ab | 5.33 (3.76) | 3.86 (1.82) | 12.24 (2.09) |
| **LT** | 4.98 (0.74) Ba | 3.62 (1.59) | 1.31 (0.68) | 11.01 (2.89) |
| **LT+AC** | 10.95 (2.08) Bb | 1.89 (1.20) | 1.23 (0.78) | 18.69 (5.18) |
| **LT+LR** | 23.31 (7.58) Bb | 1.89 (0.34) | 1.07 (0.53) | 12.05 (2.26) |
| | | | | |
| **WSA 250 - 2000 µm (small macroaggregates)** | | | | |
| **0** | 67.97 (0.67) b | 73.01 (6.36) | 65.37 (4.82) a | 97.98 (14.80) |

| | | | | |
|---|---|---|---|---|
| **AC** | 54.14 (2.06) a | 70.53 (8.01) | 59.05 (6.55) a | 87.94 (14.70) |
| **LR** | 64.28 (9.22) ab | 89.65 (18.43) | 59.45 (5.35) a | 80.24 (5.91) |
| **LT** | 62.73 (7.79) ab | 63.16 (10.95) | 57.82 (1.70) a | 68.01 (8.61) |
| **LT+AC** | 88.42 (8.38) bc | 78.73 (7.16) | 109.88 (9.04) b | 116.86 (19.47) |
| **LT+LR** | 105.18 (5.94) c | 75.11 (4.65) | 94.38 (7.52) b | 101.79 (10.81) |
| **WSA 53 - 250 µm (microaggregates)** | | | | |
| **0** | 788.12 (3.03) b | 790.21 (8.96) bc | 782.33 (4.95) bc | 755.73 (14.30) ab |
| **AC** | 809.33 (2.84) b | 799.07 (6.64) c | 816.75 (7.46) d | 770.47 (17.01) ab |
| **LR** | 808.34 (10.53) b | 770.64 (27.62) abc | 804.54 (5.50) cd | 778.04 (10.94) b |
| **LT** | 797.16 (10.04) b | 809.79 (6.50) c | 809.14 (3.12) d | 802.61 (17.3) b |
| **LT+AC** | 736.61 (16.66) a | 744.74 (4.91) a | 738.98 (16.37) ab | 710.31 (19.15) a |
| **LT+LR** | 728.35 (10.31) a | 760.6 (8.18) ab | 750.36 (6.30) a | 726.73 (8.36) a |
| **Silt and clay fraction SC <53 µm** | | | | |
| **0** | 129.97 (3.10) b | 119.70 (8.88) a | 137.39 (2.11) c | 123.29 (2.75) b |
| **AC** | 109.05 (2.64) a | 107.38 (1.67) a | 102.86 (1.62) a | 106.91 (2.91) a |
| **LR** | 104.17 (5.14) a | 110.86 (10.51) a | 116.75 (2.97) b | 114.86 (7.16) ab |
| **LT** | 113.63 (6.68) ab | 108.62 (6.60) a | 105.95 (2.32) ab | 98.56 (13.98) ab |
| **LT+AC** | 150.73 (13.86) b | 162.79 (4.77) b | 140.68 (11.93) abc | 143.54 (10.64) b |
| **LT+LR** | 134.35 (8.11) ab | 154.22 (6.13) b | 146.36 (3.68) c | 145.31 (12.81) ab |

|  | **Mixed models (F and p-values)** |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|
|  | **F** | **p** | **F** | **p** | **F** | **p** | **F** | **p** |
| **WSA > 2000 µm** |  |  |  |  |  |  |  |  |
| ***L. terrestris*** | 39.35 | ***<0.0001*** | 0.32 | 0.582 | 0.10 | 0.758 | 0.55 | 0.471 |
| **Other species** | 12.00 | ***0.001*** | 2.35 | 0.130 | 1.24 | 0.316 | 1.08 | 0.364 |
| ***L. terrestris* x Other species** | 3.31 | 0.065 | 0.78 | 0.477 | 1.07 | 0.368 | 0.67 | 0.527 |
| **WSA 250 - 2000 µm** |  |  |  |  |  |  |  |  |
| ***L. terrestris*** | 103.91 | ***<0.0001*** | 1.13 | 0.304 | 3.66 | 0.075 | 0.04 | 0.845 |
| **Other species** | 40.42 | ***<0.0001*** | 1.07 | 0.368 | 5.50 | ***0.016*** | 1.74 | 0.210 |
| ***L. terrestris* x Other species** | 9.52 | ***0.002*** | 1.78 | 0.203 | 21.59 | ***<0.0001*** | 3.43 | 0.059 |
| **WSA 53 - 250 µm** |  |  |  |  |  |  |  |  |
| ***L. terrestris*** | 42.91 | ***<0.0001*** | 76.08 | ***<0.0001*** | 1.23 | 0.284 | 8.27 | ***0.012*** |
| **Other species** | 0.26 | 0.777 | 76.22 | ***<0.0001*** | 42.03 | ***<0.0001*** | 3.01 | 0.080 |
| ***L. terrestris* x Other species** | 15.24 | ***<0.001*** | 12.23 | ***<0.001*** | 48.18 | ***<0.0001*** | 7.76 | ***0.005*** |
| **Silt and clay fraction SC < 53 µm** |  |  |  |  |  |  |  |  |
| ***L. terrestris*** | 8.94 | ***0.009*** | 223.20 | ***<0.0001*** | 0.96 | 0.344 | 5.44 | ***0.034*** |
| **Other species** | 8.37 | ***0.004*** | 14.81 | ***<0.001*** | 63.18 | ***<0.0001*** | 6.09 | ***0.012*** |
| ***L. terrestris* x Other species** | 8.21 | ***0.004*** | 20.52 | ***<0.001*** | 66.34 | ***<0.0001*** | 6.28 | ***0.010*** |

**Table 6 –** Summary of the outcomes of best fitted linear mixed model of earthworm-induced porosity (percent of porosity in relation to total soil volume after correction for porosity of control columns) after 61 days, as affected by different earthworm species and soil depth (main factors: species (*L. terrestris*, *A. caliginosa*, or *L. rubellus*), soil depths: 2.5 to 10, 10 to 20, 20 to 30, and 30 to 35 cm soil depth). N = 2.

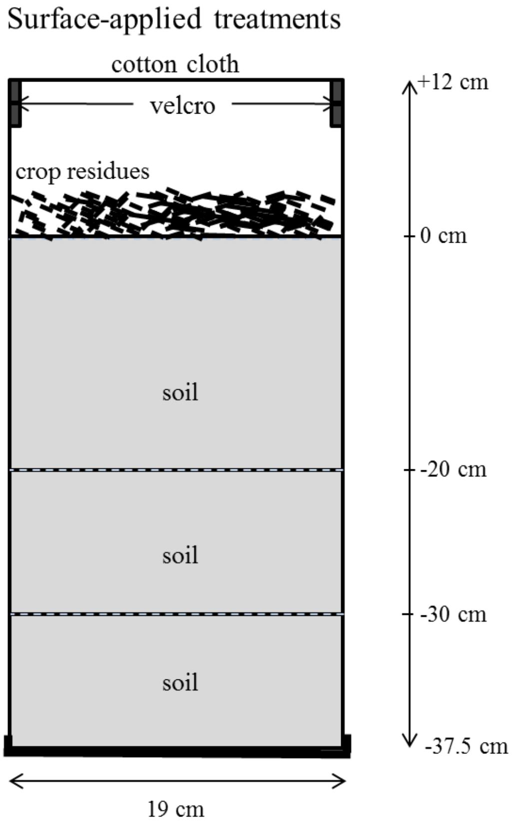
| | **Surface applied crop residues** | | **Incorporated crop residues** | |
|---|---|---|---|---|
| | **F** | **p** | **F** | **p** |
| **Species** | 0.91 | 0.429 | 5.27 | ***0.025*** |
| **Soil depth** | 7.36 | ***0.006*** | 6.03 | ***0.011*** |
| **Species x Soil depth** | 0.11 | 0.994 | 1.29 | 0.339 |

# Figure captions

**Figure 1 –** Scheme of the experimental mesocosms, showing crop residue placement treatments and earthworm treatments.

**Figure 2 -** Means and standard errors of earthworm-induced porosity (i.e. after correction for porosity of earthworm-free treatments) averaged over earthworm treatments. **A)** crop residues applied at the soil surface; **B)** crop residues incorporated between 20-30 cm depth. Different letters depict pairwise significant differences at $p < 0.05$ of porosity with soil depth layers. "*" depict mean porosity values that are significantly different from 0 (one-tailed t-test). N=2.

**Figure 3** – Mean and standard error of earthworm-induced water stable aggregates (WSA) size fractions (i.e. after correcting for WSA in earthworm-free control treatments), in treatments of single *vs.* two-species of earthworms (grey and white bars, respectively), when crop residues were surface-applied (panels A) or incorporated (panels B), per soil depth (0-20 (panels 1) and 20-30 (panels 2) cm).



**Earthworm treatments**

| | |
|---|---|
| 0 | No earthworms |
| AC | *A. caliginosa* |
| LR | *L. rubellus* |
| LT | *L. terrestris* |
| LT+AC | *L. terrestris* + *A. caliginosa* |
| LT+LR | *L. terrestris* + *L. rubellus* |

**Figure 1 –**

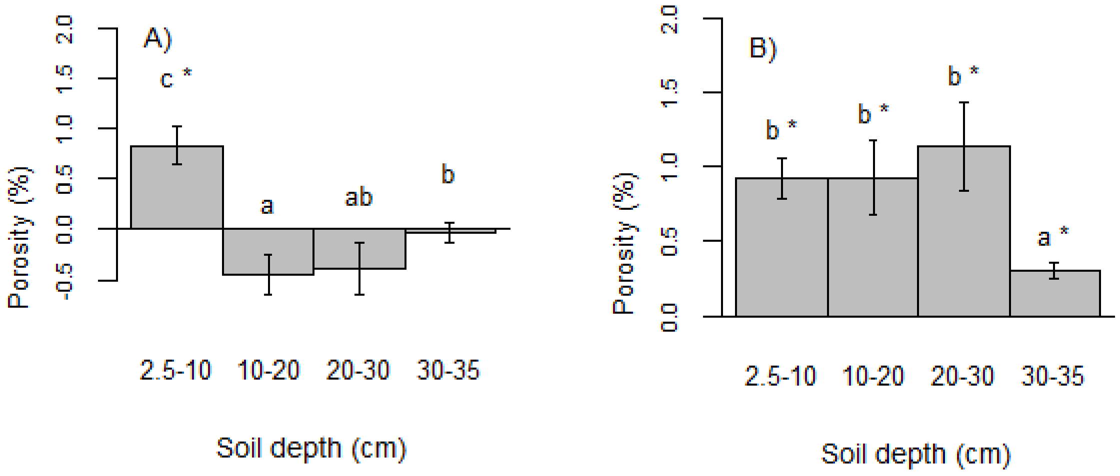
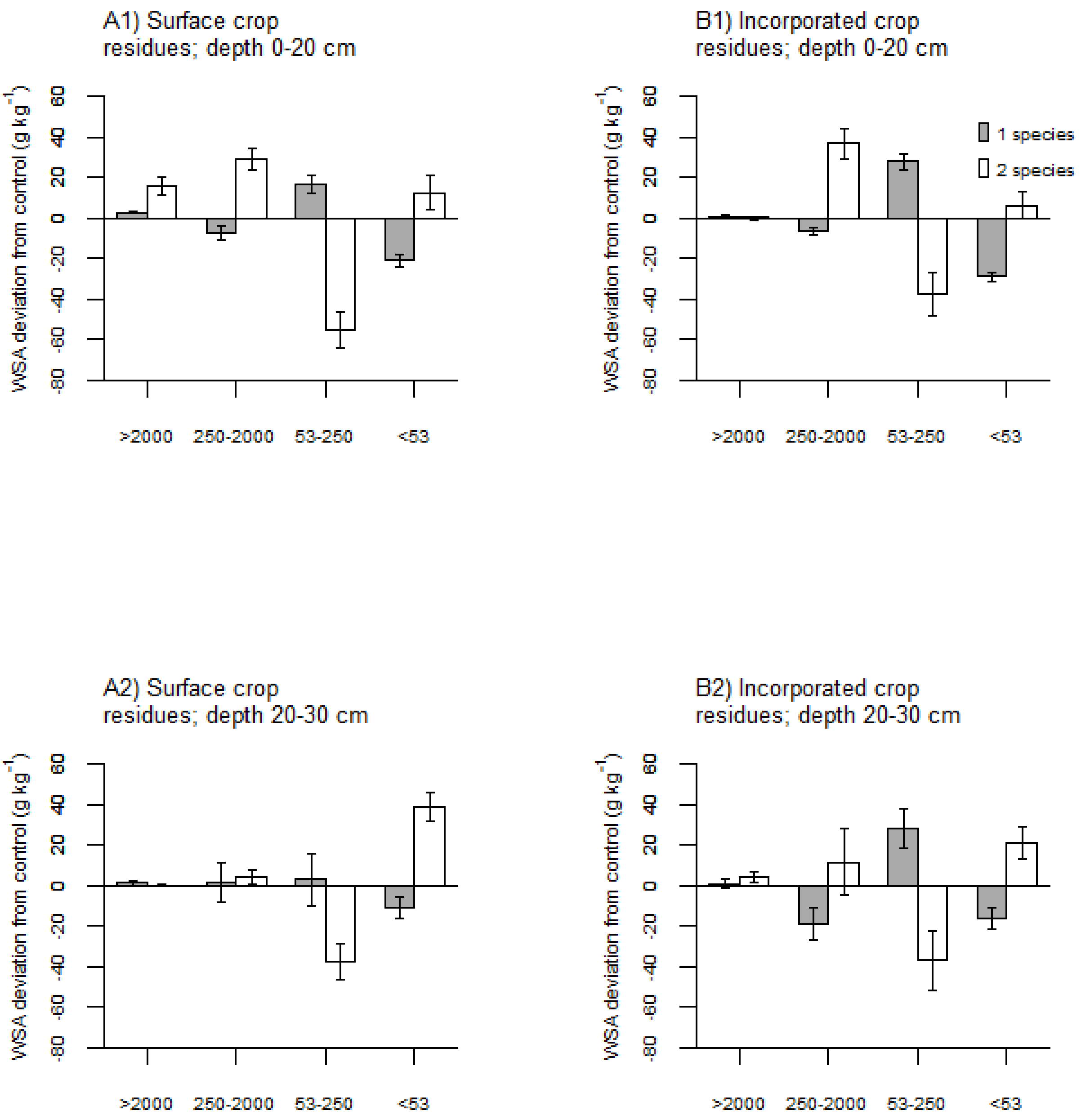

A)
Porosity (%)
-0.5
0.0
0.5
1.0
1.5
2.0
c *
a
ab
b
2.5-10
10-20
20-30
30-35
Soil depth (cm)
B)
Porosity (%)
0.0
0.5
1.0
1.5
2.0
b *
b *
b *
a *
2.5-10
10-20
20-30
30-35
Soil depth (cm)

**Figure 2 -**



A1) Surface crop residues; depth 0-20 cm

B1) Incorporated crop residues; depth 0-20 cm

A2) Surface crop residues; depth 20-30 cm

B2) Incorporated crop residues; depth 20-30 cm

**Figure 3** –