How earthworms cope with drought

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Abstract

Earthworms are an important part of ecosystems, such as meadows. They provide structure and drainage to the soil and they are a crucial food source for animals like meadow birds. It has been found that earthworm abundance decreases during dry periods of the year. Previous research investigated whether this may be attributed to vertical distribution by keeping earthworms in columns of homogeneously mixed clay soil, which showed that the earthworms hide deeper when the soil is dry. This study has elaborated on this with the aim to experimentally test how soil moisture content affects vertical of ecologically different earthworms by using in tact columns of both extensively and intensively managed farmland with homogeneously mixed clay soil as a means of control. As a sub question, this study looked at the effect of weight on vertical distribution. 18 columns (6 per sample type) underwent different moisture treatments (dry, moist and wet) for a period of 14 days in a climate chamber. It was found that vertical distribution in natural soil differs significantly from that in homogeneous clay soil with a clearer uneven distribution in the natural samples. Evidence was found for a decreased evaporation rate in extensively managed farmland compared to intensively managed. In accordance with that, the data indicates that earthworms, especially red worms, remain closer to the surface in extensively managed samples. This provides interesting implications for the increased abundance of meadow birds in extensively managed farmland compared to intensively managed fields. Further research is needed to identify possible causes of a difference in evaporation rate. Moreover, it will be important to replicate this study to improve upon sample size and experimental running time.

Introduction

Earthworms are extremely important soil organisms. They are considered to be one of the main ecosystem engineers in habitats such as meadows, because they three-dimensionally transport and recycle organic matter, providing plants and other organisms with important nutrients (Bertrand et al., 2015; Onrust & Piersma, 2019). Moreover, the production of vast systems of burrowing holes causes the soil to remain loose and well-drained, which creates opportunities for other organisms to inhabit the soil as well (Marashi & Scullion, 2003; Zaller & Arnone, 1999). Earthworms are also considered to be a crucial food source for (endangered) meadow birds (Onrust & Piersma, 2017). Over the past years, meadow birds such as the black-tailed godwit (*Limosa limosa*) have been widely studied because of their considerably rapid decline. One of the main threats that these birds are facing may be food availability (Onrust & Piersma, 2019; Wiggers et al., 2015), which increases the need for better understanding earthworms even further.

As earthworms are highly dependent on moisture, because they breathe through their skin, they are most abundant during fall and winter when the amount of rainfall is highest (Hooijmeijer et al., 2020). Climate change causes the period of high rainfall to shorten, because of which the soil will start to dry out earlier in spring (Lavalle et al., 2009). This might cause earthworm availability to decrease earlier as well, right during the period in which they are most important for breeding birds to feed on (Hooijmeijer et al., 2020). Thus, it is important to see how drought affects earthworms.

A study by Onrust et al. (2019) measured vertical distribution of two abundant species of earthworms, a detrivore (*Lumbricus rubellus*) and a geophage (*Aporrectodea caliginosa*), under different moisture levels. It was found that during drought, the worms will be located deeper in the soil, which renders them unavailable for birds to predate on. No interspecific differences were found, even though these groups are expected to show different burrowing behaviors (Bouché, 1977). This may be attributed to the fact that the worms were kept in a homogeneous mixture of clean clay soil, which would drastically change the distribution of soil organic matter compared to layered soils found under natural circumstances.

Hooijmeijer et al. (2020) show how earthworm abundance varies between differently managed soils. It would be interesting to see if the depth at which the earthworms are held up is also affected by these different types of plots to relate this back to meadow bird foraging behavior. Thus, this study will elaborate on the research question: “How does soil moisture content affect vertical distribution of ecologically different earthworm species under various management approaches?”. To do so, an intensively as well as extensively managed pasture will be used from which data was also collected by Hooijmeijer et al. (2020) (monoculture and species-rich meadow). To be able to compare the findings of this study to the experiment by Onrust et al. (2019), homogeneous clay soil will also be used as a means of control. In addition, this study will address how size affects vertical distribution under these circumstances as a sub question, because this remains poorly understood.

It is hypothesized that the results from this study will correspond to the results found by Onrust et al. (2019) in the general sense that earthworms will remain at greater depth with increasing drought. However, it is expected that vertical distribution in a homogeneous mixture of clean clay soil will significantly differ from those in naturally layered soil types, where the homogeneous mixture will result in a greater difference in distribution between the different moisture levels. The use of naturally layered soil is also expected to allow interspecific differences in vertical distribution to be conserved under laboratory conditions, where red worms are expected to remain closer to the surface compared to grey worms (Bouché, 1977). Additionally, it is expected that larger worms will be able to dig deeper in order to avoid dehydration (Hooijmeijer et al., 2020), which will cause the anecic worms to be located deeper as well. Lastly, the different soil types are characterized by different ways of management that resulted in very different vegetation compositions and densities. This is likely to have affected the availability and distribution of soil organic matter, the ability of the soil to retain water and the density of the soil itself. Hence, it is expected that the vertical distribution of the earthworms will also differ significantly between these different types of soil.

Materials & Methods

Fieldwork

The earthworms were collected from two different farmlands (intensively and extensively managed clay soil) in Ferwoude, Friesland ((53.007, 5.426) and (53.015, 5.434), respectively). These farmlands have also been studied by Hooijmeijer et al. (2020) and Onrust et al. (2019b), concerning earthworms. The two farmlands differ with regards to how they are managed. In other words, where the extensively managed farmland is often left alone, the intensively managed farmland is often visited with heavy agricultural machinery and activities such as manure injection occur. This has a clear effect on the vegetation in the pastures. The intensively managed land predominantly consists of Lolium perenne and other plant species are scarce, whereas the vegetation on the extensively managed land is more diverse with common additions like Ranunculus repens, varying species of grass and a few species of Rumex. The homogeneous soil was also retrieved from the intensively managed farmland and was mixed and pulverized prior to being put in the tubes. For the homogeneous soil, the tubes were completely filled with soil, which was then pressed down 5cm to obtain a consistent soil resistance across the different samples. The two natural soil sample types that were used for the lab experiment were collected by hitting PVC tubes (length 30cm, width 10cm) into the farmland. The tubes were vertically cut in half prior to this for ease of use later on in the experiment and thus, these halves were first put together using duct tape and tie wraps. They were taken out by digging away the surrounding soil, upon which the bottom was closed with a lid. To prevent loss of soil moisture during storage between collection and the setup of the experiment, the tubes were covered with cling film. The soil samples were stored in this way for ~60 hours in a dark and cool environment before the addition of the earthworms.

At the different farmlands, earthworms were collected by digging in the soil with a shovel, which was followed up by ripping the cut-out soil clod apart to find the earthworms. Specimens from the two different earthworm species groups were collected, the detritivorous Red worms (*Lumbricus castaneus*, *L. rubellus*, and *Satchellius mammalis*) and the geophagous Grey worms (*Aporrectodea caliginosa*, *A. longa* and *A. rosea*), and put in trays to be transferred to the laboratory. In total 306 earthworms were collected, 102 from the extensive farmland and 204 from the intensive farmland, so that for every soil sample type earthworms could be used from the same field as where these soils were collected. The earthworms were stored in the containers and some soil from the corresponding farmland was added to the containers to create an environment for the earthworms to live in. The earthworms were also stored for ~60 hours in the same place as the soil samples before the transfer to the PVC tubes.

Lab experiment

To study the vertical distribution of the Red and Grey worms in the two differently managed soil types and a homogeneous soil under different soil moisture levels, the earthworms were put into the PVC tubes filled with the differently managed soil types for 14 days (from 03-05-2021 - 17-05-2021). The PVC tubes had a length 30 cm and a width of 10 cm. To easily reach the soil after the experiment and to prevent the earthworms from redistributing, the PVC tubes were cut in half beforehand and were held together by tie wraps and sealed with a lid on the bottom. The top was sealed with a piece of fabric with tiny holes and a rubber band to prevent the earthworms from escaping.

As previously described, the tubes were already filled with clay soil in the location where the worms were collected. Because we wanted to do every combination in duplicate, this resulted in 6 tubes for each soil management approach (intensive farmland, extensive farmland and homogeneous). In each tube 17 earthworms from the corresponding sampling pasture were added: 10 Grey worms, 5 Red worms and 2 anecic worms (*Aporrectodea longa*). This ratio (10:5:2) has been retrieved from a raw dataset provided by J. Onrust, where he calculated the abundance per ecotype in different farmlands (Hooijmeijer et al., 2020; Onrust, 2021). This is depicted in figure 1. Variation in sizes was also taken into account, as this is also within the scope of this research. To make a comparison between the soil moisture before and after the treatment this was also measured, before the addition of the earthworms, with the ML3 ThetaProbe. This soil moisture measurement tool measures the soil moisture content and gives a value between 0 and 100% (± 1%) and also causes minimal soil disturbance (Eijkelkamp, 2013). The measurements were made by putting the probe of the soil moisture sensor in the top part and the bottom part of the soil.

The earthworms were kept in the tubes for a period of 14 days. The tubes were maintained in a climate chamber under a constant temperature of 15C, 80% humidity and a photoperiod of 12 hours. Unfortunately on 09-05-2021 the climate chamber had an error and the temperature reached 25C for a few hours. Soil watering occurred on a daily basis, with the exception of the 12th day. The evaporation rate in the climate chamber was measured to be 11.0mL/day by Onrust et al. (2019). Therefore, the moist treatment received 11.0mL of water and the wet treatment received twice this amount (22.0mL). The dry treatment was not supplied with water during this setup. The earthworms received no additional food supply besides the organic matter that was already present in the column after collection of the samples.

After 14 days, soil moisture was measured again in both the top and bottom part of the soil using the ML3 *Thetaprobe*. Consequently, both halves of the tubes were taken apart to quickly cut the soil into layers of 5cm. In each layer, earthworms were counted by ecotype and weighed to take into account individual size as well. Weight was chosen as an indicator of size instead of length or thickness as the earthworms can easily change their shape and thus might significantly affect such measurements.

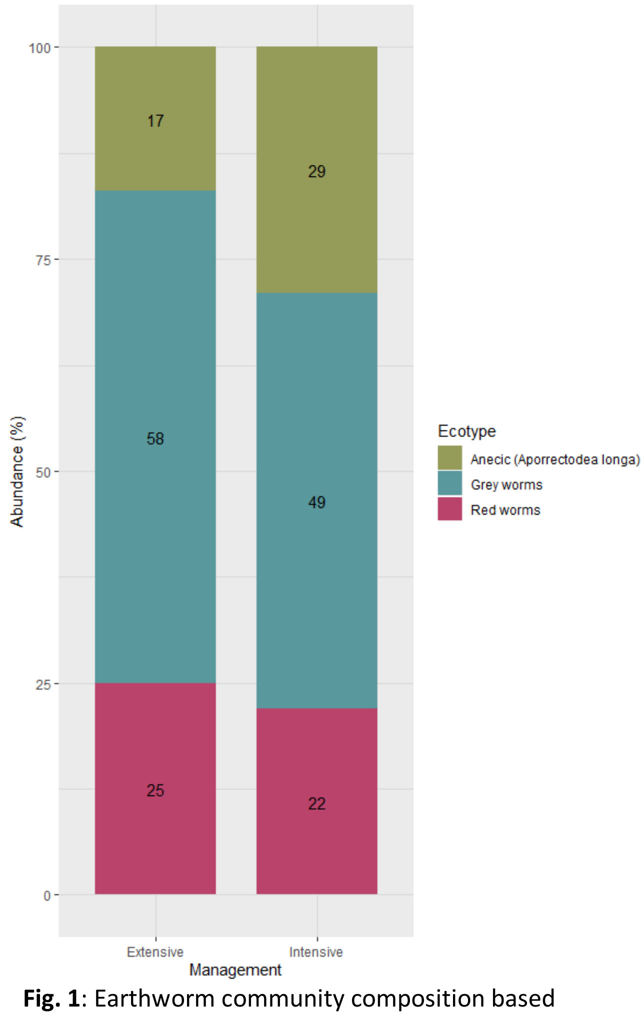

Fig. 1: Earthworm community composition based on the raw data of J. Onrust (2021).

Data analysis

The data from the lab experiment was analysed and visualised in the software environment R (R Core Team, 2017). The package "ggplot2" (Wickham, 2016) was used to visualize the data and to create graphs. During data collection often more (and sometimes less) earthworms were found than that were added at the start. Above that, the number of earthworms differed per PVC tube, because the columns were retrieved intact from the pastures and it was not known how many earthworms were present in those columns. Therefore, the proportion of earthworms was used as a measure of the amount of earthworms per layer, rather than the number of earthworms. For the weight distribution, the average weight per layer was calculated. The data was analysed via three-way ANOVA statistical tests. Moreover, post-hoc tests were applied via the Tukey method using the “emmeans” package (lenth, 2020). For the analysis of the vertical distribution data, the depth, treatment and pasture were regarded as independent variables and the proportion and the average weight of the earthworms as dependent variables. For the analysis of the change in soil moisture, the time interval (before and after), the treatment and the pasture were regarded as independent variables and the soil moisture as a dependent variable. The total proportion of earthworms in a PVC tube is by definition 100%. Hence, no significant results can be found if depth is not included in the relation. This variable was therefore always included in analyses when looking at proportions of earthworms.

Results

Soil moisture content

Figure 2 shows the soil moisture content before and after running the experiment. A significant difference between the management approaches can be seen before as well as after ($p < 0.0001$). Three-way ANOVA showed a significant difference between all three treatments ($p < 0.01$). However, a post-hoc test reveals that in relation to management approach and time only the dry and wet treatments differ from each other significantly ($p < 0.05$). The effect of the treatment on the samples was largely insignificant, except the dry treatment on the top soil of the extensive and intensive samples ($p < 0.05$). However, the data does imply a slight increase in soil moisture in the wet treatment after the experiment compared to before at both depths. Similarly, the dry treatments show some decrease in moisture, not only in extensive and intensive samples, but also in homogeneous samples. Moreover, the data suggests that deeper extensive soil seems to lose moisture slower than intensively managed soil, which can be seen in both the dry and the wet treatment.

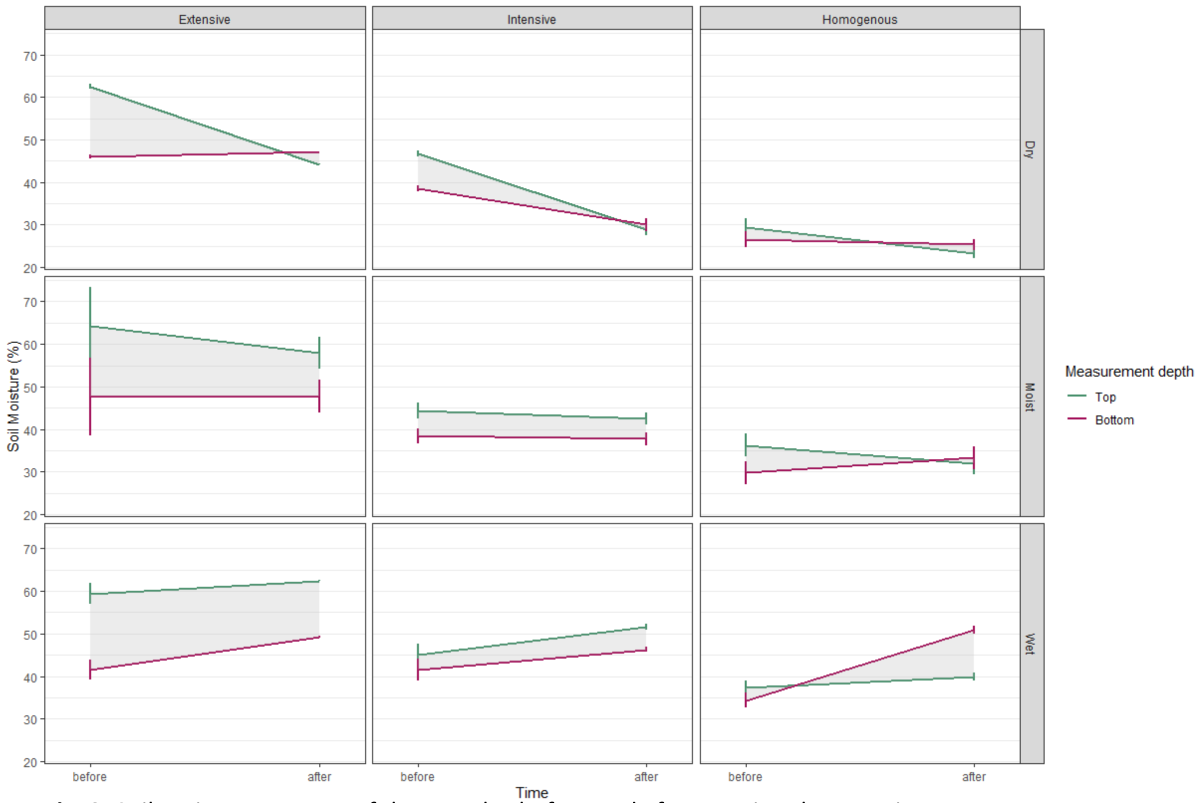

Fig. 2: Soil moisture content of the samples before and after running the experiment, divided over a grid of treatment and management approach.

Vertical distribution

Proportion

The general pattern that can be seen in the collected data is that the proportion of earthworms significantly differs with varying depth (Figure X). This is noticeable across all the worms ($p < 0.0001$) (Figure 3a), and also when focusing on the individual ecotypes ($p < 0.01$) (Figure 3b-d). Although Figure 3 suggests a difference between treatments and management approaches, this is often not statistically significant. In all ecotypes there was an interaction between the depth and the management approach ($p < 0.01$), regarding the distribution of the earthworms. This means that the effect of the depth on the distribution can be (partly) explained by the management approach. However, post-hoc tests show almost no difference between management approaches. The interaction between the depth and the treatment was only significant for the total worms and the Grey worms ($p < 0.001$). Although, again after executing post-hoc tests this was not found to be significant.

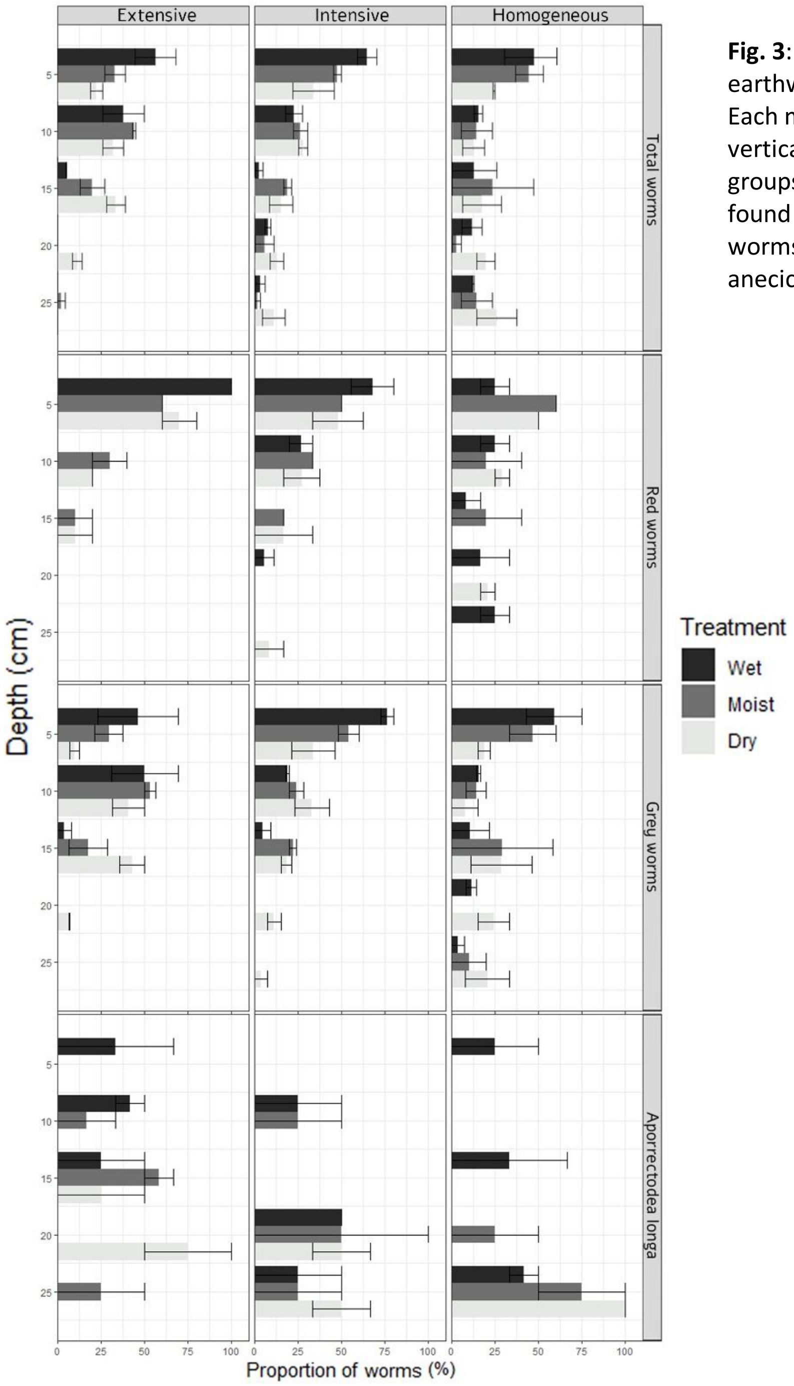

Fig. 3: Vertical distribution of the earthworms based on proportion. Each management approach is vertically lined out over the different groups of earthworms, which can be found horizontally: all worms (a), red worms (b), grey worms (c) and the anecic worm *A. longa* (d).

Weight

The vertical distribution of all earthworms based on average weight is visualized in Figure 4. Three-way ANOVA shows an overall significant difference in weight between different soil depths ($p < 0.05$). Moreover, depth in relation to management approach significantly affects average weight ($p < 0.01$). On the other hand, there is no significant effect of treatment on the weight distribution and post-hoc tests show no significant differences either. However, the data does suggest an increase in average weight with increasing depth, which is most apparent in the homogeneous soil. The weight seems to be most evenly distributed in extensively managed farmland.

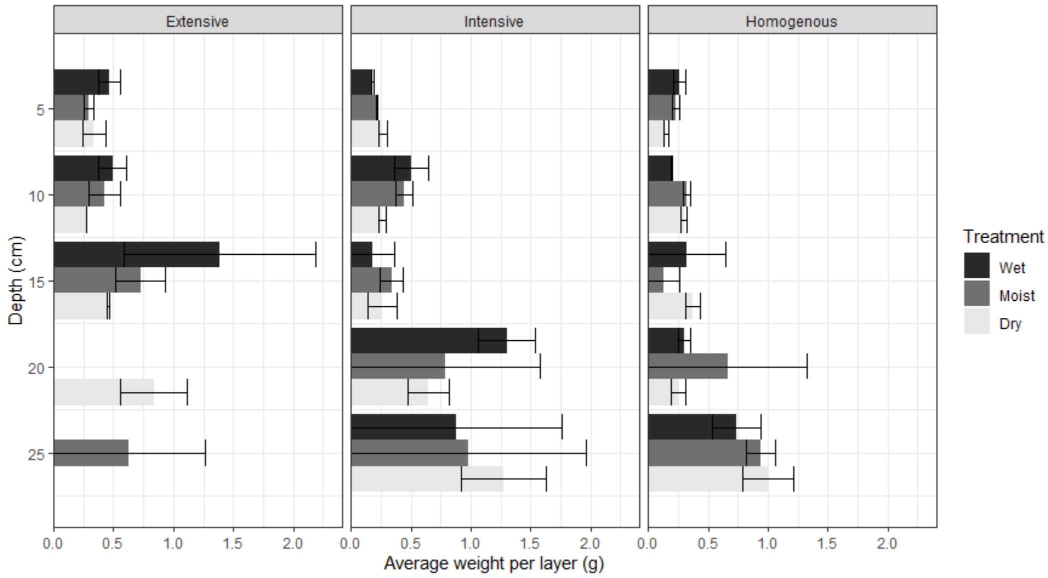

Fig. 4: Vertical distribution of the earthworms based on average weight across all ecotypes. Results are divided by management approach and treatment.

Discussion and conclusion

First of all, it is important to note that only the dry and wet treatment significantly differed from each other. No sufficient divergence had occurred between the three moisture treatments compared to the starting situation. There is, however, some divergence visible from the data. A larger sample size might have helped to increase the significance. To speed up the process of divergence, air humidity could have been decreased in the climate chamber to cause the evaporation rate to increase. Alternatively, the moist and wet treatment could have received more water, which would lead to an increased difference compared to dry as well as between moist and wet. However, it is likely preferable to increase the experimental running time, because speeding of the process of dehydration might cause stress factors to affect the behavior of the earthworms. In general, the treatments did not cause significant divergence from the starting situation, which is also somewhat visible from the data. This again suggests that a longer running time is needed. The data indicates that there might be a difference in evaporation rate between extensive and intensive, especially at depth. However, this was not significant either, so this could again be improved upon via the afore mentioned methods. This might provide important underlying causes for differences in distribution between the management types and thus implications for the presence or absence of meadow birds.

Regarding the vertical distribution, this study found that even though no significant difference was observed between the management approaches, findings within them provide evidence for significant aberration of homogeneous soil. Extensive and intensive both show significant differences in earthworm proportions between different layers, whereas homogeneous does not. This implies that worms are evenly distributed in the homogeneous soil compared to being more gathered in the other two. Results by Onrust et al. (2019) showed significant effect of soil moisture on vertical distribution. However, the results from the homogeneous samples of this experiment remain comparable to the moist treatment of Onrust et al. (2019) over all treatments. This again suggests that running the experiment for a longer period of time would be needed to acquire more significant results. This may thus well be the same for intensive and extensive. However, the results were already more apparent in these two management approaches than in homogeneous soil samples. The data suggests that red worms overall seem to remain closer to the surface than grey worms in all treatments, which would connect to the reasoning that red worms are more important prey species for meadow birds (Onrust & Piersma, 2017). Furthermore, the data implicates that worms remain closer to the surface in extensively managed farmland, which would provide a possible explanation for increased presence of meadow birds in such farmlands (Hooijmeijer et al., 2020) and it coincides with the findings of Onrust & Piersma (2019) that earthworms seem to partially avoid the upper layer of intensively managed farmland, possibly due to the injection of manure, which causes soil desiccation. However, the soil moisture content data shows that the extensive farmland was more moist in all treatments, so one could say this results in a biased observation. On the other hand, because they have been subjected to the exact same conditions, the decreased evaporation of extensive may exactly be why the distribution differs from intensive and might therefore make it more suitable for meadow birds. Lastly, a study by Felten & Emmerling (2009) show that earthworms dig much deeper than 25cm. Their data indicates that the worms can easily be found at 50cm. However, they used homogeneous soil. Our data shows that in natural soil, the worms do not even inhabit the lower layer(s) of the samples, which indicates that 25cm depth is sufficient for experiments such as performed during this study.

The anecic worm *Aporrectodea longa* did not provide useful results regarding proportion, but because they are an important asset to the weight range of earthworms, they are important to vertical average weight distribution. Even though the results are not significant, there seems to be a correlation between weight and depth, where heavier worms are distributed deeper in the soil. However, there is no apparent difference in weight between treatments, which suggests that smaller worms always remain higher than large worms, or possibly that only larger worms are capable of significant vertical migration. Average weight in the top layers seems to be higher in extensive than intensive. This would be another possible suggestion as to why meadow birds are more attracted to extensively managed fields. It could well be that not only abundance, but also biomass is increased in extensively managed farmland. This again connects well to the findings of Hooijmeijers et al. (2020).

Overall, a bigger sample size and longer running period of the experiment would provide more apparent results. Unfortunately, this was not possible withing the timeframe of this study, but this would be very interesting to improve upon in further research. To conclude on the research question of this study, a clear difference was found between natural and homogeneous samples, although this difference was exactly opposite to what was expected beforehand. The differences were more apparent in intensive and extensive instead of homogeneous. It was assumed that clean soil would have a higher evaporation rate due to the lowered soil density. However, it appears that the presence of vegetation causes more evaporation instead, even though the soil does remains more moist as a whole, compared to homogeneous. This study found an indication for a difference between intensive and extensive, especially for red worms. When relating this back to the field, this study found implications that extensively managed farmland is more resistent to long dry periods. Therefore earthworms will remain at the surface to a greater extent, providing more food to meadow birds in a time where it is most needed. Future research could focus more on further examination of differences in evaporation rate between extensively and intensively managed farmland and causes for it, such as soil density and vegetation. This may well provide answers as to why a difference in vertical distribution and abundance is found and why meadow birds are located more in extensive farmland. To finalize, this study shows the need for revision of the study by Onrust et al. (2019) by using natural soil samples, which will provide important and interesting result that can be better related back to natural circumstances.

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