Earth, worms & birds
Onrust, Jeroen

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Abstract
Earthworms (Lumbricidae) are important prey for many birds. Based on their own feeding ecology, earthworms can be distinguished in two ecotypes; the detritivores that feed on organic material and the geophages that feed on soil particles and organic matter. Detritivores collect their food on the surface during the night when they are exposed to nocturnal predators. Hungry animals tend to show more risk-prone behaviour and may therefore be more vulnerable to bird predation, so we expect well-fed detritivorous earthworms to visit the surface less frequently. In this study, we tested this hypothesis in dairy farmland in Friesland, The Netherlands. Two uniform grasslands were split, with each half receiving either an early (1 February 2014) or a late (14 March 2014) farmyard manure application. Every two weeks, nocturnal surface activity of earthworms was measured by counting surfacing earthworms from a slowly pushed cart. Furthermore, soil samples were taken for total abundances and to measure individual body conditions of earthworms. As predicted, the density of surfacing earthworms was on average 2.5 times higher in the fields before farmyard manure was applied. Immature detritivores had significantly lower body masses in fields not yet manured, suggesting that these growing earthworms must have been hungry. Differences in surfacing behaviour and body mass disappeared after all fields had been given farmyard manure. We conclude that hunger forces detritivorous earthworms to the surface. After manure application, they appear satisfied and avoid the risk of depredation by birds by staying away from the soil surface. To promote earthworm availability for meadow birds, spreading farmyard manure on the surface should occur as late in spring as possible. In this way, hungry earthworms are forced to the surface and are available as meadow bird prey for longer periods.
Introduction

As places with the most food are not necessarily the safest places, foraging animals must often trade off the rewards of feeding and the risks of becoming food themselves (Lima & Dill 1990, Sih 1992, Krebs & Davies 2007). During periods of shortage, eventually their increased need for food overrides the ‘fear’ to forage at risk (Lima 1998). The tendency for hungry animals showing for more risk-prone behaviours (Dill & Fraser 1984, Horat & Semlitsch 1994), may be relevant for the understanding of earthworm surfacing behaviour, with implications for their availability to avian predators.

Earthworms (Lumbricidae) are soil-dwelling organisms well known for their positive effects on soil functioning (Lavelle et al. 2006, Blouin et al. 2013). Earthworms feed on decaying organic material, and derive nutrition by feeding directly on bacteria and fungi that grow upon these materials, but also on the mutualistic relationship with these micro-organisms in the earthworms’ guts (Flack & Hartenstein 1984, Edwards & Fletcher 1988, Brown 1995). As litter is deposited onto the soil surface, earthworms need to visit the surface, or retrieve the food for ingestion in their burrows (Photo 4.1). Some earthworm species rely on surface foods more than others, the surfacing species being called detritivores (Hendriksen 1990, Curry & Schmidt 2007). In contrast, earthworms that primarily feed on soil particles and humified organic matter are termed geophages (Svendsen 1957, Judas 1992, Neilson & Boag 2003). According to the widely used classification of Bouché (1977) who classified earthworms into three ecological groups, the anecic (e.g. Lumbricus terrestris) and epigeic species (e.g. Lumbricus rubellus) belong to the detritivores, whereas endogeic species (e.g. Aporrectodea caliginosa) belong to the geophages.

In turn, earthworms themselves are important food for many animals (MacDonald 1983, Curry 1998). By feeding or collecting food at the surface, detritivore earthworms expose themselves to their above-ground predators. An earthworm can effectively avoid predation by visually oriented diurnal predators by remaining in the soil, or by surfacing only at night. As earthworms do not rely on visual sensory cues for feeding, they can forage in darkness (Edwards & Bohlen 1996). Surfacing for feeding, moving or mating, not surprisingly, only occurs at night (Baldwin 1917, Svendsen 1957, Butt et al. 2003). Their night crawling may explain to some degree why many earthworm-eating predators are also nocturnally active, e.g. Red Foxes (Vulpes vulpes; MacDonald 1980), Badgers (Meles meles; Kruuk & Parish 1981), adult Carabidae beetles; Jelaska & Symondson 2016), Little Owls (Athene noctua; Hounsome et al. 2004) and Golden Plovers (Pluvialis apricaria; Gillings et al. 2005, Piersma et al. 2014). Predation risk tends to be higher in moonlit nights (Galbraith 1989, Milsom 1990, Kirby 1997, Gillings & Sutherland 2007),
which possibly explains why earthworm surface activity is lower around Full Moon (Ralph 1957, Michiels et al. 2001). Although the risk predation risk is real when they are above ground, these soil-dwelling organism need to come to the surface to acquire food. Surfacing activity by detritivores would best take place when food availability is high and when the need to collect food overrides the danger of being eaten.

In this study we experimentally investigated the effect of increased food availability, in terms of surface-applied farmyard manure, on the surfacing activity of earthworms in an agricultural grassland. Through this field experiment we aim to better understand how different fertilization regimens may benefit earthworms as well as their key predators, meadow birds (Charadriiformes), in Dutch dairy farmland. Especially during the pre-breeding period (February-April), earthworms are staple food for these birds (Högstedt 1974, Galbraith 1989, Baines 1990, Beintema et al. 1995). We expect that earthworms will show more surfacing activity in areas not supplied with farmyard manure, as hunger will then force the earthworms to search for food on the surface. This would mean that the timing of manure applications would strongly affect the suitability of grassland as feeding areas of meadow birds.

Photo 4.1: With its tail anchored in its burrow, a detritivorous earthworm (Lumbricus rubellus) is searching for food at night (Koudum, 17 April 2013).
Methods

Study site
On the dairy farm of Murk Nijdam in Wommels, Fryslân, The Netherlands (N 53°5’35”, E 5°33’51”), two adjacent grasslands (A: 100 x 350 m, B: 100 x 280 m) were selected for this study. Both grasslands have the same (extensive) management, meaning that the fields are fertilized once year at the end of March with farmyard manure and mowed in June, after which grazing occurs until October/November. Farmyard manure consisted of straw mixed with cattle dung and urine, collected daily in the stable and then put on a heap outside. Here it is composted for up to a year before it is used as fertilizer. The carbon-to-nitrogen ratio at the moment of application was 14.7 ($N = 3, SD = 0.22$), which was measured according to the DUMAS method. To create a homogenous sward, the two grasslands, separated by a path of concrete and are surrounded by canals (Fig. 4.1A), had been levelled in 1999 and there are no foot drains on the surface but buried drainage pipes. The plant community of the fields was dominated by *Agrostis stolonifera*, *Alopecurus geniculatus*, *Bromus hordaceus*, *Cardamine pratensis*, *Cerastium fontanum*, *Elytrigia repens*, *Lolium perenne*, *Poa trivialis*, *Ranunculus repens*, *Rumex acetosa* and *Taraxum officinale*.

Photo 4.2: One of the experimental grasslands where the early field (left) has already received farmyard manure (Wommels, 6 March 2014).
Farmyard manure application experiment

For this experiment the two grasslands were divided in a field with an early manure application (1 February) and a field with a late manure application (14 March), creating four rectangular experimental fields (Fig. 4.1A, Photo 4.2). During an application, around 13 ton/ha farmyard manure was spread on the surface. Depending on the amount of farmyard manure available, most meadow bird reserves in The Netherlands are fertilized with 20 ton/ha once a year from 1 Feb – 1 April (van der Geld et al. 2013).

Surfacing earthworms were counted in every field every two weeks between 6 February and 3 April 2014. This was done by lying prone on a robust cart which was slowly moved forward by foot. In this way, little vibrations were created and the observer can count surfacing earthworms from a height of 50 cm and within a width of 50 cm (Onrust et al. unpubl. data). Counts were conducted at night, as earthworms only surface then, therefore a head torch (160 lumens) was used. The surfacing earthworms were counted on ten random transects per field, each transects with a length of 5 m.

When farmyard manure is put on the soil surface, it reduces the soil surface area on which surfacing earthworms can be detected by predators. To account for this ‘shading’ effect, we measured the cover of farmyard on the grass by throwing randomly a 1 × 1 m quadrat and estimate the percentage of manure cover within that quadrat. This was repeated 10 times per field for three days starting on 21 February, 21 March and 9 April 2014, respectively. One week after the first application, the farmyard manure covered 15% of the soil surface, with a rapid decline in the following weeks to 3% in the early fields and 9% in the late fields by the end of the experiment (Fig. 4.1B). We used the interpolated percentages to correct observed number of surfacing earthworms per square meter.

The overall densities of earthworms in the soil were measured on 6 March by taking randomly six 20 × 20 × 20 cm soil samples per field. These were sorted by hand. As deep-burrowing anecic species could be missed, one litre of ‘hot’ mustard solution was poured into the dig and for 15 min all emerging earthworms were collected (for a description of this method, see Lawrence & Bowers 2002).

To determine the mass of the individual earthworms in the different fields, we collected earthworms on two days. The first collecting day occurred 33 days after the first fertilizer treatment but before the second treatment was applied, and the second collecting day occurred 26 days after the second fertilizer treatment. All earthworms (detritivores and geophages) were collected and stored in a 98% ethanol before being processed. From each individual earthworm we measured ash-free dry mass (AFDM) in mg, later accounting for the length of the earthworm in mm. To do so, first dry mass was determined by drying the worms in a stove at 70 °C for 48 h after they were weighed to the nearest 0.1 mg. The ash mass was deter-
mined by burning the earthworms in a muffle oven at 500 °C for 4 hours after they were weighed again to the nearest 0.1 mg. AFDM was then determined by subtracting the ash mass from the dry mass. As mature earthworms are heavier than immature individuals of the same length, we analysed these groups separately.

**Figure 4.1:** For this study two adjacent agricultural grasslands (A in red and B in blue) were split in a field with an early (dark colour) and a field with a late (light colour) farmyard manure application (A). Cover of farmyard manure on the surface was measured at three intervals during the fieldwork period in spring 2014 (B). Six hand-sorted 20 x 20 x 20 cm soil samples were used to determine earthworm abundances per field, bars represent stacked data for geophagous and detritivorous earthworms (C). Error bars represent SE.
On the first sampling day, seven soil samples per field were taken randomly to determine the vertical distribution of both earthworm groups in the soil to a depth of 20 cm. We expect detritivores to be higher in the soil column when farmyard manure is applied. To measure this, a $20 \times 20 \times 20$ cm soil sample was horizontally cut in 4 slices of 5 cm. Each slice was then sorted out by hand and the number of earthworms per group was determined. The vertical distribution was then calculated as the proportion of earthworms per slice and per group.

Statistical analyses
As all fields were eventually fertilized with farmland manure, we analysed the data on surfacing earthworms according to the two periods; period 1 is before the second fertilization and period 2 is after that. For both periods we used a Generalized Linear Mixed Model (GLMM) with number of surfacing earthworms as response variable and grassland (A or B), manure (early or late) and time (observation day) as explanatory variables. Transect number was added as a random factor. A step-wise backward procedure was followed to find the Minimal Adequate Model (MAM) in which terms were deleted in order of decreasing $P$-value (Quinn & Keough 2005). Earthworm abundances were analysed separately per earthworm ecotype by a GLMM with grassland, sampling date and manure application as explanatory variables and soil sample as random factor and with a Poisson error distribution. For the vertical distribution of earthworms we used proportion data and therefore the results were analysed by a binomial GLMM in which the response variable was entered as a matrix where the first column is the number of earthworms found at a certain depth (“successes”) and the second column is the number of earthworms not found (“failures”). Earthworm ecotype and manure were added as fixed effects and a random intercept term was added with depth nested in soil sample. A Generalized Linear Model (GLM) with Gaussian family structure was used to analyse the data on body condition of earthworms in all four fields. A Tukey HSD post hoc analysis was then performed to reveal differences between groups by using the lsmeans package (Lenth 2016). All statistical analyses were performed in R version 3.1.2 (R Development Core Team 2017).

Results
The detritivorous earthworm species found were *Lumbricus castaneus*, *L. rubellus* and *L. terrestris*. Geophagous species were *Allolobophora chlorotica*, *Aporrectodea caliginosa* and *A. rosea*. The abundance of detritivores was higher in grassland A than in B (GLMM: $F_{1,22} = 4.890$, $P < 0.05$) and in fields with late rather than early manure application (Fig. 4.1C, GLMM: $F_{1,19} = 412.36$, $P < 0.001$). The densities of
geophagous earthworms was similar in the two grasslands (GLMM: $F_{1,20} = 0.553$, $P = 0.457$), but the abundances were somewhat higher for the early fertilized fields than the late fertilized fields (Fig. 4.1C, GLMM: $F_{1,21} = 17.742$, $P < 0.001$).

One week after the farmyard manure was spread on the surface in the early fields, the total number of surfacing earthworms was significantly lower in the early
than in the late application fields (Fig. 4.2, GLMM manure period 1: $F_{1,116} = 191.336$, $P < 0.001$), with no significant differences among the two grasslands (GLMM grassland period 1: $F_{1,113} = 0.440$, $P = 0.507$). After the second application, there was no difference between early and late fields (Fig. 4.2, GLMM manure period 2: $F_{1,77} = 2.842$, $P = 0.091$), although grassland A had higher number of surfacing earthworms than grassland B (GLMM grassland period 2: $F_{1,78} = 45.248$, $P < 0.001$).

The vertical distribution of earthworms in the soil column did not show significant differences between the two ecotypes of earthworm (Fig. 4.3, GLMM: $F_{3,162} = 0.3059$, $P = 0.577$) and between fields with or without farmyard manure (Fig. 4.3, GLMM: $F_{3,162} < 0.01$, $P = 0.928$). Immature detritivores were significantly heavier in terms of AFDM per mm in the early application fields (Fig. 4.4, Tukey post hoc analysis, $Z = 3.426$, $P < 0.05$) during the first sampling, but this effect disappeared

Figure 4.4: Body condition (expressed as ash-free dry mass (AFDM) mg per mm length) of geophagous (left) and detritivorous (right) earthworms and for different age classes (immature top panels, mature lower panels). Earthworms were collected on two sampling days in spring (6 March and 9 April 2014). On 6 March, only the early fields had farmyard manure since 1 February. The late fields received manure on 14 March. Different letters denote differences (GLM: $P < 0.05$) between timing of manure application. Sample sizes are given below the boxplots and horizontal dashed lines gives the average body mass per panel.
during the second sampling (Fig. 4.4, $Z = 1.09, P = 0.745$). In mature detritivores, mature geophages and immature geophages there was no significant difference between early and late farmyard manure application fields.

**Discussion**

We found that earthworms come to the surface more frequently in the absence of fresh farmland manure, i.e. when food availability is expected to be low (Fig. 4.2). Rapidly after the application of farmyard manure, detritivorous earthworms come to the surface to collect it and retrieve it into their burrows. They can then remain deep in the soil. As we may have missed the deepest detritivores despite the mustard treatment, the total abundances of detritivores were slightly higher in the late application fields than in the early application fields (Fig. 4.1C). Indeed, only one individual of the deep-burrowing *L. terrestris* was found in the early fields against nine in the late fields. To our surprise, the manure application did not change the measured vertical distribution of detritivores and geophages in the soil (Fig. 4.3). Although vertical distribution is mainly determined by soil moisture (Gerard 1967, Rundgren 1975, Jiménez & Decaëns 2000), we do not expect differences between the fields, all probably being moist enough throughout the fieldwork period to keep earthworms actively surfacing (Onrust *et al.* unpubl. data).

Over a period of seven weeks, the availability of food in terms of manure cover sharply declined (Fig. 4.1B). As earthworms actively collected food on the surface and pulled it into their burrows, they likely have contributed to the decomposition of farmyard manure (Hendriksen 1990). This was illustrated by the observation that a while the manure was applied, blades of straw were partly incorporated in the soil and standing straight up in the grassland (Photo 4.3). The collected manure is colonized and digested by micro-organisms in the soil, forming a high-quality food source for earthworms (Wright 1972, Bonkowski *et al.* 2000). We found a small positive effect of manure application on the body mass of immature detritivores, probably the result of the relatively high energy requirements of this category of earthworms (Elvira *et al.* 1996). The time between application of manure and our sampling of the earthworms was probably too short to allow any differences in mature detritivores. The absence of an effect in geophages is in line with the expectation that this group does not rely on organic material for feeding. As we hypothesized, we conclude that it is hunger that forces detritivores to come to the surface.

Detritivorous earthworms are known to also feed on living plant material (Cortez & Bouché 1992, Eisenhauer *et al.* 2010, Griffith *et al.* 2013). However, as earthworms depend on microorganisms for digestion and assimilation, decaying and decayed organic material is preferred (Curry & Schmidt 2007). Indeed, Griffith
et al. (2013) only found earthworms grazing on live plants in locations with little plant litter on the surface. As earthworms do not have teeth, collecting living plant material takes more time than collecting decaying plant material. Furthermore, fresh organic material is less colonized by microorganisms than decaying material and might therefore be a less nutritious food source for earthworms. Surfacing remains high only when no manure is applied. Thus, it is likely that earthworms will only feed on living organic material when they are hungry (Wright 1972). Food availability for earthworms will be low in early spring as plant growth has stopped during the winter. Furthermore, in The Netherlands fertilizing is prohibited from 1 September until 1 February. Therefore, in the period before the first fertilization in spring, detritivorous earthworms are likely to be hungry and feeding on living plant material to survive.

Feeding on living plant material of low nutritional quality for earthworms (Curry 1998) requires more surfacing. As we predicted on the basis of the literature on other animals (Lima 1998, Brown et al. 1999), hunger will make detritivorous earthworms more risk-prone and thus vulnerable to predation. Earthworms indeed seem to minimize the exposure at the surface by retrieving food into the safety of their burrows and feed there. The main predators of earthworms in our study area are meadow birds (Black-tailed Godwit Limosa limosa, Lapwing Vanellus vanellus,

**Photo 4.3:** After a while the manure treatment was applied, blades of straw were partly incorporated in the soil and standing straight up in the grassland due to the action of detritivorous earthworms (Wommels, 13 March 2014).
Oystercatcher *Haematopus ostralegus* and Redshank *Tringa totanus*). This group of birds not only use these grasslands for foraging, but also for resting and breeding. During the fieldwork period in spring 2014, 164 nests of these meadow birds were found in the studied and surrounding grasslands (40 ha). Although, earthworm abundances can decline due to predation by birds (Bengtson *et al*. 1976, Barnard & Thompson 1985), number of surfacing earthworms does not show a one to one relationship with total abundances in the soil (Onrust *et al*. unpubl. data) and therefore it is unlikely that depletion by predation influenced our results.

As earthworms always live in top 10 cm when the soil is moist, confirmed again by our study, earthworm availability for probing species such as the long-billed Black-tailed Godwits and Oystercatchers will not be too much affected by the addition of farmyard manure. For visually hunting Lapwings, however, manure application does influence earthworm availability. High numbers of surfacing earthworms during the pre-breeding period are of special importance for female Lapwings as they need to build up reserves for egg production and incubation (Högstedt 1974, Galbraith 1989, Baines 1990). To promote food conditions for Lapwings and other visual hunting species, spreading farmyard manure on the surface should occur as late in spring as possible. In this way, hungry earthworms are forced to surface and provide an easy prey for hungry birds.

The timing of manure application is thus relevant for farming policies aimed to encourage and help meadow birds, birds which are currently in strong decline across Western Europe (Busche 1994, Donald *et al*. 2006, Vickery & Arlettaz 2012, Kentie *et al*. 2016). Indeed, protection measurements that involve fertilizing with farmyard manure instead of injecting slurry manure (Kleijn *et al*. 2001, Groen *et al*. 2012) may need to be re-examined with respect to the timing of the farmyard manure applications.

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Box B: Correcting size and weight for earthworms stored in ethanol

Alcohol (ethanol, EtOH, 96–100%) is an excellent killing agent and preservative for earthworms (Sherlock 2012). Furthermore, it prevent the earthworms from becoming too brittle and therefore easier to handle for measuring. However, it extracts water from tissues and cells, and therefore it will shrink the earthworm. The mass and length of earthworms will thus be underestimated when measured after the earthworms are preserved in ethanol. To know how much it is underestimated, I have measured the length and mass of earthworms twice, just before storing in ethanol and one year after collecting (369–380 days later). This resulted in formulae that can be used to correct for the loss of weight and length in earthworms. No discrimination is made between earthworm species, as the preservative has equal

The fresh length (FL, in mm) of earthworms is underestimated by 15.01% ($N = 349$) and the fresh weight (FW, in mg) is underestimated with 36.21% ($N = 372$) when preserved in alcohol (Fig. B.1). Thus to correct for this, the following equations can be used (LL denotes lab length in mm and LW lab length in mg):

\[
FL = 1.150 \text{ LL} \quad (R^2 = 0.98, P < 0.001, N = 349).
\]

\[
FW = 1.362 \text{ LW} \quad (R^2 = 0.99, P < 0.001, N = 372).
\]

![Figure B.1: Earthworm length (A) and weight (B) after preserved in alcohol plotted against length or weight just after killing. Each data point represents one individual earthworm.](image-url)
For soil samples where all earthworms are lumped together, the difference between fresh mass and mass after preserved in alcohol is 35.70% ($N = 165$). To calculate fresh weight (in grams) from preserved soil samples the equation is as follows:

$$FW = 1.357 \, LW \, (R^2 = 0.97, \, P < 0.001, \, N = 165).$$

As the time between first measurement (fresh weight) and second measurement (lab weight) differed between samples (ranging from 7 – 452 days), I was able to look at the effect of time. There is a small, but significant effect of time (LM: $F_{1,163} = 11.13, \, P = 0.001, \, R^2 = 0.064$), with samples preserved longer in alcohol losing more biomass (Fig. B.2).

**Figure B.2:** A. Total earthworm mass from soil samples after preserved in alcohol (lab weight, LW, grams) plotted against total earthworm mass just after collecting (fresh weight, FW, grams). B. Biomass loss of earthworms from soil samples preserved in alcohol over time. Error bars denotes SE.