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Onrust, Jeroen; Piersma, Theunis

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How dairy farmers manage the interactions between organic fertilizers and earthworm ecotypes and their predators

Jeroen Onrust^{a,*}, Theunis Piersma^{a,b}

^a Conservation Ecology Group, Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, P.O. Box 11103, 9700 CC Groningen, the Netherlands

^b NIOZ Royal Netherlands Institute for Sea Research, Department of Coastal Systems and Utrecht University, P.O. Box 59, 1790 AB, Den Burg, Texel, the Netherlands

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ABSTRACT

Most Dutch dairy farmland today is fertilized with slurry, a mixture of cattle dung and urine. As a food source for earthworms, this type of organic fertilizer is of lower quality than the traditionally used farmyard manure consisting of dung with some urine and mixed with bedding material such as straw. Earthworms living in dairy farmland belong to two distinct ecotypes, the detritivores and the geophages. Detritivores rely on farmyard manure as a food source more than geophages and therefore the type of organic fertilizer is expected to determine their relative abundances. In turn, this would affect higher trophic levels, as detritivores, when they come to the surface to collect food, are particularly important as prey for birds and mammals. Here we tested the hypothesis that dairy farmland fertilized with slurry will contain fewer detritivorous earthworms (thereby becoming less attractive for earthworm predators) by quantifying the abundance of the two earthworm ecotypes in 45 grasslands fertilized with either slurry, farmyard manure, or both. To determine the availability of detritivores for earthworm predators, we quantified earthworm surface availability by counting surfacing earthworms in the field and compared these numbers with densities belowground. To study the direct effects of different organic fertilizer types on earthworms, we measured their growth rates under controlled constant conditions using either slurry or farmyard manure, with hay as a control. We found that detritivores occurred in the highest densities in grasslands only fertilized with farmyard manure and that they also grew better on farmyard manure than on slurry. These differences were not found in geophages. Detritivores made up 25% of the total earthworm abundance in the soil, but contributed 83% to the surfacing earthworms at night; detritivore earthworms will thus be the main prey for visually hunting earthworm predators. The few dairy farmers using farmyard manure to fertilize their grasslands today will thus encourage the presence and availability of an earthworm ecotype which benefits higher trophic levels such as the endangered meadow birds.

1. Introduction

Current dairy farming practices in The Netherlands involve housing cattle in stables with cubicles for resting and alleys for feeding, walking and defecating. The slotted floors enable their dung and urine to be collected as slurry which is then used multiple times a year as an organic fertilizer for grasslands. Traditionally, instead of slotted floors, the bedding material (e.g. straw) of the stables was used as fertilizer in a mix with dung and with part of the urine (which was also collected separately). This farmyard manure was composted for a while before it was spread on grassland once a year. Lumbricid earthworms play a key role in transforming all types of organic fertilizers on a field into a stabilized form that can be used throughout the soil ecosystem (Atiyeh et al., 2000; Lavelle et al., 2006). There is considerable evidence that

organic fertilizers with a high organic matter content promote earthworm abundances and biomass more than inorganic fertilizers (Edwards and Lofty, 1982; Marhan and Scheu, 2005; van Eekeren et al., 2009).

Based on their feeding ecology, earthworms can be distinguished in two ecotypes, the *detritivores* and the *geophages* (Hendriksen, 1990; Curry and Schmidt, 2007). Detritivores take senescent vegetation and litter from the surface into the soil at night (Onrust and Piersma, 2017). This material is generally less decomposed than the more humified organic matter that geophages prefer (Svendson, 1957; Judas, 1992; Neilson and Boag, 2003). As farmyard manure contains organic material that is decomposed not as much as slurry, and thus has a higher C:N ratio, it is to be expected that the type of organic fertilizer determines the distribution of these two earthworm ecotypes in dairy farmland.

* Corresponding author.

E-mail address: J.Onrust@rug.nl (J. Onrust).

Although agricultural intensification may still allow high total earthworm densities (Knight et al., 1992; de Goede et al., 2003; Curry et al., 2008), the use of organic fertilizers with low C:N ratios will benefit the geophages, perhaps at the expense of detritivores (Hansen and Engelstad, 1999; de Goede et al., 2003, van Eekeren 2009). Additionally, farmyard manure rather than slurry could benefit detritivores because of its specific nutritional quality (Edwards and Lofty, 1982). In The Netherlands, 55% of the agricultural land consists of dairy farmed grassland, of which 29% being under five years old with the majority between 10–20 years old (with a steady decline since 2000 with one percent per year; CBS, 2017). To maintain a high grass production, mainly for silage, dairy farmers frequently plough and reseed their lands with fast-growing ryegrasses (*Lolium* sp.). The increased soil disturbance will negatively affect detritivores most, as this ecotype is rare in arable fields (Smith et al., 2008). Due to a policy to reduce NH₃ emissions (Neeteson, 2000), in The Netherlands slurry has to be injected in 3–5 cm deep slots that are cut in the sward, a process that might affect near the surface living detritivores more than the deeper living geophages (de Goede et al., 2003; van Vliet and de Goede, 2006).

Any declines of detritivores in dairy farmland will be affecting other trophic levels, as earthworms are an important prey for several larger organisms (MacDonald, 1983). Rather than being abundant, prey should be available (harvestable) for predators (Zwarts and Wanink, 1993). With their surfacing behaviour to collect food at night (Baldwin, 1917; Butt et al., 2003; Onrust et al., 2017), detritivores expose themselves to predators (Onrust and Piersma, 2017). In Dutch dairy farmland, there is a wide variety of predators that feed nocturnally on surfacing earthworms, including red foxes (*Vulpes vulpes*), hedgehogs (*Erinaceus europaeus*), shrews (Soricidae), badgers (*Meles meles*), lapwings (*Vanellus vanellus*), Eurasian golden plovers (*Pluvialis apricaria*) and little owls (*Athene noctua*). A decline in detritivore numbers will likely reduce the availability of earthworms for these animals and also affect the predation on alternative prey such as the eggs and chicks of the endangered meadow bird species (Summers et al., 1998; Kentie et al., 2015). This is in contrast to predator species which do not rely on surfacing detritivores, but can catch detritivores as well as geophages in the soil (e.g. the long-billed sandpipers, Scolopacidae, Burton, 1974 and moles *Talpa europaea*, Raw, 1966).

In this study we explore how the use of slurry or farmyard manure affects the distribution of the detritivores and the geophages in the field. We also explore how the organic fertilizer type affects a critical performance measure, i.e. the individual growth of the two earthworm ecotypes in the laboratory. We then evaluate how these findings impinge on earthworm availability for earthworm predators.

2. Methods

All data were collected in a 10 km² area around the hemlock of Idzegea in Southwest Friesland (N 52°58'48, E 5°33'12). In this area the main type of agriculture consists of dairy farming on a peat soil topped with a shallow layer (< 40 cm) of clay. All fields would have been managed in rather similar ways for the last two or three decades.

2.1. Earthworm ecotypes and their abundance

We grouped earthworms in the two ecotypes: (1) detritivores and (2) geophages. The species found in the studied grasslands and belonging to the detritivores were *Lumbricus castaneus*, *L. rubellus* and *L. terrestris*. Geophagous species found were *Alloobophora chlorotica*, *Aporrectodea caliginosa* and *A. rosea*.

In September-October 2013 we measured the densities of detritivores and geophages across 45 fields measuring on average 3.12 ha (range 0.31–7.05 ha). Of these fields, 22 had been fertilized with slurry only (with an application rate of 4–5 times a year), 11 with farmyard manure only (one application in February/March), and 12 were fertilized in spring (with farmyard manure) and later in summer (3–4 times

with slurry). The fertilizer treatments were consistent for at least three years before the sampling took place. Farmyard manure has become rare due to changes in the housing of cattle, and therefore only fields that have an agri-environmental scheme to protect breeding meadow birds now receive farmyard manure. The farmyard manured fields in our study were therefore managed less intensively than the slurry fertilized fields (i.e. mowing 1–2 times a year instead of 3–4 times a year) and they had relatively high groundwater tables (10–40 cm below surface level). These fields also had not been ploughed for at least 40 years, whereas the average age since last ploughing of the slurry fertilized fields was 10.9 years, and of the mixture fields 27.3 years.

We measured the densities of earthworms by taking three to six 20 × 20 × 20 cm soil samples per field, and then sorting them by hand. The number of soil samples per field varied this much as earthworm densities from three separate sampling efforts were pooled to boost sample size. Deeper living detritivores were collected by pouring one litre of a mustard powder solution in the cavity and for 15 min all emerging earthworms were collected (for a description of this method, see Lawrence and Bowers, 2002).

To measure the relative availabilities of detritivores and geophages for earthworm predators (i.e. by comparison with the total densities in the soil), in March – May 2015 we determined their surface availability at night on 11 fields treated with slurry injection. Again we measured total densities by taking six 20 × 20 × 20 cm soil samples per field along two transects of 25 m per field, sorted out by hand. Furthermore, the number of surfacing earthworms at night were counted along the transects by lying prone on a robust and simple cart which was gently pushed forward by foot (Onrust et al., 2017). The soil surface was observed at night with a head torch (160 lumens) from a height of 50 cm and within a width of 50 cm in front of the observer. All counts were conducted on grassland with a short sward height (< 10 cm) that was not grazed or mowed since autumn 2014. One transect took about 30 min to complete and each field was studied once. Counted earthworms were identified to ecotype level mainly based on the colour of their pigmentation, with detritivores mostly being darker reddish coloured. Earthworms that could not be identified were termed unknown.

2.2. Growth experiment

To study the effect of the farmyard manure and slurry on the individual growth of earthworms belonging to the two ecotypes, we collected earthworm cocoons and soil from a dairy farm in the study area and hatched them in trays with soil under controlled conditions in climate chambers at 12 °C. Every freshly hatched earthworm was weighed and kept in a PVC tube (10 cm height, 4.5 cm diameter) filled with 9 cm of sieved soil (0.143 L) and enclosed with a lid at the bottom and a fine mesh at the top. According to Lowe and Butt (2005), earthworms should be cultured in soil with a stocking density of 3–5 individuals per litre for *L. terrestris* and 6–10 individuals per litre for *A. caliginosa*. In our experimental tubes, the density was 6.9 earthworms per litre. We studied the growth of 36 geophagous earthworms and 30 detritivorous earthworms (Table 1). Although immature earthworms are difficult to identify to species, we classified freshly hatched earthworms into the two ecotypes based on colour of their pigmentation with

Table 1
Number of earthworms followed during the growth experiment.

		Time (months)					
Ecotype	Diet	0	1	2	3	4	5
Detritivore	Farmyard	10	10	10	9	8	6
	Hay	10	9	9	9	8	6
	Slurry	10	10	9	7	7	6
Geophage	Farmyard	12	12	12	11	11	9
	Hay	12	12	11	10	10	9
	Slurry	12	12	11	11	10	9

the detritivores showing much more reddish colour than the geophages. When the earthworms matured, there were only two *A. chlorotica* and the rest being *A. caliginosa* in the geophagous group and in the detritivorous group there were six unknown *Lumbricus* earthworms, the rest being *L. rubellus*.

The two ecotypes were assigned in equal numbers to three food treatments which, in addition to farmyard manure and slurry, contained a control, i.e. hay to mimic a non-manured situation. Hay consisted of grasses and forbs that were harvested from the fields where the earthworm cocoons were collected. Earthworm cocoons, soil and food sources were all collected on the same farm (N 52°58'48, E 5°33'12). To account for differences in dry matter content, all food types were dried in an oven at 70 °C for 48 h after some grinding. We measured the carbon and nitrogen content of the two organic fertilizers according to the DUMAS method, using the EA 1110 Elemental Analyzer from Interscience with Eager 200 for Windows. Three replicates per fertilizer were analysed.

Every month the body mass of the growing, individually held, earthworms were determined by removing the lid of the tube and carefully emptying it and pick out the worm from the soil. Before weighing, the worms were rinsed with tap water, then blotted with absorbable paper and weighed to the nearest 0.1 mg. Although, the content of an earthworms' gut can account for up to 20% of total body mass (Edwards and Bohlen, 1996), we did not allow the earthworms to empty their guts before weighing, as this would probably negatively influence their growth. After weighing, the earthworms were put back in their tube with the same soil. Then 1 g of dried organic fertilizer or hay was added, which was sprayed wet with tap water and slightly mixed with the top layer of the soil. The amount of food provided was greater than the daily needs, as the maximum ingestion rates for earthworms on organic matter is 80 mg dry mass g⁻¹ fresh mass d⁻¹ (Curry and Schmidt, 2007). The experiment lasted 5 months.

Earthworm growth follows a logistic growth pattern, with a rapid phase between the third and seventh week after hatching and a slow or even declining phase when reaching maturity (Daniel et al., 1996; Flack and Hartenstein, 1984). We therefore choose to use the first month of growth for analysis on the basis that it corresponds to the rapid growth phase which is useful as an index to the nutritional quality of the consumed food (Neuhauser et al., 1980; Curry and Schmidt, 2007). To account for the non-linear growth, the growth of earthworms was analysed by calculating the instantaneous growth rate per day (IGR, d⁻¹) by using the equation:

$$\text{IGR} = \ln(W_f/W_i) / \Delta t,$$

where Δt is the number of days between the initial weight (W_i) and the final weight (W_f) (Whalen and Parmelee, 1999). The IGR was calculated for each monthly measurement.

2.3. Statistics

All statistical procedures were carried out in R (R Development Core Team, 2017). Earthworm abundances for grasslands with different organic fertilizer treatments were analysed separately per earthworm ecotype by a Generalized Linear Mixed Model (GLMM) using the 'lme4' package (Bates et al., 2015), with fertilizer type as explanatory variables and soil sample nested in field as random factor and with a Poisson error distribution. We started the statistical analysis with a full model including an interaction between all fixed effects. A stepwise backward procedure was followed to find the minimal adequate model (MAM) in which terms were deleted in order of decreasing P value (Quinn and Keough, 2005).

Earthworm body masses on different food types was analysed with a linear mixed-effects model (LME). The square-root of earthworm weight was used as the response variable and diet and ecotype as explanatory variables. To account for differences between individuals, we

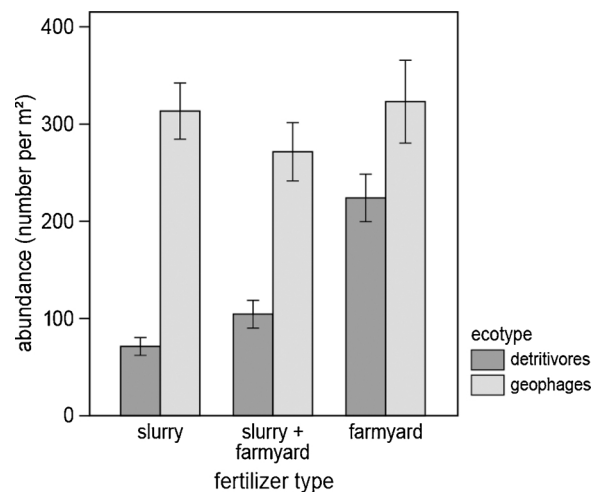


Fig. 1. Total abundances of detritivorous and geophagous earthworms in agricultural dairy grasslands that are fertilized with either slurry (N = 22), slurry and farmyard manure (N = 12) or farmyard manure (N = 11). Per grasslands 3–6 soil samples were taken.

added ID as a random intercept in the model. Furthermore, time was added as an explanatory variable and as a random slope. To test for differences in growth rates, we used a General Linear Model (GLM) with IGR as the response variable and food type as an explanatory variable for the first growth period (month 0–1). Multi-paired comparisons were then performed by using the “glht”-function of the “multcomp”-package (Hothorn et al., 2008).

We modelled the proportions of earthworms in different ecotypes within each sample in a binomial errors GLMM. Earthworm ecotype was added as fixed effect and a random intercept term was added with sample nested in grassland. The same procedure was followed to analyse the distribution of earthworms at the surface with the only difference that the random intercept was transect nested in field.

3. Results

3.1. Earthworm ecotypes and their abundance

At an average total density across all farms of 415 earthworms m⁻², there was a strong trend towards higher densities of detritivores in fields only treated with farmyard manure (Fig. 1; GLMM: $F_{2,191} = 7.980$, $P = 0.0013$). The abundance of detritivores in grasslands which were fertilized with farmyard manure was on average two times higher than in fields treated with a mixture of farmyard manure and slurry, and on average three times higher than fields treated with slurry only. There were no differences in the abundance of the geophages between fields with different organic fertilizer treatments (GLMM: $F_{2,191} = 1.415$, $P = 0.248$).

Detritivores comprised only 24% of the total number of earthworms in our soil samples ($N = 1535$, Fig. 2; GLMM: $F_{1,106} = 774.46$, $P < 0.0001$). Nevertheless, on the surface 83% of the earthworms recorded on the surface at night ($N = 2887$) were detritivores (GLMM: $F_{2,60} = 1619$, $P < 0.0001$). The abundance of earthworms in the soil of these fields was on average 544 earthworms m⁻² (SD = 289.5, $N = 11$) for geophages and 166 earthworm m⁻² (SD = 116.0, $N = 11$) for detritivores.

3.2. Growth experiment

The C:N ratio of the organic fertilizer types offered to the earthworms in the laboratory was 14.65 (SD = 0.22, $N = 6$) for farmyard manure and 9.30 (SD = 0.33, $N = 6$) for slurry. The water content averaged 52.9% (SD = 4.4, $N = 6$) for farmyard manure and 90.5%

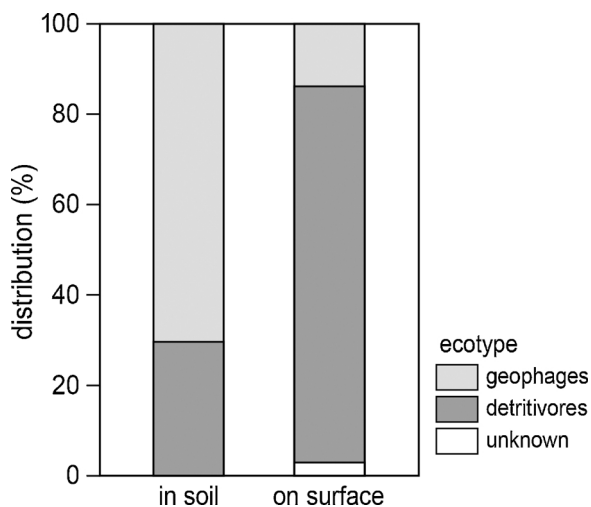


Fig. 2. Distribution of detritivorous and geophagous earthworms in the soil (left bar, $N = 1535$) and on the soil surface during the night (right bar, $N = 2887$) in 11 agricultural grasslands. All grasslands were fertilized with slurry from dairy cows. Soil distribution was determined by taking 6 soil samples per grassland. Surface distribution was determined by counting surfacing earthworms on two transects of 25 m per grassland during the night.

(SD = 0.4, $N = 6$) for slurry. After five months, 60% of the detritivores and 75% of the geophages survived (Table 1). There were no significant differences in survival between treatments. However, during the first month of growth, geophages grew fastest on slurry (IGR = 0.037 d^{-1} , Fig. 3) compared with farmyard manure (IGR = 0.022 d^{-1}) and hay (IGR = 0.022 d^{-1}). Detritivores, in contrast, grew faster on farmyard

manure (IGR = 0.040 d^{-1}) than on slurry (IGR = 0.025 d^{-1}) and hay (IGR = 0.021 d^{-1}), but only between farmyard manure and hay there was a significant difference (Tukey *post hoc* analysis, $Z = -2.365$, $P < 0.05$). The increase over time in body mass of earthworms (LME: $\chi^2(1) = 69.07$, $P < 0.0001$) did not differ between ecotypes (LME: $\chi^2(1) = 3.303$, $P = 0.069$, Fig. 3) nor between diets (LME: $\chi^2(1) = 1.828$, $P = 0.401$).

4. Discussion

Although farmyard manure and slurry are both organic fertilizers, we found detritivorous earthworms to be more abundant in fields that were fertilized with farmyard manure only (Fig. 1). Densities of earthworms have been shown to vary greatly between different types of soil, with highest densities generally found in moist soils with no disturbance and high organic matter content (Curry et al., 2002; van Vliet et al., 2007; Smith et al., 2008; Spurgeon et al., 2013). The highest abundances are usually found in permanent grasslands (Evans and Guild, 1947; Boag et al., 1997; van Eekeren et al., 2008; Rutgers et al., 2009). In our study area, fields fertilized with farmyard manure had remained unploughed and -tilted for much longer and were less intensively cropped than slurry fertilized fields. This could have influenced the distribution pattern that we found. Furthermore, detritivores seems to be affected more strongly by slurry injection than geophages (Hansen and Engelstad, 1999; de Goede et al., 2003, van Eekeren 2009). The impact is strongest under wet conditions, as under such conditions the earthworms find themselves higher in the topsoil and therefore more exposed to the injection device and/or manure (van Vliet and de Goede, 2006). In addition, the process of slit injection may also enhance the desiccation of the topsoil by opening it to the air (Onrust et al. unpublished). Together with a higher grass production and lower

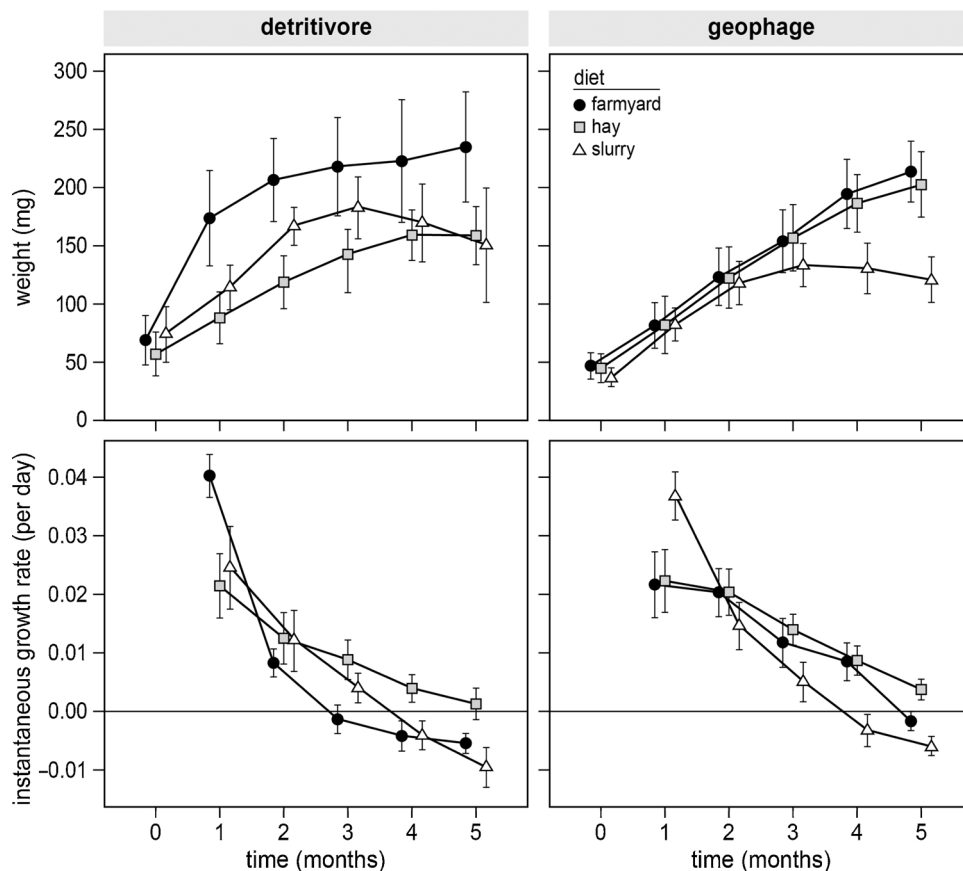


Fig. 3. Growth curves of hatchling detritivorous earthworms (*Lumbricus rubellus*, left panels) and geophagous earthworms (*Aporrectodea caliginosa*, right panels) cultured individually on farmyard manure or slurry from dairy cattle or hay for 5 months. Sample sizes are shown in Table 1.

groundwater level, slurry manured fields are thus more vulnerable to drought events which could strongly affect detritivore populations specifically (Eggleton et al., 2009). Geophages withstand drought by coiling into a tight knot and form a protective coating of secreted mucus in the soil (Edwards and Bohlen, 1996). In the intensive dairy livestock farming of today, high fertilization rates with slurry results in high grass productivity which can provide food for earthworms and thus higher populations (Edwards and Lofty, 1982; Muldowney et al., 2003; Curry et al., 2008). However, efficient utilisation of grass is likely to reduce the availability of food for detritivores: due to high mowing frequencies there will be less senescent vegetation. For these reasons it is inevitable that management practices associated with slurry applications in grasslands lead to declines in detritivore numbers, whereas geophages will be unaffected or even increase (Ivask et al., 2007; Smith et al., 2008; Bertrand et al., 2015).

The results of the growth experiment suggested why the type of organic fertilizer is an important factor determining the distribution of earthworm ecotypes; geophages grew fastest on slurry, whereas detritivores grew fastest on farmyard manure (Fig. 3) The quality of the food determines whether earthworms are able to grow (Marhan and Scheu, 2005; Butt, 2011). Just as this is the case for other decomposers, high quality food for earthworms is mostly determined by a low C:N value (Hendriksen, 1990; Bardgett, 2005). After a short period of weathering and microbial degradation, organic material becomes acceptable as a food source for earthworms, detritivores accelerate this by incorporating organic material into the soils, thereby facilitating microbial colonization. However, earthworms probably derive a large proportion of their nutrition not by feeding directly on organic material, but by grazing on the bacteria and fungi growing on these materials (Flack and Hartenstein, 1984; Edwards and Fletcher, 1988; Brown, 1995). Geophages especially eat bacteria/organic matter (Bolton and Phillipson, 1976; Neilson and Boag, 2003), whereas detritivores prefer fungi (Bonkowski et al., 2000). As organic material decreases in C:N value, a fungal-dominated situation is shifted to one being dominated by bacteria (Bardgett, 2005; van Eekeren et al., 2009). This would promote the food quality for geophages, but not for detritivores.

Of the three food types offered, only earthworms fed with hay did not show negative growth. Although hay had a much higher C:N ratio (estimated at 25:1) than the two manures (15:1 for farmyard manure and 9:1 for slurry), the nutritional value of the hay must have been low at the start of the experiment. In the experiment we did not refresh the soil after each weighing, and therefore the quality of the hay is likely to have increased as microbial activity increased. Nevertheless, Sizmur et al. (2017) found that cereal straw increased earthworm biomass more than manures. The calorific value of straw was much higher than that of manures and even paper can be used as a food source by detritivores (Wright, 1972); earthworms fed paper sludge had higher growth rates than earthworms fed horse manure (Fayolle et al., 1997). Although other studies also showed negative growth, probably as a result of maturation (Neuhauser et al., 1980; Whalen and Parmelee, 1999; Eriksen-Hamel and Whalen, 2006), the negative growth for both types of organic fertilizer in our experiment could also be caused by deteriorating environmental conditions as the fertilizers accumulated. Especially slurry could negatively affect earthworm growth as it contains high salt concentrations and toxic components (Curry, 1976; Paré et al., 1997; Reijs et al., 2003). This would also explain why body masses of all earthworms growing on slurry declined halfway the experiment, even for geophages which show no response in densities in the field (Fig. 1).

The observational data together with the experiment demonstrate that the type of dairy cattle manure influence the earthworm communities in dairy farmland. Although dairy farmland in The Netherlands still contains the highest densities of earthworms in Europe (on average 252 earthworms per m²; Rutgers et al., 2016), with the great majority of these lands being fertilized with slurry instead of farmyard manure, the high densities are likely to mainly consist of geophages. This is a

problem for the third trophic layer, the earthworm predators, as detritivores showing surface behaviour will be most available to them. Indeed, food intake rates of these predators is determined by the densities of surfacing detritivores (Onrust et al., 2017). To fully prove the beneficial effects of farmyard manure over slurry fertilization for detritivores and their nocturnal predators, we recommend a long-term split-field experiment with both fertilizer treatments applied within fields to minimize confounding factors.

Agricultural intensification in Western Europe caused earthworm predators to decline at alarming rates (e.g. meadow birds, including lapwing), whereas others were able to increase (e.g. red foxes and badgers) (Vickery et al., 2001; Evans, 2004; Donald et al., 2006; Teunissen et al., 2008; Kentie et al., 2013). Although these changes were not attributed to changes in earthworm abundances — after all, their densities in dairy farmland are high and most mammalian predators are generalist feeders (Baines, 1990; Muldowney et al., 2003; Evans, 2004) — a growing lack of (harvestable) detritivore earthworms may well have played an important indirect role in the saga. In the impoverished dairy farmland food web of today, prey like mice, voles and moles have become rare (de la Pena et al., 2003). If earthworms are also not available for generalist predators such as red foxes, they will have to rely on meadow bird eggs and chicks and then contribute to the decline of these endangered species.

Detritivorous earthworms play a key role in the dairy farmland food web, not in the first place by ingesting poorly decomposed organic material (e.g. litter, organic fertilizer etc.) and incorporating it into the soil and therefore contributing to nutrient cycling, but also as a food source for higher trophic levels. A decline in detritivores will thus alter the entire food web (Aira et al., 2008; Keith et al., 2018). Fertilizing with manures that have a higher C:N ratio, for example slurry manure mixed with coarse organic material, or increasing the quantity of litter, will benefit detritivores and therefore also the food conditions for avian and mammalian earthworm predators (van Eekeren et al., 2009; Bertrand et al., 2015).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.agee.2018.12.005>.

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