How dairy farmers unwittingly manage the tritrophic interactions between grassland fertilizers and earthworm ecotypes and their predators

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Abstract
Much of the Dutch dairy farmland today is fertilized with slurry manure, a mixture of cattle dung and urine. As a food source for soil biota, this type of manure is of lower quality than the traditionally used farmyard manure consisting of dung mixed with bedding material. Earthworms living in dairy farmland belong to two ecotypes, the detritivores and the geophages. Detritivores rely on manure as a food source more than geophages and therefore the type of manure may determine the relative abundances of the two ecotypes. This would affect higher trophic levels, as detritivores in particular are an important prey for birds and mammals; they come to the surface to collect food. Here we tested the prediction that dairy farmland fertilized with slurry manure 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 manure, farmyard manure, or both. To determine the importance of detritivores for earthworm predators, we quantified earthworm surface availability by counting surfacing earthworms in the field and compared these numbers with abundances belowground. To study the direct effects of different fertilizer types on earthworms, we measured their growth rates under controlled constant conditions using either slurry or farmyard manure, with litter as a control. We found that detritivores occur in the highest densities in grasslands only fertilized with farmyard manure and they also grew better on farmyard than on slurry manure. These effects were not found in geophages. Detritivores made only 25% of the total abundance in the soil, but contributed 83% of the surfacing earthworms at night, and 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 the earthworm ecotype which benefits higher trophic levels such as the endangered meadow birds.
Introduction

In the dairy farming of today, cattle are kept in stables with cubicles for resting and alleys for feeding, walking and defecating. The slotted floors enable their dung and urine to fall through to be collected as slurry manure which is then as a fertilizer for grasslands. Traditionally, farmyard manure was used as fertilizer, which is a mix of dung and bedding material (e.g. straw) that is composted for a while before it is spread on grassland. Lumbricid earthworms play a key role in transforming all types of manure into a stabilized form that can be used throughout the soil ecosystem (Atiyeh et al., 2000, Lavelle et al., 2006). Based on their feeding ecology, earthworms living in dairy farmland can be distinguished in two ecotypes, the detritivores and the geophages (Hendriksen, 1990, Curry and Schmidt, 2007). Detritivores feed on surface litter which is generally less decomposed than the more humified organic matter that geophages prefer (Svendsen, 1957, Judas, 1992, Neilson and Boag, 2003). As farmyard manure contains organic material that is in an earlier state of decomposition, and thus has a higher C:N ratio, than slurry manure, it is to be expected that the type of manure determines the distribution of these groups in dairy farmland.

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 manures with low C:N rations may benefit the geophages, perhaps at the expense of detritivores (Hansen and Engelstad, 1999, De Goede et al., 2003, van Eekeren et al., 2009). In addition, the increased soil disturbance for reseeding or crop rotation typical of intensive farming will negatively affect detritivores, which are absent in arable fields (Smith et al., 2008). Adding insult to injury, by a policy to reduce NH\textsuperscript{3} emissions (Neeteson, 2000), slurry manure in The Netherlands has to be injected in 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). Alternatively or additionally, farmyard rather than slurry manure could benefit detritivores because of its specific nutritional quality (Edwards and Lofty, 1982).

Any declines of detritivores in dairy farmland will be affecting other trophic levels, as earthworms are an important prey for other organisms (MacDonald, 1983). With their surfacing behaviour to collect food at night (Baldwin, 1917, Butt et al., 2003, Onrust et al., 2017), detritivores expose themselves to predators and are only then available for visually hunting predators. In Dutch dairy farmland, there is a wide variety of predators that feed on surfacing earthworms, including red foxes (Vulpes vulpes), hedgehogs (Erinaceus europaeus), shrews (Soricidae), badgers (Meles meles), lapwings (Vanellus vanellus) and little owls (Athene noctua). A decline in detritivore numbers will likely to reduce the availability of earthworms for these animals.
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 and the individual growth of the two earthworm ecotypes in the laboratory. We then evaluate how these findings impinge on earthworm availability for earthworm predators.

**Methods**

All data was collected in a 10 km² area around the village 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 with a shallow layer (<40 cm) of clay.

**Earthworm ecotypes and their abundance**

We grouped all earthworms in the two ecotypes, the detritivores and the geophages. According to the widely used nomenclature of Bouché (1977), who classified earthworms into three ecological groups, the anecic and epigeic species belong to the detritivores, whereas endogeic species belong to the geophages.

In September –October 2013 we measured the densities of detritivores and geophages across 45 fields measuring on average 3.12 ha (min = 0.31 ha, max = 7.05 ha). Of these fields, 22 had been fertilized with slurry manure only, 11 with farmyard manure only, and 12 were fertilized in spring with farmyard manure and later in summer with slurry manure. 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 housekeeping of cattle, and therefore only fields that have an agri-environmental scheme receive farmyard manure nowadays. The farmyard manured fields in our study were therefore managed less intensively than the slurry manured fields (i.e. mowing 2–3 times a year instead of 4–5 times a year) and they had a relatively high groundwater table (10–40 cm below surface level). These fields also had not been ploughed for at least 40 years, whereas the average age of the slurry manured 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. 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, in March – May 2015 we determined their surface availability at night on 11 fields treated with slurry manure injection. Again we measured total densities by taking six 20 × 20 × 20 cm soil samples per field, sorted out by hand. Furthermore, along two transects of 25 m per field, the number of surfacing earth-
worms at night were counted 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). Counted earthworms were identified to ecotype level mainly based on the colour of their pigmentation, with detritivores being darker reddish coloured. Earthworms that could not be identified were termed as unknown.

Growth experiment
To study the effect of the farmyard and slurry manure 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 litre) 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 worms per litre. We studied the growth of 36 geophagous earthworms (mainly Aporrectodea caliginosa) and 30 detritivorous earthworms (mainly Lumbricus rubellus) (Table 5.1).

The two ecotypes were equally assigned to three food treatments which in addition to farmyard and slurry manure contained a control, i.e. litter to mimic a non-manured situation. Litter consisted of grasses and forbs that were harvested and dried in an oven at 70 °C for 48 h after it was cut in pieces of 0.5 – 1 cm. Earthworm cocoons, soil and food sources were all collected on the same farm (N 52°58’48, E 5°33’12). We measured the carbon and nitrogen content of the two manure types according to the DUMAS method, using the EA 1110 Elemental

Table 5.1: Number of earthworms followed during the growth experiment.

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>Manure type</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detritivore</td>
<td>Farmyard</td>
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<td>10</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Litter</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Slurry</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Geophage</td>
<td>Farmyard</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>9</td>
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<td></td>
<td>Litter</td>
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<td>Slurry</td>
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</table>

CHAPTER 5
Analyzer from Interscience with Eager 200 for Windows. Three replicates per manure 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 empty the guts of the earthworms before weighing, as this probably influence the growth of the worms negatively. After weighing, the earthworms were put back in their tube with the same soil. Then 1 g of manure or litter was added, which was slightly mixed with the top layer of the soil. The experiment lasted 5 months.

To account for non-linear growth, the growth of earthworms was analysed by calculating the instantaneous growth rate per day (IGR, d⁻¹) by using the equation:

\[
\text{IGR} = \ln \left( \frac{W_f}{W_i} \right) / \Delta t,
\]

where \( \Delta t \) 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.

Statistics

All statistical procedures were carried out in R (R Development Core Team, 2017). Earthworm abundances for grasslands with different manure treatments were analysed separately per earthworm ecotype by a Generalized Linear Mixed Model (GLMM) using the ‘lme4’ package (Bates et al., 2015), with manure 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 added ID as a random intercept in the model. Furthermore, time was added as an explanatory variable and as a random slope. To test 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).
The distributions of earthworms in the soil were analysed by a binomial GLMM per ecotype in which the response variable was entered as a matrix in which the first column was the number of that ecotype and the second column was the number of the other ecotype. Earthworm ecotype was then 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.

Results

At an average total density of 415 earthworms m\(^{-2}\), there was a big shift in the composition of the earthworm community towards higher densities of detritivores in fields only treated with farmyard manure (Fig. 5.1; GLMM: \(F_{2,191} = 7.980, P = 0.0013\)). The abundance of detritivores was on average 2.3 times higher in grasslands which were fertilized with farmyard manure only than in fields only treated with slurry manure. There were no differences in the abundance of the geophages in fields with different manure treatments (GLMM: \(F_{2,191} = 1.415, P = 0.248\)).

Representing only 24% of the total number of earthworms (\(N = 1535\)), detritivores were much less abundant in the soil (Fig. 5.2; GLMM: \(F_{1,106} = 774.46, P < 0.0001\)). However, on the surface, 83% of the spotted earthworms (\(N = 2887\)) were detritivores (GLMM: \(F_{2,60} = 1619, P < 0.0001\)).

![Figure 5.1: Total abundances of detritivorous and geophagous earthworms in agricultural grasslands that are fertilized with either slurry manure (\(N = 22\)), slurry and farmyard manure (\(N = 12\)) or farmyard manure (\(N = 11\)). Per grasslands 3–6 soil samples were taken.](image-url)
The C:N ratio of the manure types offered to the earthworms in the laboratory was 14.65 (SD = 0.22) for farmyard manure and 9.30 (SD = 0.33) for slurry manure. The water content averaged 54% for farmyard manure and 90% for slurry manure. After five months, 60% of the detritivores and 75% of the geophages survived (Table 5.1). There were no differences in survival between treatments. However, during the first month of growth, geophages grew fastest on slurry manure (IGR = 0.037 d⁻¹, Fig. 5.3) compared with farmyard manure (IGR = 0.022 d⁻¹) and litter (IGR = 0.022 d⁻¹). Detritivores, in contrast, grew faster on farmyard manure (IGR = 0.040 d⁻¹) than on slurry manure (IGR = 0.025 d⁻¹) and litter (IGR = 0.021 d⁻¹), but only between farmyard manure and litter 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: χ²(1) = 69.07, P < 0.0001) did not differ between ecotypes (LME: χ²(1) = 3.303, P = 0.069, Fig. 5.3) and also not between diets (LME: χ²(1) = 1.828, P = 0.401).

### Discussion

There is considerable evidence that organic fertilizers promote earthworm abundances and biomass more than inorganic fertilizers (Edwards and Lofty, 1982, Marhan and Scheu, 2005, van Eekeren et al., 2009). In this study we could confirm this. Although farmyard and slurry manure are both organic fertilizers, we found detritivorous earthworms to be more abundant in fields that were fertilized with...
farmyard manure only (Fig. 5.1), and early in life they grew faster on farmyard manure than on slurry manure (Fig. 5.3). Although, growth rates for geophages were higher on slurry manure in the first month, there was no significant difference between food types, probably because geophages depend less on organic material for feeding.

Densities of earthworms have been shown to vary greatly between different types of habitat, 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 The Netherlands, 55% of the agricultural land consists of dairy farmed grassland, of which 71% (with a steady decline since 2000 with one percent per year) being over five, but often less than 10–20 year old (CBS,

**Figure 5.3:** Growth curves of hatchling detritivorous earthworms (*Lumbricus rubellus*, left panels) and geophagous earthworms (*Aporrectodea caliginosa*, right panels) cultured individually on farmyard manure, slurry manure or litter for 5 months. Sample sizes are shown in Table 5.1.
To maintain a high grass production, mainly for silage, dairy farmers regularly plough and reseed their lands with fast-growing Ryegrass (*Lolium* spp.).

In our study area, fields that were fertilized with farmyard manure were much older and less intensively used than slurry manured fields. This could have influenced the distribution pattern that we found. Furthermore, detritivores seem to be affected more by slurry injection than geophages (De Goede *et al.*, 2003, van Eekeren *et al.*, 2009). The impact is strongest under wet conditions, as under such conditions the worms 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 could also enhance the desiccation of the topsoil by opening the soil (Onrust *et al.* unpublished). Together with a lower groundwater level, slurry manured fields are thus more vulnerable to drought events which could strongly affect detritivore populations (Eggleton *et al.*, 2009). For these reasons it is inevitable that intensive land-use leads to a decline in detritivore numbers, whereas geophages seem unaffected or can even increase (Ivask *et al.*, 2007, Smith *et al.*, 2008, Bertrand *et al.*, 2015).

The growth experiment suggested why the type of fertilizer is an important factor determining the distribution of earthworm ecotypes. 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. However, earthworms probably derive a large proportion of their nutrition by not feeding directly on organic material, but by grazing on bacteria and fungi growing upon these materials (Flack and Hartenstein, 1984, Edwards and Fletcher, 1988, Brown, 1995). Geophages are more bacteria/organic matter feeding earthworms (Bolton and Phillipson, 1976, Neilson and Boag, 2003), whereas detritivores prefer fungi (Bonkowski *et al.*, 2000). Organic material that decreases in C:N value, shifts from a fungal-dominated situation to being dominated by bacteria (Bardgett, 2005, van Eekeren *et al.*, 2009). This would promote the food quality for geophages, but not for detritivores.

It is surprising that from all food types, earthworms fed with litter did not show negative growth, as this type of food did not have time for microbial degradation and probably had a much higher C:N ratio than the two manures (we did not measure it). The nutritional value of the litter for earthworms must have been low at the start of the experiment. As we did not refresh the soil after each weighing, the quality of the litter will likely have increased as microbial activity increased. Nevertheless, Sizmur *et al*. (2017) found that cereal straw increased earthworm biomass more than manures as the calorific value of straw was much higher than manures and even paper could be a food source to earthworms (Wright, 1972), resulting in
higher growth rates than on horse manure (Fayolle et al., 1997). However, the negative growth for both types of manures could also be caused by deteriorating conditions as manures accumulated. Especially, slurry manure could negatively affect earthworm growth as it contains high salt concentrations and phytotoxic components (Curry, 1976, Paré et al., 1997, Reijs et al., 2003). This might also explain why body masses of all earthworms growing on slurry manure declined halfway the experiment, even for geophages which show no response in densities in the field (Fig. 5.1).

Our results show 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), as the majority of these lands are fertilized with slurry manure instead of farmyard manure, these are likely to be mainly geophages. This is a problem for the third trophic layer, the earthworm predators, as, rather than being abundant, prey should be available (catchable) for predators (Zwarte and Wanink, 1993). When detritivores come to the surface to collect food they are available for earthworm predators that mainly hunt by sight (Fig. 5.2). Indeed, food intake rates of these predators is determined by the number of surfacing detritivores (Onrust et al., 2017).

Agricultural intensification in Western Europe caused earthworm predators to decline at alarming rates (e.g. meadow birds, including lapwings), 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 generalistic feeders (Baines, 1990, Muldowney et al., 2003, Evans, 2004) —, earthworms may well have played an important indirect role. In the impoverished dairy farmland food web of today, prey like mice, voles and moles have become rare. If earthworms are also not available for opportunistic 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 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). Fertilizing with manures that have a higher C:N ratio, for example slurry manure mixed with course organic material, will benefit detritivores and therefore also the food conditions for earthworm predators (van Eekeren et al., 2009, Bertrand et al., 2015).
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