

Earth, worms & birds

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Chapter 1

GENERAL INTRODUCTION

Earth, worms & birds

Jeroen Onrust

From a bird's eye view, the rural area of The Netherlands looks open, wet and green. Being a delta of three large rivers, The Netherlands has fertile soils and combined with good climatic conditions (temperate maritime) (Berendsen 1997), the right setting for agriculture. However, being a low-lying country, groundwater levels are relatively high and this facilitates grazing by cattle. In contrast to arable farming, dairy farming works at high groundwater levels. The Netherlands is a perfect country for dairy farming after the loss of the extensive peatlands during a long history of cultivation; grasslands for dairy farming became the most widespread habitat (de Vries 1953). This man-made habitat was often, especially on clay and clay-on-peat soils, forming vast open spaces without trees or other vertical obstructions. This formed a perfect habitat for a community of birds that we nowadays we call by the name 'meadow birds' (Beintema *et al.* 1995, van der Geld *et al.* 2013).

A closer look at these agricultural grasslands today reveals, however, that the majority of these grasslands are no longer suitable for meadow birds. Although still quite open and very green, the intensification of agriculture converted wet and herb-rich meadows into dry rye-grass monocultures. In association, numbers of meadow birds have declined dramatically during the last decades (Vickery *et al.* 2001, Donald *et al.* 2006, Kentie *et al.* 2016). Although lots of research have resulted in a better understanding of the problems meadow birds are facing nowadays (Benton *et al.* 2003, Kentie *et al.* 2013, Kentie *et al.* 2015), there is still little understanding of how modern agriculture affected the staple food of meadow birds: earthworms.

This research project aims to investigate the relationship between dairy farm management (**earth**), earthworms (**worms**) and their availability for meadow birds (**birds**). We have done this by studying earthworms from a meadow bird's perspective in differently managed dairy farmlands. By focusing on different ecotypes of earthworms, we hope to identify which group of earthworms are of importance for meadow birds and whether dairy farm management acts differently on different ecotypes (species and niche) of earthworm. To place our work in context, we first present a short history of the intensification of Dutch dairy farming and how this impacted on the whole dairy farm ecosystem.

A short history of the Dutch dairy farm ecosystem

A wide variety of bird species belong to meadow birds, from passerines (e.g. Skylark *Alauda arvensis*) to ducks (e.g. Northern Shoveler *Anas clypeata*), but generally, as well in this thesis, it is about wader species (Beintema *et al.* 1995, Dekker 2009). The 'big five' of meadow birds are: Black-tailed Godwit *Limosa limosa*, Northern Lapwing *Vanellus vanellus*, Common Redshank *Tringa totanus*, Oystercatcher *Haematopus ostralegus* and Ruff *Philomachus pugnax*. For some species, The

Netherlands is home to a large proportion of the total population, for example 85% of the East-Atlantic flyway population of Black-tailed Godwits breeds here (Kentie *et al.* 2016). However, this group of birds acquired this status recently as most meadow birds originated from natural open habitats (Voous 1965), and shifted more and more to the agricultural landscape when their natural habitats rapidly disappeared and man started to intensify its farming practices (Beintema *et al.* 1995). Although these fields had an agricultural function, they had a high natural value as they were home to a large number of different species.

An impression of what the food web of dairy farmland looked like around 1950 is given in figure 1.1. The first trophic level consists of primary producers (plants)

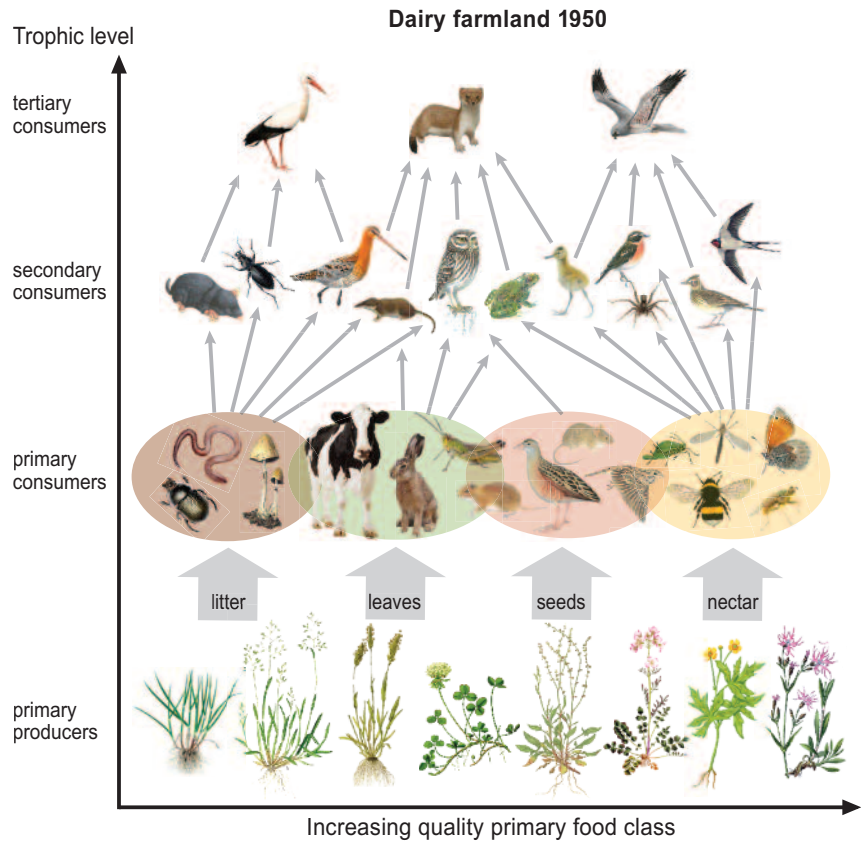


Figure 1.1: Schematic of the dairy farmland food web in The Netherlands around 1950. The x-axis represents the quality of food type that primary consumers eat, roughly classified in four groups: detritivores feeding on litter (brown), herbivores feeding on leaves (green), granivores feeding on seeds (red), and nectarivores feeding on nectar (yellow). Depiction of food web organisation along these two main axes after Olff *et al.* (2009).

and showed a high diversity with besides grasses, also forbs and legumes. Every part of a plant can be differently used by a primary consumer, for example flowers provide pollen and nectar for bees and butterflies, seeds are consumed by granivores, stems and leaves by herbivores and litter is eaten by detritivores. These primary consumers are eaten by secondary consumers, which are then eaten by tertiary consumers etc. These biodiverse grasslands were manually mown only once a year, mostly in July when grass had set seed. Furthermore, as a fertilizer they received little manure and probably only the fields closest to the farm received farmyard manure. Together with differences in groundwater levels and soil moistures, this heterogeneity in abiotic conditions resulted in a large biodiversity. With higher number of plant species and more diversity in vegetation structure, there are more niches resulting in a higher trophic diversity. It is estimated that most meadow bird species reached their highest numbers in mid twentieth century (Schekkerman 2008, Kentie *et al.* 2016). The yield of such fields was by contrast very low, but this changed rapidly in the second half of the twentieth century.

Dairy farming, and agriculture as a whole, mechanized and switched from a locally-focused production towards an efficient internationally-oriented business since 1950 (Reinders & Vernooij 2013). The European Community stimulated farmers to increase their production by giving subsidies in the form of a guaranteed minimum price for their milk. Within decades, the number of dairy cows and the production of milk increased tremendously (Fig. 1.2A) and The Netherlands became one of the world leading producers of dairy products (van Grinsven & Kooman 2017). The production was even higher than the market demands, creating 'milk lakes' and 'butter mountains' in the 1970s. To solve this problem, in 1984 the European Community introduced the milk quota, which limited the production of milk to a certain level (van Grinsven & Kooman 2017). This had the desired effect and the number of cows declined as well as the milk production (Fig. 1.2A).

Although the lakes of milk evaporated, another flood still washed over The Netherlands. The increasing livestock, including pigs and chickens, created an enormous amount of animal manures which became one of the most severe environmental problems (Heij & Schneider 1991). High input of nutrients through the use of fertilizers and manure make it possible to reach high levels of agricultural production. However, a large proportion of the applied manure in dairy farmland was not absorbed by grasses, but washed away and ended up in surface water and groundwater. Another part of the nitrogen from the manure was released in the air in the form of ammonia (NH_3). This not only caused eutrophication and eventually biodiversity loss of nearby areas, but also of natural areas further away (Heij & Schneider 1991, Bobbink *et al.* 1998, Erisman *et al.* 2015). Already in the 1970s, this problem was known, but it was not until 1987 that stricter legislation was introduced; since 1994 animal manures has to be applied to the land with supposedly low-emission

methods (Neeteson 2000, Stoate *et al.* 2009). This includes no fertilization in autumn and winter, and the manure has to be injected into the soil or into the sward. Only farmyard and other green manures are allowed to be applied on the surface.

Traditionally, dairy farmland was fertilized with farmyard manure as the cows were kept in stables with bedding material. This material, mixed with faeces, was collected and stored on a muck heap outside or a new layer of bedding material was added in the stable. After some months of composting, this farmyard manure was then applied on the surface. In modern stables, cows are kept in stables with cubicles for resting and alleys for feeding, walking and defecating (Remmelink *et al.* 2016). The slotted floors enable their dung and urine to fall through to be collected as slurry manure. This type of manure is much more liquid than farmyard manure. From the total dairy cattle manure that is produced nowadays, only 0.2% is farmyard manure, which also declined with more than 80% since 1990, whereas in the same period slurry manure increased with 31% (CBS 2017a).

Although still quite open and very glossy green nowadays, dairy farmland went through a huge metamorphosis. Large scale land re-allotments turned the landscape upside down, led to the disappearance of many smaller landscape elements (ditches, hedgerows, road verges etc.) and natural dynamics disappeared step by step. Intensive water management ensure nowadays that dairy farmland does not flood anymore and groundwater tables are manually kept low. With the help of new pumping-stations and the closing of the Zuiderzee and Lauwerszee in 1932 and 1969 respectively, outlet waterways in the Dutch province of Fryslân are kept at a constant level of -0.52 m NAP (Normal Amsterdam Water Level) (Claassen 2008). The original seasonal rhythm of higher groundwater tables in winter and lower in summer turned around, with now relatively higher groundwater tables in summer. These changes had a great impact on the functioning of grasslands. Grassland ecosystems changed from a groundwater (lithocline) dependent system towards a rainwater (athmocline) dependent system as groundwater was drained away artificially (Schotsman 1988). This affected nutrient flow and soil pH (Paulissen *et al.* 2007). The original vegetation (and likely soil fauna) of these flooded grasslands almost completely disappeared (Grootjans 1985, Schotsman 1988). Although sustained winter flooding can be detrimental for some groups of soil fauna (as earthworms), it helps to keep the sward short and open enough for meadow birds to feed and probe in the soil (Ausden *et al.* 2001). Furthermore, it retards the growth of grass and therefore the timing of mowing, promoting plant and insect diversity.

Ploughing and reseeded subsequently converted species rich grasslands into dense, homogeneous Perennial Ryegrass *Lolium perenne* monocultures (Vickery *et al.* 2001). This grass species grows fast and is a competitive dominant under nutrient-rich and frequently mown conditions, circumstances which are detrimental for many natural grassland plant species. As nitrogen is an important limiting nutrient

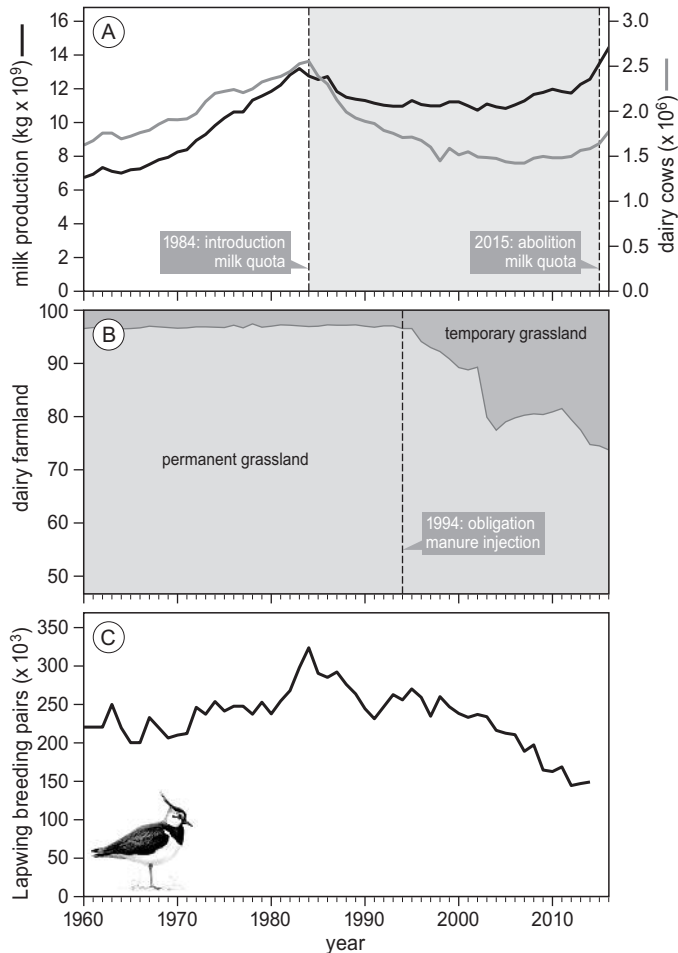


Figure 1.2: Dairy farmland in The Netherlands from 1960 to 2017. (A) Milk production in billion kg (black line) and number of dairy cows (grey line) (CBS 2017b). (B) Percentage of permanent (at least five years no crop rotation, light grey) and temporary grassland (younger than five years old, dark grey) of the total area of grassland used for dairy farming (CBS 2017b). (C). Number of pairs of Lapwing *Vanellus vanellus* breeding in the whole of The Netherlands. ©Dutch Centre for Field Ornithology (SOVON) 2017.

for plant growth in many temperate grasslands, nitrogen enrichment through intensive agriculture reduces plant species richness by favouring the few species best adapted to high nutrient levels (Stevens *et al.* 2004, Erisman *et al.* 2015); it encourages the growth of such competitive, fast growing species at the expense of slower growing species (Vickery *et al.* 2001). Insect diversity and abundance strongly declines with increasing nutrient inputs (Zahn *et al.* 2010), and increasing grazing

pressure (van Klink *et al.* 2015). Especially large insect species become rare. Under intense cutting or grazing, large insects may have difficulties completing their life-cycles (Schekkerman & Beintema 2007). With an addition of 50 kg of nitrogen per hectares per year, the dry-weight of an insect is about 1 mg. With 400 kg N ha⁻¹ yr⁻¹, the average weight declines to less than a third (Siepel 1990). Everything else being equal for meadow bird chicks this would mean that they have to consume a three-fold of insects in numbers. Also food conditions for adults are affected as larger-sized soil biota (earthworms, enchytraeids, microarthropods, and nematodes) are more sensitive to agricultural intensification than smaller-sized soil biota (protozoans, bacteria, and fungi) resulting in loss of large and profitable earthworms in agricultural lands (Wardle 1995, Postma-Blaauw *et al.* 2010). However, the increase in nitrogen content of the vegetation may promote the abundance of phytophagous and decomposing species (Andrzejewska 1979, Atkinson *et al.* 2005, Curry *et al.* 2008).

In general, however, addition of fertilizers tend to decrease the numbers and diversity of grassland invertebrates (Fenner & Palmer 1998, Zahn *et al.* 2010). This decline is also promoted by increasing regular disturbance of the soil and vegetation structure as grasslands are ploughed, graded and/or reseeded to maintain a high grass production. More often these grasslands are ploughed and tilled to create temporary arable land to grow maize for the increasing demand for energy-rich food for cattle. When dairy farmland is grassland for five consecutive years without crop rotation, it is termed as permanent grassland. The area of permanent grassland in The Netherlands has been stable for a long time at 97% of the total area of dairy farmland. When slit-injection of manure became compulsory, permanent grasslands declined to 74% at the expense of temporary grasslands (Fig. 1.2B). Nevertheless, true permanent grassland that has never been ploughed or killed by herbicides is likely to be much rarer as farmers 'improve' grassland when the botanical composition is poor (i.e. less than 50% Perennial Ryegrass cover), when the field is difficult to be worked on due to unevenness of the soil surface (e.g. ditches), or when the sward is heavily damaged, as by drought, machinery or Voles *Microtus arvalis* (Rommelink *et al.* 2016). Temporary grasslands are high-productive Perennial Ryegrass monocultures and often used for silage production. Silage is grass that after it is cut, is stored (without drying) in a large heap which is compressed to leave as little oxygen as possible in it and then covered with a plastic sheet. The resulting fermented grass is fed to the cows in the stable. Nowadays, 90% of the grass is harvested for silage production and only 3% is used for hay making. In 1960, this was 25% and 65% respectively (Klomp 1951, CBS 2017c). This is also illustrated by the fact that grass on average is mown 2.8 times per year (with a maximum of up to 6 times per year) whereas in 1960 this was 0.8 times per year on average (van der Geld *et al.* 2013, CBS 2017c).

The intensification of agriculture is affecting the dairy farmland food web at every trophic level. Efficient farming created large and monotonous monocultures where hardly anything is wasted and where very few species can survive. Increased frequency of mowing reduces flowering and seed set, and hence food availability for seed-eating animals (Vickery *et al.* 2001, Atkinson *et al.* 2005). Small mammals like rodents and shrews disappeared from the agricultural landscape (de la Pena *et al.* 2003). This group of species are also the main prey of farmland predators, such as Stoat *Mustela ermine*, Red Fox, and Barn Owl *Tyto alba*. With the loss of prey species, predators have to switch to other prey. This ‘apparent predation’ might have caused the increased predation risk on meadow bird chicks (Roodbergen *et al.* 2012, Kentie *et al.* 2015). Furthermore, the landscape have become more enclosed, with roads, wood lots, tree lines and scattered trees. Predators, may use these elements as a breeding site, perching opportunity or hiding place (van der Vliet *et al.* 2010). Together with low water tables and the absence of winter flooding (ground predators can make burrows), these changes make the meadow bird habitat more accessible for predators. Furthermore, farming practices like cutting grass during the breeding season is not only altering the protective cover for the chicks, but also the feeding conditions, resulting in chicks that are in low condition and thus an easy prey for predators (Schekkerman *et al.* 2009). Within a few decades, farmland species have declined enormously (Busche 1994, Donald *et al.* 2001, Vickery *et al.* 2001, Donald *et al.* 2006, Kentie *et al.* 2016) (Fig. 1.2C).

The ongoing intensification was still continuing when on 1 April 2015 the European regulations for a limit on milk production per farm (milk quota) came to an end. Heralded by the dairy industry as ‘liberation day’ and in anticipation of the promising long-term developments across the global dairy market, dairy farms and companies invested in capacity by increasing the number of cows (PBL 2016, van Grinsven & Kooman 2017). Already in the first year, the record of 13.2 billion kg milk in 1983 was broken (to 13.3 billion kg milk) and even increased further in 2016 (to 14.3 billion kg milk) (CBS 2017b). This production was reached with almost one million cows fewer than in 1984 (Fig. 1.2A), which illustrates how efficient dairy farming has become.

This has come at a cost, though. The impoverished food web of today’s dairy farm is represented in figure 1.3. Although many species disappeared, new species entered the food web, mostly predator species (which recovered after persecution and pollution) or competitive species. Agricultural intensification changed and simplified the food web (Tsiafouli *et al.* 2015). This is not only detrimental for organisms depending on this habitat, but it makes this habitat also more susceptible for pest and insect outbreaks. It is shown that high plant diversity in grasslands increased the stability of a diverse arthropod community across trophic levels (Haddad *et al.* 2011). The same is true for the diversity of microorganisms below-

ground and ecosystem functioning (Tsiafouli *et al.* 2015, Bender *et al.* 2016). Furthermore, the ratio between bacteria and fungi may change towards a more bacterial dominated system as intensification increases (Wardle *et al.* 2004). In grasslands, arbuscular mycorrhizal (AM) fungi is an important symbiont for plants as facilitates nutrient acquisition (especially phosphorous), and protects the plant against diseases and drought (van der Heijden *et al.* 2008). Furthermore AM fungi can suppress aggressive agricultural weeds (Rinaudo *et al.* 2010). As already mentioned, the intensification did not have a great impact on macrodetritivores as earthworms, probably because artificial high litter input (via slurry or farmyard manure) replaced the role of dung depositions by cows in the field (Leroy *et al.* 2008).

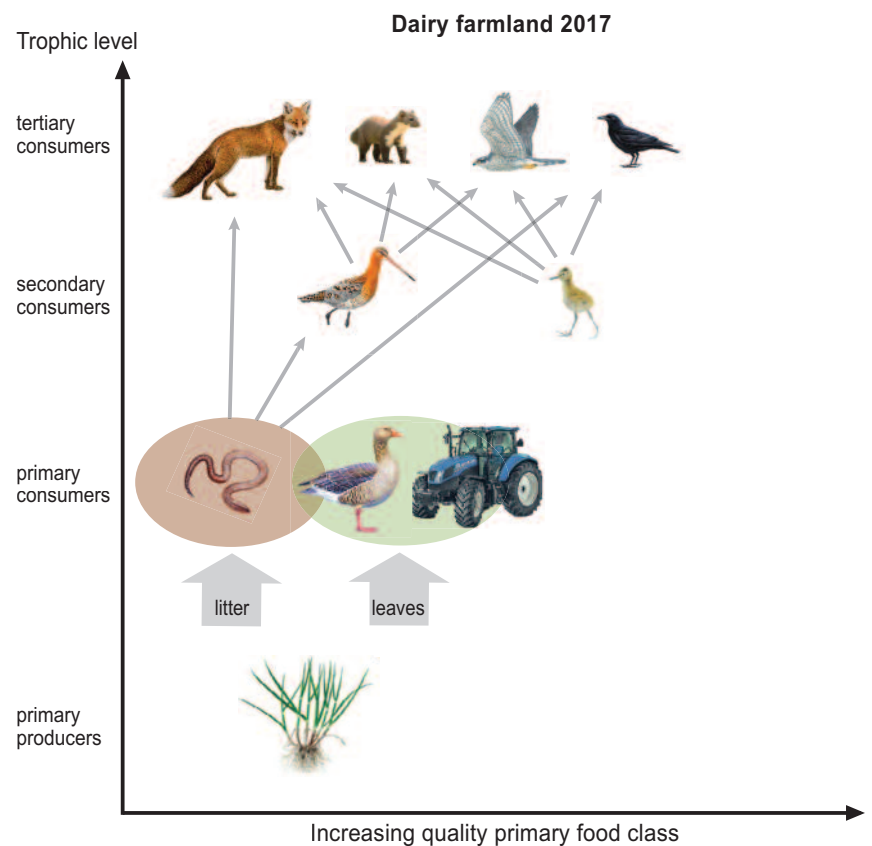


Figure 1.3: Schematic of the dairy farmland food web in the Netherlands in 2017. It represents a monoculture of *Lolium perenne* where only litter and leaves are the primary food class. Compared to figure 1, grazing cows are replaced by the tractor that mows the grass and bring it to the cows in the stable. Furthermore, geese have entered the food web as primary consumers. Most of the tertiary consumers (predators) are replaced by other species.

What worms want

Although most organism cannot cope with agricultural intensification, it does not seem to harm overall earthworm densities (Edwards & Lofty 1982, Hansen & Engelstad 1999, Muldowney *et al.* 2003, Atkinson *et al.* 2005, Curry *et al.* 2008). Highest densities of earthworms in northwestern Europe are found in The Netherlands (Rutgers *et al.* 2016), with Fryslân as the most earthworm rich province (Rutgers & Dirven-van Breemen 2012) (Fig. 1.4). Food conditions for adult meadow birds or other earthworm predators should therefore at first sight not be a limiting factor. However, as is generally true (Zwarts & Wanink 1993), for any earthworm predator it is not about how many earthworm are found in the soil (total abundance), but about how many it can catch (availability to predators).

Some meadow birds use their long bill to probe in the soil to catch earthworms by touch (Green 1988, Smart *et al.* 2006, Duckworth *et al.* 2010). Earthworms which are in top layer of the soil that matches the probing depth of a birds' bill, are available to that bird. Furthermore, depending on the strength of the bill, a bird cannot probe in soil that is too hard, for example when it is too dry. Struwe-Juhl (1995) observed that Black-tailed Godwits are unable to probe in the soil when the soil resistance exceeds the limit of 125 N/cm². Earthworm depth and soil resistance are thus limiting factors for a tactile hunting earthworm predator. There are also predators that catch earthworms which are visible to them. An earthworm is thus only available for this group of predators when it is, partly or completely, on the soil

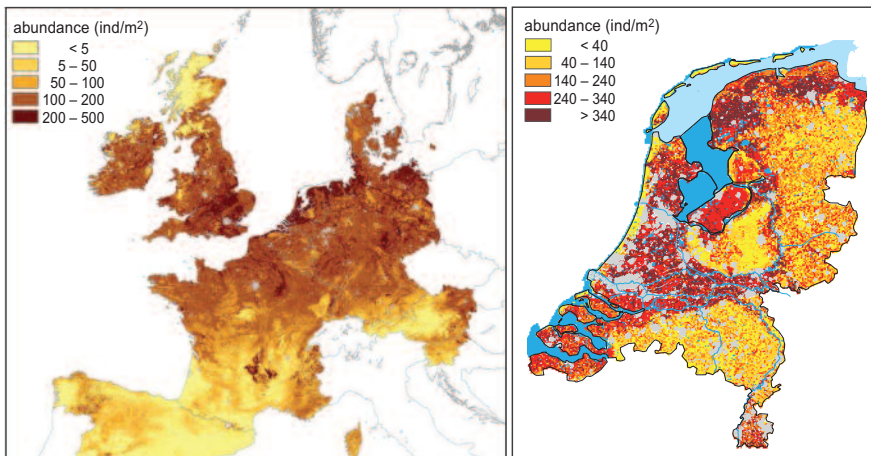


Figure 1.4: Earthworm abundances in Northwest Europa (Rutgers *et al.* 2016) and in The Netherlands (Rutgers & Dirven-van Breemen 2012).

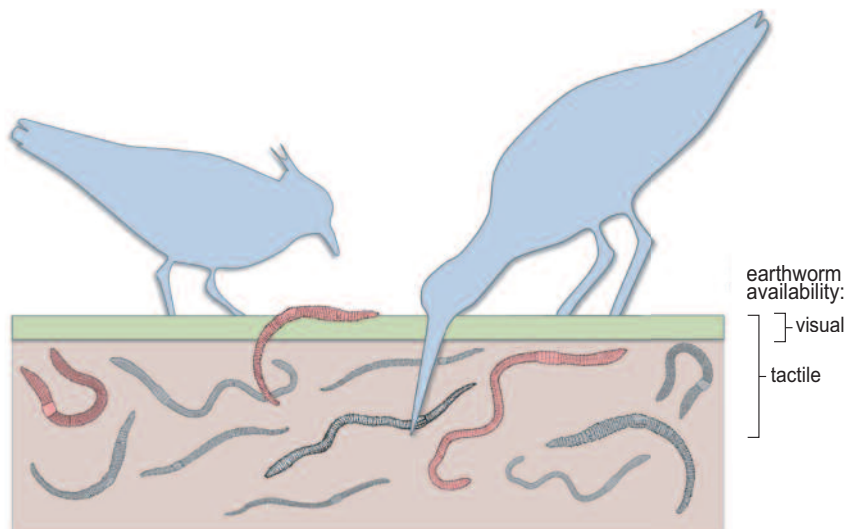


Figure 1.5: Earthworm availability for meadow birds is determined by their foraging strategy. Birds using visual cues can only catch earthworms that are near or at the surface. Tactile hunting birds can catch all earthworms which are in reach of their bill. Detritivorous (surfacing) earthworms are coloured red, geophages earthworms grey.

surface. Throughout the thesis, a discrimination is made between these two earthworm hunting strategies. A bird probing in the soil (e.g. Black-tailed Godwits, Oystercatcher) could potentially catch all earthworms that are in reach of their bill, which includes non-active earthworms. A bird using visual cues (e.g. Lapwing, Ruff), can only catch earthworm which are active on the surface. It is thus likely that earthworm availability differs between these groups (Fig. 1.5).

Since Charles Darwin wrote his last book about earthworms (Darwin 1881), the importance of these organisms is recognized, especially in agriculture. More and more agricultural scientists became interested in these 'low creatures' and with every published paper, the recognition of the importance of earthworms increased. Earthworms break down organic material and make nutrients again available to plants, they bioturbate the soil by burrowing and increase water infiltration (Lavelle 1988, Lavelle *et al.* 2006, Blouin *et al.* 2013). By performing all these ecosystem functions, they are even termed as 'ecosystem engineers' (Lavelle 1997).

Earthworm (family Lumbricidae) belong to the class of Oligochaeta (worms with few setae), which are part, together with other worm-groups, of the phylum Annelida (ringed worms) (Edwards & Bohlen 1996). They are thus worms with setae, or bristles, on each segment. Although in The Netherlands it is estimated that around 23 species of earthworms occur (van Rhee 1970), most of them are only

known by their scientific name. However, various species are functionally similar, which led Bouché (1977) to classify earthworm species in three ecological groups based on their vertical distribution in the soil and their feeding preferences. *Anecic* species form long permanent vertical burrows and emerge on the soil surface to feed or collect food which is pulled into their burrow. This group includes *Lumbricus terrestris*, the largest European earthworm species and also named as 'Nightcrawler' which reflects their nocturnal surfacing behaviour. *Epigeic* species typically live mainly in the top layer of the soil or in the litter layer and *endogeic* species inhabit the mineral soil and consume more soil than the other groups. This classification is now widely used in ecological studies of earthworms. In this work, however, we use a different and even simpler classification by dividing the species in only two groups; *detritivores* and *geophages*. Detritivores rely on surface foods and therefore show surfacing behaviour (Hendriksen 1990, Curry & Schmidt 2007). In contrast, geophages primarily feed on soil particles and humified organic matter and rarely come to the surface (Svendsen 1957, Judas 1992, Neilson & Boag 2003). According to classification of Bouché (1977), the anecic and epigeic species belong to the detritivores, whereas endogeic species belong to the geophages. For earthworm predators that hunt by using visual cues, only surfacing detritivores are available to them. Tactile hunters can feed on both groups as long as they are in reach of their bill.

Earthworm availability for an earthworm predator is of course also determined by the behaviour of earthworms themselves. Moist conditions are of vital importance for earthworms as they lack lungs and gaseous exchange with their environment requires a moist skin (Laverack 1963, Edwards & Bohlen 1996). As a response, earthworms will retreat deeper into the soil to avoid dry conditions (Gerard 1967, Rundgren 1975, Jiménez & Decaëns 2000). Therefore, earthworms are not available when the soil is frozen (winter) or desiccated (summer). Interestingly, earthworms are hermaphrodite with testes as well as ovaries that can function simultaneously, but they do need a partner for copulation and fertilization (Edwards & Bohlen 1996). *Lumbricus terrestris* mates on the surface, and copulation can take more than three hours (Nuutinen & Butt 1997), making them vulnerable for predation. By lacking lungs, a skeleton, a skin that prevent them from dehydration, and a physiology that is comparable to marine animals (Laverack 1963, Turner 2000), it is remarkable that earthworms live in the earth and not in water. Their success on earth, is mainly determined by living belowground. By digging through the soil, and excreting mucus that cements their burrows and form aggregates that increase the water binding capacity of the soil (Edwards & Bohlen 1996, Lavelle 1997, Blouin *et al.* 2013), they can create their own damp environment. Furthermore, they collect litter to form middens over the mouth of their burrows or incorporate it, which also beneficial to maintain moist conditions (Ernst *et al.* 2009). And by doing so, they have become, according to Lloyd (2009), the most influential species on earth.

However, the tragedy of earthworms is that they also encompass the whole gamut of behaviours attributed to ‘advanced’ organisms (Darwin (1881) even played piano to them!), but that in the literature they have been ‘kidnapped’ by agricultural biologists because of their role in soil functioning, rather than them being interesting organisms in their own right (there are no ‘earthworm journals’, for example) (Ghilarov 1983, Scheu 2003, Gross 2016). Also in ecology, however, earthworms are often regarded as bulk prey for other organisms where even large conservation programs are for (badgers, meadow birds, kiwi’s etc.). To understand these animals in their environment and to be able to protect them, it is of paramount importance to understand how earthworms themselves respond to their environment, specific food abundance or to the risk of being fed upon (Laidlaw *et al.* 2013), so their behavioural ecology.

Inspired by intensive research on the declining shellfish food of foraging Red Knots *Calidris canutus* in the Wadden Sea during a period of intensive cockle dredging (van Gils *et al.* 2006, Kraan *et al.* 2009), we will explore earthworms in Frisian dairy farmland to understand what determines their distribution and availability for the strongly declining meadow birds. The research is conducted mainly in the province of Fryslân in the northwest of The Netherlands. Here, 90% of the cultivated land is used for dairy farming and the highest earthworm and meadow bird densities of The Netherlands have been traditionally found there (van Dijk *et al.* 1989, Altenburg & Wymenga 2000, Rutgers & Dirven-van Breemen 2012, Nijland & Postma 2016). Furthermore, it is this group of birds that are part of the Frisian culture, with rich traditions linked with both breeding and migrating meadow birds (e.g. egg collecting (Breuker 2012) and ‘wilsterflappen’ (Jukema *et al.* 2001)).

Outline of thesis

We started this research endeavour by developing new methods to measure earthworm surface availability properly. Especially for visually hunting predators, this was a challenge as surfacing earthworms retreat quickly into their burrows before they could be observed when they notice vibrations. Duriez *et al.* (2006) and Dänhardt (2010) counted the earthworms that were crawling on the surface in grasslands and arable fields at night by walking transects whilst illuminating the soil with a torch. Walking observers still created vibrations and only large retreating earthworms can then be measured. Furthermore, in grasslands an observer has to be close to the soil to discriminate earthworms from grasses. In **chapter 2** we describe how this hurdle is circumvented by building a robust cart which is pushed slowly across the field by a prone observer. In this way, number of surfacing earthworm could be counted without disturbing them. We test this method during day

and night and in different managed grasslands and compare number of surfacing earthworm with total abundances in the soil.

After we had a good method to measure earthworm availability for visually hunting earthworm predators, we apply this method in a study to understand how Ruffs use Frisian dairy farmland during spring migration. However, we did not know how this peculiar bird find its prey exactly. Therefore, in **chapter 3** we perform an indoor feeding experiment with captive male Ruffs to study which cues they use in finding earthworms. In the field on different grasslands, intake rates of Ruffs feeding on earthworms during the day were scored as well as the number of surfacing earthworms at night. Together with transmitter data of Verkuil *et al.* (2010), we ask the question why Ruffs do not feed at night when food availability is much higher.

In **chapter 4** we study what the short-term effect of fertilizing with farmyard manure is on the availability for visually hunting earthworm predators. This type of fertilizing was common in the heydays of meadow birds halfway the 20th century, but has become rare as modern stables only produce slurry manure instead of farmyard manure. As earthworms come to the surface to collect food, we expected well-fed earthworms to present themselves on surface least to avoid the risk of being eaten by a predator. Two uniform grasslands were split with either the two halves to receive an early (1 February 2014) or a late (14 March 2014) farmyard manure application. Every two weeks, nocturnal surface activity of earthworms was measured. Furthermore, soil samples were taken for total abundances and to measure individual body conditions of earthworms.

To understand food availability for meadow birds, we also had to understand how food determines the surfacing behaviour of earthworms, and thus availability for meadow birds. Therefore, in **chapter 5** we investigate the effect of different types of dairy manure on two earthworm 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. As detritivores come to the surface to collect food, they are an important prey for birds and mammals. We test 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 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 below-ground. Furthermore, growth rates of the two ecotypes were measured under controlled conditions using either one of the two manure types.

Besides food, water is probably even more important for the moisture-loving earthworms. Dry conditions are avoided by going in diapause or by retreating

deeper into the soil. This would negatively influence earthworm availability for meadow birds. It is interesting to know, when earthworm surfacing behaviour stops in dairy farmland. In **chapter 6**, we study this by measuring weekly the number of surfacing earthworms, as well as hydrological conditions of eight intensive managed grasslands with different groundwater tables. The sensitivity of a detritivorous and a geophagous earthworm species to variation in the vertical distribution of soil moisture was experimentally studied.

Finally, I will synthesize the results in **chapter 7** by placing them in the broader context. To do so, I use data collected in Flevoland, where we studied the role of earthworms in a natural grassland, as well as on a conventional intensive dairy farm and a dynamic-organic dairy farm. With a controlled indoor experiment, complete sods were collected in the three areas and received either earthworms (*Lumbricus rubellus*), cow dung, both or nothing and for three months, grass production was measured. This experiment showed the importance of earthworms, not only as a prey, but also as an ecosystem engineer.

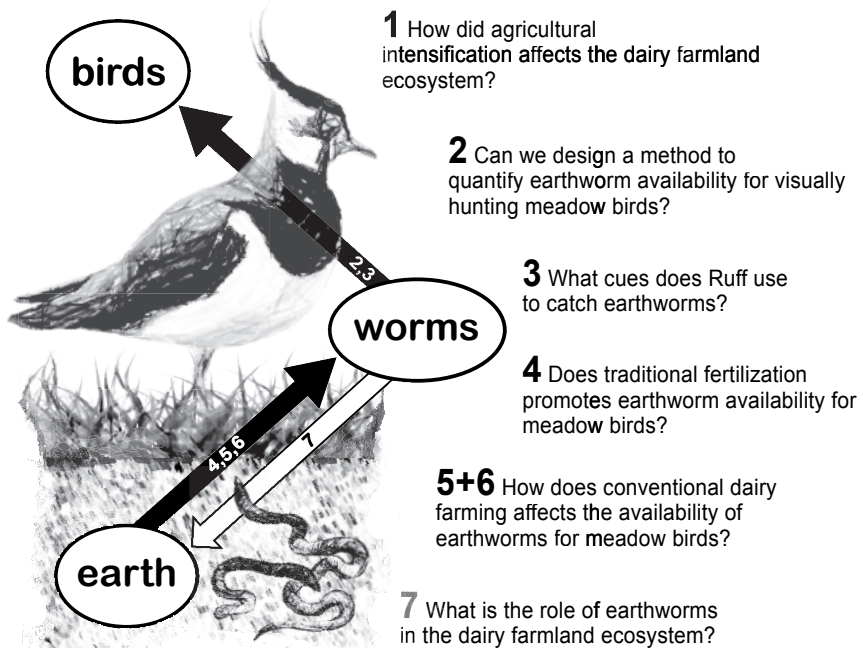


Figure 1.6: Outline of the thesis “Earth, worms & birds”: How does dairy farm management (**earth**) affects earthworms (**worms**) and their availability for meadow birds (**birds**)? In the synthesis chapter 7, we study the role of earthworms (**worms**) in the dairy farmland ecosystem and how dairy farm management (**earth**) is affecting this.



Determining earthworm availability for visually hunting predators; a novel method versus standard sampling

Jeroen Onrust, Sjoerd Hobma, & Theunis Piersma

Abstract

Studies of the interactions between earthworm prey and their visually foraging predators required a field method that measures the density of surfacing earthworms. Here we present such a method. Surfacing earthworms were counted at night by an observer lying prone on a cart that was self-propelled across measured distances at constant low speed. The method was applied in the Netherlands in October 2011 to study surfacing numbers relative to total abundance in agricultural grasslands on clay and peat soils and with an intensive or extensive management. We found contradictory correlations between availability and total abundance, emphasizing the importance of directly measuring earthworm availability in studies to explain the behaviour of visual earthworm predators.

Introduction

Earthworms (Lumbricidae) play a critical role in soil ecology and nutrient cycling (Darwin 1881, Edwards and Bohlen 1996). At the same time, they are important as food for many animals (MacDonald 1983, Curry 1998). These protein-rich prey are found in many habitats around the world and can be very abundant in fertile soils (Edwards and Bohlen 1996).

As earthworms are soil-dwelling organisms, they can be caught by predators that probe deeply in the soil (e.g. the long-billed sandpipers, Scolopacidae (Burton 1974)) and by pursuit in predators that dig themselves through the soil (e.g. moles (*Talpa europaea*) (Raw 1966)). Soil samples can be taken to assess the abundance of earthworms (Römbke *et al.* 2006, Coja *et al.* 2008), and such samples can then be subdivided in different depth layers to obtain measures of availability for a probing predator (Rundgren 1975). However, many predators only catch earthworms on the surface, especially reptiles and amphibians (Hamilton 1951, MacDonald 1983), some mammal species (e.g. badger (*Meles meles*) (Kruuk and Parish 1981, Madsen *et al.* 2002)) and some bird species (e.g. little owls (*Athene noctua*) (Hounscome *et al.* 2004, Romanowski *et al.* 2013), golden plovers (*Pluvialis apricaria*) (Bengtson *et al.* 1978) and blackbirds (*Turdus merula*) (Chamberlain *et al.* 1999)). Therefore, the abundance or biomass of earthworms derived from soil samples taken during the day at best will give a biased estimate of earthworm availability from the predator-point of view, or perhaps no estimate at all (Duriez *et al.* 2006). In studies on the foraging ecology of visual earthworm predators it would be important to directly measure the density of surfacing earthworms.

Earthworm availability is defined as the number of visible earthworms per unit surface. Darwin (1881) already noticed nocturnal activity of earthworms on the soil surface, and others showed that the highest activity is measured in the first hours after sunset (Baldwin 1917, Butt *et al.* 2003). Earthworms come to the surface to scavenge for living and decaying organic material (Edwards and Bohlen 1996). This behaviour differs between species and is determined by their feeding ecology (Lowe and Butt 2002). Surface-dwelling earthworms mostly belong to the epigeic and anecic, rather than the endogeic ecological group (Bouché 1977, Curry and Schmidt 2007).

Earthworm availability for visual predators has previously been assessed indirectly using climatic variables to calculate 'worm nights' (including temperature, humidity and time since last rain) (MacDonald 1980, Kruuk and Parish 1981, Baubet *et al.* 2003). A more direct method was used by MacDonald (1980) who counted emergent earthworms on grids in gardens using a torch fitted with a red filter. A similar method was employed by Dänhardt (2010), who measured earthworm availability for golden plovers in croplands in southern Sweden by walking transects of

30 meter and observing the surface of about 60–70 cm in front of the observer. However, as we were interested in earthworm surface availability in grasslands, an observer had to be close to the soil to discriminate earthworms from grasses. Furthermore, in studies aimed at understanding the feeding distribution of woodcock, (*Scolopax rusticola*), Duriez *et al.* (2006) counted the earthworms that were crawling on the surface at night, but noticed that earthworms were sensitive to vibrations and retreated in their burrows when a walking observer approached.

Here we describe a new method to measure surfacing earthworm densities in grassland habitats. We then apply the method in four types of agricultural grasslands in the Netherlands, which are commonly used by wide variety of visually hunting earthworm predators. Although agricultural intensification of these grasslands might promote earthworm abundances (Curry *et al.* 2008), it is not clear whether earthworms are also more available for predators. Extensification of agricultural practices is often used to promote habitat suitability for the strongly declining meadow birds, the question remains, however, whether this also promotes earthworm availability.

Study area

This study was performed on 48 grasslands throughout the province of Friesland, the Netherlands, across an area spanning about 20 by 40 km. All grasslands were used for dairy farming and were selected based on their soil type (clay or peat) and degree of agricultural use (monocultures vs. species rich grasslands). Monocultures consisted predominantly of fast growing rye grass species (*Lolium* sp.) and are mowed 5–6 times a year, in most turns followed by treatment with injected slurry manure. Furthermore, these grasslands have a relative low groundwater table (80–120 cm below surface level) and a monotonous vegetation (Groen *et al.* 2012). Species-rich grasslands had a management agreement to protect meadow birds, meaning that these grasslands are mowed less often (2–3 times), later in spring and are fertilized with farmyard manure only and therefore tend to have (many more) forbs.

Methods

The movable earthworm observation platform (the ‘cart’) consisted of a robust rectangular metal frame with four fixed tires (100 mm width), with the frame being half closed with a shelf (Fig. 2.1). In this way, the legs of the observer could touch the ground and move freely while in prone position and with the head in front of

the cart. The soil surface could then be observed from a height of 50 cm and within a width of 50 cm in front of the observer. At night, a headlight (160 lumens) without any filter was used. All counts were conducted on grassland with a short sward height (<10 cm).

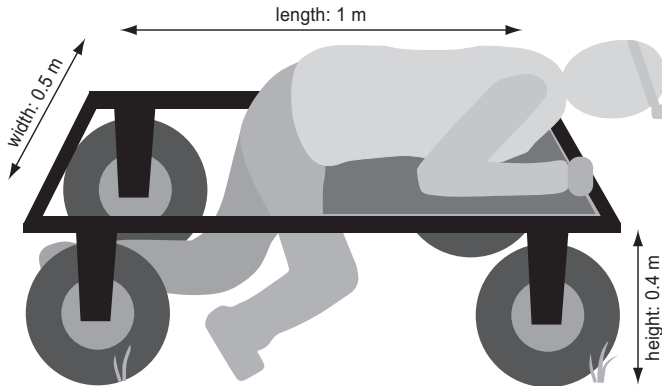


Figure 2.1: Representation of the method described in this paper to count earthworms on the surface.

First, we determined activity patterns in the surfacing behaviour of earthworms. In autumn 2010 we counted surfacing earthworms from 16:00 CEST until 8:00 CEST. Every hour the same transect of 100 m was counted, but the counts were divided in three periods of 4–5 hours over three days. This transect was in an agricultural grassland on clay soil near Akkrum, Friesland (N 53°3.367, E 5°52.012). As the hourly counts were divided over three days, we used the relative numbers of the maximum number counted per time period.

To test whether the management classification of the 48 grasslands resulted in distinct type of grasslands, we surveyed the vegetation composition of each field and determined a weighted Ellenberg's indicator value for soil fertility and moisture (Ellenberg *et al.* 1991). These values indicate the ecological preference of plants and is scored on a scale of 1–9 for fertility (9 represents extreme nutrient-rich situations) and on a scale of 1–12 for moisture (12 represents submerged conditions) (Ellenberg *et al.* 1991). Vegetation surveys took place in November 2011 by randomly placing five times a 1×1 m quadrat and determine the plant species (rosettes of most herbs still visible in this time of year) and abundance within that frame.

In October 2011, earthworms were counted by a single observer (JO) at two random placed transects of 50 m with a speed of about 0.3 m s^{-1} . Counts were conducted during night time between 21:00 and 24:00 CEST, as this is the period with the highest surface activity (own observations, Butt *et al.* 2003). We consid-

ered every earthworm seen a potential prey for an eye-hunting predator. Therefore, all earthworms were counted and no distinctions were made between species, small and large earthworms and earthworms which were either completely or partially out of their burrows. Over a period of 20 nights, all fields were counted once. In the morning after the night-time surveys, four soil samples of $20 \times 20 \times 20$ cm were excavated at the transects (two per transect, four in total per field). All earthworms were counted by sorting out the samples by hand. There might be a sampling effect as some deeply burrowing anecics could be missed when handsorting soil samples, although this method generally yields the most individuals and highest biomass of earthworms (Coja *et al.* 2008).

Hourly weather conditions during observations were obtained from the nearest weather station in Leeuwarden, Friesland (N $53^{\circ}13'$ E $05^{\circ}46'$, www.knmi.nl). For the analysis we used the following average values for the 21:00–24:00 h CEST period: temperature in $^{\circ}\text{C}$ at 10 cm above ground level, atmospheric humidity, total precipitation during the observations in mm, and total precipitation during daytime.

Statistical analyses were performed using R (R Development Core Team 2016). As two transects per grassland were counted in 2011, we were able to calculate repeatability of this method by estimating the Intraclass Correlation Coefficient (ICC) by using the R package 'ICC' (Wolak *et al.* 2012). For all analyses we performed a linear mixed effects analysis for nested data with the package 'nlme' (Pinheiro *et al.* 2016), as type of soil (clay or peat) and type of grassland (monocultures or herb-rich meadows) are the fixed effects and field is the random effect. Data exploration for this multivariate dataset showed that earthworm availability and earthworm abundance contained outliers and violation of homogeneity. A log-transformation for availability and a square root transformation for abundance solved these problems. For each model, also a random intercept model and, when multiple measurements were taken on the same field, a random slope model was built. The model with the lowest Akaike's Information Criterion (AIC) was then used for further analysis. P-values were obtained by likelihood ratio test of the full model with the effect in question against the model without the effect in question. We checked the normality of the residuals by visual inspecting the QQ plots (Miller 1986). *Post hoc* comparisons were made by using the R package 'lsmeans' (Lenth 2016).

Results

Earthworms only came to the surface in darkness, with numbers rising rapidly after sunset and declining equally rapidly before sunrise (Fig. 2.2). The Intraclass Correlation Coefficient for this method is 0.69 with 95% CI (0.36, 0.85), which shows considerable agreement between the two transects in 2011.

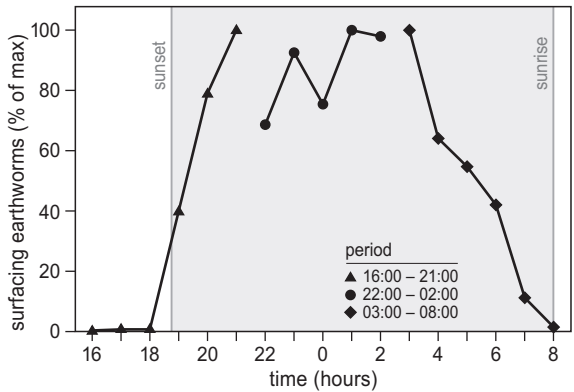


Figure 2.2: Earthworm availability at a single transect of 100 m in agricultural grassland from 3 counts at different time periods. The relative numbers of the maximum number counted in one time period is plotted as the counts were done on different days.

Grassland characteristics of the 48 studied grasslands are summarized in Table 2.1. Compared with monocultures, species-rich grasslands had a lower Ellenberg value for fertility ($\chi^2(1) = 61.536, P < 0.001$), but there was no effect of soil type ($\chi^2(1) = 0.580, P = 0.446$). In addition, species-rich grasslands had a higher value for moisture ($\chi^2(1) = 42.426, P < 0.001$), but soil type was also slightly significant ($\chi^2(1) = 6.097, P = 0.014$). These results show that our classification clearly distinguished grasslands based on management type, but not on soil type.

Table 2.1: Grassland characteristics according to soil and vegetation type. Earthworm availability, abundance, and number of species for grasses and forbs are all in numbers per m². For each variable the average for 12 grasslands is shown with standard deviation in brackets. Data was collected in October and November 2011.

Soil type: Grassland:	Clay		Peat	
	Species-rich	Monoculture	Species-rich	Monoculture
Earthworm				
Availability	1.22 (0.85)	1.10 (0.49)	0.44 (0.21)	1.76 (1.60)
Abundance	264.06 (132.91)	353.65 (187.85)	371.35 (220.83)	543.23 (305.76)
Vegetation				
Grasses	3.50 (1.05)	1.92 (0.65)	3.25 (1.22)	1.83 (0.70)
Forbs	4.70 (1.58)	1.71 (0.86)	4.83 (1.32)	2.56 (1.02)
Ellenberg value				
Fertility	6.10 (0.35)	7.11 (0.46)	6.05 (0.40)	7.03 (0.37)
Moisture	6.17 (0.64)	5.34 (0.35)	6.47 (0.79)	5.42 (0.36)
pH	5.92 (0.70)	6.11 (0.61)	5.52 (0.31)	5.70 (0.51)

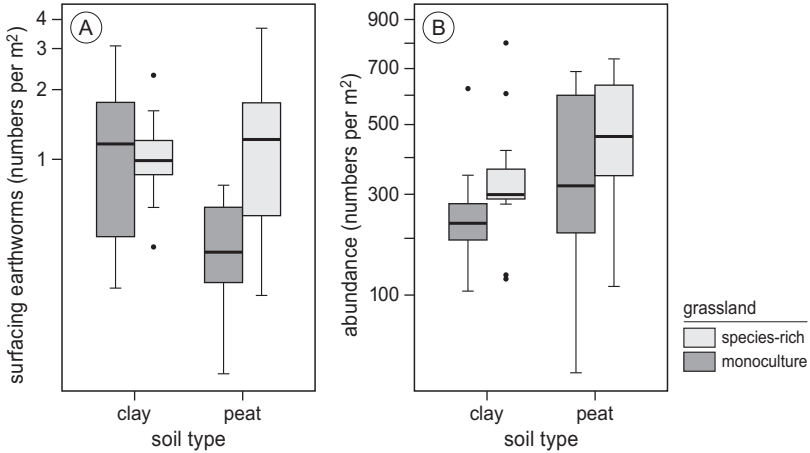


Figure 2.3: Boxplots of nocturnal counts with number of available earthworms per 100 meter (A, in number per m²) and total earthworm abundances in the soil (B, in number per m²), derived from soil samples taken from the same transects. Each boxplot represents 12 grasslands. Note that the y-axes are scaled to log (A) and square root (B).

In the period of observations, sward height was short for all grasslands (7.5 cm, SD = 2.8, $N = 48$). The density of surfacing earthworms varied between 0.12 and 3.66 earthworms m⁻² with on average 1.04 earthworms m⁻² (SD = 0.81, $N = 48$, Table 2.1). Most earthworms were only partly out of their burrow and in the process of collecting food items, others were mating or crawling around. There was not a significant effect of soil type on number of surfacing earthworms ($\chi^2(1) = 3.087$, $P = 0.079$), but grassland type ($\chi^2(1) = 8.296$, $P = 0.004$) and the interaction were significant ($\chi^2(1) = 7.262$, $P = 0.007$). However, a *post hoc* comparison revealed only a significant difference between species-rich grasslands on peat soil with all other grasslands at $P < 0.05$ (Fig. 2.3A).

There was large variation in number of earthworms collected from soil samples, with numbers ranging between 18.8 and 800.0 earthworms m⁻² (Table 2.1). Although earthworm abundance was highest in monocultures ($\chi^2(1) = 4.244$, $P = 0.039$) and in peat soils ($\chi^2(1) = 4.196$, $P = 0.041$) (Fig. 2.3B), the interaction was not significant ($\chi^2(1) = 0.403$, $P = 0.525$). A scatterplot of numbers of earthworms on the surface on total abundance (Fig. 2.4) showed a lack of relationship for species-rich grasslands on both clay ($R^2 = 0.06$, $F = 0.34$, $P = 0.573$) and peat soil ($R^2 = 0.02$, $F = 1.216$, $P = 0.296$). For monocultures, however, there was a positive relationship for clay soils ($R^2 = 0.49$, $F = 11.48$, $P = 0.007$), but a negative relationship for peat soils ($R^2 = 0.33$, $F = 5.856$, $P = 0.039$). None of the weather variables during observations explained the number of surfacing earthworms ($F_{4,43} = 1.091$, $P = 0.373$).

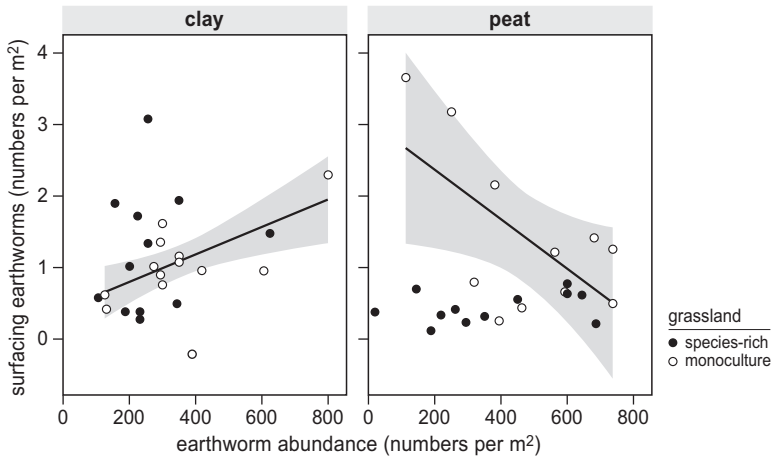


Figure 2.4: Earthworm availability at night as a function of the total abundance in the soil. For intensive farmed grasslands only, there is a significant positive relationship at clay soil ($R^2 = 0.49$, $F = 11.48$, $P = 0.007$), but a significant negative relationship at peat soil ($R^2 = 0.33$, $F = 5.856$, $P = 0.039$).

Discussion

We describe a method that yields a direct measure of earthworm availability for visually hunting earthworm-eaters in grassland habitats. As earthworm abundance in the soil did not consistently predict the numbers of surfacing earthworms (Fig. 2.4), direct measurement of the densities of surfacing earthworms are certainly a requirement in studies in which prey availability for visual hunting predators is a key variable. Earthworms might come up or go down as a result of vibrations applied to the soil (Mitra *et al.* 2009). Only when the cart was close (a few centimeters) to an earthworm, would it retract in its burrow. Thus although, the cart may have caused vibrations, the large wheels and the slow and constant speed did not appear to affect the earthworms much. During the nocturnal counts, earthworms did react to the bright luminescence of the headlight, but only after 2–3 sec, which gave us enough time to spot and count them (Darwin 1881, Svendsen 1957).

Surfacing behavior of earthworms is greatest during nocturnal hours (Fig. 2.2) (Darwin 1881, Baldwin 1917, Butt *et al.* 2003) and is dependent on soil moisture (Kretzschmar 1991), ambient light and temperature (Darwin 1881, Baldwin 1917, Edwards and Bohlen 1996, Butt *et al.* 2003). However, the lack of relationship between earthworm abundance and number of surfacing earthworms could be caused by species-specific surfacing behavior. Surfacing occurs most in epigeic and

anecic species that scavenge for food on the soil surface (Svendsen 1957, Curry and Schmidt 2007). This explains why Cuendet (1983) found proportionally more epigeic than endogeic in the gut content of black-headed gulls (*Chroicocephalus ridibundus*), accounting for numerical presence in the soil. The results of the nocturnal observations in this study might thus reflect different species composition at the four types of grasslands. We only found a positive relationship in monoculture grasslands on clay soil. Although, we did not identify earthworms to species level, we do not expect that in these grasslands more epigeic or anecic species occur than in the other types of grasslands as these species are normally to be found in undisturbed soils with high organic matter content (de Vries *et al.* 2007, van Eekeren *et al.* 2010). However, as we also did not find a relationship in the species-rich grasslands (which are generally older and less disturbed), it is unlikely that the number of earthworms in the soil determines the numbers on the soil surface.

Management implications

We developed and field-tested a quantitative research tool to measure the densities of surfacing earthworms in grasslands, a method that is easy to perform and replicable. We have shown that only a small fraction of the total earthworms surface during the night and earthworm abundance does not predict the numbers of surfacing earthworms. Therefore taking soil samples will give no, or at least a biased, estimate of earthworm availability for a predator. Using this method, new insights in the ecology of earthworms and their relationship with visually hunting nocturnal predators have come within reach.

Acknowledgements

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Box A: How many earthworms does a meadow bird need?

Although earthworms can be very abundant in fertile soils (Edwards & Bohlen 1996), the question remains how many earthworms a meadow bird actually needs to meet its daily energetic requirements? To answer this question I use a series of formulae from literature that estimate the birds' daily energy expenditure and I use my own data about the ash-free dry mass (AFDM) of earthworms from different species and different size classes (from 8 – 141 mm).

Methods

Earthworms were collected at four different agricultural grasslands at the farm of Klaas Oevering (Idzegea; N 52°58'48, E 5°33'12) at 20 November 2014. From each field three 20 × 20 × 20 cm soil samples were taken and sorted out by hand. All (intact) earthworms found were used for this analysis. For the calculations, I use the data of all earthworms species combined, but also from detritivores and geophages separately.

In total 577 earthworms (142 detritivores; 435 geophages) were measured individually. First, fresh weight was determined by rinsing the earthworms with tap water, then blotted with absorbable paper and weighed to the nearest 0.1 mg. After weighing, the earthworm was euthanized by putting it in a tube with 98% Ethanol solution. This killed the earthworm within seconds. Then, the length was measured in mm. By killing the earthworm shortly before measuring the length, it gave the most reliable measure of length as all earthworms were measured in relaxed state. Dry mass was determined by drying the earthworms in a stove at 70 °C for 48 h after they were weighed to the nearest 0.1 mg. The ash mass was determined 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.

When fresh length (FL, in mm) or fresh weight (FW, in mg) of an earthworm is known, AFDM (in mg) can be calculated by using the following equations:

$$\text{Fresh length: AFDM} = 0.0063 \text{ FL}^{2.2972}, R^2 = 0.955, P < 0.001$$

$$\text{Fresh weight: AFDM} = 0.1727 \text{ FW}, R^2 = 0.976, P < 0.001$$

There are several calculations that have to be made to arrive at the number of earthworms a bird need. First we need to determine the daily energy expenditure (DEE, in kJ per day) which can be calculated for waders using the following formula:

$$DEE = 1092 * BM^{0.729}$$

In which DEE stands for the daily energy expenditure (in kilojoules per day) and BM stands for birds's body mass (in kilograms) (Kersten & Piersma 1987). For these calculations we use body mass data of breeding female Lapwings *Vanellus vanellus* (197.3 g) and Black-tailed Godwits *Limosa limosa* (286.4 g) from Hegyi & Sasvári (1998).

Second, we need to know the energy content of an earthworm. Bolton & Phillipson (1976) measured this for six earthworm species. The average energy content of an earthworm is 23.00 kJ per gram AFDM. For detritivores this is 23.16 and for geophages 22.84. Most food does not yield the total energy content, as the digestive tract is not able to process all the energy consumed. The digestive efficiency of birds feeding on terrestrial invertebrates is on average 74.2% (Bairlein 1999).

Third, the required daily energy intake for a bird (DEI, in gram AFDM) can be calculated with the above values by using the equation: $DEI = DEE / 0.742 / 23.00$, which becomes:

$$DEI = 63.99 * BM^{0.729}$$

Fourth, the number of earthworms can then be calculated by dividing DEI with the average AFDM of an earthworm. For all earthworms this is 0.0353 g and for detritivores 0.0612 and for geophages 0.0268 (Table A.1). Biomass can be calculated with the allometric relationship between fresh weight and AFDM: $FW = 0.1731^{-1} AFDM$, which can be rewritten as: $FW = 5.790 C_d$. For detritivores: $FW = 5.618 C_d$ and for geophages: $FW = 6.383 C_d$.

Table A.1: The average length in mm and weight in mg of different species of earthworms and its ash-free dry mass (AFDM) in mg

	N	Fresh length (mm)	Fresh weight (mg)	AFDM (mg)
<i>Allolobophora chlorotica</i> ¹	52	29.1	149.1	26.6
<i>Aporrectodea caliginosa</i> ¹	369	35.2	151.2	23.3
<i>Lumbricus rubellus</i> ²	133	32.6	172.4	28.6
<i>Lumbricus terrestris</i> ²	15	102.6	2127.2	381.6
all species	569	35.8	206.1	35.3
Detritivores	148	39.7	365.3	61.2
Geophages	421	34.4	150.9	26.8
¹ geophagous species, ² detritivorous species				

Results

A female Lapwing requires 19.60 g AFDM each day and a female Black-tailed Godwit 25.72 g AFDM. As detritivores have higher AFDM values (Table A.1), a bird can consume fewer numbers of these earthworms to meet their daily energetic requirements (Table A.2). However, the larger AFDM value for detritivores is mainly determined by the large species *Lumbricus terrestris* (Table A.1).

Table A.2: Number of earthworms a meadow bird needs to meet their energetic requirements, with total biomass in grams between brackets. Calculations are based on female Lapwings of 197.3 g and female Black-tailed Godwits of 286.4 g.

	all earthworms	detritivores	geophages
Lapwing	555 (113.5)	320 (110.1)	730 (125.1)
Black-tailed Godwit	728 (148.9)	420 (144.5)	957 (164.2)



Detection of earthworm prey by Ruff *Philomachus pugnax*

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Abstract

Ruff *Philomachus pugnax* staging in the Netherlands forage in agricultural grasslands, where they mainly eat earthworms (Lumbricidae). Food intake and the surface availability of earthworms were studied in dairy farmland of southwest Friesland in March–April 2011. Daily changes in earthworm availability were quantified by counting visible earthworms. No earthworms were seen on the surface during daytime, but their numbers sharply increased after sunset and remained high during the night. Nevertheless, intake rates of individual Ruff in different grasslands measured during daytime showed the typical Holling type II functional response relationship with the surfacing earthworm densities measured at night. Radiotagging of Ruff in spring 2007 revealed that most, if not all, feeding occurs during the day, with the Ruff assembling at shoreline roosts at night. This raises the question of why Ruff do not feed at night, if prey can be caught more easily than during daytime. In March–May 2013 we experimentally examined the visual and auditory sensory modalities used by Ruff to find and capture earthworms. Five males were kept in an indoor aviary and we recorded them individually foraging on trays with 10 earthworms mixed with soil under various standardized light and white noise conditions. The number of earthworms discovered and eaten by Ruff increased with light level, but only when white noise was played, suggesting that although they can detect earthworms by sight, Ruff also use auditory cues. We suggest that although surfacing numbers of earthworms are highest during the night, diurnal intake rates are probably sufficient to avoid nocturnal foraging on a resource that is more available but perhaps less detectable at that time.

Introduction

To understand the interactions between predator and prey, it is necessary to know about the sensory ecology of both actors, i.e. how a predator detects and catches its prey and how the availability of the prey changes over time (Zwarts & Wanink 1993, Barbosa & Castellanos 2005, Piersma 2011). Earthworms (Lumbricidae) are soil-dwelling organisms that are important food for a wide variety of predators (MacDonald 1983). Earthworms can be caught by probing the soil surface (e.g. the long-billed sandpipers, (Burton 1974)) or digging through the soil (e.g. Moles *Talpa europaea*, (Raw 1966)). However, as Darwin (1881) already observed, earthworms also come to the soil surface themselves and then are fed upon by visual hunters including birds (e.g. Golden Plovers *Pluvialis apricaria*, (Bengtson *et al.* 1978)); and Blackbirds *Turdus merula*, (Chamberlain *et al.* 1999), reptiles and amphibians (Hamilton 1951, MacDonald 1983).

A migratory sandpiper, the Ruff *Philomachus pugnax* (Linnaeus, 1758), is virtually extinct as a breeding species in the Netherlands (Boele *et al.* 2016), but still stages there during the migration period (Jukema *et al.* 2001, Verkuil *et al.* 2010), albeit in greatly diminished numbers (Schmaltz *et al.* 2015). Ruff use freshwater wetlands and agricultural grasslands, but deterioration of these habitats may have caused declining numbers of staging birds in the Netherlands and a shift towards a more easterly migration route (Verkuil *et al.* 2012). Ruff are opportunistic feeders and can feed on plant materials as well as invertebrates (Ezealor & Giles 1997, Baccetti *et al.* 1998). In the Netherlands, Ruff primarily use moist grasslands for feeding (Verkuil & de Goeij 2003, Schmaltz *et al.* 2016), and their main prey then are earthworms (van Rhijn 1991), sometimes supplemented by leatherjackets (Tipulid larvae) (Beintema *et al.* 1995). When earthworms become less available due to desiccation of the soil and with increasing sward height, Ruff can switch to eating insects by picking them from the foliage if these become available on warm spring days (Verkuil & de Goeij 2003, Schmaltz *et al.* 2016).

How they detect the earthworms remains unclear. Routinely deep probing of the soil has been observed (Verkuil & de Goeij 2003, Krupa *et al.* 2009), which suggests that they can use tactile foraging strategies or that they merely chased retreating prey they had detected otherwise. Indeed, van Rhijn (1991) and Barbosa (1995) identify the Ruff as a tactile forager. Hoerschelmann (1970), on the other hand, suggests that the Ruff is a typical visual forager based on the shape and structure of the bill. Ruff have relatively short bills (30–31 mm for females, 34–35 mm for males, (Meissner & Ziêcik 2005)), and the tip of a Ruff's bill contains fewer sensory cells than that of more tactile foraging wader species (Ballmann 2004). Nevertheless, Thomas *et al.* (2006) state that Ruff use a mixture of both techniques with no bias towards visual or tactile foraging.

Earthworms may come to the surface during the night (Butt *et al.* 2003) and can then be detected by sight under low illumination. Given their nocturnal surfacing behaviour, at least for visual foragers with good night vision, it would be beneficial to forage nocturnally (McNeil & Rodríguez 1996, Lourenço *et al.* 2008). This seems to be the case for Golden Plovers, which have relatively large eyes and probably also a high rod/cone ratio for good night-vision (Rojas *et al.* 1999, Martin & Piersma 2009). Ruff, however, have relatively small eyes (Thomas *et al.* 2006). Surprisingly, Cramp and Simmons (1983) state that Ruff mainly forage during twilight and at night. It is possible that, depending on ecological context, they switch from visual hunters by day to tactile feeders by night as is observed in other shorebird species (Mouritsen 1994, Burton & Armitage 2005). At night, they could also use aural cues to locate a digging earthworm, as is done by thrushes during daytime (Montgomerie & Weatherhead 1997) and possibly by Golden Plovers as well (Lange 1968).

On the basis of these conflicting statements, we predicted that Ruff use visual cues to catch earthworms, but might switch using aural cues in darkness. We used field observations of earthworm-eating Ruff to look at feeding performance during the day in relation to available prey densities at night, and used radio-telemetry data to establish whether Ruff are indeed diurnal foragers at our study site. We then performed a controlled indoor experiment to examine the capacity of Ruff to use visual and aural cues in the detection of earthworms.

Methods

The predator and its prey: field observations

All fieldwork was conducted in southwest Friesland, the Netherlands (N 52°55 E 5°26 with a radius of about 10 km). In this area the total land area consists mainly of grasslands which are used for dairy farming (Groen *et al.* 2012). These grasslands are used by Ruff to forage and the numerous lakes and shorelines are used as roosting sites (Verkuil & de Goeij 2003, Schmaltz *et al.* 2016).

From 21 March 2011 to 15 April 2011 foraging Ruff were studied in relation to the earthworm conditions in selected fields. The fields were selected on the basis of the presence of flocks of Ruff (with numbers ranging between 40 and 450 individuals). On 12 different fields (all between 2 and 6.5 ha and all used for dairy farming and with a loamy clay soil), between 6 and 11 different birds each were observed between sunrise and sunset. Bird observations involved the counting of numbers of foraging birds and the scoring of individual prey intake rates. Intake rate was defined as number of eaten earthworms per minute. Intake rates of a focal individual were scored for five min by using a 20–60x magnification telescope. Intake rates were scored for exactly 100 different Ruff. Although earthworms could be positively

identified as prey (their colour, size and behaviour), not every prey item or swallowing action could be identified and therefore only definitely consumed earthworms were counted. This leads to an underestimation of the intake rate. Field observations were stopped when Ruff switched to eating insects. Ruff feeding on insects can clearly be distinguished from earthworm-eating Ruff as their pecking at insects on the foliage results in a very different posture, head movements and gait.

Visual counts of earthworms were made a day later in the fields where the intake rate observations were made. Surfacing earthworms were counted by lying prone on a robust and simple cart which was gently pushed forward by foot. This cart provided the earthworm observer with a good view of the soil (head ca. 40 cm above surface) and it created little vibrations. Visual counts consisted of counting the surfacing earthworms along two transects of 75 m per grassland. Every earthworm within 50 cm of the central transect line was counted. In this way, about 75 m² was covered per sampling event. One transect took about 45 min to complete. The counts were repeated five times throughout the day at 7:00, 10:00, 14:00, 18:00 and 21:00 h CEST, with the second transect starting an hour after the first. Sunrise during the observation period was between 6:22 and 7:08 h CEST and sunset between 20:08 and 20:44 h CEST. Light intensity during observations was not measured. A head torch (160 Lumens) and a hand-held counter were used to see and count the earthworms after sunset. Earthworms sometimes reacted to the bright light of the head torch, but they retracted in the soil only after 1–3 s (J.O.). As we show below, we never saw any surfacing earthworm during the day and therefore we correlated our measurements of intake rate by Ruff with nocturnal surface availability of visual counts performed after sunset (21:00 and 22:00 CEST). We used the Type 2 response model of Holling (1959) to describe the relationship in a biologically sensible way (Duijns *et al.* 2015).

In spring 2007, 46 male Ruff were caught and applied with 1.8 g radio transmitters (BD-2 transmitters, Holohil Systems Ltd. Carp, ON, Canada). This was part of a study determining departure dates on migration (Verkuil *et al.* 2010). Receiver stations were placed at nine roosts throughout the study area (for a map with the roosts locations, see Schmaltz *et al.* 2016). Data was collected between 25 March and 8 May 2007. As the transmitters had a detection range of about 500 m, the receiver stations could potentially also record nearby foraging birds. To be certain that birds on a roost were not foraging, we only used data of the four offshore roosts where Ruff cannot forage (see for locations the map in Verkuil *et al.* 2010, the used roosts in this paper are: Bocht fan Molkwar, Makkumer Noardwaard, Makkumer Súdwaard and Mokkebank). This selection decreased the number of radio-tagged birds to 19. For the whole time period, we calculated the hourly percentage of birds present on a roost from the total number of birds present per hour and the maximum number of birds that were observed at the roosts.

Sensory capacity: prey detection trials

Five male Ruff were caught in southwest Friesland by standard *wilsternetter* procedures (for description and routines, see (Rogers & Piersma 2005)). To prevent sexual interactions during the experiments, we selected only adult males. After capture, the birds were individually colour-ringed and transported to an indoor aviary of $2 \times 2.6 \times 4$ m (width, height, depth) at the Groningen Institute for Evolutionary Life Sciences in Groningen, the Netherlands, 100 km from the site of capture. To acclimate the birds to human presence and to reduce the effects of sudden human sounds, a radio station with human voices and music was broadcast continuously. As male Ruff become competitive in spring, wooden dividers were placed in the aviary so that the birds could avoid each other; still, they could move freely through the room and engage in social interactions. During the off-trial days Ruff were provided *ad libitum* with commercially obtained live mealworms (*Tenebrio molitor* larvae), earthworms (*Dendrobaena veneta* and *Eisenia fetida*), and fresh water.

The prey detection trials started when the birds seemed to have fully adjusted to captivity conditions, 2 weeks after capture. Experimental trials were carried out in the mornings. To motivate Ruff to feed during a trial, birds were deprived of food for 12 hours before the start of each trial. Fresh water remained available *ad libitum*. On an experimental day, all birds were caught simultaneously, kept in dark boxes, and randomly assigned a sequence number. Trials were carried out in the same aviary in which the Ruff were housed. Thereafter, Ruff were placed in a small cage (width = 0.8, height = 0.4, depth = 0.4 m), which was divided in two equally sized compartments using a wooden baffle. While the ground layer present in the left side was the same as in the cage (wooden chips) and did not contain prey items, the right side was covered with a shallow layer of 1 cm clean potting soil (ingredients: 70% peat, 20% compost, 10% of an unknown fertilizer) and contained 10 earthworms (length = 50 mm) which were placed in the compartment 10 min before a trial, enabling them to cover in soil and show more or less natural behaviour, but did not allow them to create burrows or casts that might help Ruff in finding them in the field. We chose to use a shallow depth of only one cm to be sure that the earthworms presented to the birds in every trial was more or less equal. Only *E. fetida* earthworms were used in the experiment, as *D. veneta* actively jumped upon being touched, a behaviour that could probably make them more available than the more timid earthworm species encountered in the field (J. Onrust unpubl. obs.). After each trial the soil was removed and the number of earthworms left over was scored. Each trial we started with a new set of earthworms.

During a trial, a bird was first placed in the left side of the cage under experimental light and noise condition. After a habituation period of 5 min we removed the wooden baffle. The bird was then able to feed for 15 min in the experimental compartment. However, full adaptation to darkness often takes about an hour in

most animals (Martin 1990, Dusenbery 1992). Therefore the visual sensitivity of the Ruff under dark conditions was probably not optimal in this experiment. However, the birds were kept 20 – 100 min in dark boxes prior to the trials.

A full factorial design with the two factors light and noise was designed to examine the effects of either visual cues or auditory cues (Table 3.1). In addition, in Treatment 1 all cues were available and in Treatment 6 both types of cues were absent. Treatments were repeated twice for each individual. Treatments were randomly assigned to the birds following the throw of a die. Visual cues were reduced by decreasing the amount of available light; Ruff were allowed to forage under light conditions ranging from 1000, 0.01 and 0 Lux, which correspond to daylight, twilight and complete darkness (Dusenbery 1992).

To exclude auditory cues, we followed Montgomerie & Weatherhead (1997) and Cunningham *et al.* (2010), and used white noise to mask any sounds made by earthworms moving in the soil. White noise was generated using two speakers (output 100–18 000 Hz) placed on either side of the compartment. The sound level used to generate the white noise was 61 dB. As Ruff did not always consumed every found prey, we recorded all trials on video (Sony Handycam HDR-SR12E with infrared function) with an extra infrared illuminator (wavelength 850 nm, range 30 m). The camera and illuminator did not create any visible light.

Videos were analysed in Windows Media Player (Windows 10). As we were primarily interested in whether Ruff were able to find an earthworm, we noted the number of worms found and eaten (denoted W_{f+e}). The results were analysed in R version 3.1.2 (R Development Core Team 2017) using Generalized Linear Mixed Models with each bird (BirdID) representing a random intercept. The response variable was W_{f+e} and the explanatory variables were light and noise levels, both categorical. To control for a learning effect between the first and second repetition, we also added repetition as a variable. The package “lsmeans” was used for a *post hoc* analysis (Lenth 2016).

Table 3.1: Overview of the different experimental treatments during tests to examine the visual and audial sensory modalities used by Ruff to find and capture earthworms.

Treatment	Background noise	Amount of light	Light level (lux)
1	Silence	Daylight	1000
2	White Noise	Daylight	1000
3	Silence	Twilight	0.01
4	White Noise	Twilight	0.01
5	Silence	Complete Darkness	0
6	White Noise	Complete Darkness	0

Results

Field observations

The intake rate of Ruff showed a slight increase around noon ($F_{2,97} = 3.58$, $R^2 = 0.069$, $P = 0.032$, $N = 100$; Fig. 3.1A). Surprisingly, during 28 h of 'carting', covering 0.21 ha of grassland, not a single surfacing earthworm was observed during day-time (Fig. 3.1B). Earthworms appeared on the surface only after sunset. However, when plotted per field, the average intake rate of foraging Ruff during the day was a function of the densities of surfacing earthworms measured in darkness (the average of transects at 21:00 + 22:00 h CEST), showing the positive but steadily flattening relationship typical of a Holling type 2 functional response (Fig. 3.2) (Holling 1959).

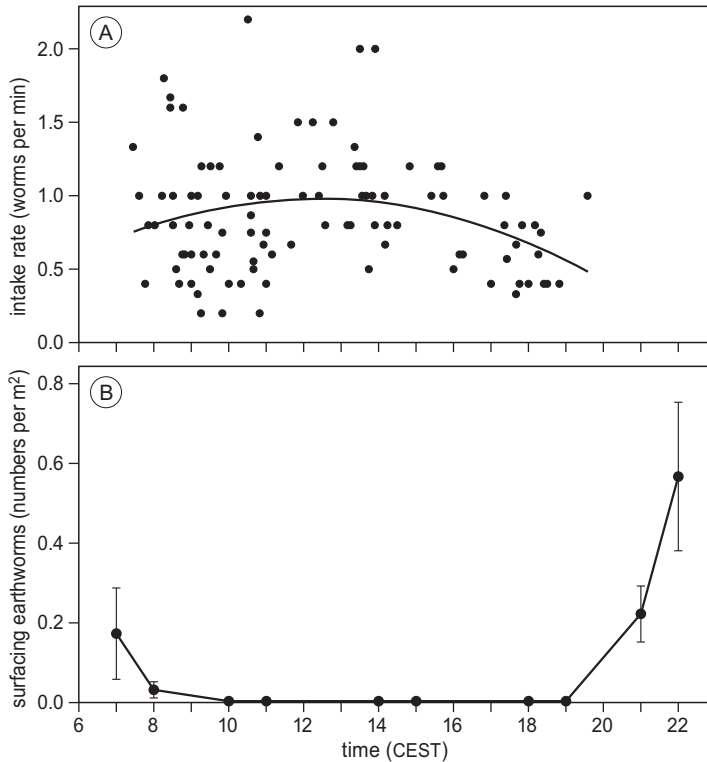


Figure 3.1: (A) Intake rate of Ruff feeding on earthworms is highest around noon and (B) earthworms only come to the surface during the night. Each point in (A) is an individual observation. Means and se of 12 different grasslands are shown in (B).

At any time of the night 90–100% of the 19 birds were present at the roost (Fig. 3.3). By 08:00 h more than 90% of the birds had left the roosts and by noon about 60% were back at the roost for a daytime rest ((Schmaltz *et al.* 2016); Fig. 3.3). Around 16:00–17:00 h, 80% of birds had left the roost again, but at twilight the majority had returned (Fig. 3.3).

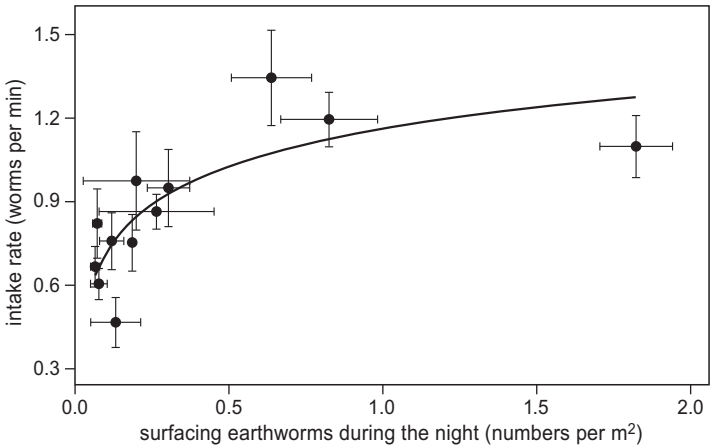


Figure 3.2: Intake rate on earthworms by Ruff during daytime shows a Holling type II functional response with the number of available earthworms during the night. Each point represents the average intake rate of 6–11 Ruff and the average number of earthworms counted in each of 12 fields. The equation for the fitted curve: intake rate = $1.1556 + 0.1903 * \ln(\text{earthworm availability})$, $R^2 = 0.619$, $P = 0.002$.

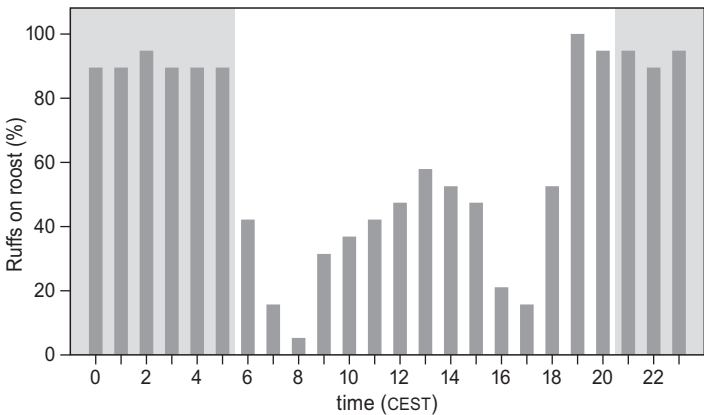


Figure 3.3: Ruff roost during the night and around noon. Each bar represents the hourly percentage of 19 Ruff that were present on four offshore roost in Lake IJsselmeer, Friesland, between 28 March and 8 May 2007. Shaded areas represent the night (20:30 – 5:30 h CEST).

Prey detection trials

The prey detection trials showed that prey intake under daylight was similar at the two noise levels, but in twilight and darkness, earthworms were found and eaten more in the absence of white noise (Fig. 3.4, Table 3.2). This indicates that Ruff use auditory cues to find earthworms in twilight and darkness. A *post hoc* analysis revealed, however, that only the darkness treatment with white noise was significantly different from the two daylight treatments, and twilight with white noise was

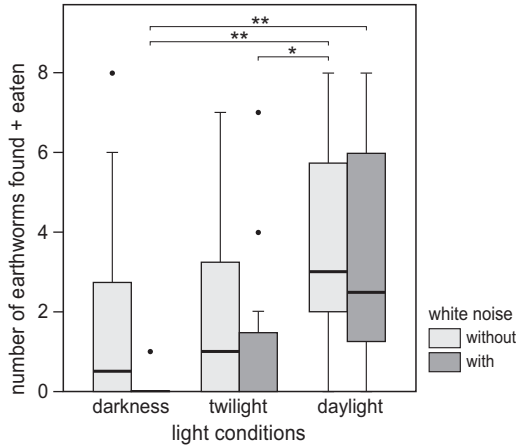


Figure 3.4: Results of the prey detection trials. Boxplots represents the data of five captive male Ruff under three light conditions (darkness, twilight and daylight which corresponds to 0, 0.01 and 1000 Lux, respectively) and with or without white noise. Per bird, all treatments were repeated twice. Significant differences between treatments are indicated with an asterisk (* = $P \leq 0.05$).

Table 3.2: Coefficient estimates β , standard errors SE (β), associated Wald's z-score ($=\beta/SE(\beta)$) and significance level p for all predictors in the analysis derived from a generalized linear mixed model (GLMM) with number of earthworm found + eaten as the response variable and light conditions and white noise (Y/N) as explanatory variables (fixed effects). Bird identity is fitted as a random effect. Reference level for white noise was 'no noise', for light levels it was darkness, and for the interaction terms it was no noise*darkness.

Predictor	Coef. β	SE (β)	Z-value	P-value
Intercept	-0.140	0.651	-0.214	0.830
Repetition	0.189	0.271	0.697	0.486
White Noise	-2.906	1.081	-2.689	0.007
Twilight	0.067	0.458	0.146	0.884
Daylight	0.903	0.428	2.109	0.035
White noise*Twilight	2.362	1.191	1.984	0.047
White noise*Daylight	2.702	1.144	2.361	0.018

significantly different from daylight without white noise (Fig. 3.4). As indicated by an absence of a difference between the first and second repetition of a treatment there was no significant effect of learning (Table 3.2).

Discussion

To explain how animals maximize their intake rate, we must consider how animals find their prey and sense the availability of prey in the field (MacArthur & Pianka 1966, Piersma 2011). We predicted that Ruff use visual cues to detect and catch earthworms in grasslands, but could switch to using audial cues at night when food availability is highest in terms of surfacing earthworms (Fig. 3.1B). However, Ruff still found earthworms during daytime when human observers could not (Fig. 3.1), and radio-tagged Ruff did not forage during the night (Fig. 3.3). This was unexpected, as we found the expected Holling type II functional response relationship between intake rate measured during daytime and earthworm availability measured at night (Fig. 3.2). This suggests that earthworms, of which some species surface during the night (Baldwin 1917), remain close to the surface during the day, so that nocturnal measurements of their surface abundance are closely correlated with their daytime availability. For example, Ruff can see parts of the earthworm, use other visual cues such as fresh earthworm casts, or indeed hear them move. Thus, the most accurate method for measuring earthworm availability for this species should indeed be based on the counting of visible earthworms but also on locating invisible earthworms based on the sound they produce.

The prey detection trials with five birds and two replicates per treatment indicated that Ruff can discover earthworms in twilight and even in total darkness, with the suggestion that white noise reduces performance. This indicates that Ruff find earthworms mainly on the basis of visual and auditory cues, but in principle could also modulate the use of these cues under different light conditions. Such switches between foraging strategies in the day and night have been described previously for several different shorebirds (Hulscher 1976, Robert & McNeil 1989). In the present case, it would be mostly a switch from visual feeding during the day to the tactile feeding at night, previously suggested by van Rhijn (1991), Barbosa (1995), Thomas *et al.* (2006). However, these studies were based on observations under field conditions, whereas we forced birds to forage in the absence or presence of cues that limited them to using either a visual or an auditory strategy. Even though our initial experimental setup was not designed to test whether Ruff use tactile cues, Ruff were not able to find worms when both visual and auditory cues were eliminated (treatment 6). This suggests that we successfully eliminated all the cues used by Ruff. Although the difference between white noise in darkness and no white noise is not

significant, if Ruff primarily use tactile cues to find prey, they should also have found earthworms in darkness when white noise was played (Fig. 3.4).

Over the last two decades the numbers of staging Ruff have declined considerably in the Netherlands (Jukema *et al.* 2001, Verkuil *et al.* 2010, Verkuil *et al.* 2012). Agricultural intensification has resulted in grasslands that are less attractive for feeding. Although earthworms can profit from higher manure input (Hansen & Engelstad 1999), earthworm availability for Ruff might have declined because of generally drier conditions (Ausden & Bolton 2012). To avoid the drought, earthworms in drained grasslands retreat deeper into the soil (Gerard 1967). Furthermore, tipulid larvae are also susceptible to desiccation and will avoid drained grasslands (Pritchard 1983, Carroll *et al.* 2011). This may provide part of the reason why Verhulst *et al.* (2007) found a positive relationship between groundwater level and meadow bird numbers and prey density. High groundwater levels also have a positive effect on the penetrability of the soil for a birds' bill, making it easier to catch earthworms (Green *et al.* 1990, Duckworth *et al.* 2010, Ausden & Bolton 2012).

In staging areas, food conditions need to be sufficient to allow migrants to gain the fuel stores for onward migration and breeding (Piersma & Baker 2000). Biometric data of Ruff that were caught as part of a long-term study monitoring the population of Ruff staging in southwest Friesland (Hooijmeijer 2007) indicated that the fuelling rates of male Ruff declined between 2001 and 2008 (Verkuil *et al.* 2012) and that birds may have had lower departure masses in recent years (L.E. Schmaltz, unpubl. data). Verkuil *et al.* (2012) argues that this is caused by a loss of moist grasslands. Indeed, the distribution in recent years of the remaining staging Ruff also hints at the importance of wet grasslands (Schmaltz *et al.* 2016).

According to McNeil *et al.* (1992), shorebirds forage at night to meet their daily energy requirements (i.e. supplementary hypothesis), or because food conditions at night are better and predation risk is lower (i.e. preference hypothesis). After sunset, food conditions for Ruff should be better as earthworms start to surface then (Fig. 3.1B). Ruff can still find earthworms in darkness probably by hearing. However, our data showed that Ruff are not nocturnally active and therefore rarely make use of auditory cues to exploit an abundant resource during the night (Fig. 3.4). During their migratory staging in southwest Friesland, Ruff, therefore, rarely if ever forage nocturnally. This implies that food conditions during the daytime feeding are sufficient.

In conclusion, a combination of field and experimental indoor observations on the relationships between Ruff and earthworms indicated that although we measured only surfacing earthworms during the night, Ruff predominantly fed during the day. We propose that they use indirect visual and auditory cues to detect earthworm that are already close to the surface.

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The hungry worm feeds the bird

Jeroen Onrust & Theunis Piersma

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*; Hounscome *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 × 350 m, B: 100 × 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.

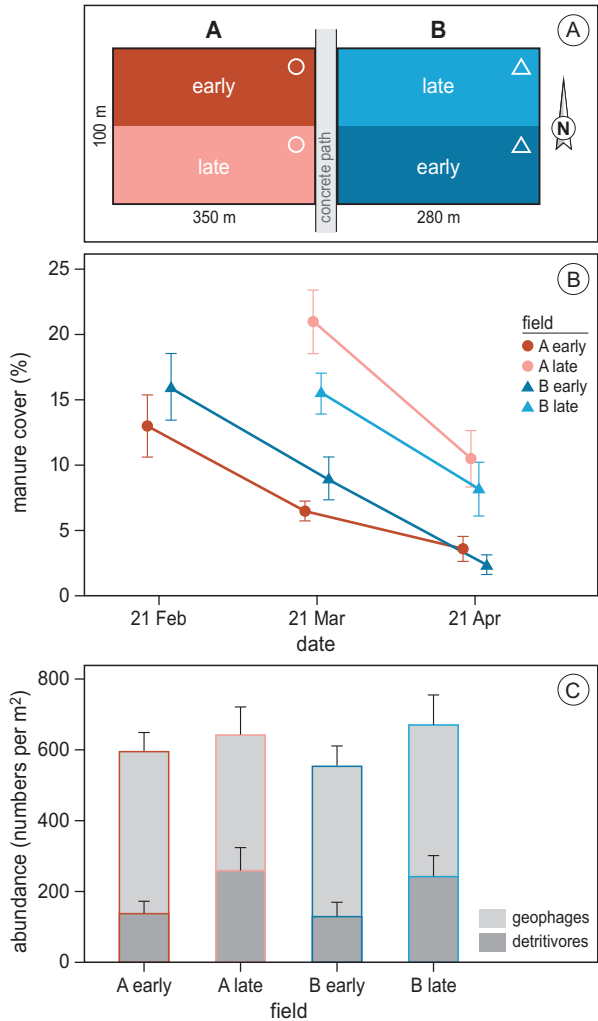


Figure 4.1: For this study two adjacent agricultural grasslands (A in red and B in blue) were split in a field with an early (dark colour) and a field with a late (light colour) farmyard manure application (A). Cover of farmyard manure on the surface was measured at three intervals during the fieldwork period in spring 2014 (B). Six hand-sorted 20 × 20 × 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 × 20 × 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

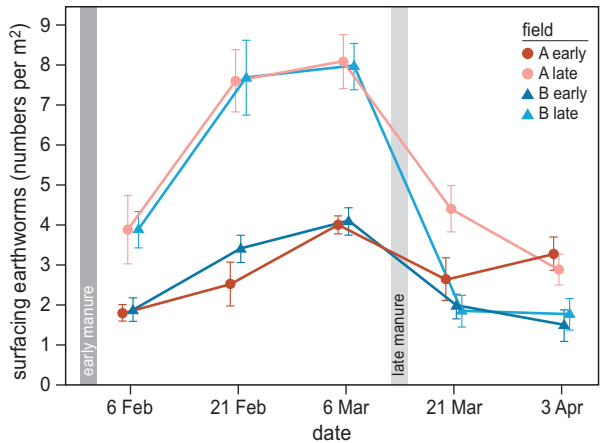


Figure 4.2: Number of surfacing earthworms in spring 2014 at two grasslands (A in red and B in blue) that were split in a field with an early (1-Feb, dark colour) and a field with a late (14-March, light colour) application with farmyard manure. Error bars represent SE. Application dates are indicated with a vertical grey bar.

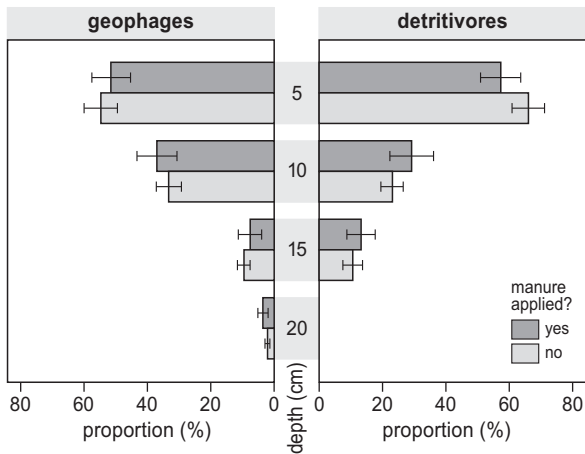


Figure 4.3: Vertical distribution of geophagous (left) and detritivorous (right) earthworms sampled 6 March 2014 in fields with (dark bars) and without (light bars) farmyard manure applied on the surface. Per field 14 soil samples were taken divided over two grasslands. Error bars represent SE.

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

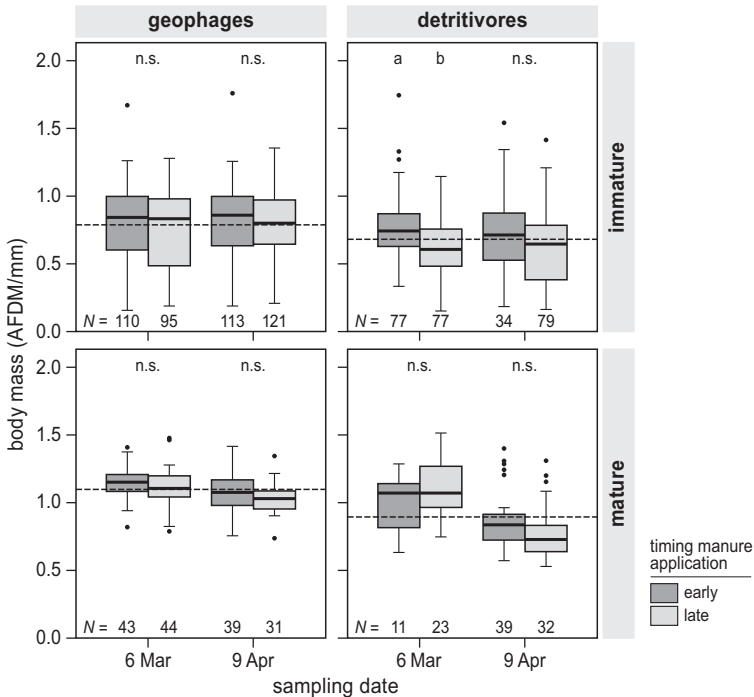


Figure 4.4: Body condition (expressed as ash-free dry mass (AFDM) mg per mm length) of geophagous (left) and detritivorous (right) earthworms and for different age classes (immature top panels, mature lower panels). Earthworms were collected on two sampling days in spring (6 March and 9 April 2014). 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 *Heamatopus 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.

Acknowledgments

This project owes a lot to the wisdom and foresight of farmer Murk Nijdam, who helped with the experimental design and allowed us to perform the experiment on his land. This work is part of a small research programme on dairy farmland ecology financed by the Province of Fryslân (University Campus Fryslân support through the Waddenacademie), with additional financial support from the University of Groningen.

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 prevents 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):

$$FL = 1.150 LL \ (R^2 = 0.98, P < 0.001, N = 349).$$

$$FW = 1.362 LW \ (R^2 = 0.99, P < 0.001, N = 372).$$

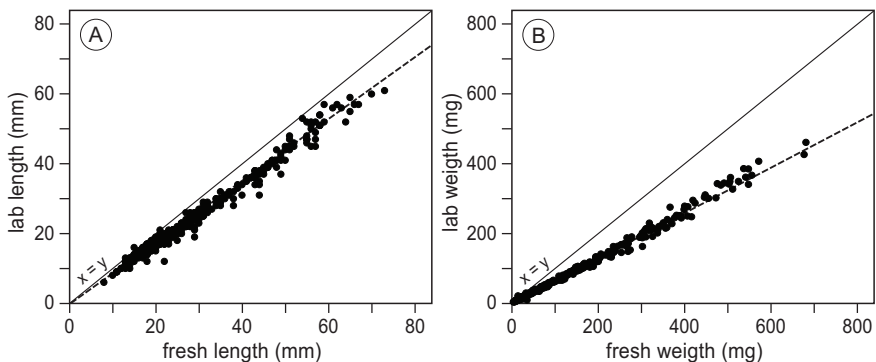


Figure B.1: Earthworm length (A) and weight (B) after preserved in alcohol plotted against length or weight just after killing. Each data point represents one individual earthworm.

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).

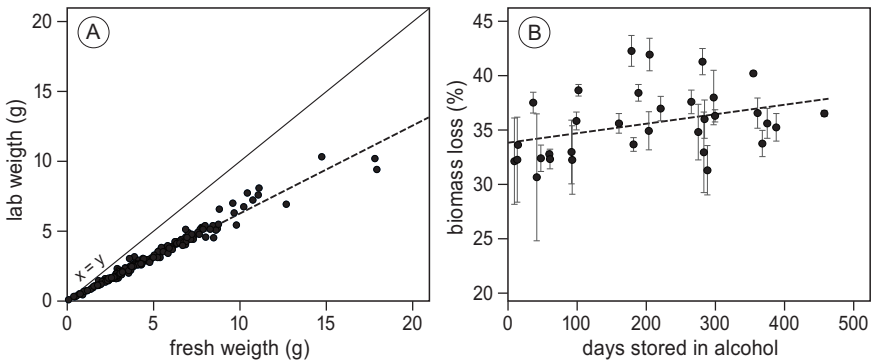


Figure B.2: A.Total earthworm mass from soil samples after preserved in alcohol (lab weight, LW, grams) plotted against total earthworm mass just after collecting (fresh weight, FW, grams). B. Biomass loss of earthworms from soil samples preserved in alcohol over time. Error bars denotes SE.



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

Jeroen Onrust & Theunis Piersma

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 ratios 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 NH₃ 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.

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

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:

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

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.

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: $F_{2,60} = 1619$, $P < 0.0001$).

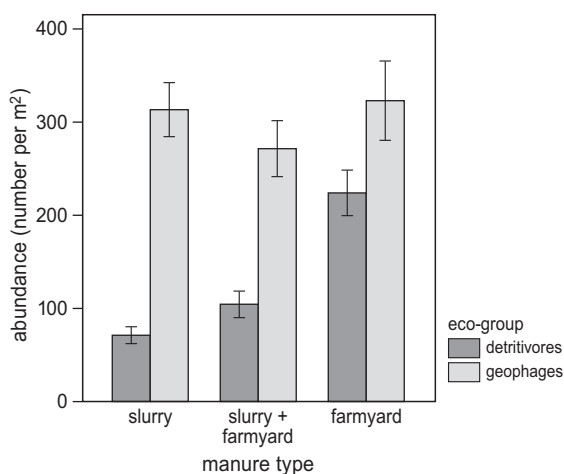


Figure 5.1: Total abundances of detritivorous and geophagous earthworms in agricultural grasslands that are fertilized with either slurry manure ($N = 22$), slurry and farmyard manure ($N = 12$) or farmyard manure ($N = 11$). Per grasslands 3–6 soil samples were taken.

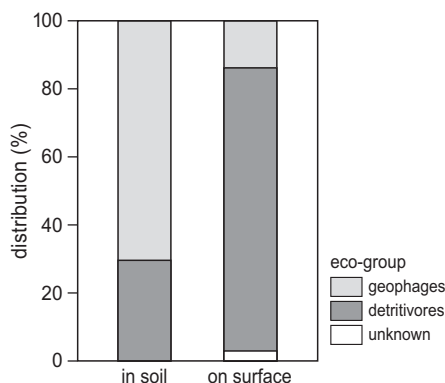


Figure 5.2: Distribution of detritivorous and geophagous earthworms in the soil (left bar, $N = 1535$) and on the soil surface during the night (right bar, $N = 2887$) in 18 agricultural grasslands. All grasslands were fertilized with slurry manure only. Soil distribution was determined by taking 6 soil samples per grassland. Surface distribution was determined by counting surfacing earthworms on two transects of 25 meter per grassland during the night.

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 ($\text{IGR} = 0.037 \text{ d}^{-1}$, Fig. 5.3) compared with farmyard manure ($\text{IGR} = 0.022 \text{ d}^{-1}$) and litter ($\text{IGR} = 0.022 \text{ d}^{-1}$). Detritivores, in contrast, grew faster on farmyard manure ($\text{IGR} = 0.040 \text{ d}^{-1}$) than on slurry manure ($\text{IGR} = 0.025 \text{ d}^{-1}$) and litter ($\text{IGR} = 0.021 \text{ d}^{-1}$), 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: $\chi^2(1) = 69.07$, $P < 0.0001$) did not differ between ecotypes (LME: $\chi^2(1) = 3.303$, $P = 0.069$, Fig. 5.3) and also not between diets (LME: $\chi^2(1) = 1.828$, $P = 0.401$).

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

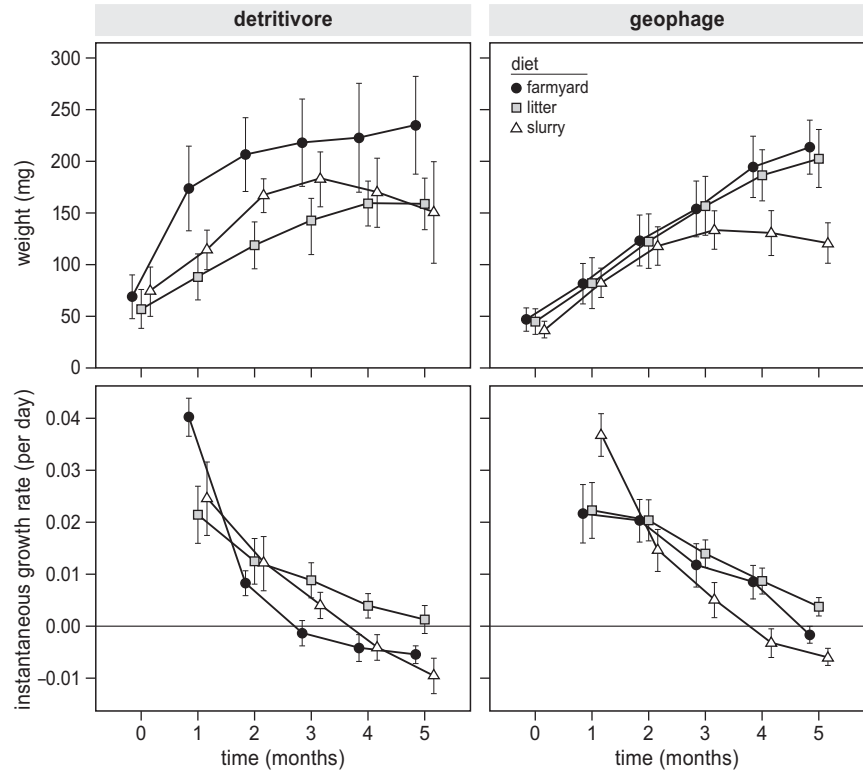


Figure 5.3: Growth curves of hatchling detritivorous earthworms (*Lumbricus rubellus*, left panels) and geophagous earthworms (*Aporrectodea caliginosa*, right panels) cultured individually on farmyard manure, slurry manure or litter for 5 months. Sample sizes are shown in Table 5.1.

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,

2017). 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 seems 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 (Zwarts 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 coarse 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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Intensive agricultural use of grasslands restricts earthworm activity and their availability for meadow birds through topsoil drought

Jeroen Onrust, Eddy Wymenga, Theunis Piersma & Han Olff

Abstract

All meadow birds of the wet agricultural grasslands in north-west Europe are declining throughout the last decades. Earthworms are an important prey for these species, and although the intensive grassland management with high manure inputs so characteristic of today's dairy farming generally promotes overall earthworm abundances, it may reduce the effective food availability for meadow birds through drying out the topsoil, causing earthworms to remain deeper in the soil.

We studied the responses of both detritivore (*Lumbricus rubellus*) and geophage (*Aporrectodea caliginosa*) earthworm species to soil moisture profiles in the field and under experimental conditions. During spring 2015, surfacing earthworms were counted in eight intensively managed grasslands with different groundwater tables in southwest Friesland, The Netherlands. At each count, soil penetration resistance, soil moisture tension and groundwater level were measured in these fields, while air temperature and humidity were obtained from a weather station 15 km away. The response to variation in the vertical distribution of soil moisture was experimentally studied in a detritivore and geophage earthworm species.

In the field, surfacing activity at night of earthworms was negatively associated with soil moisture tension and positively by relative air humidity. Surprisingly, there was no effect of groundwater level, an important management variable in meadow bird conservation. Under experimental conditions, both the detritivores and the geophages moved to deeper soil layers (>20 cm) in drier soil moisture treatments, avoiding the upper layer when its moisture level dropped below 30%.

We find that current intensive grassland management in dairy farming mainly reduces earthworm availability for meadow birds through topsoil desiccation. This can be counteracted by keeping soil moisture tensions of the top soil above -15 kPa. We suggest that the mechanical manure injection practices are a key factor in explaining increased topsoil desiccation, thus decreasing earthworm availability. Meadow bird conservation populations thus requires changes in manure application methods that promote earthworm activity near and at the soil surface.

Introduction

In northwest Europe, agricultural intensification has caused breeding populations of meadow birds to decline during the last decades at alarming rates (Donald *et al.* 2001, Stoate *et al.* 2009, Vickery & Arlettaz 2012). Despite considerable conservation attention and efforts, the declines are still continuing (Kleijn *et al.* 2004, Donald *et al.* 2006, Kentie *et al.* 2016), indicating that main drivers of this decline have been insufficiently identified. Changes in food conditions have received little attention and when this was the case, have not been studied with an eye on the importance of prey availability (Zwarts & Wanink 1993, Piersma 2012) rather than total abundance (Ausden *et al.* 2001, McCracken & Tallowin 2004, Leito *et al.* 2014).

Most meadow bird species depend on earthworms as their main food source (Beintema *et al.* 1995). The currently high manure input in dairy farmland could promote overall earthworm abundances (Hansen & Engelstad 1999, Atkinson *et al.* 2005, Curry *et al.* 2008), which might explain why this factor has been little investigated. However, food availability for meadow birds is not only determined by the total abundance of earthworms in the soil, but also by their vertical distribution in the soil profile and their activity on the surface. Tactile hunting meadow birds can only capture earthworms within reach of their bill in the upper soil layer (e.g. for Black-tailed Godwits *Limosa limosa*; (Duijns *et al.* 2015)), or when they can be seen at the surface for visually hunting meadow birds (e.g. for Ruffs *Philomachus pugnax*; (Onrust *et al.* 2017)). Under desiccating conditions, earthworms might retreat deeper into the soil and stop their surfacing behaviour, which will negatively affect the food availability for meadow birds.

We suggest that topsoil humidity (an associated agricultural management) is an important determinant of the availability of earthworms for meadow birds. Despite their name, and although common in many terrestrial habitats around the world, earthworms are evolutionary and functionally closely related to the oligochaete worms living in freshwater environments (Edwards & Bohlen 1996, Turner 2000). Their respiration and the maintenance of their hydrostatic pressure necessitate moist living conditions (Edwards & Bohlen 1996, Turner 2000). Previous work suggests that earthworm growth and activity depend strongly on the moisture content of the soil (Presley *et al.* 1996, Berry & Jordan 2001, Wever *et al.* 2001, Perreault & Whalen 2006). As their skin does not have the ability to prevent dehydration in dry conditions, lack of water is hazardous (Laverack 1963). To overcome desiccation, earthworms spend most of their time belowground. Under humid and not too cold conditions, the majority of earthworms are found near or at the soil surface (thus being available to meadow birds), while they migrate to lower depths at lower temperatures and when the topsoil is too dry (Gerard 1967, Rundgren 1975, Jiménez & Decaëns 2000). These vertical movements likely reflect a constant balancing

between access to food on the surface, and the risk of desiccation and freezing.

The capacity to cope with drier topsoil conditions likely differs between earthworm species belonging to different ecological groups (Roots 1956, El-Duweini & Ghabbour 1968). Generally, geophagous, substrate-eating, earthworms are more tolerant to desiccation than detritivorous, litter-eating, earthworms (Ernst *et al.* 2009, Eggleton *et al.* 2009). Geophages have a thicker skin than detritivores and go into diapause by curling into a small knotted ball in the soil and form a protective coating of secreted mucus (El-Duweini & Ghabbour 1968, Edwards & Bohlen 1996). Detritivores regularly surface at night to scavenge for food which is pulled into their burrows (Baldwin 1917, Butt *et al.* 2003). These earthworms are therefore also likely to be more sensitive to the aboveground microclimate. Although little is known about the conditions under which earthworms come to the surface, there are observations that earthworms avoid dry surface conditions (Parker & Parshley 1911); high numbers of surfacing earthworms are usually counted during or after rainfall (Darwin 1881, MacDonald 1980). This suggests that precipitation and relative air humidity near the soil surface are important.

Regions in northwest Europe that are important for meadow birds often have a history as wetland (i.e. riverine floodplains, marshes) that became drained and cultivated into dairy farmland. In The Netherlands, these agricultural grasslands are amongst the most intensively managed in the world in order to maximize the transformation of grass into dairy products (Bos *et al.* 2013). This led to two major changes in agricultural practices: (i) the lowering of water tables through landscape-level drainage measures, promoting longer growing seasons and higher grassland productivity through less water logging, and (ii) increased nutrient supply to grasslands, including the recent practice of manure injection. Although these grasslands have high densities of earthworms (Edwards & Lofty 1982, Muldowney *et al.* 2003), it may be expected that their activity and availability for meadow birds is reduced by the damage to soil structure and soil desiccation created by intensive agricultural practices.

In this study we investigated the influence of soil water conditions in intensively used grasslands on the behaviour of detritivorous and geophagous earthworms and their resulting surface availability for meadow birds. In the field, we measured earthworm surface activity and correlated this with soil water conditions and the moisture of the air. Under controlled conditions we compared the vertical distribution of detritivores and geophages under different soil moisture conditions. This helps us understand how hydrological conditions influence the surface activity and vertical movements of earthworms and hence food availability for meadow birds, and can thus inform farmers and conservation managers about measures that promote food availability for the meadow birds in the wet pastures of north-west Europe.

Methods

Study site and observations in the field

The field study was conducted in a 10 km² area of dairy farming bordered by the villages of Oudega, Gaastmeer and Heeg in south-west Friesland, The Netherlands (N 52°58'48, E 5°33'12). From 1990 until 2010, this area was subject to land 'rationalisation' schemes which included drainage improvements and rearrangement and readjustment of grasslands to create highly productive ryegrass (*Lolium* sp.) monocultures. We selected eight of these grasslands with similar management and history/age, but differences in groundwater level (ranging from 10 to 90 cm below surface level). All grasslands had a peaty soil covered with a layer of clay (<40 cm).

The intensive management practices of these grasslands are intended to harvest grass multiple times per year. Fertilization includes injection of manure, for which slots are cut (typically 3–5 cm deep and 15–25 cm apart) and filled with slurry manure (about 20 m³ per ha). In The Netherlands this type of fertilizing became compulsory in 1994 and is allowed from 16 February until 1 September and occurs 5–6 times a year. All grasslands were manured this way 2–4 weeks before the fieldwork started and mowing occurred 1–2 weeks afterwards. The observation period took place from mid-March to late April 2015, coinciding with the transition period in which the amount of evaporation becomes higher than the amount of precipitation in The Netherlands (Colenbrander *et al.* 1989, Jacobs *et al.* 2007). As March and April generally are the months with the lowest rainfall of the year (Colenbrander *et al.* 1989), we expected desiccating conditions during fieldwork.

In each field, earthworms were counted along two transects of 25 m and all measurements took place on the same day. Measurements were repeated five times per field. Prior to the observations (from 9–13 March 2015), earthworm abundance at each transect was determined by taking three soil samples of 20 × 20 × 20 cm which were cut in slices of 5 cm depth. Each slice was sorted by hand and number and species were determined. Earthworm activity was measured after sunset by counting surfacing earthworms (see Onrust *et al.* submitted for a detailed description). To measure groundwater level in cm below surface level during the moment of observation, a 100 cm deep and 5 cm wide 'well' was made in the middle of each transect.

Even at the same soil moisture content, soils can have different soil moisture tensions due to differences in physical properties such as texture, structure, pore size and organic matter content (Collis-George 1959). Above a critical moisture tension, the soil will extract water from the body of earthworms causing first their diapause and then their mortality (Holmstrup 2001). Soil moisture tension is thus a direct measure of what matters to earthworms, and probably a main determinant of their behaviour (Doubé & Styan 1996). Using a Quick draw tensiometer (Eijkelkamp,

Giesbeek, 14.04.05.01) soil moisture tension of the soil was determined at three points on the transect. The tensiometer measures the suction pressure of the soil in KiloPascals (-kPa, negative as tension is a negative pressure).

Tactile hunting birds should be able to probe in the soil, therefore soil resistance to penetration was measured along the transect at every five meters using a penetrometer (Eijkelkamp, Giesbeek, 06.01.SA). The instrument measures the force in Newton per cm² that is required to push a probe through the soil at a constant velocity to a depth of 10 cm. All variables were measured on the same day and repeated weekly. Hourly meteorological data were obtained from a weather station 15 km from the study area. We used air temperature in Celsius degrees at 10 cm above surface level and relative air humidity (%) measured during the times the earthworm surfacing observations were made.

Laboratory experiment

To study the vertical distribution of detritivores and geophages under different soil moisture contents, we kept earthworms of both ecological groups for 24 days in 10 cm diameter PVC tubes with a length of 30 cm. The tubes were split lengthwise, to allow us to open the tubes at the end of the experiment without this disturbance causing the earthworms to redistribute. The two parts of the tube parts were held together by tie wraps; the lower opening was closed with a lid.

To each tube, 25 cm of sieved clay soil and 16–18 earthworms were added on the surface. There were no plants growing in the top of the tubes and the soil contained no root structures. In 18 tubes we enclosed a geophagous species (*Aporrectodea caliginosa*) and in 18 tubes a detritivorous species (*Lumbricus rubellus*). Prior to being added to the tubes, total earthworm fresh weight per tube was determined by rinsing the earthworms with tap water, carefully blotting them with absorbable paper and weighing them to the nearest 0.001 g. Both the earthworms and the soils were collected from the agricultural grasslands in south-west Friesland where we also carried out the field observations.

The tubes were placed in climate chambers with a constant temperature of 12 °C, air humidity of 80% and light regime of 12/12 h. The tubes were randomly assigned to either one of three treatments; wet, moist and dry. We used 12 tubes per treatment, divided over the species. The tubes of the wet treatment every day received the amount of water that was equal to the evaporation in the chamber, which was 11 mm per day. The moist treatment received half of the evaporation, and the dry treatment received no water during the 24 day experiment. The earthworms were not fed. Surfacing earthworms were not scored in the laboratory experiment.

When the tubes were opened, the soil column was immediately cut in 5 slices of 5 cm depth and the total number and fresh weight of the earthworms per slice was determined. Earthworm survival per tube was determined by calculating the

proportion of earthworms that were still alive at the end of the experiment from the number at the beginning of the experiment. Furthermore, the average weight per earthworm in each tube was calculated by dividing the total fresh weight by the total number of earthworms. The soil moisture content of every slice was determined by oven-drying a weighted amount of soil at 70 °C for 48 h after it was weighed again. The relative change in weight was used as soil moisture content.

Data analyses

Data was analysed in R version 3.1.2 (R Development Core Team 2017) using generalized linear mixed modelling (GLMM) by using the package “lme4” with the glmer function and family=poisson (Bates *et al.* 2015). As the number of earthworms at the end of the experiment differed between the tubes, we used the proportion of earthworms for every depth. A binomial GLMM was built to analyse the data of the lab experiment. The response variable was entered as a matrix where the first column is the number of earthworms found (“successes”) and the second column is the number of earthworms not found (“failures”). Species, treatment and depth were added as fixed effects in the model with an interaction between treatment and depth as we expected earthworms to go deeper in dry soils, but move to the surface in wet soils. Furthermore, a random intercept term was added with depth nested in tube ID. To analyse the survival data, the same procedure was followed, but with species and treatment as the only fixed effects.

A GLMM was also used to analyse the field data. To account for differences between fields and transects, we added them as a random intercept in the model in which transect was nested in field. In order to control for a temporal effect between the repeated observations, we added observation day as a variable and as a random slope. The response variable was the number of surfacing earthworms per transect and the explanatory variables were soil moisture tension, observation day, earthworm abundance, air temperature at 10 cm above surface level during observations and air humidity during observations. We started the statistical analysis with a full model including all fixed effects. We controlled for over-dispersion by adding an observation level random factor (X). Furthermore, the explanatory variables were rescaled. 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 & Keough 2005). We checked the normality of the residuals by visual inspecting the QQ plots (Miller 1986).

Results

Surface presence activity of earthworms in the field

As the majority of earthworms in the field were found in the top 5 cm of the soil and no earthworms were found between 15 and 20 cm depth (Fig. 6.1), the studied grasslands apparently were moist enough at the beginning of the field study. There was no differences in the vertical distribution between detritivores and geophages (Fig. 6.1). During the fieldwork period of six weeks, fields became drier with ground-water levels declining from 10 – 85 cm (min – max) below surface level at the beginning to 42 – 90 cm below surface level at the end. Soil moisture tension increased from -12.1 kPa (SD = -7.0) to -45.5 kPa (SD = -14.5) and soil resistance increased from 94.3 N/cm² (SD = 34.28) to 218.8 N/cm² (SD = 41.44).

The surfacing activity of earthworms was best explained by soil moisture tension as well as aboveground (air humidity). Low soil moisture tension and high air humidity during the observations increased the number of surfacing earthworms at night (Fig. 6.2 and Table 6.1). Air temperature at 10 cm above soil surface level ranged from $0.7 - 7.6$ °C. Temperature during observations, observation day and earthworm abundance did not explain the number of surfacing earthworms (Table 6.1). We found that more than 80% of the surfacing earthworms were counted on soils with a moisture tension value of less than -15 kPa.

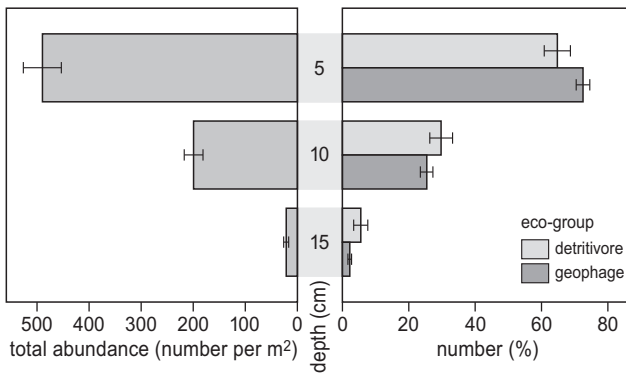


Figure 6.1: In March, the majority of earthworms in the field was found in the top 5 cm of the soil (left panel). Proportionally there was no difference in the vertical distribution between detritivorous and geophagous earthworm species (right panel). Data is collected on 8 grasslands in south-west Friesland from 9 – 13 March 2015. Per grassland, 3 soil samples at two transects of 25 m were taken.

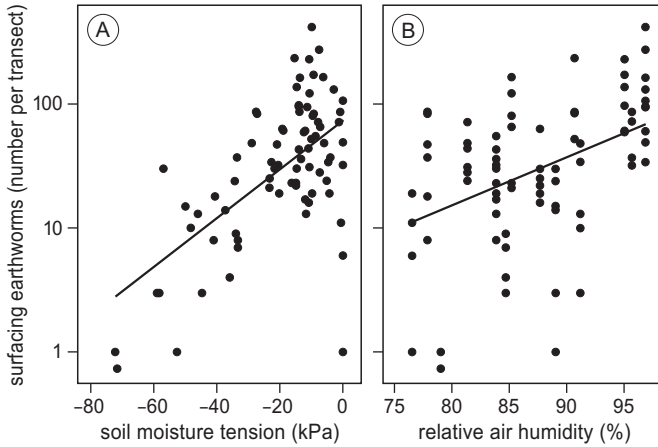


Figure 6.2: A. Low soil moisture tensions increases the number of surfacing earthworms at night ($F_{1,78} = 52.04$, $R^2 = 0.400$, $P < 0.0001$). B. High air humidity during observations increases the number of surfacing earthworms ($F_{1,78} = 20.52$, $R^2 = 0.208$, $P < 0.0001$). Note: the number of surfacing earthworms is plotted on a log-scale. Surfacing earthworms were counted on 8 grasslands and repeated five times in spring 2015.

Laboratory experiment

In all three treatments, soil moisture content increased with depth (Fig. 6.3). However, the soils in the wet treatment at every depth were always wetter than the soils in the drier treatments. In the wet treatment most earthworms were found in the upper layers, while the earthworms retreated to greater depths in the drier treatments (Fig. 3.3 and Table 3.2). Surprisingly, but consistent with the similar depth profiles in the field (Fig. 3.1), there were no differences in the depth response between the two ecological types of earthworm. In both species/types, earthworms mostly selected the soil layers with a soil moisture content of around 30%, irrespective of the moisture treatment (Fig. 3.4). At the end of the experiment, the survival of geophages was significantly higher than that of detritivores (93% and 75% respectively, Fig. 3.5A). Furthermore, whereas the geophages increased in weight, the detritivores lost weight in all treatments (Fig. 3.5B).

Table 6.1: Coefficient estimates β , standard errors SE (β), associated Wald's z-score ($=\beta/\text{SE}(\beta)$) and significance level P for all predictors in the analysis derived from a Generalized Linear Mixed Model (GLMM) with number of surfacing earthworms at night as the response variable and soil moisture tension and air humidity during the observations as explanatory variables (fixed effects). Transect nested in field are the random effects and observation day is added as random slope. An observation level random factor (X) was added to the model to correct for over-dispersion.

Full model: AIC = 741.0				
Fixed effects	Coef. β	SE (β)	z-value	P-value
(Intercept)	3.400	0.157	21.647	<2e-16 ***
Soil moisture tension	-0.847	0.158	-5.356	8.50e-08 ***
Air humidity	0.450	0.078	5.767	8.08e-09 ***
Temperature	0.111	0.097	1.155	0.248
Observation day	0.138	0.151	0.919	0.358
Abundance	0.226	0.143	1.573	0.116
Random effects	Variance	Std.Dev.	Cor	
X	0.399	0.632		
transect : field	0.012	0.111		
observation day	0.001	0.024	-1.00	
Field	0.144	0.379		
observation day	0.038	0.195	0.63	
Full model: AIC = 751.8				
Fixed effects	Coef. β	SE (β)	z-value	P-value
(Intercept)	3.330	0.193	17.235	<2e-16 ***
Soil moisture tension	-0.814	0.119	-6.862	6.77e-12 ***
Relative air humidity	0.448	0.079	5.694	1.24e-08 ***
Random effects	Variance	Std.Dev.	Cor	
transect : field	3.104e-05	0.006		
observation day	2.982e-06	0.002	0.89	
Field	2.346e-01	0.484		
observation day	8.073e-02	0.284	0.45	
Full model: worms ~ moist + U.o + T10.o + time + abundance + (1 X) + (time field/transect)				
AIC = 752.5, BIC = 783.4, logLik = -363.2, deviance = 726.5, DF residuals = 67				
MAM: worms ~ moist + U.o + time (1 X) + (time field/transect)				
AIC = 751.8, BIC = 775.6, logLik = -365.9 deviance = 731.8, DF residuals = 70				

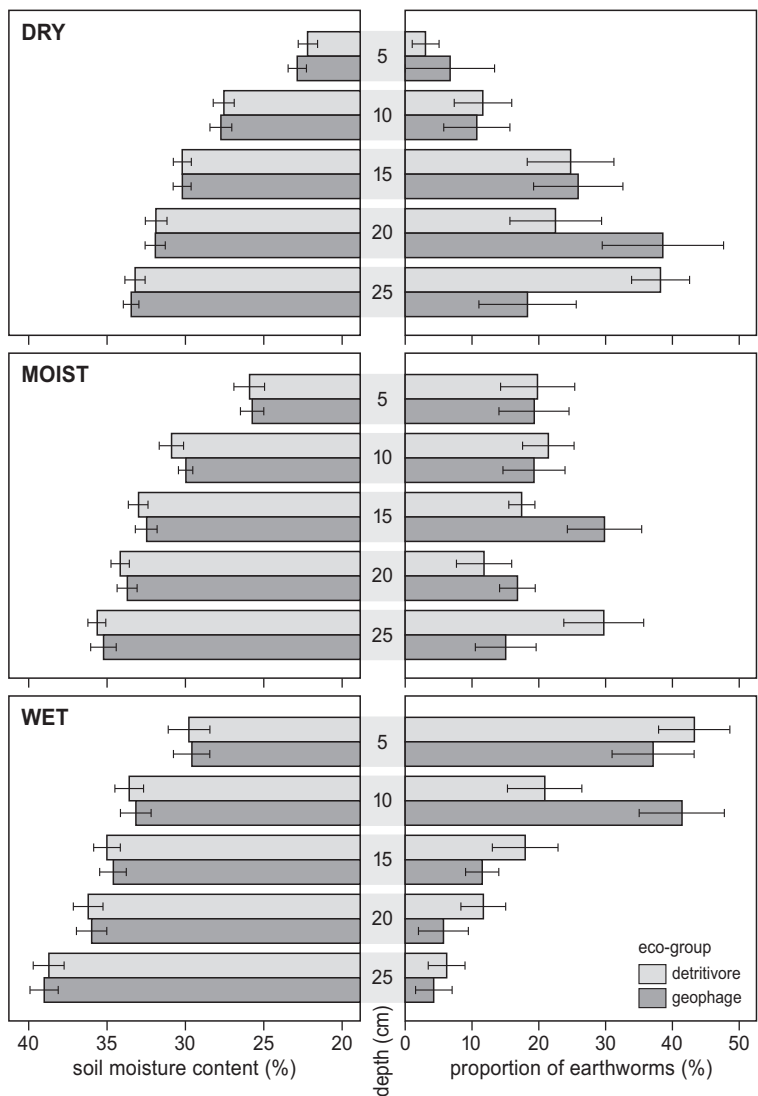


Figure 6.3: Under experimental conditions earthworms move deeper in dry conditions ($F_{4,40} = 9.235$, $R^2 = 0.43$, $P = <0.001$) and remain in the top soil in wet conditions ($F_{4,40} = 29.2$, $R^2 = 0.72$, $P = <0.001$). In the medium treatment earthworms are evenly distributed over the soil column ($F_{4,40} = 1.477$, $R^2 = 0.04$, $P = 0.227$). There was no significant difference between detritivores (*Lumbricus rubellus*) and geophages (*Aporrectodea caliginosa*).

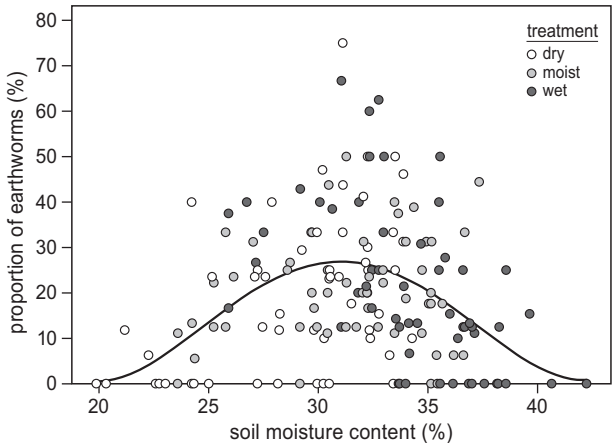


Figure 6.4: The majority of all earthworms were found in soil with a moisture content between 30–34%. A quartic polynomial is plotted through the points ($F_{4,175} = 11.14$, $R^2 = 0.185$, $P = <0.001$). Per species, 18 tubes divided over three treatments were used, each tube contained 16–18 earthworms.

Table 6.2: Coefficient estimates β , standard errors SE (β), associated Wald’s z-score ($=\beta/\text{SE}(\beta)$) and significance level P for all predictors in the analysis derived from a generalized linear mixed model (GLMM) with proportion of earthworms at different depths as the response variable and treatment (dry, medium, wet) and depth as explanatory variables (fixed effects). Depth is nested in tube ID and are added as random effects. Reference level for treatment is dry and for the interaction it is dry:depth.

Fixed effects	Predictor	Coef. β	SE (β)	z-value	P-value
(Intercept)		-2.755	0.277	-9.961	< 2e-16 ***
Treatment:	medium	1.473	0.351	4.191	2.78e-05 ***
	wet	3.008	0.353	8.519	2.78e-05 ***
Depth		0.421	0.074	5.686	1.30e-08 ***
Interaction:	medium depth	-0.456	0.099	-4.594	4.34e-06 ***
	wet depth	-1.041	0.111	-9.339	< 2e-16 ***
Random effects	Predictor	Variance	Std.Dev.		
	depth : tube ID	0.000	0.000		
	tube ID	0.000	0.000		

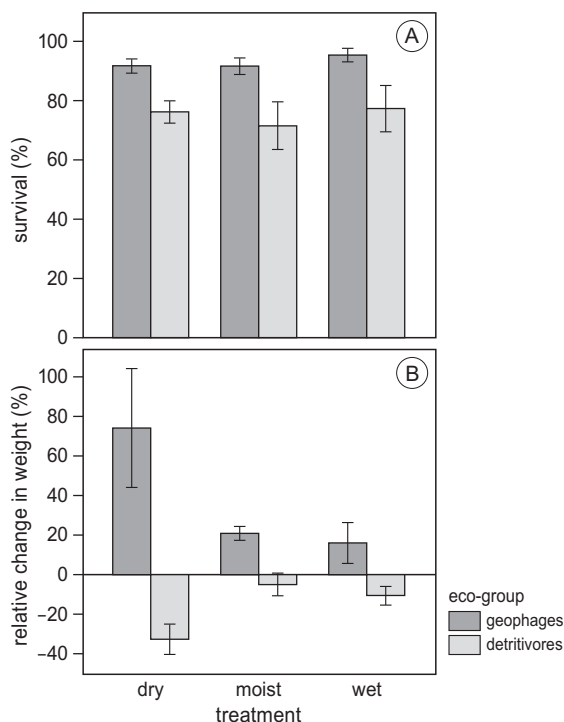


Figure 6.5: A. The survival of detritivorous earthworms (*Lumbricus rubellus*) was lower than geophagous earthworms (*Aporrectodea caliginosa*), irrespective of treatment. B. The average weight per earthworm decreased for detritivores, but increased for geophages. Per species, 18 tubes divided over three treatments were used, each tube contained 16–18 earthworms.

Discussion

The strong observed positive effect of soil moisture on earthworm vertical distribution and surface activity establishes a firm link between meadow bird food availability and the meadow-level hydrology. This conclusion is in line with other studies that find a clear impact of soil moisture on earthworms in the soil, (Evans & Guild 1947, Gerard 1967, Nordström 1975, Baker *et al.* 1992), but the new aspect in our study is the direct link to earthworm activity at the soil surface and thus to meadow bird food availability. Desiccation (either by lower groundwater tables or by topsoil desiccation through manure injection) will thus impair the food availability for breeding meadow birds as well as staging birds, like Golden Plover *Pluvialis aprinaria* and wintering Lapwing *Vanellus vanellus*. Although probing meadow birds might still catch earthworms in diapause, hardening of the soil prevents this (Green

1988, Smart *et al.* 2006, Duckworth *et al.* 2010). Struwe-Juhl (1995) observed that Black-tailed Godwits do no longer probe the soil when the soil resistance exceeds the limit of 125 N/cm². Soil moisture is thus the driving factor behind food availability for meadow bird.

The degree of desiccation of a soil is determined in part by the capillary rise from the groundwater level. As water in the soil will rise to the height where the gravity and the matric potential are in balance, higher groundwater levels generally result in higher capillary rise (Bos *et al.* 2008), but this depends also on the hydrological properties of the locations. As all studied grasslands desiccated, the capillary rise was probably not strong enough to maintain a moist topsoil and thus surfacing earthworms.

Also grasslands with a high groundwater level (less than 25 cm below surface level) desiccated as quickly as the other studied grasslands. An explanation for this shallowly desiccation may be found in the type of management in the studied grasslands. The process of slit injection early in the season, disturbs the topsoil and could therefore enhance the desiccation of the topsoil later in the season. In addition, by cutting through the soil, aggregates and fungal hyphae, which are both beneficial for the water binding capacity of a soil, are broken and therefore the drainage of water from the soil will increase (Beare *et al.* 1997, Franzluebbers 2002, Pulleman *et al.* 2003, Bronick & Lal 2005, Bittman *et al.* 2005). Ploughing and reseedling of these grasslands every 5–10 years will further decline the fungal biomass and aggregates stability, and therefore reduce the hydrological properties of the soil (de Vries *et al.* 2007, van Eekeren *et al.* 2008, Abid & Lal 2009, de Vries *et al.* 2012).

The timing of raising the groundwater table may have affected the seasonal drying of the soils too. In The Netherlands, ditchwater levels are usually kept higher in summer than in winter. The switch from winter to summer level occurs mostly at 1 April, after the farmers have manured their land. However, in April evaporation starts to become larger than precipitation, causing the top-layer of the soils starting to desiccate (Colenbrander *et al.* 1989, Jacobs *et al.* 2007). Raising the water level in that period, especially on clay soils, probably does not have the desired effect of increasing soil moisture in the topsoil as the topsoil is already starting to desiccate (Armstrong 1993).

Not only soil structure, but also earthworms themselves could alter the soil moisture, with contrasting effects between ecological groups. Ernst *et al.* (2009) showed that *A. caliginosa* and *Lumbricus terrestris* enhance the drying of the topsoil by intensive burrowing, whereas *L. rubellus* enhance the storage of soil moisture in the topsoil by incorporating more organic carbon into the soil. This fact explains why fungal biomass in soil decreases with geophages, but increases with detritivores (McLean & Parkinson 2000, Butenschoen *et al.* 2007). Under dry conditions, *A. caliginosa* even increases its burrowing activity by exploring a larger volume of soil

(Perreault & Whalen 2006). Numbers of detritivores can reduce sharply by drought events (Eggleton *et al.* 2009). Furthermore, this group of earthworms seems to be affected more by slurry injection than other groups (De Goede *et al.* 2003, van Eekeren *et al.* 2009). The impact is strongest under wet conditions, as they are then higher in the topsoil and therefore more exposed to the injection device and/or manure (van Vliet & de Goede 2006).

Although geophages are thus more drought tolerant than detritivores (El-Duweini & Ghabbour 1968) and are therefore likely to show a slower response to drying soils, we did not find a difference in the vertical distribution between the detritivorous *L. rubellus* and the geophagous *A. caliginosa* in the field (Fig. 6.1), nor in the experiment (Fig. 6.3). However, the survival of *L. rubellus* was significantly lower than *A. caliginosa* in the experiment (Fig. 6.5A). As this effect was equal between the treatments, it is not the soil moisture content of the soil in this experiment that determined the survival. It is likely that food availability during the experiment caused this pattern. *A. caliginosa* feeds on soil particles, whereas *L. rubellus* requires organic material, which was not present in the experimental tubes (Bouché 1977, Curry & Schmidt 2007). This is supported by the observation that *L. rubellus* lost weight in all treatments, whereas *A. caliginosa* increased in weight (Fig. 6.5B). Daniel *et al.* (1996) showed that *L. terrestris*, a detritivore, loses weight when kept in containers with equal soil moisture content, but without food. Earthworms can also lose considerable weight by excreting large amounts of body water in response to drought (Grant 1955, Roots 1956, Kretzschmar & Bruchou 1991). As the weight response of the earthworms in our experiment was not correlated with treatment and as the geophages even increased in weight, the soils in all treatments were probably not dry enough to cause weight loss due to low soil moisture content.

Although being a freshwater oligochaete, soils fully saturated with water are avoided by earthworms (Fig. 6.3 + 6.4) (Darwin 1881, Roots 1956, Laverack 1963). In our experiment, both species moved to soil with a moisture content of about 30–34 % (Fig. 6.4). Grant (1955) performed a similar experiment and found for *A. caliginosa* a soil moisture preference of 20–30% in sandy loam soil. Also for another geophagous species, *A. tuberculata*, the optimum soil moisture for growth was 25% (Wever *et al.* 2001). Berry and Jordan (2001) found that *L. terrestris* grows optimally with a soil moisture of 30% for silty clay loam soil, but still grows in soil with a 20% soil moisture content when food was *ad libitum* available. Although most species in grasslands can survive up to 17 to 50 weeks submerged in water (Roots 1956, Ausden *et al.* 2001, Zorn *et al.* 2005), their survival depends on the oxygen level of the water and the ability to withstand prolonged starvation (Roots 1956, Turner 2000). Also in the field, earthworms vacate flooded soils, especially when the water is warm and contains decaying organic material resulting in low oxygen values (Zorn *et al.* 2005, Plum & Filser 2005).

Agricultural intensification is always associated with strong declines of meadow bird numbers (Vickery *et al.* 2001, Groen *et al.* 2012). Protection measures often involve maintaining high groundwater levels or create other wet features in the grassland (Armstrong 2000, Ausden *et al.* 2001, Kleijn & van Zuijlen 2004, Smart *et al.* 2006, Groen *et al.* 2012). As a result, grass growth is retarded and this not only creates a better sward for bird locomotion, but is also likely to promote earthworm availability (McCracken & Tallowin 2004, Atkinson *et al.* 2005). Indeed, Verhulst *et al.* (2007) found a positive relationship between groundwater table, prey density

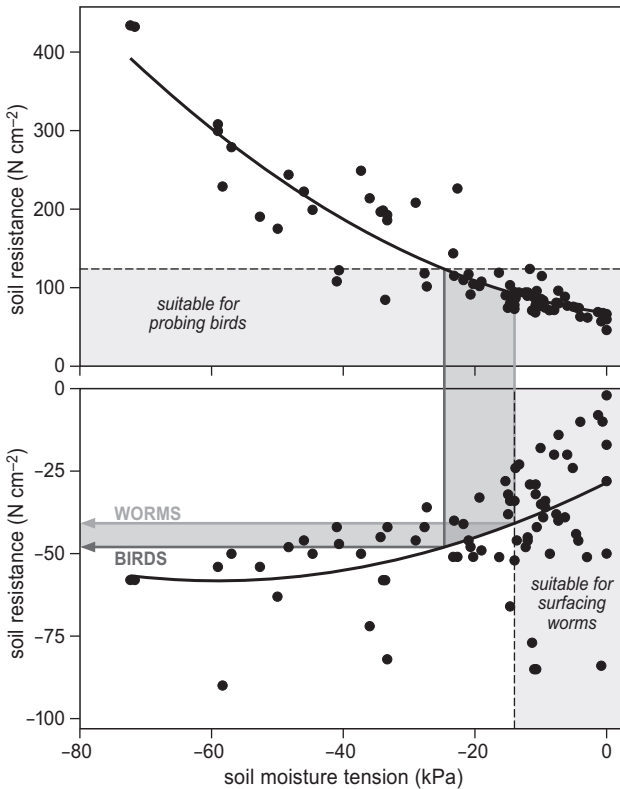


Figure 6.6: A soil should have a maximum soil resistance of 125 N cm^{-2} (dashed line in upper box) to allow meadow birds to probe in the soil. Furthermore, the soil moisture tension should not be higher than -15 kPa as surfacing earthworms rapidly decline above this values (dashed line in lower box). As soil resistance and groundwater table are strongly correlated with soil moisture tension (for soil resistance: $F_{3,76} = 25.87$, $R^2 = 0.505$, $P < 0.0001$, for groundwater level: $F_{2,77} = 13.91$, $R^2 = 0.265$, $P < 0.0001$), we plotted the maximum groundwater level that is required to allow meadow birds to probe in the soil (dark grey line) and earthworms to surface (light grey line). As soil moisture tension values are soil type specific, these values are specific for our studied grasslands (a clay-on-peat area in southwest Friesland).

and meadow bird numbers. As a soil should not exceed a soil resistance of 125 N/cm^2 to allow tactile hunters to probe in the soil (Struwe-Juhl 1995), and the soil moisture tension should not be higher than -15 kPa as surfacing earthworms rapidly decline above this values (Fig. 6.2), we calculated the maximum groundwater level that is required to maintain these functions.

Groundwater levels should not exceed -42 cm to maintain surfacing earthworms, and should not be lower than -46 cm to maintain a soil that is suitable for probing (Fig. 6.6). It should be noted that soil moisture tension values are soil type specific (Collis-George 1959), these values therefore only corresponds to peat grasslands with a layer of clay in our study area. Raising groundwater levels generally occurs by manipulating the ditchwater level, but in peat soils with a damaged soil structure this groundwater level will not be effectively raised (Armstrong 2000), or at least not result in a higher soil humidity comparable to the capillary rise in undisturbed soils.

The intensively managed and drained dairy grasslands in The Netherlands impair the important role of earthworms by promoting dry soil conditions during the growing season. If earthworms are not active, they do not take part in the grassland food web, and perform their work as ‘ecosystem engineers’ (Lavelle 1988, Blouin *et al.* 2013). Maintaining moist soil conditions will therefore not only promote biodiversity (Milsom *et al.* 2002, Atkinson *et al.* 2004), but could also lead to more sustainable agricultural systems for the positive effects of earthworms (van Groenigen *et al.* 2014, Erisman *et al.* 2016).

Acknowledgements

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Chapter 7

SYNTHESIS

Ecological consequences of conventional dairy farming

Jeroen Onrust

Throughout this thesis, I have explored how dairy farm practices (**earth**) influence earthworms (**worms**) and their availability for predators such as meadow birds (**birds**). The underlying question was whether agricultural intensification affected earthworms in the same way as it did other organisms. Several previous studies (Edwards & Lofty 1982b, Muldowney *et al.* 2003, Atkinson *et al.* 2005, Curry *et al.* 2008) had suggested that today's current dairy farming systems, rather than having negative impacts on earthworm populations had positive effects.

As populations of meadow birds, for which earthworms are a staple food, strongly declined throughout Western Europe (Busche 1994, Donald *et al.* 2001, Vickery *et al.* 2001, Donald *et al.* 2006, Kentie *et al.* 2016), the possibility remained open that agricultural intensification negatively affected earthworm *availability* for these predators. We indeed found this to be the case. In chapters 4, 5 and 6 we show that the impact of agricultural intensification is ecogroup-specific, with the surfacing detritivores being negatively affected, and the subsurface-living geophages not being affected (Postma-Blaauw *et al.* 2006, van Eekeren *et al.* 2008). Because of their surfacing behaviour, detritivores are of special importance for higher trophic levels as they then can be caught by visually hunting predators, e.g. Lapwings *Vanellus vanellus* (chapters 2 and 3).

Now, having the chance for extended synthesis, I would like to move a step further. Earthworms are not only prey for predators, but also provide crucial ecosystem services (Lavelle 1997, Lavelle *et al.* 2006). In this thesis we showed the negative effect of intensive agriculture on detritivorous earthworms, but did this also affect the important role of earthworms in the dairy farmland ecosystem?

Darwin (1881) already noticed the positive effect of earthworms on plant growth and later many studies have indeed showed this (Curry & Boyle 1987, Lavelle 1997, Scheu 2003, van Groenigen *et al.* 2014). Earthworms improve the structure and the aeration of the soil and increase decomposition rates by incorporating litter into the soil, ingesting and fragmenting it and by excreting nutrient-rich faeces, this all provides favourable conditions for microbial activity and eventually accelerates the release and uptake of nutrients for plants (Edwards & Fletcher 1988, Lavelle 1997). It is the detritivores, not the geophages, which perform this latter function (Postma-Blaauw *et al.* 2006) and thus play a crucial role in rotating the dairy farmland ecosystem wheel faster (Fig. 7.1). So, although total earthworm densities may increase under intensification, their positive role in the dairy farmland ecosystem might diminish as detritivores decline.

To study the effect of detritivore earthworms in differently managed grasslands, we collected data during two studies in 2013 and in 2015. In both studies, intact sods were collected in the field and placed in a greenhouse. This allowed us to measure ecological relevant responses, and by placing them under controlled conditions and adding or excluding earthworms and or manures, we studied the effect of

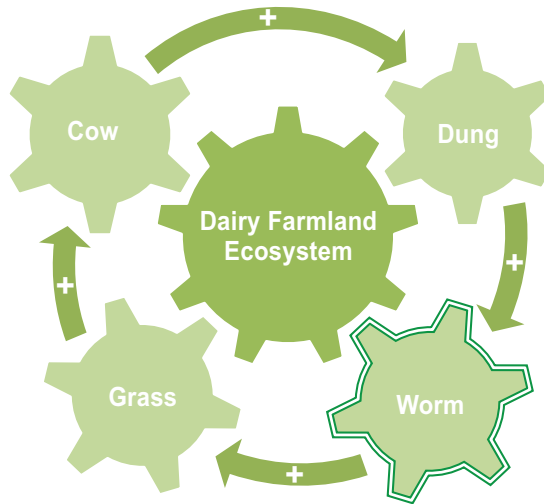


Figure 7.1: Graphical illustration of how dairy farmland ecosystem is driven by the interaction between earthworms that process cattle dung into nutrients for grass which is grazed by cows which then produce dung again. Earthworms (especially detritivores) play a crucial role in rotating this wheel faster as they accelerate the step between dung and grass.

earthworms on sward productivity. In 2013 we examined, with the help of master student Siwen Tang, whether earthworms from one site can accelerate the production faster as they are adapted to their own system, the so called home-field advantage (Rashid *et al.* 2013). We compared sods from the extensively managed dairy farm of Murk Nijdam in Wommels, Fryslân with sods from a naturally grazed grassland in the Oostvaardersplassen, Flevoland. Native earthworms and dung were exchanged with earthworms and dung from the other site and productivity of the sward was measured.

We chose to use sods from the Oostvaardersplassen as it is one of the few natural grasslands in The Netherlands that is grazed throughout the year. Furthermore, it is an ecological interesting area as this very young area (it is located in a polder that was reclaimed from a freshwater lake in 1968) and is home to a population of 4555 (2016 count) freely roaming large herbivores (Heck Cattle *Bos taurus*, Konik Horses *Equus ferus caballus* and Red Deer *Cervus elaphus*) in an area of less than 1600 ha of grassland (Cornelissen 2017). Compared with conventional dairy farming, the number of large grazers per hectares is 1.5 times higher and for organic dairy farming it is even 3 times higher (Fig. 7.2). Furthermore, large flocks of 1000s to 10,000s of geese (Greylag Goose *Anser anser*, Barnacle Goose *Branta leucopsis* and White-fronted Goose *Anser albifrons*) also use this area for grazing. The primary productivity of this grassland must be high to support such high numbers of herbi-

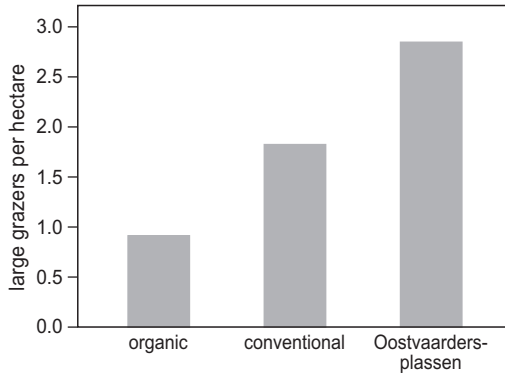


Figure 7.2: Grazing pressure in terms of number of large grazers per hectare for organic and conventional dairy farming in The Netherlands and for the Oostvaardersplassen. Grazing pressure for the dairy farms is calculated by dividing the total number of cows with the total area of grassland from data obtained from CBS.

vores without any supplementary feeding or intervention. Earthworms might thus play a crucial role in this system which can give valuable insights for dairy farming systems.

The productivity of an ecosystem is highly variable and is defined by multiple factors, both biotic and abiotic (Bardgett 2005). Previous studies already showed significant differences on soil productivity depending on the land historical use (Olf *et al.* 1994). The Oostvaardersplassen is much younger than the centuries old marine clay landscape where the farm of Murk Nijdam is situated. As this might influence the results of this experiment, in 2015 with the help of two master students, Eduard Mas and Aaron te Winkel, we conducted a similar experiment as in 2013, but now with sods only from Flevoland close to the Oostvaardersplassen. This time, the sward production with different earthworm and/or dung treatments of the Oostvaardersplassen was compared with grasslands from the biodynamic dairy farm ‘Zonnehoeve’ in Zeewolde and from the conventional intensive used dairy farm of Jeroen van Maanen next to the Zonnehoeve. In this experiment only native earthworms were used.

For this synthesis, we only selected from both experiments those sods that had native earthworms and or dung and sods that received neither earthworms nor dung (control), in total thus four treatments. Unfortunately, we ended up with only three sods per treatment per location, which is too low to find significant differences, but might give interesting patterns.

Methodological intermezzo

Study sites

Two datasets from 2013 and 2015 are used. In 2013 we compared a natural grassland with an extensive managed dairy farm. The natural grassland is located in the Oostvaardersplassen nature reserve (N52°25'11; E5°21'5). In this area there is a part with open water and reed beds (3600 ha) and a drained, dry area, consisting mainly of short-grazed grassland (1600 ha). It was originally designated for industrial and agricultural use before the surprise emergence of several endangered breeding birds in the wettest part, after it was decided that it became a nature reserve. Since then three populations of large grazers were introduced to the area: 35 Heck Cattle in 1983, 27 Konik Horses in 1984 and 54 Red Deer in 1992–93. The number of herbivores are not controlled by culling, no supplementary feeding is given during winter and no management intervention is implemented to maintain vegetation. This management resulted in an enormous increase of large grazers. In 2016 180 Heck Cattle, 975 Konik Horses and 3400 Red Deer were counted. The area of short grazed grassland also increased at the expense of shrubs and trees (Cornelissen *et al.* 2014). The grassland is dominated by *Lolium perenne*, *Poa trivialis* and *Trifolium repens*.

The other grassland in 2013 was located at Murk Nijdam's dairy farm in Wommels, Fryslân (N53°5'30; E5°33'51). This grassland is fertilized once a year at the end of March by spreading farmyard manure on the surface. Mowing occurs in June, after which grazing occurs until October/November. The grassland has a diverse plant community including *Agrostis stolonifera*, *Alopecurus geniculatus*, *Cardamine pratensis*, *Lolium perenne*, *Poa trivialis*, *Ranunculus repens*, *Rumex acetosa* and *Taraxum officinale*.

In 2015, the same grassland in the Oostvaardersplassen was compared to a biodynamic and a conventional dairy farm close to the Oostvaardersplassen. The biodynamic grassland was located 13 km south of the Oostvaardersplassen (N52°18'22; E5°22'55) at the mixed-farming biodynamic company 'De Zonnehoeve'. The fields used for this experiment are part of a crop-rotation regime, with cereals and legumes alternated with grass-clover every three years. At the time of our sampling, the grasslands were two years old. The dominant species were *Lolium perenne*, *Trifolium repens* and *Trifolium pratense*. From spring to autumn, dairy cattle grazed on the grasslands and fertilized the fields. No other form of fertilizer was used, and neither were antibiotics used to cure sick cows. The conventional grassland was located 1.3 km to the south of the biodynamic grassland (N52°17'38; E5°22'44) and is part of the intensive dairy farm of Jeroen van Maanen. This grassland was also part of a rotation regime with tulips and during sampling this field was two years old. This grassland was a monoculture of *Lolium perenne* which are

not grazed by cattle, but is mown 4–5 times a year, from which the harvest is fed to the cows in the stable. The grasslands are fertilized with slurry manure and artificial fertilizer in February and after each mowing.

We also measured soil organic matter content as it is an important factor in the nutrient cycling and the distribution of earthworms (Riley *et al.* 2008, Crittenden *et al.* 2015). We randomly took 15 soil samples of approximately 5 g from upper 10 cm of the soil per location. We also collected 1 sample per site from 10 – 20 cm depth. These soil samples were first mixed thoroughly before 1 g was oven-dried at 60 °C for 24 h. After drying, the samples were weighed again to calculate soil moisture content as a percentage of weight loss and then burned in a muffle furnace at 440 °C for 4 hours. The cooled samples were weighed again and the percentage organic matter was calculated based on the weight difference. These measurements were only performed in 2015.

The sods were collected by using a corer with a diameter of 19 cm that was pushed slowly into the soil to a depth of 10 cm (Photo 7.1A). Then the corer with the sod was carefully excavated and the sod was placed inside a fitting PVC ring within a square plastic basin (Photo 7.1B). All sods were collected on two days in October 2013 and over 5 days in October 2015.

Greenhouse experiment

The sods were grown in a greenhouse at the University of Groningen. The temperature was kept at approximately 20 °C and water was given daily. Before the treatments were applied to the sods, all earthworms inside the sods were removed. We did this by first watering the sods and then sending electrical pulses through it for 10 minutes. Earthworms were chased out of the sod and could be collected easily. In this way, the sod remained intact and did not had to be destructed, which would influence the microbial community in the sod. After all sods were treated with electricity, we clipped the vegetation to 3 cm height and applied earthworms or dung according the treatment schedule. In 2013, the worm treatments received 10 *Lumbricus rubellus* earthworms (total biomass on average 4.36 grams) at the start of the experiment and another 5 (total biomass on average 2.18 grams) 40 days later to replace any escaped earthworms. In 2015, the worm treatments received only at the beginning 13–16 (approximately 3 grams) *Lumbricus rubellus* earthworms. We chose to use only *Lumbricus rubellus*, as it is a detritivore and therefore feeds on organic material which is pulled into the soil. Furthermore, this species was found on every location. Each worm-sod received earthworms from its own location to avoid detrimental effects of changing habitat type.

In 2015 the application of dung was standardized by applying PokonTM organic dried dung pellets to all dung treatments. These pellets are made of a mixture of chicken and cattle dung and without any other additives. Before applying to the

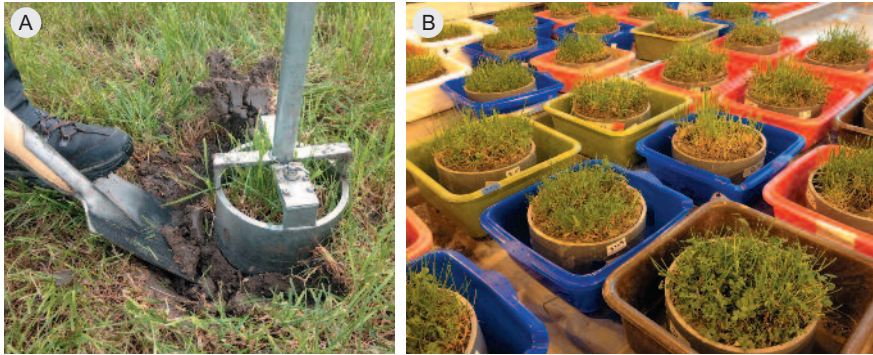


Photo 7.1: (A) Taking intact sod samples with a corer. (B) SodS in the greenhouse. Using intact sods is based on a study by Olff & Pegtel (1994) that investigated nutrient limitation in different grasslands.

sods, the pellets were processed by moistening it with water to create slurry and to allow microbial growth. This slurry was left in the greenhouse and kept moist for a week before 15 g was applied to the dung-sods. The dung and worm-sods received the same amounts of earthworms and dung as the worm and dung treatments. The control received nothing.

After a habituation period of one month, the experiment in 2013 started on 12 December and lasted 13 weeks until 13 March 2014. The sods were clipped at 13 January and 13 February. Frequent clipping was needed to maintain growth. In 2015, the experiment started on 2 November and lasted 10 weeks until 11 January 2016. The sods were clipped twice at 26 November and 21 December. During a clipping event, all sods were clipped at 3 cm height and the harvest was dried in a stove at 70 °C for 24 hours after it was weighed to the nearest 0.001 gram. The growth rate was determined by dividing the total dry weight of a sod by the number of days since the previous clipping.

Statistics

The two datasets were analysed separately. For both years, the data of the last clipping was used and analysed with a Two-way ANOVA in R (R Development Core Team 2017). Sward production was entered as the response variable, with an interaction between location and treatment as explanatory variables. The logarithm of sward production was used for the 2015 dataset. 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 & Keough 2005). Soil moisture and soil organic matter data were analysed with a General Linear Model using a quasi-binomial family structure as proportions were used.

Results & discussion of these exploratory experiments

In the comparison between the natural grassland of the Oostvaardersplassen and the extensively managed dairy farmland of Murk Nijdam in 2013, there was no significant effect of treatment on biomass production (Two-way ANOVA; $F_{3,19} = 2.828$, $P = 0.067$), but production was almost twice as high in the natural sods than in the dairy farmland sods (Two-way ANOVA; $F_{1,22} = 20.982$, $P < 0.001$). The interaction between location and treatment was not significant (Two-way ANOVA; $F_{3,16} = 2.112$, $P = 0.139$) and there were also no statistical differences between treatments within locations (Fig. 7.3). The treatment earthworms plus dung increased the production with 70% for the extensive dairy farm sods and 40% for the natural sods.

In 2015, the conventional intensive dairy farm sods showed the lowest biomass production (Two-way ANOVA; $F_{2,30} = 4.124$, $P = 0.026$). Treatment had an overall

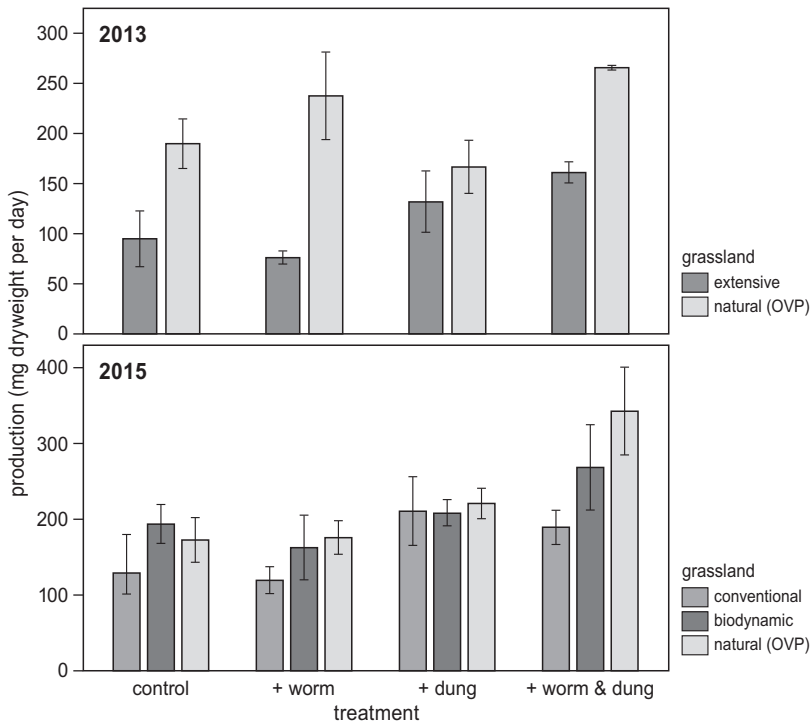


Figure 7.3: Biomass production in milligram dry weight per day of grassland sods and receiving either only earthworms, only dung, dung and earthworms, or nothing (control). In 2013 extensive corresponds to the extensively managed dairy farm of Murk Nijdam in Wommels, Fryslân and natural is the Oostvaardersplassen, Flevoland. In 2015, all locations were in Flevoland. Each bar represents the average growth rate of three sods with error bars representing SE.

significant effect (Two-way ANOVA; $F_{3,30} = 6.310$, $P = 0.002$), with highest production in sods with earthworms and dung. The interaction between location and treatment was not significant (Two-way ANOVA; $F_{6,24} = 0.467$, $P = 0.826$) and there were also no statistical differences between treatments within locations (Fig. 7.3). Nevertheless, production in the earthworms plus dung treatment was 47%, 39% and 98% higher for conventional, biodynamic and natural sods respectively.

Compared to the two grasslands used for dairy farming, the Oostvaardersplassen grassland had a threefold higher soil organic matter content in the upper 10 cm of the soil compared to the two dairy farm grasslands (GLM; $F_{2,42} = 597.08$, $P < 0.001$, Fig. 7.4A). This effect disappeared at lower soil layers (GLM; $F_{2,6} = 1.601$, $P = 0.277$, Fig. 7.4A). The high soil organic matter content is also reflected in 150% higher soil moisture content in the Oostvaardersplassen (GLM; $F_{2,42} = 169.64$, $P < 0.001$, Fig. 7.4B).

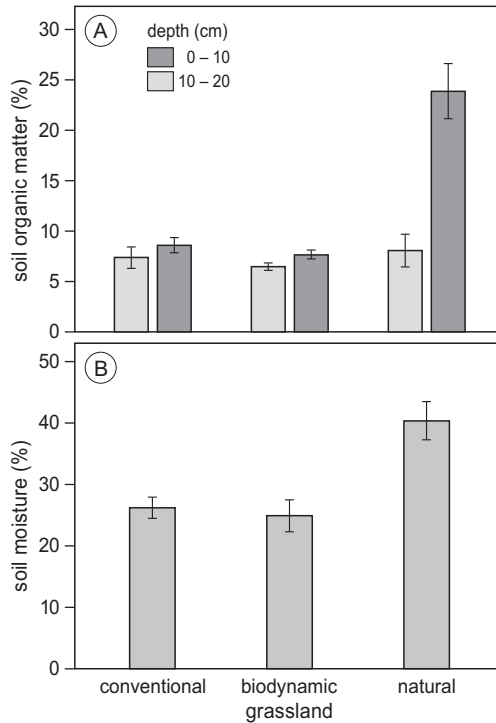


Figure 7.4: (A) Soil organic matter content of the Flevoland grasslands in 2015. (B) Percentage of moisture in the top 10 cm of the soil. 15 samples were taken at 0–10 cm depth and 3 at 10–20 cm depth. Error bars represent SD.

The method of collecting and using intact sods showed that the functioning of a grassland can be measured experimentally while maintaining ecological relevant functions. With only 3 repeats per treatment the statistical power of the test was rather low, but even with such low sample sizes, the experiment in 2015 showed a significant overall effect of treatment. Furthermore, in both years, there was a clear trend of increasing production in sods with earthworms and dung. However, the big disadvantage of this method is that it is unknown how many earthworms were in the sod. The best method of extracting earthworms from the sods without destructing it and affecting other organisms, is the use of electricity. However, it was uncertain how successful this method would be, as probably not all electrically paralysed earthworms would be able to crawl out the sod (Coja *et al.* 2008, Pelosi *et al.* 2009). When in 2013 the sods were hand-sorted after the experiment, earthworms were still found in most of the sods, including the non-earthworm treatments. Although these were mainly geophagous *A. caliginosa*, it still could have blurred the pattern. To eliminate this in the clearly necessary, and clearly promising, future studies, sods could be collected in periods when earthworm activity is low, i.e. when they have migrated to deeper soil layers during drought or frost.

The difference in biomass production between the natural grassland of the Oostvaardersplassen and the two dairy grasslands in 2015 was striking. This difference is likely to be a reflection of the soil organic matter and soil moisture values in the top 10 cm of the soil which are for the Oostvaardersplassen extremely high. This 'peat-on-clay' soil type is formed by high input of organic material and low soil disturbance. The area is now grassland, but was first dominated by reed and shrubs of mainly Black Elderberry *Sambucus nigra* (Cornelissen *et al.* 2014), which resulted in high input of litter. No earthworms occurred in the freshly reclaimed soil of Flevoland, and after a few years litter accumulated at the surface and formed a thick slowly decomposing layer (Hoogerkamp *et al.* 1983). Soon after earthworms were introduced, soil fertility improved as litter was incorporated into the soil (Hoogerkamp *et al.* 1983). The same occurred in New Zealand, where fast growing European grasses also created a thick mat of litter, which quickly was incorporated into the soil after European earthworms were introduced (Stockdill 1982).

Since the Oostvaardersplassen became a nature reserve in 1974 (but probably already since their reclamation in 1968), the soil has never been tilled. This must have helped the maintenance or build-up of soil organic matter. Soil organic matter is important as it provides a primary food source for soil biota. When micro-organisms only need carbon to meet their energy needs (organic matter with low C:N ratio), the excess nitrogen is released in a form that is available to plants (mineralization), this is a key process for an ecosystem because it determines the productivity of plants (Bardgett 2005). The addition of earthworms to this system, animals which fragment and mix organic input with the soil, accelerated the decomposition

by micro-organisms and thus quicker availability of nutrients for plants. The high soil organic matter content of the Oostvaardersplassen sods enhances the cycling of nutrients leading to very high productivity when the crop of herb-rich grass is continuously harvested.

The experiments showed that earthworms play an important role, but the conditions of the soil are of paramount importance as this will influence earthworm populations. Undisturbed permanent grasslands contain much higher number of earthworms than fields that are regularly disturbed by tillage (Evans & Guild 1948, Parmelee *et al.* 1990, Paoletti 1999). Especially detritivorous earthworms are negatively affected (Nuutinen 1992, Ernst & Emmerling 2009, Crittenden *et al.* 2014, Pelosi *et al.* 2014a). Edwards & Lofty (1982a) also found a negative effect of tillage on the deep-burrowing species such as *Lumbricus terrestris* (a detritivore), but not on shallow working species such as *Aporrectodea caliginosa* (a geophage).

Scaling up

As we have shown in chapter 5, dairy farmland fertilized with slurry manure only has much lower densities of detritivores than farmland fertilized with farmyard manure. Although the lower quality of the manure for these earthworms compared to farmyard manure is likely to be involved (Edwards & Lofty 1982b, De Goede *et al.* 2003, van Eekeren *et al.* 2009, Bertrand *et al.* 2015), these slurry fertilized fields were also more often disturbed than the farmyard fertilized fields. Fertilizing by slit-injection, reseeding and ploughing are all practices that occur regularly in intensive managed dairy farmland. As already mentioned in the introduction of this thesis, grasslands that have never, or at least not for decades, experienced these disturbing farming practices are rare. In the province of Fryslân, only grasslands managed by nature organizations such as it Fryske Gea, Staatsbosbeheer and Natuurmonumenten now belong to these rare undisturbed soils, but comprise only 3.5% of the total grassland area (Fig. 7.5).

These farming practices are negatively acting on two essential factors for earthworms: water and food. The physical damage of soil disturbance not only destroys earthworm burrows, but also breaks down soil aggregates and fungal hyphae that are of importance in the water binding capacity of a soil (Beare *et al.* 1997, Franzluebbers 2002, Pulleman *et al.* 2003, Bronick & Lal 2005). Parmelee *et al.* (1990) showed that fungal biomass in untilled fields were higher than in fields that were regularly tilled and during a drought event earthworms populations were more resilient in untilled than in tilled fields. Drought events particularly harm detritivore populations (Eggleton *et al.* 2009), probably because geophages go into diapause by curling into a small knotted ball in the soil and form a protective coat-

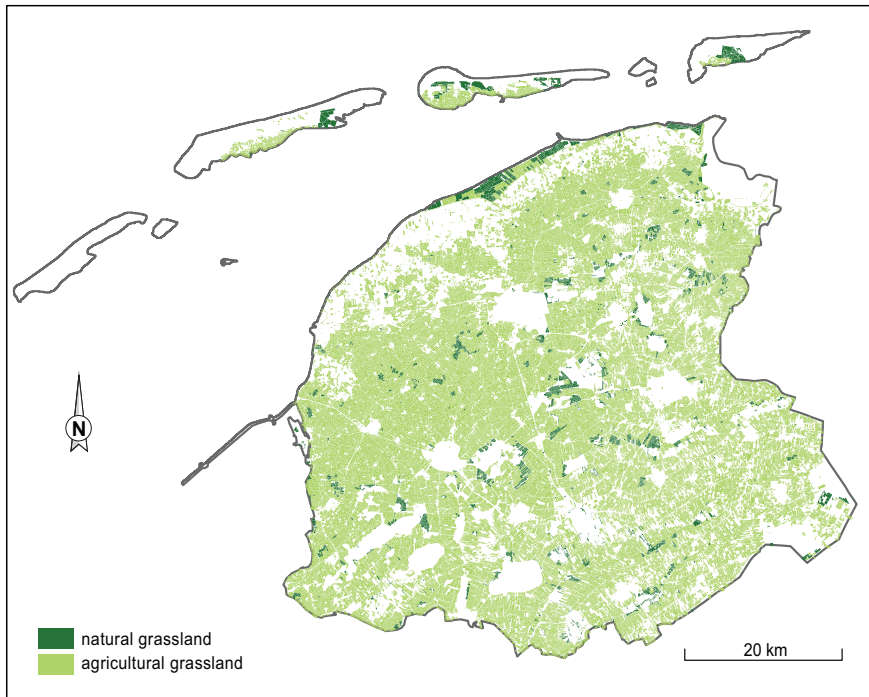


Figure 7.5: Map of Fryslân showing the area of natural grasslands (dark green) which are mostly managed by nature organizations and intensively used agricultural grasslands (light green) which are managed by dairy farmers. Map made by Ruth Howison based on data from NGR (2016).

ing of secreted mucus (El-Duweini & Ghabbour 1968, Edwards & Bohlen 1996). Experimentally measured drought tolerances for the detritivorous *Lumbricus rubellus* and the geophagous *Aporrectodea caliginosa* did not show differences between these species (A. Ooms & M.P. Berg, pers. comm.). Detritivores can increase the moisture content of the soil by collecting litter in the soil and at the surface, geophages, on the other hand, induce water runoff by their burrowing behavior (Ernst *et al.* 2009).

The negative effect of soil disturbance on earthworm food resources is mainly caused by declining amounts of surface litter, which is again detrimental for detritivores (Nuutinen 1992). Eventually, this will also lead to a decline in soil organic matter which is also negative for geophages which feed on it (Parmelee *et al.* 1990, Riley *et al.* 2008, Crittenden *et al.* 2015). So any soil disturbance is negative for earthworms, but more importantly, it is affecting the whole dairy farmland ecosystem as specifically detritivores are affected. Tillage changes the whole detritus food

web by favouring bacteria and potworms (Enchytraeidae) at the expense of fungi and earthworms (Hendrix *et al.* 1986, Wardle 1995, Wardle *et al.* 2004). Injection of slurry manure in dairy farmland probably has the same effect and by increasing bacterial biomass it promotes food conditions for geophages, but not for detritivores which generally prefer fungal degraded litter (chapter 5). This might be the reason why fungicides are also toxic to detritivores (Pelosi *et al.* 2014b).

The intensive land use of conventional dairy farming, will push aside the beneficial detritivores, and thus destroy the accelerating step in the dairy farmland ecosystem wheel between manure and grass production (Fig. 7.1). The use of inorganic fertilizer can take-over this step and indeed, increasing use of N inorganic fertilizer will decrease the positive effect of earthworms (van Eekeren *et al.* 2009, van Groenigen *et al.* 2014). The low biomass production in the sods from the conventional grassland, might be a result of this dependence on inorganic fertilizers. The loss of detritivores can lead to a deterioration of the soil structure as high abundances of solely geophages can result in sticky lumps that forms cement-like plates on the surface, a phenomenon that occurred in intensively used fields in Flevoland (Ester & van Rozen 2002).

Back-tracking the thesis

In **chapter 1** we gave an overview of the ecological impact of agricultural intensification in Dutch dairy farmland and asked ourselves whether the availability of earthworms is negatively affected by agricultural intensification as it does not seem to harm earthworm abundances, in contrast to other organisms. For meadow birds, however, it is not about abundances, but about the detection and availability of earthworms (Zwarts & Wanink 1993). Therefore, we needed a method to measure earthworm availability properly. Taking soil samples will only give an estimation of earthworm availability, when taking the bill length into account, for tactile hunting meadow birds such as Black-tailed Godwit *Limosa limosa*. Visually hunting meadow birds such as Lapwings *Vanellus vanellus*, rely on surfacing earthworms and taking soil samples alone will thus give a biased estimation of earthworm availability.

In **chapter 2** we described how surfacing earthworms could be counted by using a simple cart that is easy to perform and replicable. We have shown that only a small fraction of the total earthworms surface during the night and earthworm abundance does not predict the numbers of surfacing earthworms. Therefore taking soil samples will give no, or at least a biased, estimate of earthworm availability for a visually hunting meadow bird.

The method to count earthworms by using a cart was tested in a study to unravel the foraging strategy of Ruff *Philomachus pugnax* (**chapter 3**). With indoor feeding

experiments, we showed that Ruffs mainly use visual cues to detect earthworms. Although Ruffs only feed during the day, intake rates were strongly correlated with number of surfacing earthworms at night. This study illustrated that using the method described in chapter 2 gives indeed a good measure of earthworm availability for visual hunting meadow birds.

After we had developed a good method to measure earthworm availability we switched our focus to earthworms to understand what determines the surfacing behaviour and thus earthworm availability. In **chapter 4** we studied the effect of surface-applied farmyard manure on the availability of earthworms for meadow birds. This traditional way of fertilizing is generally thought to promote food conditions for meadow birds, however, it reduces the availability of earthworms for meadow birds in the short term. From an earthworm view, this is not surprising, as it surface to collect food. To avoid being food itself, it remains in the soil when it is satiated.

The long-term effect of fertilizing with different types of manure was studied in **chapter 5**, where earthworms were collected on differently managed dairy farmland. This showed, that fertilizing with farmyard manure will benefit detritivorous earthworms, and thus it will promote food conditions for meadow birds. However, perhaps the most important factor determining earthworm availability for meadow birds, is soil moisture. In **chapter 6** we showed that not only the surfacing behaviour of earthworms stops when the soil desiccates, but also the penetrability of the soil decreases which is detrimental for tactile feeding meadow birds.

Final words: ‘Oil’ versus ‘worms’

The main question of this thesis was: How does dairy farm management affects earthworms and their availability for meadow birds? As I have shown throughout this thesis, detritivore earthworms are key organisms in the dairy farmland ecosystem, but they are also susceptible to agricultural intensification in several different ways. Food conditions for earthworm predators will deteriorate under intensification, not only because detritivores decline, but also because earthworms become less available due to desiccating conditions making the soil harder and earthworms less active (chapter 6). Furthermore, larger-sized earthworms are most severely affected (Wardle 1995, Postma-Blaauw *et al.* 2010, Tsiafouli *et al.* 2015) and therefore predators have to consume more smaller-sized earthworms to meet energy requirements (Box A).

To promote detritivorous earthworms, soil disturbance should be minimized and (coarse, i.e. high C:N ratio) organic material should be applied on the surface. The positive effect of earthworms on plant productivity is indeed larger when more

litter is applied (van Groenigen *et al.* 2014). These actions not only promote detritivores, but could also be the beginning of a self-reinforcing system where the input of organic material promotes detritivores and improves soil structure and soil organic matter cycling including the beneficial interactions with micro-organisms (Fig. 7.6) (Bertrand *et al.* 2015, Bender *et al.* 2016). In turn, soil moisture content increases, which keeps earthworms active and available to meadow birds and other predators, but it will also stimulate sward production (Fig. 7.5). The energy driving this system does not rely on oil fuelling the machines of the farmer, but on the green energy of some humble creatures living belowground.

Earthworms are not only prey for endangered species or agents for improving agricultural production, they are a fascinating group of organisms that is part of a complex food web and thus should be studied like any other organisms in a natural ecosystem. We believe that looking with an ecological, rather than an agricultural, perspective at the dairy farm ecosystem, will yield valuable insights to help the development of much more environmentally friendly dairy farming and the conservation of meadow birds and other farmland species (Tsiafouli *et al.* 2015, Bender *et al.* 2016, Erisman *et al.* 2016).

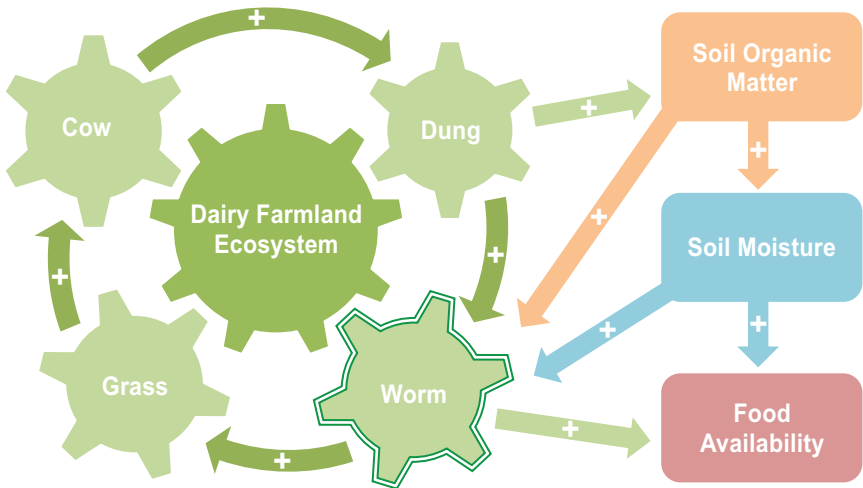


Figure 7.6: The dairy farmland ecosystem flywheel including the factors that boost rotating this wheel (soil organic matter and soil moisture) and will eventually also promote earthworm availability for other organisms.

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SAMENVATTING

Boeren, wormen & vogels

Jeroen Onrust

Wanneer je Nederland bekijkt vanuit een vogelperspectief, zie je een groen, nat en weids landschap. In Nederland is grasland dat gebruikt wordt voor de melkveehouderij het meest voorkomende landschap. Dit landschap dat door mens gecreëerd werd, creëerde tevens een gemeenschap van vogels dat we tegenwoordig 'weidevogels' noemen. Tot deze groep vogels behoren allerlei soorten, van zangvogels tot eenden, maar over het algemeen hebben we het over steltlopers, waaronder de Grutto, Kievit, Tureluur, Scholekster en Kemphaan.

Halverwege de vorige eeuw hadden die graslanden een hoge rijkdom aan allerlei soorten planten en dieren. Hoewel nog steeds weids en groen, is van die rijkdom tegenwoordig weinig meer over (**hoofdstuk 1**). Grootschalige ruilverkavelingen hebben het landschap binnenste buiten gekeerd en ontdaan van zijn natuurlijke dynamiek. Ploegen en doorzaaien hebben van kruidenrijke graslanden monoculturen van raaigras gemaakt, dat meerdere keren per jaar gemaaid en bemest kan worden. Niet meer met ruige stalmest, maar met kunst- en drijfmest dat in de bodem wordt geïnjecteerd. Deze veranderingen hebben een negatieve invloed gehad op bijna elke soort in het grasland-voedselweb, resulterend in een sterke afname van aantallen en soorten.

In tegenstelling tot al die soorten die zijn verdwenen of op het punt staan om te verdwijnen uit het agrarische grasland, lijken regenwormen een uitzondering op de regel. De hoogste dichtheden aan regenwormen worden in Nederland gevonden, met Friesland als meest wormenrijke provincie. Op het eerste gezicht lijkt het dus niet waarschijnlijk dat voedselomstandigheden voor volwassen weidevogels of andere regenwormeneters is afgenomen. Wat echter belangrijker is, zijn niet de dichtheden aan regenwormen, maar hoeveel regenwormen een weidevogel van die dichtheden werkelijk kan vangen. Met andere woorden, het gaat om de *beschikbaarheid* en niet om de dichtheid aan regenwormen.

In dit proefschrift onderzoeken we regenwormen in het Friese weidelandschap om te begrijpen wat hun verspreiding en beschikbaarheid voor weidevogels bepaald en hoe de intensivering van de landbouw dit heeft beïnvloedt. Het onderzoek wordt uitgevoerd in Friesland omdat hier 90% van het agrarische land gebruikt wordt voor de melkveehouderij en tevens ook de hoogste dichtheden van weidevogels voorkomen. Het doel van het onderzoek is om te verkennen hoe het beheer van graslanden in de melkveehouderij (**boeren**) regenwormen (**wormen**) beïnvloedt en hoe dit weer van invloed is op de wormenbeschikbaarheid van weidevogels (**vogels**). We hebben dit gedaan door in graslanden die verschillend beheerd worden naar regenwormen te kijken vanuit het perspectief van een weidevogel. Daarnaast kijken we ook specifiek naar verschillende soorten regenwormen om te begrijpen welke regenwormen belangrijk zijn voor weidevogels en of ze ook anders beïnvloedt worden door boerenbeheer.

“Worms are nocturnal in their habits, and at night may be seen crawling about in large numbers, but usually with their tails still inserted in their burrows”

- Charles Darwin (1881)

Tast- en zichtjagende weidevogels vangen andere regenwormen

Om iets te kunnen zeggen over de wormenbeschikbaarheid voor een weidevogel, moeten we ook weten hoe een weidevogel een regenworm vangt. In dit proefschrift delen we weidevogels grofweg op in twee groepen: de tastjagers en de zichtjagers. De tastjagers (bijv. Grutto) hebben een lange snavel en prikken daarmee in de bodem om op tast een regenworm te pakken. Daarmee kunnen ze alle regenwormen vangen die in het bereik van hun snavel liggen. De wormenbeschikbaarheid voor deze groep kan bepaald worden door bodemmonsters te nemen waarvan de diepte overeenkomt met de lengte van de snavel. Voor weidevogels die regenwormen vangen op zicht (bijv. Kievit), zijn alleen die regenwormen beschikbaar die ze kunnen zien en die dus zich dus aan het oppervlak begeven. Het nemen van een bodemmonster geeft dus geen goed beeld voor deze groep en dus moesten we met een nieuwe methode komen.

Om de wormenbeschikbaarheid voor zichtjagers te bepalen hebben we een kar ontwikkeld waarmee regenwormen die zich aan het oppervlak begeven geteld kunnen worden zonder ze te verstoren (**hoofdstuk 2**). Een waarnemer ligt met zijn buik op de kar en duwt zichzelf met zijn benen naar voren. Op deze manier kan heel rustig, en met goed zicht op de bodem, een transect gemonitord worden op rondkruipende regenwormen. De meeste regenwormen komen slechts gedeeltelijk naar het oppervlak en blijven met hun staart in hun holletje om bij gevaar weer terug te kunnen schieten. Door op meerdere tijdstippen te tellen, kwamen we er achter dat regenwormen alleen 's nachts naar het oppervlak komen. Van zichtjagers zoals Kieviten en Goudplevieren is ook bekend dat ze vooral 's nachts jagen. De nieuwe methode hebben we toegepast in verschillende type beheerde graslanden waar we tevens ook bodemmonsters namen. Daar kwam uit dat de dichtheid aan regenwormen in de bodem niks zegt over het aantal regenwormen dat 's nachts naar het oppervlak komt. Het nemen van bodemmonsters is dus geen goede maat om de wormenbeschikbaarheid voor zichtjagers te meten.

“The habit of lying near the surface leads to their destruction to an immense extent.”

- Charles Darwin (1881)

De Kemphaan is een zichtjager, maar 's nachts niet

De nieuwe methode hebben we getest met Kemphanen, een weidevogel die bijna is uitgestorven als broedvogel in Nederland, maar nog wel doortrekt en dan foeraagt in agrarische graslanden op regenwormen. Ik observeerde foeragerende Kemphanen en scoorde hoeveel regenwormen per minuut ze vingen (**hoofdstuk 3**). Vervolgens ging ik met de kar naar datzelfde stukje weiland en telde de regenwormen. Echter, zoals we in hoofdstuk 2 al lieten zien, zijn er overdag geen regenwormen aan het oppervlak te vinden. Blijkbaar vangen Kemphanen regenwormen toch niet op zicht of gebruiken ze andere signalen. Ik telde echter ook door in het donker en wanneer we die aantallen gebruiken, blijkt er wel degelijk een verband te bestaan tussen de hoeveelheid regenwormen die Kemphanen vangen en de hoeveelheid die 's nachts rondkruipen.

Van gezenderde Kemphanen weten we dat ze 's nachts op hun slaapplek zitten en alleen overdag actief zijn. Maar waarom zou een Kemphaan niet 's nachts foerageren, wanneer de regenwormen voor het oprapen liggen? Om daar achter te komen moesten we begrijpen hoe Kemphanen nou precies regenwormen vangen en daarvoor hebben we foerageerexperimenten gedaan met gevangen Kemphanen. Daaruit bleek dat Kemphanen vooral zichtjagers zijn, maar dat ze, in tegenstelling tot Kieviten of Goudplevieren, 's nachts niet goed kunnen zien. Ze kunnen dan ook regenwormen vangen op gehoor, maar dat lijkt ons in het veld onwaarschijnlijk. Ze zijn dus aangewezen op het foerageren overdag wanneer de wormenbeschikbaarheid veel lager ligt, maar wellicht dat ze wormenhoopjes gebruiken of dat ze regenwormen toch net onder het oppervlak kunnen horen rondkruipen.

“The half-decayed or fresh leaves which worms intend to devour, are dragged into the mouths of their burrows to a depth of from one to three inches.”

- Charles Darwin (1881)

Voedsel lokt rode wormen naar het oppervlak

In Nederland komen ongeveer 23 soorten regenwormen voor, waarvan ongeveer zes algemeen in agrarische graslanden zijn. Op basis van hun voedsleecologie kunnen regenwormen ingedeeld worden in twee ecotypen: de *detritivoren*, die van grof organisch materiaal leven, en de *geofagen*, die van bodemdeeltjes en organische stof leven. Aangezien detritivore soorten over het algemeen donker gepigmenteerd zijn en daardoor roder van kleur dan geofage soorten, noemen ik detritivore soorten *rode wormen* en geofage soorten *grijze wormen*.

Rode wormen verzamelen 's nachts hun voedsel aan het oppervlak en stellen zich daarmee bloot aan nachtelijke predatoren. Hongerige dieren zijn geneigd om meer risico's te nemen en zijn daardoor ook kwetsbaarder voor predatie. We verwachten dan ook dat goed doorvoede rode wormen zich niet, of minder, aan het oppervlak zullen begeven. In **hoofdstuk 4** testen we deze hypothese op het melkveebedrijf van Murk Nijdam in Friesland. Twee uniforme graslanden werden opgesplitst in twee percelen die of een vroege (1 februari 2014) of een late (14 maart 2014) bemesting van ruige stalmest kregen toegediend. Elke twee weken werden 's nachts de regenwormen geteld die aan het oppervlak kwamen. Bodemonsters werden genomen om regenwormen dichtheden en diepte te bepalen en om te kijken naar lichaamscondities van regenwormen.

Zoals verwacht, waren de aantallen regenwormen 's nachts aan het oppervlak 2.5 keer zo hoog in de percelen waar nog niet bemest was. In die waren ook de onvolwassen rode wormen significant lager in lichaamsgewicht. Deze effecten verdwenen nadat alle percelen waren bemest. We concluderen daarmee dat de oppervlakte activiteit van rode wormen vooral bepaald wordt door honger. Na bemesting is er geen noodzaak meer om aan het oppervlak te komen en daarmee voorkomen ze om zelf gegeten te worden. Dat betekent dus ook dat de beschikbaarheid van regenwormen voor weidevogels vergroot kan worden door bemesting in het voorjaar zo lang mogelijk uit te stellen om zo de regenwormen hongerig en aan het oppervlak te houden.

Ruige stalmest is goed voor rode wormen en dus voor weidevogels

Om te begrijpen hoe het beheer van een grasland regenwormen beïnvloedt, hebben we gekeken naar het effect van bemesting op de verspreiding van de twee ecotypen regenwormen (**hoofdstuk 5**). Tegenwoordig worden graslanden bemest met drijfmest, een mengsel van uitwerpselen en urine dat geïnjecteerd moet worden in de bodem of tussen het gras. Dit type mest heeft, vanwege een lage verhouding tussen koolstof en stikstof, een lagere kwaliteit als voedselbron voor bodemdieren dan de traditioneel gebruikte stalmest. Ruige stalmest bevat naast de uitwerpselen van een koe, ook het stro waar de koe op staat in de stal. Deze mest wordt buiten op een hoop verzameld waar het composteert en veelal in het voorjaar wordt gebruikt als bemesting van het grasland. In tegenstelling tot drijfmest wordt het juist bovengronds uitgereden.

Aangezien rode wormen meer afhankelijk zijn van mest en ander organisch materiaal als voedselbron dan grijze wormen, is de verwachting dat de type bemesting de verspreiding van deze twee ecotypen bepaald waarbij rode wormen meer voorkomen in graslanden die bemest worden met ruige stalmest. Dit heeft

dan weer effect op wormeneters, omdat juist rode wormen door hun oppervlaktegedrag een belangrijke prooi voor regenwormen zijn. Daarnaast hebben we ook uitgerekend dat een rode worm een hogere voedselwaarde heeft dan grijze wormen en weidevogels dus minder regenwormen hoeven te eten om aan hun dagelijkse behoefte te voldoen wanneer ze alleen rode wormen zouden eten (**box A**).

Om dit te onderzoeken hebben we in 45 graslanden bodemonsters genomen om te kijken naar de dichtheid aan rode en grijze wormen. Een deel van deze graslanden werd bemest met alleen drijfmest, een deel met alleen ruige stalmest en een deel werd bemest met zowel ruige stalmest als drijfmest. We vonden inderdaad dat rode wormen in hogere dichtheden voorkwamen in graslanden die met ruige stalmest werden bemest. De dichtheden aan grijze wormen was overal gelijk.

Het directe effect van de twee typen mest op regenwormen is onderzocht door naar de groei van rode en grijze wormen te kijken. Jonge wormen zijn verzameld en individueel in een potje gestopt waar ze of ruige mest, of drijfmest of strooisel (als controle) kregen toegediend gedurende zes maanden. Iedere maand zijn de regenwormen gewogen en is de groei bepaald. Hoewel er uiteindelijk geen verschil in gewichten was tussen de verschillende groepen, groeiden rode wormen wel sneller op ruige stalmest. Grijze wormen daarentegen deden het juist beter op drijfmest. Deze resultaten laten zien dat de type bemesting die een boer gebruikt, uiteindelijk bepaald welke soorten regenwormen in een grasland voorkomen en uiteindelijk dus ook de voedselomstandigheden voor weidevogels en andere regenwormeneters.

“Earth-worms must be considered as terrestrial animals, though they are still in one sense semi-aquatic.” - *Charles Darwin (1881)*

Wormenbeschikbaarheid wordt bepaald door droogte

Naast mest als voedsel, is water ook ontzettend belangrijk voor regenwormen. Regenwormen zijn fysiologisch gezien waterdieren. Omdat regenwormen geen longen hebben vindt gaswisseling plaats door de huid, voor de opname van zuurstof en de afgifte koolstofdioxide hebben ze daarom een vochtige huid nodig. Regenwormen vermijden daarom droge omstandigheden door dieper in de grond weg te kruipen of door in rust te gaan, waarbij ze zich tot een balletje oprollen in afwachting op betere/vochtige omstandigheden. Een grasland dat in het voorjaar of zomer begint uit te drogen zal dus ook de activiteit en dus beschikbaarheid aan regenwormen voor weidevogels doen afnemen. Wij waren echter benieuwd of deze respons ook verschilt tussen rode en grijze wormen en dus ook of er uiteindelijk ook een verschil is tussen de wormenbeschikbaarheid voor zicht- en tastjagers (**hoofdstuk 6**).

In het voorjaar van 2015 zijn op 8 graslanden in Zuidwest Friesland wekelijks de hoeveelheid regenwormen geteld die naar het oppervlak kwamen. De graslanden werden allen regulier/intensief beheerd, maar verschilden in grondwaterstand. Tijdens een telling werden metingen gedaan aan de vochtigheid van de bodem en zijn ook de weersomstandigheden genoteerd van een KNMI weerstation. Voor tastjagers is het ook van belang dat ze in de grond kunnen prikken met hun snavel en daarom is ook de doordringbaarheid van de bodem elke keer gemeten. Het aantal oppervlakte actieve nam sterk af door droogte van de lucht (lagere luchtvochtigheid) en van de toplaag van de bodem (hogere indringingsweerstand). Opvallend was dat er geen verschil was tussen graslanden, elk grasland liet eenzelfde patroon zien. Graslanden met een hoge grondwaterstand droogden net zo snel uit als graslanden met een lage grondwaterstand. Waarschijnlijk komt dit doordat de toplaag van de bodem regelmatig wordt verstoord door mestinjectie of doorzaaien waardoor het vermogen van de bodem om nog water op te nemen vanuit het grondwater is afgenomen. Daarnaast kan ook meespelen dat de grondwaterstand in het voorjaar te laat om hoog wordt gezet wanneer de bodems al beginnen uit te drogen.

In het lab hebben we ook gekeken naar de verticale verdeling van regenwormen in buizen met een verschillende bodemvochtigheid. Na drie weken zaten de regenwormen in de natte buizen bovenin de bodem, in de droge behandeling onderin, en in de vochtige behandeling zaten ze verspreid door de hele buis. Er was geen verschil tussen de twee soorten ecotypen. Dit experiment laat zien dat regenwormen zowel droge als te natte omstandigheden vermijden. Echter is droogte een groter probleem in Nederlandse graslanden. De oppervlakte activiteit van rode wormen stopt dan en voor zichtjagende weidevogels neemt de wormenbeschikbaarheid af. Een tastjagende weidevogel zou nog wel grijze wormen of regenwormen in rust kunnen vangen, maar alleen wanneer de regenwormen nog in bereik van de snavel zijn en als de bodem nog doordringbaar is voor die snavel.

“The plough is one of the most ancient and most valuable of man’s inventions; but long before he existed the land was in fact regularly ploughed, and still continuous to be thus ploughed by earth-worms.” - Charles Darwin (1881)

Rode wormen: de belangrijkste spelers in het grasland ecosysteem

In dit proefschrift heb ik verkend hoe het beheer van graslanden in de melkveehouderij (**boeren**) regenwormen (**wormen**) beïnvloedt en hoe dit weer van invloed is op de wormenbeschikbaarheid van weidevogels (**vogels**). De onderliggende vraag was of de intensivering van de landbouw hetzelfde negatieve effect heeft op regenwormen als het heeft op andere planten en dieren. Andere studies lieten zien dat

het huidige intensieve landbouwsysteem helemaal niet zo negatief uitpakt voor regenwormen met hogere dichtheden aan regenwormen in intensief bemeste graslanden.

Omdat weidevogels, die veel regenwormen eten, wel sterk afnamen door de intensivering van de landbouw, bleef de vraag of de *beschikbaarheid* van regenwormen wellicht is veranderd. We vonden inderdaad dat intensief landgebruik de beschikbaarheid van regenwormen voor weidevogels negatief beïnvloedt, waarbij met name de oppervlakte actieve rode wormen het meest negatief worden beïnvloedt en daarmee dus ook de voedselbeschikbaarheid voor zichtjagende weidevogels zoals de Kievit.

In de synthese van dit proefschrift (**hoofdstuk 7**) ga ik een stap verder door regenwormen niet alleen als een prooi voor weidevogels te zien, maar ook als belangrijke speler in het grasland ecosysteem. Door hun gegraveerd door de bodem verbeteren ze de bodemstructuur. Daarnaast dragen ze bij aan de afbraak van mest en strooisel en door dat materiaal de bodem in te trekken, op te eten en weer uit te poepen, creëren ze perfecte omstandigheden voor micro-organismen die er vervolgens voor zorgen dat voedingsstoffen weer vrijkomen voor het gras dat vervolgens weer gegeten kan worden door de koe. Vanwege deze functies, worden regenwormen ook wel biobouwers (*ecosystem engineers*) genoemd. Maar zoals ik in het proefschrift al heb laten zien, zijn het vooral de rode wormen die organisch materiaal aan het oppervlak verzamelen en in de grond brengen en dus zijn het vooral deze wormen die belangrijk zijn in het grasland ecosysteem. Maar het zijn juist deze regenwormen die door intensiever landgebruik het steeds slechter doen. Wordt daarmee dan ook de positieve rol van rode wormen teniet gedaan?

Om daar achter te komen hebben we een pilot experiment opgezet met uitgestoken plaggen (stukken intact grasland). Deze plaggen zijn afkomstig uit Flevoland, aangezien de bodem daar nog jong en homogeen is. Op drie plekken hebben we plaggen verzameld, van een regulier intensief melkveebedrijf, van een biodynamisch melkveebedrijf en uit natuurgebied de Oostvaardersplassen. Deze plaggen hebben we onder gecontroleerde omstandigheden gehouden in Groningen in kassen van de Linnaeusborg. Een deel van deze plaggen kreeg of alleen rode wormen, of alleen mest, of rode wormen én mest of niks. Vervolgens lieten we de plaggen drie maanden groeien en knipten we het gras regelmatig om de productie te meten.

In plaggen met rode wormen én mest was de productie het hoogst. Hoewel we door de lage steekproefgrootte geen significant effect vonden tussen de gebieden, lieten de resultaten wel een duidelijke trend zien met de hoogste productie in de natuurlijke graslanden en de laagste productie in de intensieve graslanden. Dit resultaat kan verklaard worden doordat in de Oostvaardersplassen het organische stofgehalte in de bovenste vijf centimeter drie keer zo hoog was als in de andere gebieden. Organische stof is positief voor het bodemleven omdat het een voedsel-

bron is, maar ook omdat het vocht en voedingsstoffen vasthoudt. Intensief landgebruik is negatief voor het organische stofgehalte door regelmatige bodemverstoring en het gebruik van kunstmest. Dit zorgt er tevens voor dat de voedselomstandigheden voor rode wormen afneemt waardoor rode wormen minder goed gedijen in intensief beheerd grasland en dus wordt ook de belangrijke rol van deze groep regenwormen teniet gedaan.

In dit proefschrift heb ik laten zien dat rode wormen een sleutelrol spelen in het grasland ecosysteem, maar dat juist deze groep regenwormen kwetsbaar is voor intensief landgebruik. De voedselomstandigheden voor weidevogels neemt door intensivering af, niet alleen omdat rode wormen afnemen, maar ook omdat regenwormen minder beschikbaar zijn door drogere omstandigheden. Om het aantal rode wormen te bevorderen, zou de bodem zo min mogelijk verstoord moeten worden en zou er bemest moeten worden met grof organisch materiaal (mest gemengd met stro of ander strooisel). Dit zal er niet alleen voor zorgen dat het aantal rode wormen zal toenemen, maar het zal tevens de bodemstructuur verbeteren, organische stofgehalte doen toenemen en uiteindelijk dus ook het vochtgehalte van de bodem dat de regenwormen actief houdt en dus beschikbaar voor weidevogels. Door met een weidevogel-bril naar regenwormen te kijken hebben we een uniek kijkje gekregen in de wereld van de regenworm in agrarische graslanden dat hopelijk bijdraagt aan een meer natuur-inclusieve landbouw en de bescherming van weidevogels en andere soorten.

Hijskraans in de verte
 Takeln de toekomst
 Boben de bomen uut
 Zo kan iederiene zien
 Dat het ons hier goed giet
 Gegrilde lucht
 Op 'n bedje van
 fiengesneden landschap

Altied bennen der lui west
 die het zeden
 Vanaf de aller eerste stienen steden
 Alle eeuwen hebben der welken zegt
 Beweging kan ok
 achteruutgang weden

De horizon komp dichterbij – Daniël Lohues (2009)



Dankwoord

Toen ik in de zomer van 2010 een project zocht om op af te studeren, had ik nooit gedacht om een kwart van mijn leven te spenderen aan dit onderwerp. Het begon met uitzoeken of we ook wormenbeschikbaarheid voor Goudplevieren konden meten in grasland. We wisten dat het wel eens gedaan is door met een zaklamp rond te lopen, maar dat was praktisch gezien onmogelijk in grasland omdat je dicht bij de grond moet zijn om wormen van grassprietjes te kunnen onderscheiden. Daarnaast moesten trillingen zo veel mogelijk voorkomen worden omdat regenwormen dan weer hun holletje inschieten. Kruipen leek ons, naast dat het arbotechnisch niet erg verantwoord is, ook geen goed idee. In een hostel in Brazilië tijdens het International Ornithological Congress viel het kwartje en schetste ik ergens op een papiertje een simpele kar. Thuis ben ik naar mijn oom Jan de Jonge gegaan en die heeft er een prachtige robuuste kar van gemaakt. Jan, ik ben je daar ontzettend dankbaar voor, zonder die kar was dit boekje er nooit gekomen. Ik heb die loodzware kar vaak vervloekt als ik hem midden in de nacht door de modder moest trekken of een damhek over moest zien te krijgen omdat de boer die weer eens met een stuk touw en een onmogelijke knoop had dichtgezet. Maar ik vergeet nooit meer die eerste keer. Toen de kar klaar was en ik hem bij jullie ophaalden ben ik daarna meteen doorgereden naar Friesland om hem te testen. Op het land van Jan Peenstra in Nes openbaarde zich een nieuwe wereld voor me. Ik telde tot diep in de nacht duizenden regenwormen die rondkropen, aan grassprietjes trokken of aan het paren waren. Kleine wormen, grote wormen en op de achtergrond het geluid van Kieviten en Goudplevieren. Het was magisch.

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