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Long-term effects of fertilisation regime on earthworm abundance in a semi-natural grassland area

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Summary

Environmental protection organisations involved in farmland-bird conservation promote the use of organic fertilisers, especially farmyard manure, to enhance the availability of earthworms, which are an important prey for farmland-birds. We studied changes in earthworm numbers in a field experiment on a semi-natural grassland with three different types of fertilisation; no fertilisation (NF), and application of slurry manure (SM) or farmyard manure (FM). Samples were taken in April, yearly from 1982 to 1990 and in 2005. On average, the SM treatment had a lower ($\geqslant$29%) earthworm abundance and a higher mean individual body weight than the FM and NF treatments. No statistically significant differences were observed between the FM and NF treatment. In 2005, earthworm abundance did not differ between the SM and NF treatment. The yearly variation in earthworm numbers and biomass was high, and significantly related to winter coldness. Colder winters resulted in lower earthworm abundances in the next spring. Especially the number of endogeic earthworms varied with winter coldness. The large variation between years stresses the need to be careful with the interpretation of short-term field experiments, or comparisons between areas based on single sampling events.

Introduction

Food availability for adult farmland birds might be one of the factors affecting their reproductive success, and earthworms are important prey for

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many of them (Beintema et al., 1995). Some predators of earthworms, such as Black-tailed Godwit (*Limosa limosa*) and Lapwing (*Vanellus vanellus*) have shown a downward trend in numbers over the past decades in Europe (Tucker and Heath, 1994). Environmental protection organisations (EPOs) are interested in the food availability, among other factors, for these endangered farmland birds. Therefore, EPOs look for ways to enhance earthworms populations in grasslands that are managed for farmland birds (Brandsma, 1999; Vickery et al., 2001). Fertilisation with farmyard manure, rather than slurry manure or inorganic fertilisers, is thought to be beneficial to high earthworm numbers. Climatic factors, soil type, and vegetation will also affect earthworm populations (Curry, 2004), but are not as easily manipulated as fertiliser application.

It has been shown that with an increase in nutrient availability, earthworms generally reach higher biomass and numbers (Edwards and Lofty, 1982; Hansen and Engelstad, 1999), though very high levels of fertilisation may inhibit their proliferation (Haynes and Naidu, 1998). This is especially true when soils become acidic (Ma et al., 1990); earthworms are generally scarce in soils with pH$<$4.5 (Curry, 2004). Many studies have shown that organic fertilisers favour earthworms more than inorganic fertilisers (Cuendet and Ducommun, 1990; Estevez et al., 1996; Whalen et al., 1998). Moreover, there is a general feeling that farmyard manure enhances earthworms more than slurry manure, and some evidence to support this was presented by Hansen and Engelstad (1999). Farmyard manure increases the pH and the organic matter content of the soil, which might explain its positive effects on earthworm abundance (Haynes and Naidu, 1998). The negative effects of slurry manure are related to its high salt concentrations and the occurrence of substances that might be toxic to earthworms (Curry, 1976). Furthermore, in The Netherlands, farmers are obliged to inject their slurry manure into slits cut into the soil to reduce atmospheric NH_3 emissions. This process is likely to kill earthworms, although experimental data show conflicting results. Negative effects of slit injection were reported by De Goede et al., 2003, whereas Kruk (1994) and Schekkerman (1997) failed to demonstrate short-term, negative effects of the application of slurry manure on earthworm biomass. Endogeic and epigeic worms, seen as different functional groups or morpho-ecological categories of worms, may react differently to fertilisation treatments (De Goede et al., 2003). Epigeic earthworms are red-pigmented and live close to the soil surface, while endogeic are unpigmented earthworms inhabiting deeper layers.

We investigated the long-term effects of two fertiliser application methods (farmyard and slurry manure) in addition to a control (i.e., no fertiliser) on the population dynamics of earthworms. The objective was to test the hypothesis that application of farmyard manure results in a higher earthworm abundance than application of slurry manure or no fertilisation, and that numbers of earthworms decrease with no fertilisation treatment.

Materials and methods

Study area

The study was performed in the grassland reserve Hempensermeer in the Netherlands (5°51′, 53°10′, Fig. 1) that was managed as a wet grassland with farmland birds as an important component of the biodiversity. The reserve is a small polder (85 ha), managed by a nature conservation organisation since 1978. The water table is high throughout the year, rarely falling below 10 cm. The soil type was clay over peat on top of a sandy clay soil. The soil is moist and the upper layer, 0–20 cm, has a very low penetration resistance. Below this upper layer

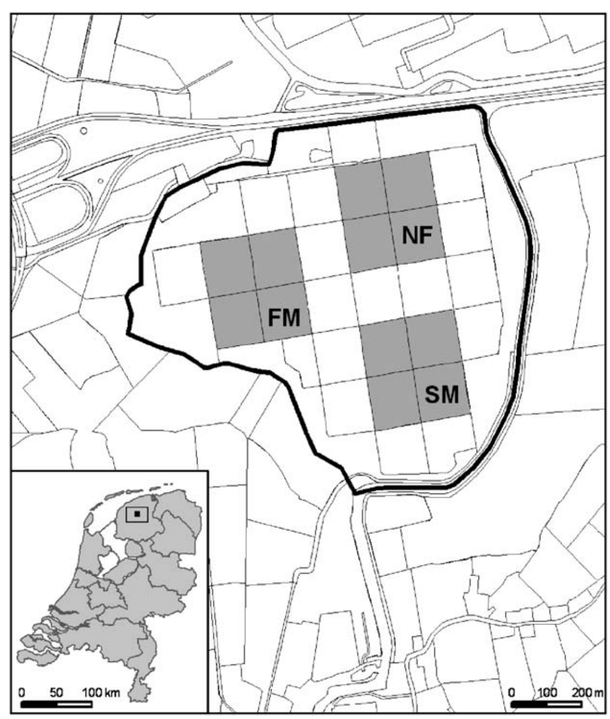

Figure 1. Map of the study area, with the layout of the experimental fields. NF = no fertilisation, SM = slurry manure and FM = farmyard manure.

however, the clay is hard and often impenetrable. There is a continuous supply of base-rich seepage groundwater, which cannot reach the surface everywhere due to this clay. pH of the water in the ditches of the polder ranged between 7 and 8, and soil pH was about 5.7 in April 2005. The vegetation was characterised by *Anthoxanthum odoratum*, *Ranunculus repens* and *Cardamine pratensis*. *Carex riparia* was locally abundant (cover: 5–40%), and *Carex nigra* fringed the many ditches. Locally, moss cover was up to 30%. Sward height in April was low (5.7 cm $\pm$ 0.3 SE in 2005). The breeding density of waders was very high with an average density of 253 pairs per 100 ha between 1997 and 2002 (Hut and Helmig, 2003).

Treatments

In 1982 three different treatments were established: (1) no fertilisation (NF), (2) application of slurry manure (SM), and (3) application of farmyard manure (FM). Each treatment comprised 16 plots, positioned regularly in adjacent fields, with a total surface area of 8 ha per treatment (Fig. 1). The NF treatment, used for hay making, was ungrazed between 1982–1990. The other two treatments were grazed after a first harvest of grass. The SM treatment received 20 tons of slurry ha^{-1} after the first harvest in June or July. The slurry was applied to the soil surface, without cutting the soil. In the FM treatment, farmyard manure (25 ton ha^{-1}) was applied yearly after the first harvest, until the mid 1990s. Thereafter, this treatment was discontinued. Since the second half of the 1990s the NF treatment changed to some extent with cattle grazing allowed at the end of the season. By accident, two out of the four parcels of the NF treatment received slurry manure in 1996.

Until the experiment started all the fields had been subject to standard agricultural practice: extensive use of fertiliser (ca. 200–300 kg N ha^{-1}), haymaking and cattle grazing. For practical reasons (i.e., the treatments were managed by local farmers), the plots were spatially clustered per treatment. At the start of the study, there were no substantial systematic differences between the fields (authors' observation). The data on clay content and pH of the fields indicate no differences between the fields by the end of the study period. Between 1999 and 2002, pH-values fluctuated around five in each of the treatments without a consistent pattern. Fluctuations in the height of the water table were similar for the whole polder throughout the study period and did not differ between the fields. Therefore, we considered our data appropriate, also because of the relatively large size of the experimental fields.

Sampling and analysis

Samples were collected every April between 1982 and 1990 as well as in 2005. In each plot six (1982–1989) or three (1990 and 2005) cores (corer surface 52 cm^2) of the 0–10 cm soil were collected at random and bulked into one sample, resulting in 16 samples per treatment. The 0–10 cm soil depth generally covers the entire root zone in this area. In 2005 only the NF and SM treatments were sampled. The samples were taken to the laboratory; worms, leatherjackets and other fauna (>1 mm) were handsorted within four days. Based on pigmentation, earthworms were assigned to different functional groups and counted. The volume of fresh worms, leatherjackets and caterpillars was measured to 0.5 ml. In 2005 we also measured the biomass (fresh weight) of earthworms in each sample, and constructed a calibration line to link fresh biomass to volume. This volume includes the gut content, since there was no time allowed for voiding of the gut. Using this relationship (fresh weight (g) $= 1.18 \times$ volume (ml), $R^2 = 0.98$) we converted the volumetric measures from the earlier years to fresh weight. This procedure was complicated by the fact that the volumetric measures from 1982–1990 included leatherjackets and caterpillars. Although we can state that earthworms accounted by far for the largest proportion in biomass, our biomass data slightly overestimates earthworm abundance during the 1982–1990 interval. Biomass data were analysed nonetheless to support our interpretation of the data. The pH of the soil (upper 5 cm, measured using 1 molar KCl) was determined, following standard procedures (Schlichling and Blume, 1966). To characterise the winters preceding a sampling season we used the figure of Hellmann (H), which is a measure for coldness in the period November 1st until March 31st (http://www.knmi.nl/klimatologie/lijsten/hellmann.html). It is obtained by summing all daily average temperature values below 0 °C, while omitting the negative sign.

Statistical analyses

Data where analysed with generalised linear modelling (GLM) with treatment as a fixed factor. Fresh biomass, number of earthworms and the proportion of epigeic worms were taken as independent variables in three separate analyses.

Winter coldness (H) was entered as covariate. We investigated whether a year effect, independent from winter coldness, was detectable by analysing the residuals of the models against year. Using linear regression we separately investigated the relation between numbers of earthworms and winter coldness per functional group. Data were transformed when necessary to improve homogeneity of variances using a square-root (for count data; $x' = \sqrt{(x + 0.5)}$) or arcsine (for % values; $x' = \text{arcsine}(x)$) transformation (Zar, 1996). Untransformed data are presented in the figures. Significance of differences between treatments was assessed using pair wise comparisons (Tukey's HSD).

Results

In 2005 earthworms made up the largest part of the soil faunal biomass (85%). Leatherjackets accounted for 12% on average, while the mass of other fauna was negligible. Total number of earthworms fluctuated substantially with up to four-fold differences between years (Fig. 2). There was a statistically significant effect of winter coldness with low population sizes after severe winters ($F_{1,460} = 56$, $P < 0.001$). Earthworm numbers were also statistically significantly affected by treatment ($F_{2,460} = 15$, $P < 0.001$). Biomass and numbers, averaged per treatment and year, were highly correlated (Pearson $R = 0.8$, $P < 0.01$, $n = 29$). Consequently, biomass shows the same pattern as total numbers in relation to winter coldness ($F_{1,460} = 90$, $P < 0.001$) and treatments ($F_{2,460} = 4$, $P < 0.05$). The fields that had been treated with farmyard manure (FM) or that received no fertilisation (NF) had on average $\geqslant$29% higher numbers and 18% higher biomass than those treated with slurry manure (SM, Fig. 3; Tukeys HSD, $P < 0.05$). This implies that on average the earthworms were slightly heavier under SM (0.28 g $\pm$ 0.01 SE) than, for example, under NF (0.24 g $\pm$ 0.01 SE). Surprisingly, earthworm numbers in the NF treatment were high and did not differ from the FM

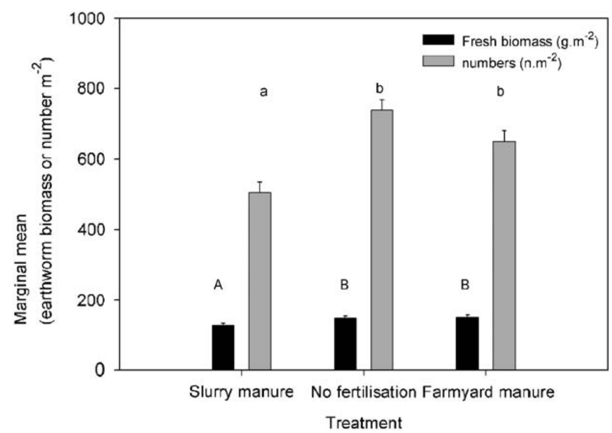

Figure 3. Marginal means (i.e. corrected for the covariable 'winter coldness') of fresh biomass (g m^{-2}) and numbers (m^{-2}) of earthworms per fertiliser treatment. Error bars refer to standard errors.

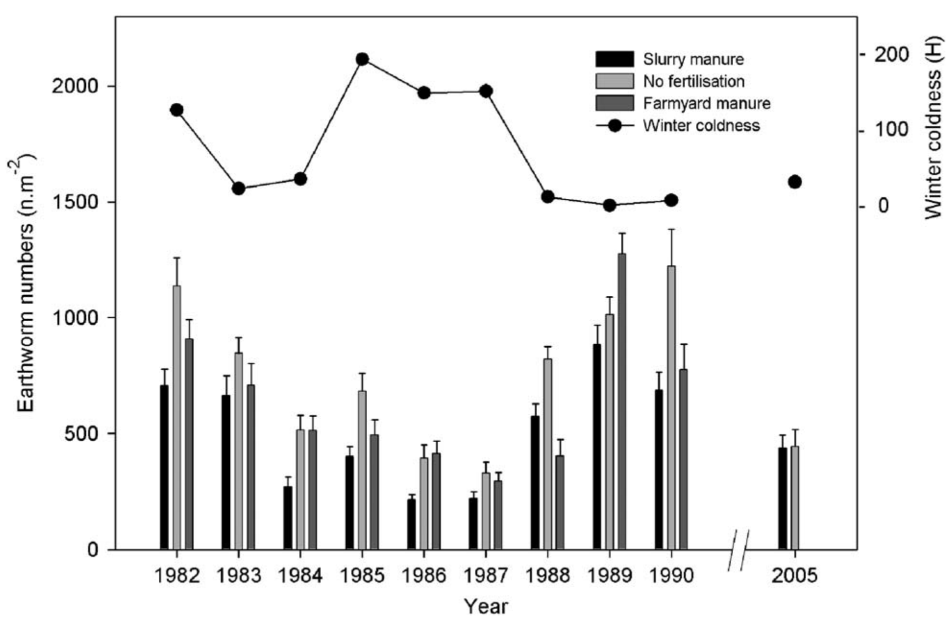

Figure 2. Total numbers of earthworms (m^{-2}) per year and per fertiliser treatment. Winter coldness is quantified using the Hellmann figure (H). High values of H indicate severe winters. In 2005 there were no data for farmyard manure. Error bars refer to standard errors.

treatment. Even after 25 years, numbers and biomass under NF did not differ (*t*-test, n.s.) from those in the SM treatment. Furthermore, there was no apparent downward trend between 1982 and 1990 in any of the treatments.

The effect of winter coldness was larger than that of fertiliser treatments. However, winter coldness could not explain all of the variation between years. Our tests on the residuals indicated a significant effect of year (residuals for numbers $F_{9,454} = 16$, $P < 0.001$ and residuals for biomass $F_{9,454} = 28$, $P < 0.001$).

The proportion of epigeic worms ranged from 16 to 48% (on average 28% $\pm$ 1 SE). There was no difference between treatments ($F_{2,458} = 2.4$, n.s.), but a statistically significant relation to winter coldness ($F_{1,458} = 9$, $P = 0.003$). The number of endogeic worms declined after severe winters (linear regression $F_{1,462} = 70$, $P < 0.001$, see Fig. 4), whereas epigeic worms were not affected by winter coldness at all ($F_{1,462} = 0.1$, n.s.).

Discussion

Differences between years were large, and much of this variation can be explained by winter temperatures. The endogeic earthworms were relatively more abundant after mild winters. In our opinion this is probably a causal relationship, as few earthworm species can tolerate temperatures below 0 °C (Curry, 2004). Mortality will be greater in severe environments. Our research area had a very high ground water table that hardly ever came below 10 cm soil depth. Therefore, earthworms

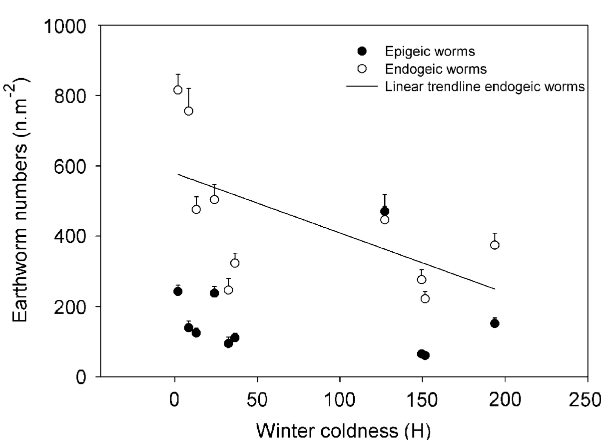

Figure 4. Relationship between winter coldness (Hellmann figure, H) and earthworm numbers (m^{-2}) in the subsequent spring per functional group. Endogeic worms are negatively related to winter coldness $y = 579 - 1.7 \times H$, $R^2 = 0.132$. Error bars refer to standard errors.

could likely not escape low winter temperatures by downward migration into the soil profile. We hypothesize that epigeic earthworms rely on other strategies to cope with low temperatures, which could explain why winter coldness did not affect this functional group. In spite of the fact that some variation may be explained by winter coldness, there was still unexplained variation between years. Together, these findings underline the need for cautious interpretation of short-term field experiments or comparison between areas based on single sampling events.

Given that the winter preceding the 2005 sampling was characterised as a 'gentle winter' ($H = 32$), the numbers and biomass of worms were relatively low that year. However, because of the above-mentioned yearly fluctuations, these low numbers are not of concern. Only 2 years earlier (April 2002, after a 'very gentle winter' ($H = 22$)), Hut and Helmig (2003) demonstrated that our study area, the Hempensermeer, was rich in earthworms. The Hempensermeer ranked second among the 33 grassland reserves in their study, with 700 $\pm$ 136 (SE) worms per m^2 and 156 g $\pm$ 38 SE fresh weight per m^2 (0–20 cm deep). Also, pH values of ditch water and soil were within the range that is optimal for most earthworm species. Thus, no evidence exists to suspect a systematic downward trend in earthworm abundance in the Hempensermeer between 1990 and 2005. Rather, it may illustrate that variation between years is common.

Our results showed that long-term management without fertilisation leads to neither detectable long-term decrease in earthworm numbers nor to lower numbers compared to the fertilizer treatments. In fact, earthworm numbers in the NF treatment were higher than in the slurry manure treatment. This is in contrast with the general observation that earthworm abundance is higher under fertilised conditions (Edwards and Lofty, 1982; Jordan et al., 2004; Curry, 2004). The relatively high numbers of earthworms in the no fertilizer treatment are hard to explain, but might be related to the hydrology and soil conditions of the study area. The area has a very high water table and the seepage of base-rich ground water results in a relatively high natural soil pH. Whether the observed negative effect of slurry manure application on earthworm numbers is biologically relevant for farmland bird conservation is unclear, given the fact that yearly fluctuations are so much greater. Our data show that the abundance of earthworms can be very high under each of the treatments. The mean number of 504 ($\pm$30 SE) earthworms per m^2 in the SM treatment, for example, is higher than many reported values in literature for flourishing populations (Edwards and Lofty, 1982; Curry, 2004). From the point of view of earthworm abundance, there is not a good reason to ban SM. When fertilising, there may be good arguments for preferring farmyard manure over slurry manure though, such as the availability of nesting material for birds, and effects on soil structure or entomofauna, but these have not been covered in this study. Grassland management should aim at soil conditions that are suitable for earthworms. Depending on the local circumstances this often implies counteracting acidification and impoverishment of soil nutrients. Managers thus need to monitor the status of their soils, but need not be surprised to see earthworm populations fluctuate.

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