Anecic earthworms and associated ecosystem services in a ley-arable crop rotation

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Abstract
Earthworms in general and anecic earthworms in particular, play a key role in the ecosystem service of water regulation through enhancing water infiltration and stimulating root growth to deeper soil layers by earthworm burrows. A crop rotation of grass and maize can be sustainable in terms of efficient nutrient use, but there is lack of information on the effect on earthworms. Earthworms were sampled over three years in a 36 years old experiment. Permanent arable land was compared with permanent grassland and with a ley-arable crop rotation. In the first year of arable cropping in the rotation, the number of earthworms was already low and not different from continuous cropping. In the three-year grass ley, the abundance of earthworms returned to the level of permanent grassland in the second year. The restoration of earthworm biomass took a minimum of three years. However, the anecic species did not recover in the three-years grass ley to the dominance they had in the permanent grassland. The number of earthworm burrows was related to earthworm biomass and was highest in permanent grassland. Our data suggest that anecic earthworms are under pressure in a ley-arable crop rotation, which may have a negative impact on the ecosystem service of water regulation.

Key Words
Ecosystem services, earthworms, crop rotation, grassland, water regulation.

Introduction
The rainfall in the Netherlands has increased in the last 100 years by 18\% and the number of days with more than 50 mm of rainfall has increased in the last 50 years from 5.4 to 9.0 (Anonymous, RNMI; Boxel and Cammeraat, 1999). On the other hand the length of drought periods in summer increases. Therefore, the ecosystem service of water regulation by soil biota becomes more important. Earthworms play a key role in water regulation (Hoogerkamp et al.,1983; Clements et al., 1991): epigeic and endogeic earthworms increase water infiltration in the topsoil through their burrowing activity, while the deep, vertical burrows of anecic earthworms increase water infiltration and root growth (Logsdon and Linden, 1992; Edwards and Shipitalo, 1998). Bouché and Al-Addan (1997) measured an average infiltration rate of 282 mm h\textsuperscript{-1} per 100 g m\textsuperscript{-2} for anecic species and 150 mm h\textsuperscript{-1} per 100 g m\textsuperscript{-2} of all the other earthworms.

Recent legislative restrictions on the use of organic and artificial N fertilizers and a quest for sustainable farming systems, have brought attention back to crop rotations with grass and maize. A crop rotation of grass and maize can be sustainable in terms of efficient nutrient use (Nevens and Reheul 2002; 2003). Furthermore, ley farming guarantees a high clover content and provides an opportunity to control perennial weeds on organic farms (Younie and Hermansen 2000). However, there is lack of information on the effect of such a crop rotation on abundance and ecological group composition of earthworms.

Earthworms were sampled over three years in a 36 years old experiment in which permanent arable land was compared with permanent grassland and with a ley-arable crop rotation. Our objectives were (1) to determine the long-term effects of a ley-arable crop rotation on earthworms in comparison with permanent grassland and continuous arable cropping, and (2) to assess the short-term recovery of soil biota in a ley-arable crop rotation.

Methods
Sampling site and experimental design
In 1966, a crop rotation experiment was established on a sandy loam soil at the experimental farm of Ghent University at Melle (50° 59’N, 03°49’E; 11 m above sea level) (Nevens and Reheul 2001; 2003). Four
treatments were established in a complete randomised block design with four blocks:
PG: Permanent grassland since 1966;
TG: Temporary ley-arable crop rotation, started in 1966 with three years of grass ley followed by three years of arable land cropped with forage crops;
TA: Temporary arable crop-ley rotation. This treatment is comparable to TG but started in 1966 with three years of arable cropping followed by three years of grass ley;
PA: Permanent arable cropping since 1966.

In the seventh rotation of the trial the TG treatment was established in April 2002 after rotavating the maize stubble of the preceding three years of arable cropping. The seed mixture used was 40 kg *Lolium perenne* L. ha⁻¹ ( cvs. Plenty and Roy) and 4 kg *Trifolium repens* L. ha⁻¹ (cv. Huia). In the seventh rotation the TA treatment was established in 2002 after rotavating the former grass ley on 9 April. In addition to nitrogen fertilizer (ammonium nitrate 27%), all plots received a basal fertilizer application of triple super phosphate (45% P₂O₅) and potassium chloride (40% K₂O). Herbicides were used in the treatments with maize, according to good agricultural practices. No pesticides were used on the grassland plots.

**Earthworm sampling**

In the first three years of the seventh rotation, earthworms were sampled in 2 blocks (20x20x20 cm) per plot on 30 October 2002, 7 October 2003 and 15 October 2004. The blocks were transferred to the laboratory where the earthworms were hand-sorted, counted, weighed and fixed in alcohol prior to identification. Numbers and biomass were expressed per m². Adults were identified according to species. A distinction was made between (1) epigeic species (pigmented, living superficially in the litter layer, little burrowing activity), (2) endogeic species (living in burrows at approximately 10-15 cm depth) and (3) anecic species (relatively large worms, living in vertical burrows from which they collect dead organic matter at the surface at night) (Bouché 1977). In 2004, before the blocks were sorted for earthworms, the earthworm burrows with a diameter >2 mm were counted on horizontal surfaces (20x20 cm) at 10 cm and 20 cm depth.

**Statistical analysis**

The data were analysed with GENSTAT (8th Edition, VSN International, Hemel Hempstaed, UK) using a two-way ANOVA in randomised blocks with treatment (PG, TG, TA and PA) and year of sampling as factors.

**Results**

The number of earthworms was highest in the PG treatment followed by the TG treatment (Table 1). On arable land (TA and PA) the number of earthworms was 12-24% of PG. The significant interaction of treatment and year is mainly due to the recovery in the number of earthworms in the TG treatment (Figure 1). In October 2003, the second year after the establishment of grass (TG) in the arable-ley crop rotation, the number of earthworms reached the same level as in the PG treatment.

The body biomass of the earthworms in TG was significantly lower (P<0.001) than in PG, and therefore the recovery of the total biomass was not as spectacular as the total numbers. Even in October 2004, the final year of the three-year period of grass ley, the earthworm biomass in TG was different from PG: 96 g m⁻² compared to 163 g m⁻² (P=0.002). In total numbers and biomass the TA plots resembled the PA plots. Numbers and especially biomass in the TA treatment already reached a low level in the first year of the rotation, suggesting a rapid decrease in earthworms after rotavating the grass ley.

Species of earthworms found in the trial were *Lumbricus rubellus, Aporrectodea caliginosa, Allolophora chlorotica, Aporrectodea rosea and Aporrectodea longa*. PG had the highest number of species and the arable treatments the lowest number. The interaction between treatment and year was mainly due to an increase of the number of species in the TG treatment from 2002 to 2003. As with the total numbers, the number of species in TG almost recovered within two years in comparison with the PG treatment. Among the adult earthworms in the PG plots, the anecic species were dominant (52 % anecic species). In TG and the arable treatments (TA and PA), the endogeic species were most common: 62 %, 88 % and 100 %, respectively. The epigeic species were mainly found in the grass treatments.

The number of earthworm burrows showed a clear decrease in the order PG>TG>TA>PA (Table 1). The variation in the number of burrows at 10 cm depth, measured in October 2004, was explained by a regression
model ($R^2=0.93$) with treatment and earthworm biomass measured in 2004 as fitted terms (Figure 2). There was a significant positive relation between the biomass and the number of burrows for all treatments (slope=0.058, $P=0.044$). In the model all four individual treatment levels differed significantly ($P<0.001$).

Table 1. Earthworm numbers, biomass, species, functional groups and earthworm burrows in permanent grassland (PG), temporary grassland (TG), temporary arable land (TA) and permanent arable land (PA): averages from three consecutive years (2002-2004).

<table>
<thead>
<tr>
<th>Earthworms</th>
<th>Units</th>
<th>Treatments</th>
<th>Year</th>
<th>Treat.*year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>n m$^{-2}$</td>
<td>PG: 256 a, TG: 187 b, TA: 62 c, PA: 30 c</td>
<td>&lt;0.001</td>
<td>NS: 0.008</td>
</tr>
<tr>
<td>Body biomass</td>
<td>g worm$^{-1}$</td>
<td>PG: 0.65 a, TG: 0.25 b, TA: 0.23 b, PA: 0.12 b</td>
<td>&lt;0.001</td>
<td>0.033: NS, 0.001: NS</td>
</tr>
<tr>
<td>Total biomass</td>
<td>g m$^{-2}$</td>
<td>PG: 166 a, TG: 52 b, TA: 14 bc, PA: 5 c</td>
<td>&lt;0.001</td>
<td>NS: NS, 0.001: NS</td>
</tr>
<tr>
<td>Number of species</td>
<td>n 20x20x20cm$^{-1}$</td>
<td>PG: 2.0 a, TG: 1.3 b, TA: 0.5 c, PA: 0.2 c</td>
<td>&lt;0.001</td>
<td>NS: &lt;0.001</td>
</tr>
<tr>
<td>Epigeic adults</td>
<td>n m$^{-2}$</td>
<td>PG: 20 a, TG: 25 a, TA: 1 b, PA: 0 b</td>
<td>0.016</td>
<td>0.011: NS, 0.031: NS</td>
</tr>
<tr>
<td>Endogeic adults</td>
<td>n m$^{-2}$</td>
<td>PG: 46 ab, TG: 49 a, TA: 22 bc, PA: 7 cd</td>
<td>0.009</td>
<td>NS: 0.031</td>
</tr>
<tr>
<td>Anecic adults</td>
<td>n m$^{-2}$</td>
<td>PG: 71 a, TG: 4 b, TA: 2 b, PA: 0 c</td>
<td>&lt;0.001</td>
<td>NS: NS</td>
</tr>
<tr>
<td>Earthworm burrows*</td>
<td></td>
<td>10 cm depth: PG: 388 a, TG: 238 b, TA: 106 c, PA: 6 d</td>
<td>&lt;0.001</td>
<td>--: --, --: --</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 cm depth: PG: 356 a, TG: 206 b, TA: 100 c, PA: 6 d</td>
<td>&lt;0.001</td>
<td>--: --, --: --</td>
</tr>
</tbody>
</table>

*Earthworm burrows were counted in 2004 only

Values followed by the same letter within a row are not statistically different at the 5% error level for the main treatment effect.

![Figure 1. Mean number (± SE) of earthworms (n m$^{-2}$) in permanent grassland (PG), temporary grassland (TG), temporary arable land (TA) and permanent arable land (PA) in the 7th rotation of a 36 years old experiment](image)

**Discussion and conclusions**

It is well known that grassland contains more earthworms than arable land (Edwards and Bohlen 1996). In this experiment, the number of earthworms in the PA treatment was as low as 12% of the number in the PG treatment. Edwards and Bohlen (1996) mention two reasons for a decreased number of earthworms, besides the mechanical damage and predation after cultivation: the loss of an insulating layer of vegetation and a decreased food supply. The small number of earthworms in the TA treatment, six months after rotavating the grass ley, suggests that the decrease in earthworm numbers in our experiment was rapid. Growing grass over several years favours the growth of earthworm populations and the best way of maintaining a large earthworm fauna in agricultural land is to include ley farming (Edwards and Bohlen 1996). In fact, in the ley phase of this experiment, earthworm biomass increased from 8 g m$^{-2}$ in the first year to 51 g m$^{-2}$ in the second year and to 96 g m$^{-2}$ in the third year. This is a biomass increase of 40-45 g m$^{-2}$ per year. Assuming a temporal constant increase, the grass ley were to last for 4 to 5 years in order to reach similar biomass levels as found in PG. A more lasting difference between the PG treatment and the remaining ley-arable crop rotation treatments, is the dominance of the anecic species in PG. The data suggest that especially anecic earthworms are under pressure in a ley-arable crop rotation which may have a negative impact on the ecosystem service of water regulation under future grassland.
Figure 2. Relation between earthworm biomass (g m⁻²) and earthworm burrows at 10 cm depth (n m⁻²) in 2004 for the four treatments (R²=0.93). Earthworm burrows (n m⁻² at 10 cm depth) = treatment (P<0.001) (intercept 5 for PA, 95 for TA, 182 for TG, 293 for PG) + 0.58 * earthworm biomass (g m⁻²) (P=0.044). PG=permanent grassland, TG=temporary grassland, TA=temporary arable land, PA=permanent arable land.

References
Anonymous, Royal Netherlands Meteorological Institute, Risk analysis of heavy rain.