Effects of temporary grassland introduction into annual crop rotations and nitrogen fertilisation on forage production and earthworm communities

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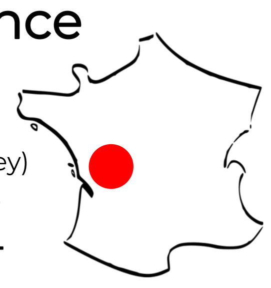
Introduction

Earthworms contribute to a wide range of ecosystem services, especially valuable in croplands (Bertrand *et al.,* 2015). Nevertheless, crop management can strongly modify earthworm communities. Earthworms functional role is known at the level of four ecological categories (Bouché 1977; Larsen *et al.,* 2016), epigeics: live and feed on surface litter or animals dungs; endogeics: live in horizontal burrows and feed on soil organic matter; *Lumbricus* anecics: live in few vertical burrows and feed on surface litter, *Aporrectodea* anecics: live in several sub-vertical burrows and feed on soil organic matter. The development of earthworm communities is highly dependent on ecological categories and the species that compose them (Satchell 1980; Butt 1993). Most studies focusing on the impact of agricultural practices on earthworm communities are related to soil tillage, fertilisation or pesticides, while the effect of temporary grassland introduction into a crop rotations remains largely unknown.

In this context, the aim of the present study was to determine the effects of grassland presence and duration in a crop rotation, as well as grassland fertilisation, on earthworm communities.

Materials & methods

Long Term Observatory in Lusignan, France



4 treatments
- Fertilised annual crop rotation, AC (grain maize, wheat, barley)
- 3-year-old grassland, highly fertilised (230 kg ha⁻¹), G3N+
- 6-year-old grassland, highly fertilised (230 kg ha⁻¹), G6N+
- 6-year-old grassland, lowly fertilised (30 kg ha⁻¹), G6N-

The four treatments were replicated in four blocks

	2005	6	7	8	9	10	11
AC	c1	c2	c3	c1	c2	c3	c1
G3N+	c1	c2	c3	1	2	3	c1
G6N+	1	2	3	4	5	6	c1
G6N-	1	2	3	4	5	6	c1

Earthworm sampling in march 2011

Crops and grass were sown after ploughing. Grain maize was sown in April and harvested in October, and its residue was crushed and left on the soil surface. Wheat was sown in October and harvested in July. Barley was sown in November and harvested in July; after barley harvest, soil remained bare until maize was sown in March. The straw of wheat and barley was exported. Grass was sown in November and cut three or four times per year (depending on its productivity). Grasslands were sawn as a mixture of *Lolium perenne* cv. Milca, *Festuca arundinacea* cv. Soni and *Dactylis glomerata* cv. Ludac.

Forage production and composition

In 2010, grass was cut three times during the year. Above-ground biomass was estimated by cutting an area of 1.5 × 5.0 m with an experimental harvester (Haldrup, Germany). Grass was dried in an oven at 70°C and weighed to determine dry matter content. It was then ground for chemical analysis. Total N and C concentrations were determined by the Dumas method using an elemental analyser (Carlo Erba EA 1108).

Earthworm sampling and laboratory analyses

Earthworms were sampled in march 2011 according to the ISO 23611-1 modified according to Pérès *et al.* (2010). It combines chemical with physical extraction. In each plot, earthworms were sampled at three different locations spaced more than 10 m apart and at least 10 m from the edge. Each earthworms sampling consisted in applying three watering of 10 L with an increasing concentration of formaldehyde (0.08%, 0.08% and 0.12%) on one square meter. After earthworm collection, to recover earthworms unable to reach the surface, a block of soil (25 x 25 x 20 cm) was hand-sorted inside the sample square corresponding to a surface of 1/16 square meter.

Statistical analysis

We first used linear mixed-effects models, followed by Tukey HSD tests for post hoc pairwise comparisons, to test effects of the four treatments (annual crop, highly fertilised 3- and 6-year-old grassland and lightly fertilised 6-year-old grassland) on each earthworm community parameter. To account for pseudo-replication, the random part of the model specified that earthworm samplings were nested within blocks We then used separate one-way ANOVAs, followed by Tukey HSD tests for post hoc pairwise comparisons, to assess differences in forage production and composition (C and N) between the treatments.

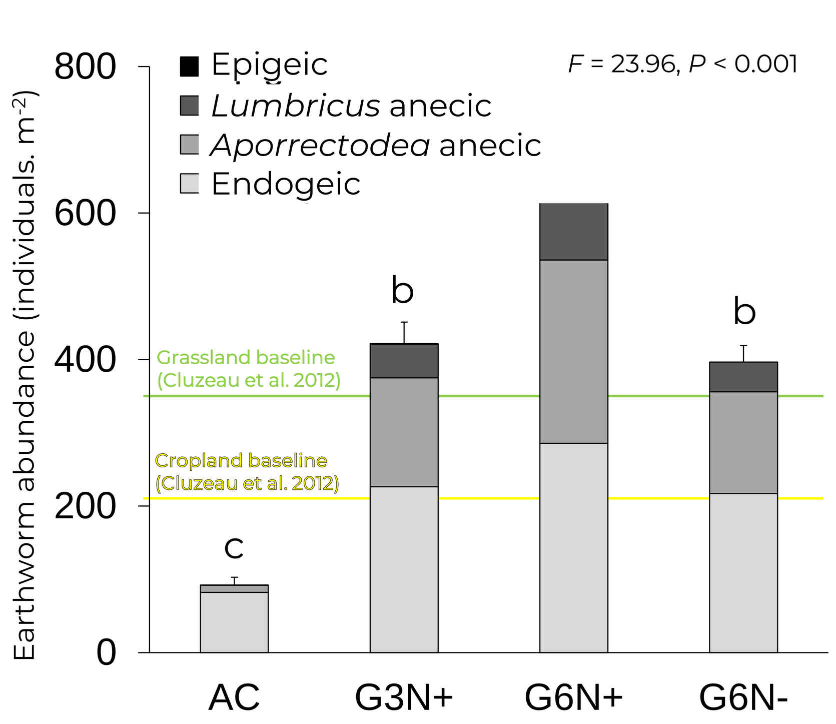
Results

Forage production and composition

Forage production and composition (C and N) were not significantly different between the highly fertilised grasslands (G3N+ and G6N+). Nonetheless, forage production was significantly higher in the highly (G6N+) than in the lightly fertilised 6-year-old grassland (G6N-) but without any difference in term of composition (C and N).

	G3N+	G6N+	G6N-	
Forage production (t DMY ha–1)	5.37^{a} (±0.21)	5.50^{a} (±0.35)	1.69^{b} (±0,07)	F = 80.01, P < 0.001
Carbon (mg/g)	434.5^{a} (±0.7)	432,9^{a} (±1.3)	431.0^{a} (±1.8)	F = 0.78, P = 0.489
Nitrogen (mg/g)	27.1^{a} (±1.7)	25.3^{a} (±2.2)	22.4^{a} (±1.6)	F = 0.88, P = 0.448

Earthworm abundance, biomass and richness



Compared to the annual crop (AC), grasslands treatments (G3N+, G6N+ and G6N-) increased significantly earthworm abundance and biomass

➔Negative effect of soil tillage (Briones and Schmidt 2017), pesticide application (Pelosi *et al.,* 2014) and low plant litter supply (Schmidt *et al.,* 2001).

Nonetheless, earthworm abundance and biomass in the 3-year-old grassland (G3N+) were significantly lower than in the 6-year-old grassland (G6N+).

➔Can be explained by the time needed for earthworms population to reach their maximum.

Grassland fertilization (G6N- *vs* G6N+) enhanced significantly earthworm abundance and biomass.

➔Attributed to the increase of the plant litter and dead roots quantity and therefore a greater supply of food for earthworms (Curry *et al.,* 2008) which was confirmed by the forage production 3.3 higher in the highly than in the lighly fertilised 6-year-old grassland.

Earthworm richness and Shannon index were not different between the 3 grassland treatments but significantly higher in the 3 grassland treatments than in the annual crop.

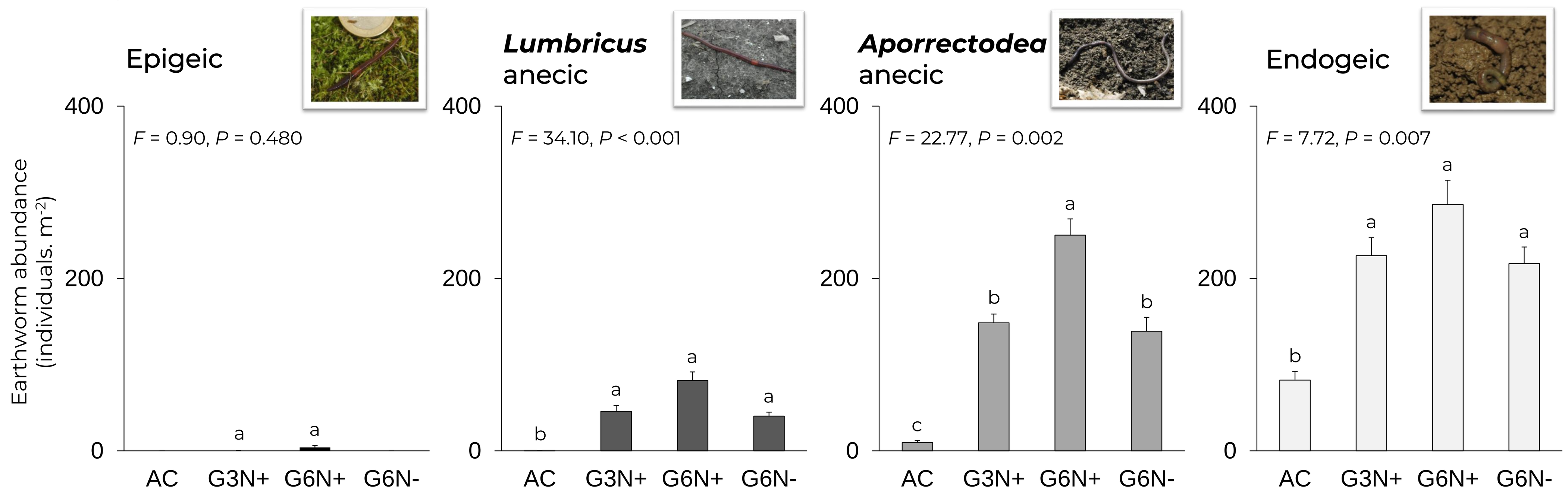
In particular due to the presence of *Aporrectodea giardi*, *Ethnodrilus zajonci* and *Octolasion cyaneum* species in grassland.

Earthworm ecological categories

Regardless of the treatments, endogeic were the most abundant and epigeic earthworms were the least abundant.

➔Commonly observed in agricultural soils (Cluzeau *et al.,* 2012).

Epigeic was dominated by *Lumbricus castaneus*, *Lumbricus* anecic by *Lumbricus centralis*, *Aporrectodea* anecic by *Aporrectodea longa longa* and endogeic by *Allolobophora chlorotica chlorotica* and *Aporrectodea caliginosa caliginosa*. Epigeic earthworms were represented only by *L. castaneus* in highly fertilised grasslands (G3N+ and G6N+) and absent in the other treatments



Aporrectodea anecic abundance enhanced by grassland duration (G3N+ *vs* G6N+)

➔Probably due to their slower growth rate than *Lumbricus* anecic or endogeic earthworms (Satchell 1980; Butt 1993) thus increasing the required time to reach their maximum population.

Aporrectodea anecic abundance enhanced by mineral fertilisation (G6N- *vs* G6N+).

➔The lower *Aporrectodea* anecic abundance and biomass in lightly than in highly fertilised 6-year-old grassland means that the development of these populations has been constrained by a lower food supply (plant litter and roots). *Aporrectodea* anecic earthworms ingest a mixture of degraded organic matter mainly from plant origin, microorganisms and soil mineral fraction (Schmidt. *et al* ,1999; Larsen *et al.,* 2016). Thus, it is possible that the amount of soil organic matter was lower in the lightly fertilised grassland.

Aporrectodea anecic earthworms with their higher burrow network than *Lumbricus* anecic earthworms (Bastardie *et al.,* 2003) allow a better soil water flux. Thus, grassland duration and fertilisation could have significant consequences on soil functioning.

Conclusions

Grassland introduction into a crop rotation significantly increases earthworm abundance, biomass and species richness. It also improves the functional structure, especially for *Lumbricus* and *Aporrectodea* anecic earthworms.

Grassland duration (G3N+ *vs* G6N+) and fertilisation (G6N+ *vs* G6N-) increase earthworm abundance and biomass and are beneficial for *Aporrectodea* anecic, without affecting *Lumbricus* anecic earthworms.

3 years of highly fertilised grassland into a crop rotation seems to be a good compromise between grassland duration and fertilisation because it (i) significantly increases earthworm abundance and improve the functional structure and (ii) leads to the same forage production as 6 years of highly fertilized grassland.

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Funded by:
European Commission
Bundesministerium für Bildung und Forschung
Eesti Teadusagentuur
Estonian Research Council
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