Hierarchization of the effect of agricultural practices in cropland on earthworm communities in Brittany

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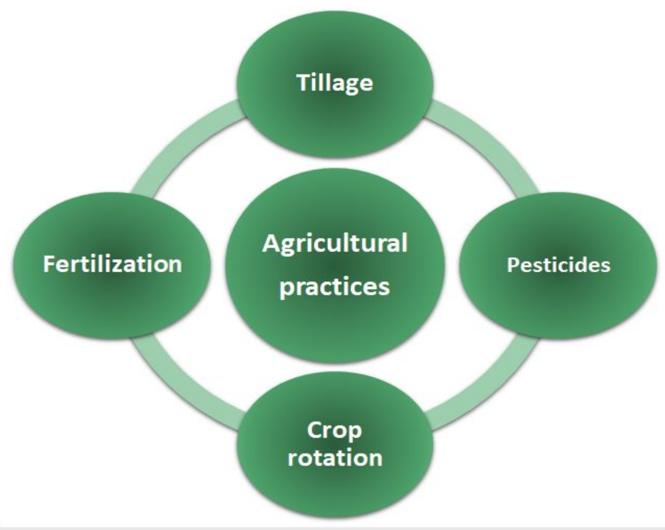
Context & problematic

Earthworms contribute to a multitude of ecosystem services in agricultural soils. However, earthworms are impacted by agricultural practices and most studies that have evaluated the effect of agricultural practices on earthworm communities have been conducted on a reduced combination of practices (Overstreet *et al.*, 2010; Bai *et al.*, 2018; Torppa *et al.*, 2022) specific to tillage (Chan, 2001; Curry *et al.*, 2002; Briones and Schmidt, 2017), fertilization (Marhan *et al.*, 2005; van Eekeren *et al.*, 2009; Niswati *et al.*, 2022), pesticides application (Yasmin *et al.*, 2010; Pelosi *et al.*, 2014; Yatoo *et al.*, 2022) and crop rotation (Pérès *et al.*, 2003; Crotty *et al.*, 2016) whereas the combination of certain practices can compensate or aggravate their effect on earthworm communities.

The aim of the present work was to hierarchize and study conjointly the effects of

I. **tillage (either ploughing or a simplified cultivation technique),**
II. **nitrogen (N) fertilization (either mineral or organic),**
III. **pesticide application (Treatment Frequency Indices for herbicides and without herbicides),**
IV. **number of plant species in the rotation (from 3 to 5) on earthworm communities.**



Materials & methods

Study site → **Agricultural practices** → **Earthworm sampling and lab. analysis** → ***Statistical analysis***

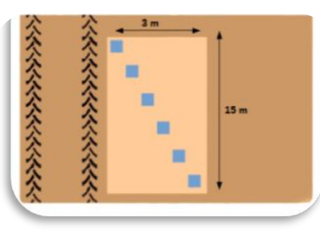
The study was conducted during 4 years on a field network of 26 annual crops plots in Brittany (France).

The agricultural practices were collected from the farmers. Then, we performed correlation matrices to eliminate auto-correlated variables. In the end, we selected 6 agricultural variables which are summarized in the table below.

Agricultural practices	MIN	MAX	MEAN	SD
Type of tillage	Ploughing = 63		SCT = 41	
Mineral fertilization (N.ha)	0	180	60.26	50.98
Organic fertilization (N.ha)	0	320	96.20	70.49
Herbicide TFI	0	4	1.62	0.88
Without herbicides TFI	0	3.23	0.52	0.63
Number of crops	3	5	3.87	0.53

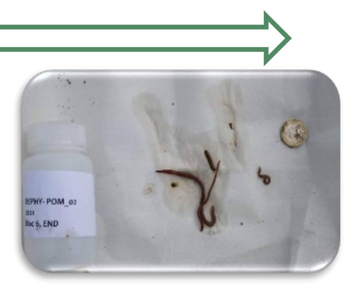
TFI = Treatment Frequency Index
SCT = simplified cultivation techniques

4 years of sampling from 2017 to 2020



Design of soil blocks

Extraction of soil blocks



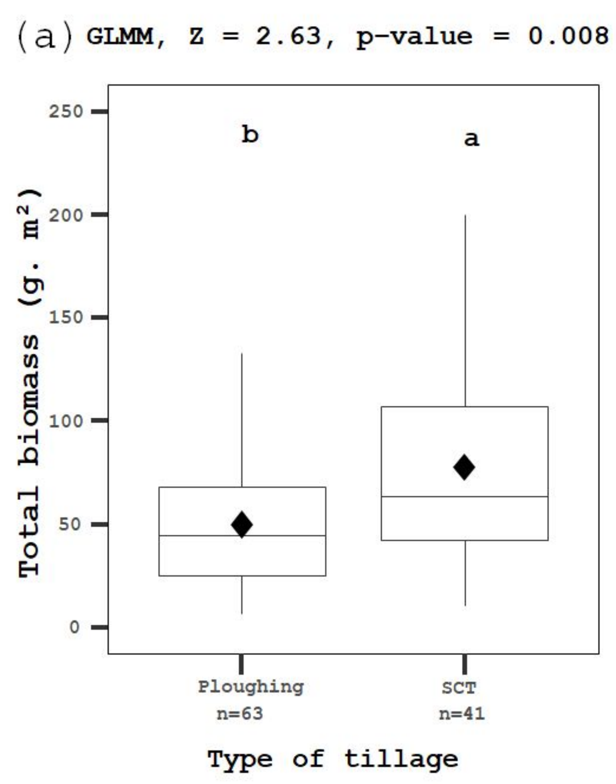
Storage in ethanol

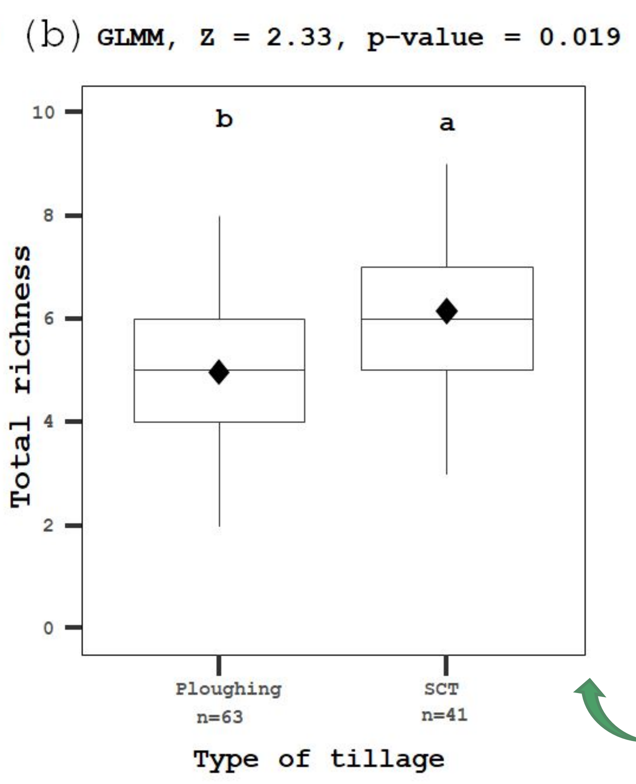
The 26 crop plots were sampled each spring by the modified protocol (ISO 23611-1:2018) which consists to extract 6 blocks of soil (20x20x25 cm) and sorted manually the earthworms.
In the laboratory, earthworms were counted, weighed by ecological categories and soil block, assigned to a developmental stage (juvenile, sub-adult, and adult), identified at the lowest taxonomic level possible (sub species, species or genus) and assigned to an earthworm category: Epigeic, *Lumbricus* anecic, *Aporrectodea* anecic, and endogeic (Bouché, 1972, 1977 ; Jégou et *al.*, 1998 ; Hoeffner et *al.*, 2019).

We used generalized linear mixed models (GLMM, Bolker *et al.*, 2009; Brooks *et al.*, 2017) to test the effect of agricultural practices (tillage, fertilization, pesticides application and crop rotation) on all the parameters of earthworm communities (total earthworm abundance, biomass and richness, ecological category abundance and biomass, and Equitability Index).

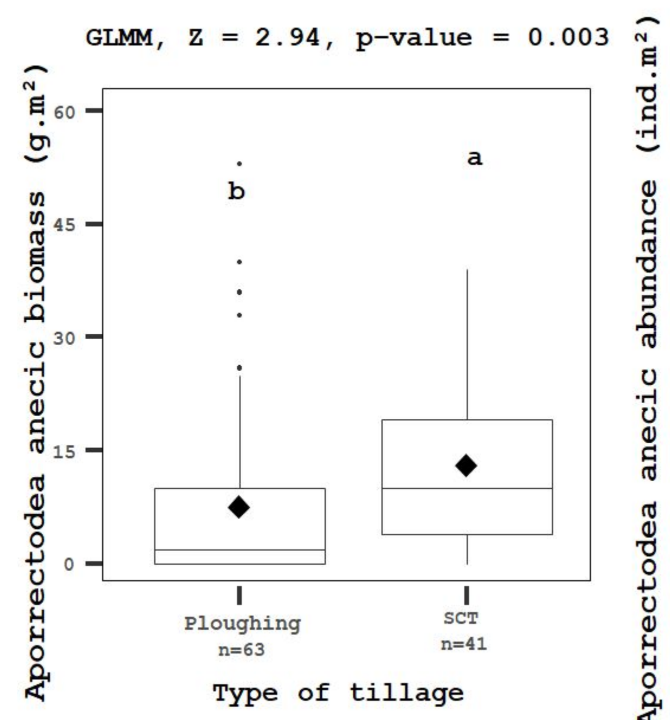
Results & discussion

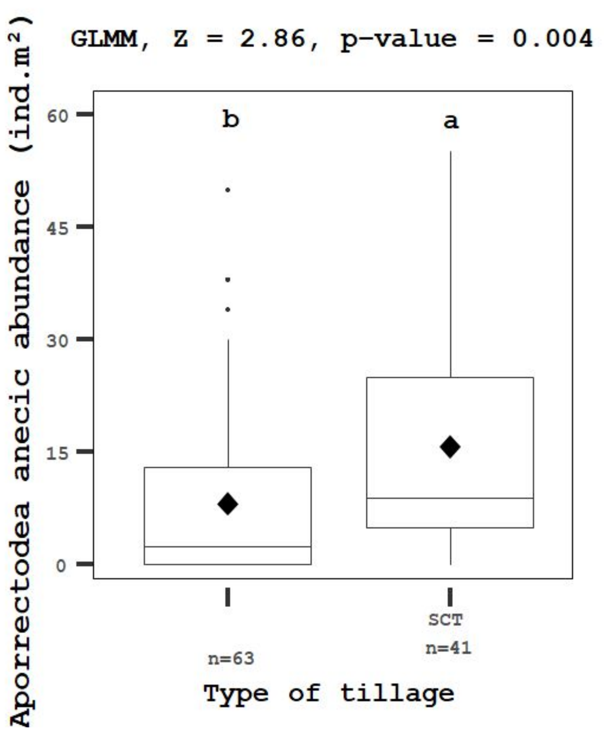
Earthworm biomass and richness





Focus on *Aporrectodea* anecic earthworms





1) Tillage is the most influential agricultural practice for earthworms

Ploughing had a significant negative effect on total earthworm biomass, earthworm species richness and *Aporrectodea* anecic.
These results are consistent with the work of Chan, (2001); Briones and Schmidt, (2017). This, can be explained by direct and indirect mortality caused by the plow and the fact that anecic earthworms with vertical galleries are more sensitive to tillage than epigeic earthworms living on the soil surface or endogeic earthworms digging horizontal galleries (Kuntz *et al.*, 2013; Pelosi *et al.*, 2014).

	Epigeic abundance			*Lumbricus* anecic abundance			*Aporrectodea* anecic abundance			Endogeic abundance		
	SE	Z	P	SE	Z	P	SE	Z	P	SE	Z	P
Type of tillage				0,28	1,91	0,056	0,42	2,86	**0,004**			
Mineral fertilization	0,004	2,52	**0,011**									
Organic fertilization							0,001	-1,92	**0,049**			
Herbicide TFI				0,1	-1,81	0,071						
Without herbicides TFI												
Number of crops	0,82	1,71	0,086									

	Epigeic biomass			*Lumbricus* anecic biomass			*Aporrectodea* anecic biomass			Endogeic biomass		
	SE	Z	P	SE	Z	P	SE	Z	P	SE	Z	P
Type of tillage				0,31	1,96	0,05	0,51	2,94	**0,003**	0,19	1,85	0,063
Mineral fertilization	0,004	2,74	**0,006**	0	2,13	**0,033**						
Organic fertilization												
Herbicide TFI				0,12	-1,79	0,0732	0,15	1,02	0,307			
Without herbicides TFI							0,24	2,11	**0,034**			
Number of crops	0,65	1,87	0,062									

2) Organic and mineral nitrogen fertilization improve epigeic and anecic earthworms

Organic nitrogen fertilization (kg/ha) significantly increased the abundance of *Aporrectodea* anecic species. The epigeic earthworms and the biomass of *Lumbricus* anecic species increased significantly when the amount of mineral nitrogen fertilization (kg/ha) increases.

In agreement with the work of Jin *et al.*, (2022) and Niswati *et al.*, (2022), this result can be explained by the fact that fertilization is a secondary food resource readily available to earthworms (Leroy *et al.*, 2008, 2009; Zhu and Zhu, 2015).

3) Pesticide application frequency indices negatively impact *Aporrectodea* anecic earthworms

Treatment Frequency Indices (TFI) of pesticides application (without herbicides) had significant negative effects on biomass of *Aporrectodea* anecic earthworms.
The studies of Collange *et al.*, (2010) and Yatoo *et al.*, (2022) confirm this result. Indeed, pesticides can cause individual earthworm mortality directly or indirectly by disrupting enzymatic activities, affecting reproductive functions or modifying earthworm behavior (Pelosi *et al.*, 2014b; Datta *et al.*, 2016).
In addition, these effects particularly impact epigeic and anecic earthworms, which are the most exposed to chemical molecules due to their presence on the upper surface of the soil (Datta et *al.*, 2016a; Yatoo et *al.*, 2022)

Conclusions

- ✔ Conventional tillage is the most influential agricultural practice on earthworms, followed respectively by fertilization, pesticide application and crop rotation.
- ✔ Ploughing impacts earthworms biomass, richness and *Aporrectodea* anecic species.
- ✔ Fertilization (organic and mineral) increased epigeic and anecic earthworms.
- ✔ Treatment Frequency Indices (TFI) of pesticide application (without herbicides) influence negatively the biomass of *Aporrectodea* anecic species.

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