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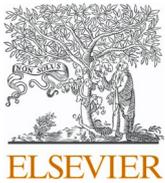
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## A complex relationship between cropping systems and soil macrofauna: Influence of practice intensity, taxa and traits

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### ABSTRACT

Larger soil organisms have often been reported as the most sensitive to disturbances caused by cropping practices. However, soil macrofauna comprises groups with a wide diversity of morphological and ecological features, which may respond differently to applied practices. In order to further assess the effect of cropping systems on soil macrofauna, macrofauna organisms were extracted from soil blocks over 21 fields (each comprising three plots) located in the Paris basin, in autumns 2020 and 2021. Fields belonged to conventional, conservation or organic systems, either long-established ( $\geq 7$  years) or in transition ( $\leq 3$  years). Tillage, pesticide treatment and organic matter input intensity were assessed in each field using composite indexes of practice intensity. Macrofauna density and diversity, earthworm ecological categories, species richness and functional traits were investigated. Our results showed that the density and diversity of macrofauna demonstrated few differences regarding different cropping systems, with highly variable effects across groups and years. Specific macroarthropod groups responded differently to tillage, pesticide treatment and organic input intensity, but not over the two years of the study. Regarding earthworms, high tillage intensity had a negative effect on the density and biomass of epi-aneic juveniles and on species with a small body size. Higher organic matter inputs had a negative effect on the density and biomass of endogeic earthworms, and could be related to several earthworm functional traits (body length, mass/length ratio, carbon preferences). Effects of pesticide treatments were less clear, although they could have impacted some earthworm species. More generally, taxonomic and functional trait approaches of earthworm community led to similar conclusions. Overall, our results support the need to account for (i) the actual intensity of practices in cropping systems and (ii) the different taxonomic, trophic and ecological groups of macrofauna, in order to assess the effects of cropping systems on soil biodiversity.

### 1. Introduction

Intensive management of agroecosystems is a major cause of soil biodiversity loss (Gardi et al., 2013). However, some groups of soil organisms could respond faster to disturbances caused by agricultural practices in relation to their size or their trophic position (Coudrain et al., 2016; Coller et al., 2022). In particular, larger organisms were shown to be the most impacted by agricultural intensification and to be more sensitive to disturbances caused by practices (Postma-Blaauw et al., 2010, 2012).

Soil macrofauna comprises the largest soil invertebrates ( $> 2$  mm) and includes many taxa (Lavelle and Spain, 2001). Macroarthropods are involved in several functions at the soil surface such as litter decomposition (Frouz, 2018; Chassain et al., 2021) and pest regulation through

predation (Kromp, 1999). Among them, the extensively studied Carabidae (i.e. ground beetles) are considered as useful bioindicators to compare different cropping systems, as their density and diversity vary with applied practices (Kromp, 1999; Kotze et al., 2011; Burgio et al., 2015). Earthworms are major drivers of soil properties and functioning through their activities of burrowing, tunneling, feeding and casting, that influence organic matter decomposition (Barrios, 2007) and stabilization (Bertrand et al., 2015), microbial activity (Kladivko, 2001) and soil structure (Joschko et al., 1989; Young et al., 1998). They are considered as bioindicators of soil quality (Pères et al., 2011), soil biodiversity (Bispo et al., 2009) and soil disturbances caused by different cropping systems (Paoletti, 1999; Masin et al., 2020).

Conventional cropping systems reportedly have negative effects on soil macrofauna density, biomass, diversity and activity (Eggleton et al.,

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2005; Yin et al., 2022), particularly on earthworms (Young and Ritz, 2000; Tsiafouli et al., 2015; Briones and Schmidt, 2017). Alternative systems, such as organic farming (i.e. absence of pesticides and synthetic fertilizers) and conservation agriculture (i.e. no-tillage, permanent soil cover and diversification of the crop rotation), may benefit soil macrofauna density and diversity. In particular, organic systems demonstrated positive effects on total macroarthropod density (Maeder et al., 2002; Hole et al., 2005; Pelosi et al., 2015), as well as on the density, biomass and diversity of earthworms (Bettiol et al., 2002; Hole et al., 2005; Pelosi et al., 2015), but variable results were reported depending on taxonomic groups (Bengtsson et al., 2005; Henneron et al., 2015; Patterson et al., 2019). Conservation agriculture also demonstrated benefits, in particular on earthworm density and biomass (Mele and Carter, 1999; Hernández et al., 2017; Dulaurent et al., 2022). However, few studies have been conducted on soil biodiversity in conservation systems (Christel et al., 2021), which prevents assessing the actual variability of soil organisms' responses. In addition, few studies assessed the effect of the transition from conventional to alternative systems, or from one alternative to the other, on soil macrofauna. The first years of the transition to organic agriculture were found to benefit ground-dwelling macroarthropod (Lundgren et al., 2006), but depending on the newly applied practices (Schipanski et al., 2014; Jabbour et al., 2016; Gareau et al., 2019) and on taxa (Tsutsui et al., 2018). Similarly, earthworm density reportedly increases in the first year of the transition to organic (Irmiler, 2010) and to no-tillage systems (Stubbs et al., 2004), however this might be transitory (Pelosi et al., 2015, 2016).

Management practices are highly variable between and within cropping systems categories, and have the potential to influence soil macrofauna. Macroarthropods were reported to respond unevenly to tillage (Stubbs et al., 2004; Patterson et al., 2019), crop rotation (Patterson et al., 2019), organic matter inputs (de Souza et al., 2016; Růžicková et al., 2020), and pesticide treatments (Wardle et al., 1999; Burgio et al., 2015; Pearsons and Tooker, 2021). Regarding earthworms, several studies demonstrated a negative effect of tillage on abundance (Wardle, 1995; Roger-Estrade et al., 2010; Diallo et al., 2023), although this effect is not always observed (Capowiez et al., 2009a), can be modulated by soil properties and varies for different ecological categories (Chan, 2001). Pesticides reportedly have a negative effect on earthworm density (Pelosi et al., 2014a; Datta et al., 2016), while organic matter inputs may benefit both earthworm density (Birkhofer et al., 2008; Ponge et al., 2013; Bertrand et al., 2015) and biomass (Capowiez et al., 2009b; D'Hose et al., 2018).

Overall, the effects of cropping systems and practices on soil macrofauna seem highly variable, with previous studies showing inconsistent results. Therefore, the development of alternative systems relying on strong soil macrofauna communities requires a finer level of description than system categories or single practices (Roger-Estrade et al., 2010). To that aim, some authors have suggested using indicators on the intensity and frequency of soil disturbances to assess the effects of management intensity on soil macrofauna (Gareau et al., 2019; Masin et al., 2020). Indicators of practice intensity recently developed by Büchi et al. (2019), and used for soil mesofauna (Chassain et al., 2023, 2024), could help assessing the effects of cropping systems on soil macrofauna.

Understanding responses of soil organisms to disturbances, such as the ones caused by agricultural practices, also requires an adapted description of their diversity. This increasingly relies on the study of organism's functional traits (Hedde et al., 2012; Pey et al., 2014; dedeCastro-Arrazola et al., 2022). The effects of agricultural practices on macrofauna traits were mostly studied for ground beetles (Cole et al., 2002; Liu et al., 2012; Boinot et al., 2019) and in a lesser extent for earthworms (Pelosi et al., 2014; Pelosi et al., 2016; Frazão et al., 2019). In addition, earthworm communities are often characterized by assigning species to ecological categories (i.e. anecic, endogeic and epigeic). These categories were historically defined by Bouché (1972), (1977) and were recently redefined in Bottinelli et al. (2020). The use of these redefined ecological categories could have an important impact on

understanding the effects of cropping practices on earthworm communities.

Overall, the objectives of our study were 1) to assess the effects of various cropping systems, long-established or in transition, on soil macrofauna, 2) to test if assessing practice intensity (tillage, pesticide treatments, organic matter inputs) allows for a better characterization of the effects of cropping systems on soil macrofauna, and 3) to investigate the relevance of the taxonomic and functional approaches of earthworm diversity to determine the effects of cropping systems and practices on macrofauna. We hypothesized that systems with lower physical (i.e. tillage) and chemical (i.e. pesticide treatments) disturbances present higher macrofauna density and diversity as well as a modified community composition compared to intensive systems, and that most groups benefit from higher organic matter input. We expected variable effects of practices depending on taxonomic, ecological or functional groups of macroarthropods or earthworms, and a main effect of tillage intensity on earthworm species and functional traits.

## 2. Materials and methods

### 2.1. Study sites and sampling design

The study was conducted over 21 fields owned by farmers in the Paris area (Yvelines, Eure-et-Loir and Essonne departments), France. Climate was temperate with 600–700 mm of annual precipitations and a mean annual temperature of 11°C. Soils were silty or clayed, with a pH of 5.5–8.2 and a bulk density of 1.1–1.5 at 0–20 cm depth (Table S1, for more details see Chassain et al., 2024). Fields were cropped with winter wheat in 2020–2021 and with various crops or cover crops in 2021–2022 (Table 1).

Three replicate plots were defined in each field, located at the corners of an equilateral triangle with a side length of 25 m, and set at least 25 m apart from the field margins. Plots were positioned to avoid tractor traffic tracks. Samplings were performed in each plot in autumn 2020 from October 19th to December 2nd, and in autumn 2021 from October 25th to November 22nd. They took place minimum one week after sowing to allow for a partial recovery of soil organisms after mechanical operations.

### 2.2. Agricultural practices and intensity indexes

Fields were assigned to one of the six studied cropping systems, namely long-established systems ( $\geq 7$  years) under conventional (Conv,  $n = 6$  fields), organic (OA,  $n = 3$ ) or conservation agriculture (CA,  $n = 3$ ), and systems in transition ( $\leq 3$  years) from conventional to organic (Conv-OA,  $n = 3$ ), conventional to conservation (Conv-CA,  $n = 3$ ) or conservation to organic agriculture (CA-OA,  $n = 3$ ) (Table 1).

Information regarding practices applied to each field (e.g. tillage, organic matter inputs, pesticide treatments, crop rotation, dates of seedling and harvest) was collected by conducting a survey amongst the farmers. Primary indicators and composite indexes of practice intensity were selected from Büchi et al. (2019) to describe practices associated to crop rotation, tillage, pesticide treatments and organic inputs in each field (Table 1, Table S2). Composite indexes of tillage, pesticide treatment and organic input intensity were computed following the methodology presented in Chassain et al., (2024) (Table S2). The composite indexes of tillage intensity (Itill) and organic matter input intensity (Iorg) were obtained by an additive combination of the normalized values of primary indicators divided by the number of indicators. The composite index for pesticide treatment intensity (Itreat) was the normalized value of the total number of applied treatments (e.g. herbicides, fungicides, insecticides).

### 2.3. Soil and macrofauna sampling

Composite soil samples were collected on each plot at 0–10 and

**Table 1**  
Cropping systems, age, crops and practice intensity indexes characterizing the 21 fields of the study.

System	Field	Age	Crop		Itill		Itreat		Iorg	
			2020	2021	2020	2021	2020	2021	2020	2021
Conv	A5	20	wheat	barley	0.3	0.3	0.5	0.8	0.58	0.58
	A9	20	wheat	rapeseed	0.9	0.8	0.4	0.8	0.50	0.50
	A10	20	wheat	alfalfa	0.4	0.1	1.0	0.9	0.50	0.50
	A12	20	wheat	cover	0.5	0.5	0.6	0.6	0.42	0.42
	A16	20	wheat	mustard	0.4	0.3	0.9	0.7	0.67	0.67
	A21	20	wheat	barley	0.4	0.5	0.5	0.6	0.50	0.50
CA	A2	17	wheat	cover	0	0	0.7	0.8	0.58	0.58
	A8	7	wheat	mustard	0.1	0	0.2	0.2	0.50	0.50
	A17	10	wheat	rapeseed	0	0	0.9	0.8	0.58	0.58
Conv-CA	A1	3	wheat	wheat	0.1	0.1	0.8	1.0	0.50	0.50
	A14	3	wheat	cover	0.1	0	0.8	0.5	0.08	0.08
	A15	3	wheat	wheat	0.1	0.1	0.8	0.8	0.29	0.29
OA	A3	20	wheat	wheat	0.4	0.7	0	0	0.75	0.75
	A4	19	wheat	bare	0.9	0.7	0	0	1.00	1.00
	A11	20	wheat	cover	0.8	0.7	0	0	0.63	0.63
Conv-OA	A7	2	wheat	rye	0.8	0.9	0	0	0.38	0.38
	A18	3	wheat	bare	0.7	0.5	0	0	0.38	0.38
	A19	2	wheat	clover	0.5	0.5	0	0	0.71	0.71
CA-OA	A6	2	wheat	bare	0.7	0.5	0	0	0.75	0.83
	A13	2	wheat	rye, lentil	0.3	0.3	0	0.1	0.67	0.75
	A20	2	wheat	cover	0.7	0.5	0	0	0.67	0.54

Conv: conventional agriculture, CA: conservation agriculture, OA: organic agriculture, Conv-CA: transition from conventional to conservation ( $\leq 3$  years), Conv-OA: transition from conventional to organic ( $\leq 3$  years), CA-OA: transition from conservation to organic ( $\leq 3$  years), Itill: tillage intensity index, Itreat: pesticide treatment intensity index, Iorg: organic matter input intensity index.

10–20 cm depth by mixing eight soil cores obtained with an auger. A fraction of soil sampled in 2020 was sieved at 4 mm, air dried and analyzed by the INRAE Laboratory of Arras to characterize the main soil properties.

A soil block of 25 × 25 × 25 cm was extracted on each plot and hand-sorted to collect earthworms and other macrofauna organisms. Earthworms were preserved in 4 % formol and other organisms in 70 % ethanol.

All macrofauna organisms were identified at the order level under binoculars. Coleoptera were further identified at the family level. Earthworms were sorted depending on their development stage (adults, sub-adults, juveniles) and their ecological category (endogeic, epigeic, anecic, epigeic, intermediate) following Bottinelli et al. (2020). Adults and sub-adults were identified at the species level.

The assessment of ecological categories of earthworms was refined by using for each species the percentages by which it belongs to the three main ecological categories (anecic, endogeic, epigeic) according to Bottinelli et al. (2020) (Table 2). We considered these three categories as three attributes of the same variable and calculated the community-weighted means (CWM) using the *FD* package on R (Laliberté et al., 2022). This conducts to obtain a fuzzy estimate of the share of the different ecological categories in the community of adult earthworms (as only adults were identified at the species level).

**Table 2**  
Attribution of collected earthworm species to the different ecological categories and percentage of belonging of each species to the three main categories after Bottinelli et al. (2020).

Species	Ecological categories	% of belonging to categories		
		% epigeic	% anecic	% endogeic
<i>A. chlorotica</i>	Intermediate	31	31	38
<i>A. caliginosa</i>	Endogeic	16	4	80
<i>A. giardi</i>	Epi-anecic	30	70	0
<i>A. icterica</i>	Endogeic	0	8	92
<i>A. longa</i>	Epi-anecic	32	68	0
<i>A. rosea</i>	Endogeic	15	0	85
<i>L. castaneus</i>	Epigeic	90	10	0
<i>L. friendi</i>	Epi-anecic	34	66	0
<i>L. terrestris</i>	Epi-anecic	30	70	0

All earthworms were weighed by species or by groups to obtain an average biomass ( $\text{g.m}^{-2}$ ). Earthworm taxonomic diversity was estimated with species richness and by calculating Shannon and Pielou's evenness indexes.

#### 2.4. Earthworm functional traits

Earthworm functional traits were selected as parameters that could be influenced by the effects of practices and associated changes in soil properties. We thus selected four morphological (body length, body mass/length ratio, cocoon diameter, epithelium type), one ecological (carbon preferences) and one behavioral traits (vertical distribution in soil) (Pelosi et al., 2014; Briones and Álvarez-Otero, 2018; Frazão et al., 2019). The body length and cocoon diameter may reflect physical disturbances, as the larger the earthworms or cocoons are, the more they risk to be impacted by soil physical disturbances such as tillage (Pelosi et al., 2014; Frazão et al., 2019). Epithelium type represents the elasticity or strength of earthworm's skin, thus its resistance to different types of pressures, and may respond to tillage intensity (Pelosi et al., 2014; Frazão et al., 2019). Species with a preference for environments that are rich in organic carbon are expected to be present in fields with a high quantity of organic matter and higher organic input intensity. Finally, the vertical distribution in soil may help to determine the depth at which different practices are impacting soil organisms. All traits for earthworm species were collected from the BETSI database (CESAB/FRB) (Pey et al., 2014) (Table S3).

For each trait, the community-weighted means (CWM) were calculated as the weighted mean of trait classes in communities (Lavorel et al., 2008). Functional richness (Fric) and evenness (Feve) were computed as indexes of the functional diversity of earthworms (Villéger et al., 2008) using the *FD* package on R (Laliberté et al., 2022).

#### 2.5. Statistical analyses

Prior to analyses, the values of the three plots were averaged to obtain one value per field. The macrofauna density was obtained by dividing abundance data by the area of extraction ( $0.0625 \text{ m}^2$ ).

Macroarthropod community composition (also including gastropods) was compared in different cropping systems by performing a

principal component analysis (PCA). Earthworm species community composition was assessed using a principal coordinate analysis (PCoA) with Bray-Curtis distance, followed by a permutational analysis of similarities (ANOSIM) to test for significant differences in composition between cropping systems. Species present on a single plot and in a single year were not included in the analysis.

The effects of cropping systems or practice intensity indexes on soil macrofauna density and diversity were assessed for each sampling year separately. The normality and homogeneity of variances were tested with a Shapiro-Wilk test ( $\alpha > 0.05$ ) and a Bartlett test ( $\alpha > 0.05$ ). Relations between cropping systems (i.e. system categories - discrete variable) or practice intensity (i.e. each composite index or primary indicators separately - continuous variables) as explanatory variables and soil macrofauna (i.e. macroarthropod density and order diversity, earthworm diversity, biomass, functional traits and percentages of belonging to ecological categories) as response variables were assessed using generalized linear models (GLM) with a quasi-Poisson error distribution and an identity link. Differences between cropping systems were tested using Tukey HSD post-hoc tests.

All the analyses were performed using R software version 3.6.3 (R Development Core Team 2020) and the *stats* (R Development Core Team 2020), *ade4* (Dray and Dufour, 2007), *vegan* (Oksanen et al., 2022) and *multcomp* (Hothorn et al., 2022) packages.

### 3. Results

#### 3.1. Effects of cropping systems on macroarthropod density and diversity

Six classes including 12 orders of macroarthropods were collected in 2020 and 2021 (Table S4). The mean total densities of macroarthropods were 88 ind.m<sup>-2</sup> and 123 ind.m<sup>-2</sup> in 2020 and 2021 respectively. The main macroarthropod groups were Coleoptera (33–68 % depending on cropping systems), Diplopoda (3–52 %), Araneae (4–15 %) and Chilopoda (0–14 %) (Table S4). In addition to macroarthropods, Gastropoda (mostly Limacidae) were found in three fields belonging to three different systems in 2020 and in 12 fields from all systems in 2021 (Table S4).

The results of the PCA for 2020 showed that macroarthropod communities in CA and Conv-CA differed from communities in Conv-OA and CA-OA (Fig. 1a.2), both of which had a low density of all macroarthropod groups (Fig. 1a.1). In addition, communities were highly variable within CA fields in 2020 (Fig. 1a.2), and within CA-OA fields in 2021 (Fig. 1b.2).

The total macroarthropod density and number of orders did not differ significantly between cropping systems (Table S4). In 2020, we observed a significantly higher density of Chilopoda in CA than in CA-OA and of coleopteran larvae in CA than in Conv ( $P < 0.05$ , Table S4). In 2021, the density of Carabidae was significantly higher in Conv-CA than in Conv and OA (Table S4).

As for cropping systems, total macroarthropod density was not

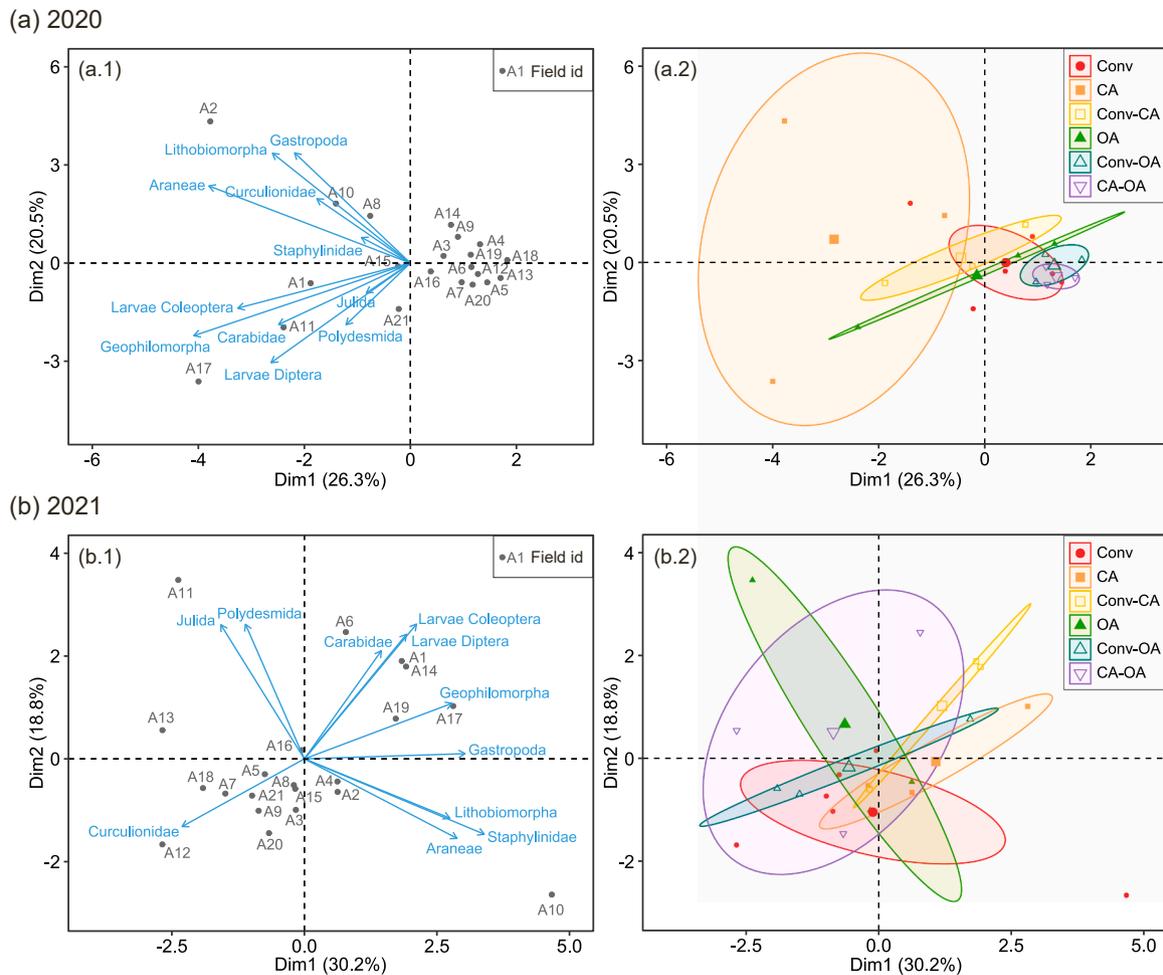


Fig. 1. PCA on the density of macroarthropod groups in (a) 2020 and (b) 2021. Gastropod density is also included in the analysis. Points represent fields and ellipses represent 95 % confidence estimates for the different cropping systems. Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions.

significantly influenced by practice intensity (Table 3a). However, the number of orders was significantly higher under high pesticide treatment intensity in 2020, and lower under high tillage intensity in 2021 (Table 3b). In addition, practice intensity indexes showed various effects depending on macroarthropod groups and years. An increase in tillage intensity was related to an increase in the density of Diplopoda (i.e. Polydesmida) in 2020 and of Curculionidae in 2021, and to a decrease in the density of Chilopoda in 2021 (Table 3a). On the other hand, an increase in pesticide treatment intensity was associated with an increased density of Araneae in 2020 and Chilopoda (i.e. Lithobiomorpha) in 2021, and with a decrease in Diplopoda (i.e. Julida) density in 2020 (Table 3a). Organic input intensity had a positive effect on the density of Diplopoda (i.e. Julida) in 2020 and Chilopoda (i.e. Geophilomorpha) in 2021, and a negative effect on the density Chilopoda (i.e. Lithobiomorpha) in 2020 (Table 3a). Additional effects were observed using primary practice indicators (Table S5).

### 3.2. Effects of cropping systems on earthworm density, biomass and species diversity

On average, the density of earthworms was 271 ind.m<sup>-2</sup> in 2020 and 265 ind.m<sup>-2</sup> in 2021 (Table S6). More juveniles than adults were observed in all systems (Table S6). Earthworm total density, total biomass and diversity (species richness, Shannon and evenness indexes) did not differ significantly between systems in 2020 (Fig. 2a, Table S6). In 2021, total earthworm biomass was significantly higher in Conv-CA than in Conv, Shannon index was higher in Conv-CA than in CA-OA, and evenness index was lower in CA-OA than in the other systems (Table S6). The ratio of total adults/juveniles biomass was higher in OA than in Conv-OA in 2021 (Table S6). Earthworm total density, biomass and diversity were not significantly related to practice intensity indexes (Fig. 2a, Table 4a and b).

Collected earthworms were mostly endogeic or epi-aneic, and one species was attributed to the intermediate ecological category (i.e. epi-endo-aneic) (Table 2). As almost no epigeic individuals were collected (five juveniles in A13 in 2020, one adult in A3 in 2021), they were grouped with epi-aneics for the analyses. The density of epi-aneic juveniles was higher in CA than in OA in 2020, but we found no other difference neither in 2020 nor in 2021 (Table S6). An increase in tillage intensity was associated with a significant decrease in the density and biomass of epi-aneic juveniles in 2020, with similar results

**Table 3**

Effects of practice intensity on macroarthropod (a) density (ind.m<sup>-2</sup>) and (b) diversity in 2020 and 2021. Results for gastropod density are also provided. *t*-values and *P*-values were obtained using GLM with a quasi-Poisson error distribution. Bold values indicate significant effects (*P* < 0.05 \*, < 0.01 \*\*, < 0.001 \*\*\*). Additional results using primary indicators are reported in Table S5.

	2020						2021					
	Itill		Itreat		Iorg		Itill		Itreat		Iorg	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
(a) Density												
Total macroarthropod	-0.58	0.569	0.89	0.381	0.47	0.645	-1.50	0.151	0.60	0.555	1.10	0.286
Araneae	-1.94	0.067	2.66	<b>0.015*</b>	0.18	0.858	-1.30	0.209	1.50	0.150	0.67	0.510
Chilopoda	-1.47	0.158	2.09	0.050	-3.21	<b>0.005**</b>	-3.04	<b>0.007**</b>	1.66	0.112	0.75	0.462
Geophilomorpha	-1.51	0.149	1.89	0.074	-0.67	0.514	-0.64	0.532	0.25	0.809	3.27	<b>0.004**</b>
Lithobiomorpha	-0.62	0.545	1.30	0.208	-2.66	<b>0.016*</b>	-2.09	0.050	2.77	<b>0.012*</b>	-1.76	0.094
Diplopoda	1.93	0.069	-0.76	0.456	2.32	<b>0.031*</b>	1.32	0.203	-1.09	0.292	1.78	0.091
Julida	1.63	0.120	-2.64	<b>0.016*</b>	2.29	<b>0.034*</b>	1.08	0.293	-1.29	0.211	2.04	0.056
Polydesmida	2.27	<b>0.035*</b>	-0.22	0.828	1.97	0.063	1.48	0.154	-0.65	0.523	1.87	0.077
Coleoptera	-1.78	0.091	1.18	0.254	-0.39	0.702	-2.00	0.060	0.53	0.605	0.17	0.865
Carabidae	-0.50	0.624	0.57	0.577	-0.31	0.758	-2.01	0.059	0.03	0.975	-1.53	0.142
Curculionidae	-1.40	0.179	1.59	0.129	0.11	0.915	2.23	<b>0.038*</b>	-1.80	0.088	-2.05	0.054
Staphylinidae	0.87	0.393	0.32	0.754	-0.31	0.761	-1.83	0.082	1.20	0.245	0.00	0.997
Larvae	-1.85	0.080	0.87	0.395	-0.36	0.724	-0.74	0.465	-0.08	0.938	0.97	0.344
Gastropoda	-1.97	0.063	0.98	0.338	-0.22	0.824	-4.48	<b>&lt;0.001***</b>	1.45	0.164	-0.09	0.929
(b) Diversity												
Nb macroarthropod orders	-1.50	0.149	2.48	<b>0.022*</b>	0.04	0.971	-2.89	<b>0.009**</b>	1.40	0.177	1.36	0.189

Itill: tillage intensity index, Itreat: pesticide treatment intensity index, Iorg: organic input intensity index.

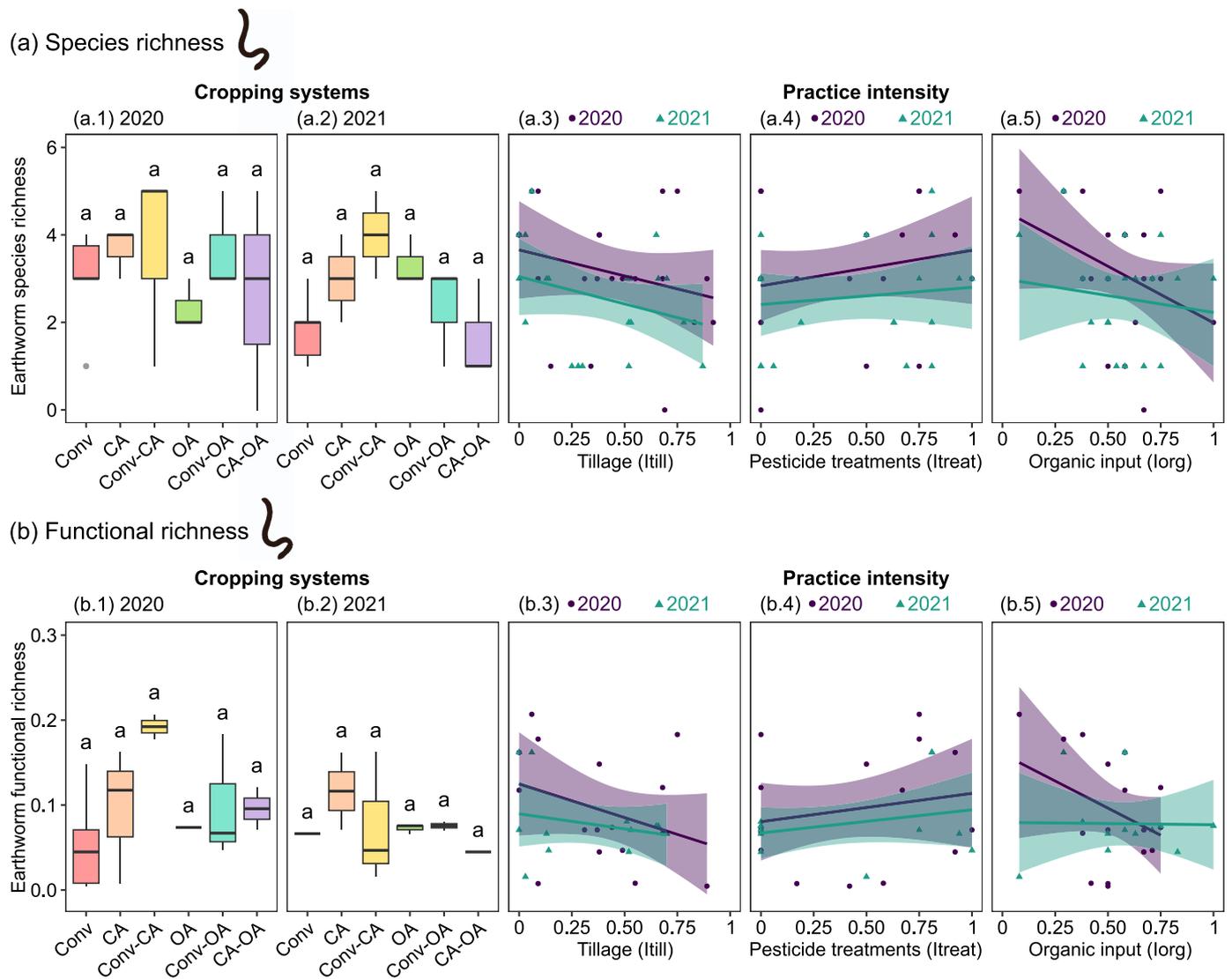
for biomass in 2021 (Table 4a and b). Pesticide treatment intensity had no significant effect on earthworms groups. Organic input intensity had a negative effect on the density of endogeic earthworms in 2020 and on their biomass in both years, especially regarding juveniles (Table 4a and b). In contrast, it had a positive effect on the biomass of epi-aneic adults in 2021 (Table 4b). Additional effects observed with primary indicators are presented in Table S5.

Eight species of earthworms were identified each year. Species with the higher occurrence were *Allolobophora chlorotica* (Savigny, 1826), *Aporrectodea caliginosa* (Savigny, 1826) and *Aporrectodea longa* (Ude, 1885), followed by *Aporrectodea rosea* (Savigny, 1826) and *Aporrectodea icterica* (Savigny, 1826). Only one individual of *Lumbricus friendi* Cognetti de Martiis, 1904 was collected in 2020 and one of *Lumbricus castaneus* (Savigny, 1826) in 2021. *A. chlorotica*, *A. caliginosa* and *A. longa* were encountered in all systems in both years (Table S7). *A. rosea* was absent in almost all organic sites (except in one CA-OA field in 2020 and one Conv-OA field in 2021). *Lumbricus terrestris* Linnaeus, 1758 was absent from all long-established systems in both years (Table S7).

We found no significant effect of cropping systems on the density or biomass of earthworm species (Table S7). In addition, the PCoA showed no difference in earthworm species community between years or cropping systems (Figure S1). However, the density and biomass of *A. icterica* (in 2021 only) and *A. rosea* decreased as tillage intensity increased (Table 4a and b). The density and biomass of *A. caliginosa* and biomass of *L. terrestris* decreased as pesticide treatment intensity increased in 2020 (Table 4a and b). An increase in organic input intensity had a negative effect on the density and biomass of *A. icterica* and *A. rosea* in both years (Table 4a and b). In 2020, it also had a negative effect on the density of *L. terrestris* (Table 4a) and on the biomass of *A. caliginosa* (Table 4b). Inversely, organic inputs had a positive effect on the density of *A. longa* in 2021 (Table 4a).

### 3.3. Effects of cropping systems on earthworm percentages of belonging to the main ecological categories

The percentages of belonging to the three ecological categories of earthworm communities were similar in all cropping systems (GLM, *P* > 0.05) and were not influenced by tillage and pesticide treatment intensity (Table 4c). However, in 2020, we observed a decrease in the percentage of endogeics in the community as organic input intensity increased (Table 4c), which is consistent with the decrease in endogeic



**Fig. 2.** Effects of cropping systems and practice intensity on earthworm (a) species richness and (b) functional richness in 2020 and 2021. Results of GLM showed no significant difference between systems (indicated by similar lower-case letters) nor significant effect for intensity indexes ( $P > 0.05$ ). Conv, CA and OA: conventional, conservation and organic agriculture; Conv-CA, Conv-OA and CA-OA: initial-recent system transitions; Itill: tillage intensity index, Itreat: pesticide treatment intensity index, Iorg: organic input intensity index.

adults.

### 3.4. Effects of cropping systems on earthworm functional traits

Indexes of earthworm functional diversity did not differ significantly between systems and did not respond to practice intensity, as it is reported here for the functional richness (Fig. 2b). Furthermore, the PCA on earthworm trait CWM did not show difference between functional communities in different cropping systems (Figure S2). However, in 2020, Conv-CA systems had a significantly higher proportion of earthworm species with a short body (20–50 mm) and producing small diameter cocoons (< 4 mm) than Conv-OA systems (GLM,  $P < 0.05$ ).

Short-bodied earthworms (20–50 mm) were less present as tillage and organic input intensity increased and as pesticide treatment intensity decreased in 2020, with similar effects of organic inputs in 2021 (Table 4d). In addition, earthworms with small body mass/length ratio (1–7 g.mm<sup>-1</sup>) and medium carbon preference (20–33 mg.kg<sup>-1</sup>) were disfavored under high organic input intensity in 2020 (Table 4d).

A synthesis of the observed effects of cropping systems and practice intensity on macroarthropods and earthworms is provided as [supplementary material](#) (Table S8).

## 4. Discussion

### 4.1. Overall effects of cropping systems on soil macrofauna and need to take into account the intensity of practices

In this study, total density and diversity of macroarthropods and earthworms were not significantly different between alternative and conventional systems, nor between long-established and transitioning systems. Tillage, pesticide treatment and organic input intensity indexes did not reveal more effects on total density nor on diversity. However, they allow to better characterize the effects of cropping systems on macroarthropod taxa and earthworm ecological categories, species and traits.

Our results showed several macroarthropod groups to be influenced by tillage, pesticide treatment or organic input intensity, whereas earthworms were influenced mostly by tillage and organic input intensity. Practices have direct and indirect effects that can influence all the representatives of soil macrofauna depending on their intensity.

Tillage widely influenced the macrofauna community, with effects observed in one or both years of the study. Observed influence of tillage on macrofauna could be due to direct effects, such as physical damage

**Table 4**

Effects of practice intensity on earthworm (a) density (ind.m<sup>-2</sup>), (b) biomass (g.m<sup>-2</sup>), (c) belonging to ecological categories (%) and (d) functional traits in 2020 and 2021. *t*-values and *P*-values were obtained using GLM with a quasi-Poisson error distribution. Bold values indicate significant effects (*P* < 0.05 \*, < 0.01 \*\*, < 0.001 \*\*\*). Percentages of belonging to ecological categories and functional traits were assessed using CWM. Only trait attributes showing significant results are presented. Additional results using primary indicators are reported in Table S5.

	2020						2021					
	Itill		Itreat		Iorg		Itill		Itreat		Iorg	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
<b>(a) Density</b>												
Total	-1.93	0.069	0.89	0.384	-1.51	0.149	-0.53	0.604	-0.26	0.799	-0.40	0.691
Adults	-0.60	0.553	-0.36	0.723	-1.18	0.252	-0.59	0.563	-0.37	0.717	-0.15	0.886
Juveniles	-2.60	<b>0.017*</b>	1.46	0.160	-1.47	0.157	-0.48	0.639	-0.22	0.828	-0.44	0.665
Endogeic	-0.06	0.950	-0.27	0.788	-2.92	<b>0.009**</b>	-0.19	0.856	0.32	0.756	-1.66	0.114
Adults	-0.05	0.963	-1.15	0.263	-3.63	<b>0.002**</b>	-0.79	0.441	-0.07	0.942	-1.43	0.169
Juveniles	-0.07	0.944	0.23	0.822	-2.91	<b>0.009**</b>	-0.03	0.974	0.34	0.738	-1.68	0.109
Epi-aneic	-3.20	<b>0.005**</b>	0.73	0.475	-0.18	0.863	-1.44	0.167	-0.06	0.949	-0.06	0.950
Adults	-0.42	0.677	-0.48	0.637	0.92	0.372	-0.88	0.392	0.57	0.573	1.63	0.120
Juveniles	-3.56	<b>0.002**</b>	0.95	0.353	-0.47	0.641	-1.40	0.179	-0.14	0.894	-0.21	0.836
Intermediate	-0.88	0.392	1.41	0.174	0.05	0.964	0.29	0.773	-1.01	0.327	0.22	0.830
Adults	-0.69	0.501	1.10	0.285	-0.48	0.638	0.15	0.886	-0.60	0.555	0.35	0.728
Juveniles	-1.04	0.311	1.14	0.267	0.39	0.700	0.35	0.732	-1.13	0.271	0.34	0.738
<i>A. chlorotica</i>	-0.69	0.501	1.10	0.285	-0.48	0.638	0.15	0.886	-0.60	0.555	0.38	0.709
<i>A. caliginosa</i>	1.00	0.330	-2.54	<b>0.020*</b>	-1.97	0.064	0.59	0.562	-1.20	0.244	0.36	0.723
<i>A. giardi</i>	-0.07	0.944	-1.08	0.292	0.81	0.426	-1.50	0.151	0.60	0.559	1.31	0.206
<i>A. icterica</i>	-0.80	0.434	1.72	0.102	-3.74	<b>0.001**</b>	-2.72	<b>0.013*</b>	1.96	0.065	-2.63	<b>0.017*</b>
<i>A. longa</i>	-0.50	0.623	-0.27	0.790	1.23	0.234	-0.84	0.412	0.71	0.488	2.11	<b>0.048*</b>
<i>A. rosea</i>	-3.66	<b>0.002**</b>	1.61	0.125	-3.15	<b>0.005**</b>	-2.81	<b>0.011*</b>	1.31	0.206	-3.03	<b>0.007**</b>
<i>L. terrestris</i>	0.15	0.882	-0.40	0.694	-2.23	<b>0.038*</b>	1.67	0.110	-1.92	0.070	-1.56	0.136
<b>(b) Biomass</b>												
Total	-1.25	0.226	0.01	0.990	-0.12	0.904	-1.39	0.180	-0.01	0.993	0.02	0.987
Adults	0.21	0.836	-0.86	0.403	0.17	0.870	-0.18	0.860	-0.75	0.464	0.80	0.435
Juveniles	-2.80	<b>0.011*</b>	1.10	0.284	-0.47	0.644	-1.82	0.084	0.44	0.665	-0.48	0.636
Endogeic	-0.16	0.875	-0.47	0.646	-4.02	<b>&lt;0.001***</b>	-0.45	0.657	0.23	0.825	-2.14	<b>0.046*</b>
Adults	0.33	0.745	-1.05	0.309	-3.84	<b>0.001**</b>	-0.59	0.564	-0.21	0.834	-1.34	0.195
Juveniles	-1.27	0.221	0.53	0.600	-3.01	<b>0.007**</b>	-0.12	0.906	0.51	0.615	-2.40	<b>0.027*</b>
Epi-aneic	-1.24	0.229	0.08	0.936	0.72	0.483	-1.94	0.067	0.24	0.811	0.39	0.698
Adults	0.24	0.813	-0.55	0.592	0.99	0.335	0.13	0.895	-0.35	0.730	2.34	<b>0.030*</b>
Juveniles	-2.34	<b>0.030*</b>	0.95	0.352	-0.06	0.954	-2.49	<b>0.022*</b>	0.59	0.564	-0.24	0.815
Intermediate	-0.90	0.382	0.94	0.358	-0.13	0.895	0.41	0.686	-1.26	0.225	0.68	0.507
Adults	-0.69	0.496	0.66	0.515	-0.26	0.796	0.30	0.768	-1.15	0.265	1.08	0.294
Juveniles	-1.75	0.096	1.04	0.311	0.32	0.751	0.57	0.574	-1.42	0.171	0.38	0.708
<i>A. chlorotica</i>	-0.70	0.493	0.67	0.510	-0.26	0.796	0.30	0.768	-1.14	0.270	1.08	0.294
<i>A. caliginosa</i>	1.25	0.227	-2.28	<b>0.034*</b>	-2.89	<b>0.009**</b>	0.66	0.516	-1.36	0.190	0.57	0.576
<i>A. giardi</i>	0.24	0.813	-0.88	0.389	-0.22	0.832	0.21	0.833	-0.21	0.834	1.58	0.131
<i>A. icterica</i>	-1.11	0.282	1.82	0.085	-3.47	<b>0.003**</b>	-2.85	<b>0.010*</b>	2.05	0.055	-2.74	<b>0.013*</b>
<i>A. longa</i>	-0.15	0.886	-0.21	0.837	1.43	0.168	-0.11	0.913	0.06	0.950	2.04	0.056
<i>A. rosea</i>	-4.01	<b>&lt;0.001***</b>	2.08	0.051	-3.54	<b>0.002**</b>	-2.64	<b>0.016*</b>	1.42	0.172	-3.04	<b>0.007**</b>
<i>L. terrestris</i>	1.11	0.281	-2.21	<b>0.040*</b>	-1.56	0.136	1.67	0.112	-1.92	0.071	-1.57	0.132
<b>(c) Ecological categories</b>												
%epigeic	-0.01	0.988	0.69	0.502	1.48	0.156	0.83	0.414	-0.54	0.597	1.85	0.079
%aneic	0.20	0.845	0.45	0.661	1.26	0.224	-0.22	0.830	0.08	0.933	0.74	0.467
%endogeic	-0.17	0.867	-0.66	0.517	-2.46	<b>0.024*</b>	-0.07	0.942	0.08	0.935	-0.90	0.378
<b>(d) Functional traits</b>												
Body length 20–50 mm	-3.14	<b>0.006**</b>	2.35	<b>0.030*</b>	-2.58	<b>0.019*</b>	-1.21	0.240	1.24	0.230	-2.59	<b>0.018*</b>
Mass/length ratio 1–7	-0.94	0.358	-0.22	0.828	-5.41	<b>&lt;0.001***</b>	0.43	0.669	-0.24	0.814	-0.62	0.543
Corg 20–33 mg.kg <sup>-1</sup>	0.40	0.694	-0.01	0.990	-2.52	<b>0.021*</b>	-0.95	0.355	-0.19	0.847	-1.57	0.132

Itill: tillage intensity index, Itreat: pesticide treatment intensity index, Iorg: organic input intensity index, Corg: organic carbon preferences.

(Wardle, 1995; Chan, 2001; Pelosi et al., 2014b), and indirect effects including the destruction of habitats (i.e. burrows, surface residues), changes in soil parameters (e.g. organic matter distribution, soil water content and temperature) and predation by bird as organisms are brought to the surface during tillage (Kladivko, 2001; Roger-Estrade et al., 2010; Pelosi et al., 2017).

Pesticide treatments influenced several groups of macrofauna but never in both years. Pesticide treatments are reported to have direct toxic effects on various soil organisms (Pelosi et al., 2014a; Pearson and Tooker, 2021) and can affect the availability of food source through a decrease in weed biomass (i.e. herbicides). The variability of the effects of pesticide treatment intensity between years probably depends on standing crops and annual climatic conditions that drive pest outbreaks and plant diseases.

Organic input intensity was associated to the diversity of macrofauna

groups. This may be explained as organic inputs have a wide range of effects on soils and on food availability. Organic amendments (e.g. manure, compost) and residue retention in soil increase trophic resources directly available for soil organisms (Ponge et al., 2013), benefit soil structuration and water and nutrient retention (de Souza et al., 2017; Olayemi et al., 2022) and provide additional microhabitats (de Souza and Freitas, 2018). Inversely, mineral fertilizer inputs can affect soil properties causing for instance a decrease in soil pH (e.g. N fertilizers) and in soil organic carbon, and promote plant growth without being directly edible by macrofauna organisms (Birkhofer et al., 2008; de Souza and Freitas, 2018).

#### 4.2. Influence of crop management on soil macroarthropods

Our results regarding the density of macroarthropods confirmed that

responses to cropping systems vary among macroarthropod taxa (Fuller et al., 2005; Hernández et al., 2017; Tsutsui et al., 2018) and across years. Long-established conservation systems showed the highest density of Coleoptera larvae, mostly identified as Carabidae larvae, suggesting that they could promote Carabidae through an increase in the number and survival of larvae (Menalled et al., 2007; Henneron et al., 2015). This is consistent with the higher density of Carabidae observed in recent conservation system in the second year of the study, which may show a progressive increase in Carabidae larvae in fields after conversion.

Macroarthropod groups were also influenced by the intensity of different practices, with the significance and direction of the response varying according to taxa and year. It is noteworthy that none of the observed effects were significant in both years. Therefore, the usefulness of practice intensity indicators for assessing the effect of various cropping systems on soil macroarthropods deserves further investigation.

#### 4.3. Influence of crop management on earthworm density, diversity and species

Total earthworm density, biomass and diversity were similar in conventional and long-established organic and conservation systems. This contrasts with previous studies reporting a higher total biomass (Hernández et al., 2017) and diversity of earthworms (Pelosi et al., 2009; Dulaurent et al., 2022) under conservation than under conventional systems. However, we found a higher density of epi-aneic juveniles in conservation than in organic systems in 2020. Similarly, Pelosi et al. (2009) reported higher density and biomass of anecic and epigeic earthworms in conservation than in conventional and organic systems, but with an important variability between years.

Our results showed earthworms to be influenced by transitional more than by long-established systems. Indeed, systems recently converted to conservation agriculture tended to have a higher total biomass and species diversity of earthworms than conventional systems. In addition, some recent organic systems had a lower ratio of adults/juveniles biomass than long-established organic systems in association with a higher density and biomass of epi-aneic and endogeic juveniles. This higher proportion of juvenile earthworms may be transitory and reflect changes occurring during the transition process (Irmeler, 2010). Moreover, we found *L. terrestris* only in transitioning systems, which could suggest that this species is occurring following the emergence of new disturbances, but is not in agreement with previous studies (Pelosi et al., 2015).

Tillage intensity reportedly affects earthworm abundance and diversity with highly variable responses (Chan, 2001), and a negative effect mostly on large epi-aneic species (Briones and Schmidt, 2017). In our study, tillage intensity had a negative effect on the density and biomass of epi-aneic juveniles, which strongly influenced the total density of juveniles and the ratio of adults/juveniles biomass. This negative effect was observed for both deep and surface tillage. In agreement with this, juvenile earthworms from all ecological groups were reported to feed mostly at the soil surface, which could partly explain their high sensitivity to all types of tillage (Briones and Schmidt, 2017). However, at the species level (i.e. adult stage), we detected a negative effect of tillage only for *A. rosea* and *A. icterica* (i.e. two endogeic species). Therefore, this suggests that adults and juveniles of the same ecological category do not respond equally to tillage intensity.

Pesticide treatments, especially insecticides and fungicides, were reported to affect earthworms through direct effects, namely a decrease in the rate of survival and reproduction or changes in feeding behavior (Pelosi et al., 2014a). Therefore, we expected a decrease in earthworm density as the use of pesticide treatments was more important. However, we observed negative effects of pesticides to be species specific and associated to both fungicides and herbicides. A negative effect of pesticide treatment intensity was indeed observed on the density and biomass of *A. caliginosa*, and on the biomass of *L. terrestris*, whose

sensitivity to pesticides was reported in previous studies (Dittbrenner et al., 2011; Pelosi et al., 2014). *A. caliginosa* was notably suggested to be a good model for pesticide risk assessment (Pelosi et al., 2013).

Organic inputs (i.e. organic amendments and residues) were reported to influence the total earthworm community, with benefits from organic matter inputs (Birkhofer et al., 2008), but the distinction of these effects for different ecological categories of earthworms were rarely investigated (Betancur-Corredor et al., 2023). Here, organic input intensity was observed to influence mostly endogeic earthworms, with a negative effect on both juveniles and adults. This was associated in particular to a decrease in *A. icterica* and *A. rosea* density and biomass. Organic matter inputs are associated to tillage intervention to mix organic matter with soil, which was observed to have a negative effect on *A. rosea*, thus suggesting a confounding effect. However, we observed neither total endogeic adults nor total endogeic juveniles to be influenced by tillage intensity. Thus, other explanations are required to explain the negative effect of organic inputs on endogeic earthworms, such as a detrimental changes in soil properties or a short-term toxic effect of some organic matter inputs (e.g. manure) (Curry, 1976). The effects of organic matter inputs on earthworms depend on the quality of organic matter, with possible higher benefits of manure and cattle slurry than some types of compost on earthworm abundance and biomass (Leroy et al., 2008). Inversely, we observed benefits of organic inputs for epi-aneic adults, *A. longa* or *L. terrestris* notably, depending on years. This may be related to the accumulation of organic matter inputs and crop residues at the soil surface which increases food resource.

#### 4.4. Use of recently redefined ecological categories of earthworms and percentage of belonging to the main categories

Ecological groups of earthworms as determined in Bottinelli et al. (2020) allowed observing differences that were hidden while considering categories commonly used in the literature. In particular, the reattribution of *A. chlorotica* to the intermediate ecological category, otherwise attributed to the endogeic category, enabled to observe the impact of organic inputs on endogeic species. Indeed, *A. chlorotica* is one of the most common species encountered in cropping systems (Schmidt et al., 2001; Pelosi et al., 2015) and obviously does not react to practices like endogeic species.

Percentages of belonging to the different groups did not bring more information than ecological categories. These might be relevant for more diverse communities than the one observed in cropping systems in our study.

#### 4.5. Are species traits useful predictors of the effect of disturbances on earthworms?

The functional approach of diversity was alternatively reported to be useful (Decaëns et al., 2011; Pelosi et al., 2014b; Frazão et al., 2019) or poorly efficient (Hedde et al., 2012; Pelosi et al., 2016) to assess the effects of disturbances on earthworm communities. In our study, we found similar earthworm functional richness and functional community composition between cropping systems. However, functional traits, considered individually, brought some insight on the role of practices in the functional changes occurring in earthworm communities. Contrary to our initial assumptions and as observed in previous studies (Pelosi et al., 2014), we found a higher representation of species with a small body length in systems recently converted to conservation agriculture than in organic systems. Earthworm species traits also brought information on the morphological characteristics and preferences of the earthworm community depending on practice intensity. Tillage, pesticide treatment and organic input intensity all influenced species body length. Small species were notably favored by low tillage. In addition, a high organic input intensity had a negative effect on the presence of small species, with small mass/length ratio and preference for low organic carbon concentration in soil (20–33 mg.kg<sup>-1</sup>), all of which are

characteristics of endogeic species.

Overall, the low number of collected earthworm species, although consistent with the usual diversity in agricultural areas in Europe (Cluzeau et al., 2012; Rutgers et al., 2016), implies that trait attributes are associated to one or two species only and follow similar trends than species density. Therefore, results obtained with the functional approach could have been discussed with the sole taxonomic approach. This shows that the effects of cropping systems on earthworms could be reliably studied using traits (mostly morphological traits) when the identification knowledge is missing.

## 5. Conclusion

The effects of cropping systems on soil macrofauna are better described by considering the intensity of applied practices and specific macrofauna groups (i.e. taxa, ecological categories, trophic groups) rather than the entire community. Macrofauna plays an essential role in soil functioning, with different taxa providing different functions and having potential cascading effects on other taxa. Therefore, it is paramount to take into account the effects of cropping systems and practices at a finer level than the whole community. Regarding earthworms, taxonomic and functional traits approaches of communities yielded the same conclusions. However, the updated earthworm ecological categories proved to be relevant to assess the effects of disturbances on earthworm communities, hence we would like to emphasize on the need to use these categories in forthcoming studies. Overall, the variability in macrofauna density and diversity remains high in agricultural soils. Intensity indexes are the first step for a better characterization of cropping systems, but long-term trials are now required as effects of practices on soil macrofauna can be transitory.

## CRedit authorship contribution statement

**Laure Vieublé Gonod:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Sophie Joimel:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Juliette Chassain:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Juliette Chassain reports financial support was provided by INRAE, AgroParisTech and the Office Français de la Biodiversité. Juliette Chassain reports financial support was provided by AgroParisTech National Institut of Forestry Agriculture and Environmental Engineering. Juliette Chassain reports financial support was provided by French Biodiversity Office. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

## Data availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.pedobi.2024.150974](https://doi.org/10.1016/j.pedobi.2024.150974).

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