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Soil properties, grassland management, and landscape diversity drive the assembly of earthworm communities in temperate grasslands

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ABSTRACT

Earthworms are widespread soil organisms that contribute to a wide range of ecosystem services. As such, it is important to improve our knowledge, still scanty, of the factors that drive the assembly of earthworm communities. The aim of the present study was to conjointly evaluate the effects on the assembly of earthworm communities of i) soil properties (texture, organic matter content, and pH), ii) grassland management (grassland age, livestock unit, and type of fertilization), iii) landscape diversity (richness, diversity of surrounding habitats, and grassland plant diversity), and iv) presence of hedgerows. The study was conducted in temperate grasslands of Brittany, France. Earthworms were sampled in 24 grasslands and, in three of these grasslands, they were sampled near a hedgerow or near a ditch (control without a hedgerow). Soil properties explained the larger portion of the variation in the earthworm community parameters compared to grassland management or landscape diversity. The increase in soil organic matter content and pH were the most favorable factors for earthworm abundance and biomass, in particular for endogeic species. Regarding grassland management, the increase in the livestock unit was the most damaging factor for earthworm communities, in particular for the anecic earthworm biomass and endogeic species richness. Surprisingly, landscape diversity negatively affected the total earthworm abundance and epigeic earthworm biomass, but it was related to an increase in the epi-anecic species. At a finer scale, we also demonstrated that the presence of hedgerows surrounding grasslands enhanced earthworm species richness, especially within the epigeic and anecic ecological categories. This study highlights that the earthworm ecological categories respond specifically to environmental filters; further studies need to be conducted to elucidate the factors that drive the assembly of earthworm communities at this ecological category level. We recommend that policymakers should act on landscape management to favor earthworm diversity in order to improve the ecosystem services they drive.

Key Words: ecological category, field margin, hedgerow, livestock unit, soil organic matter, soil PH, trampling

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INTRODUCTION

Earthworms are widespread soil organisms that constitute the most important terrestrial biomass in temperate climate zones (Hole, 1981; Bar-On *et al.*, 2018). In general, they are classified into three main ecological categories depending on their physiology, morphology, and behaviour: epigeic, anecic, and endogeic species (Bouché, 1972, 1977). Briefly, epigeic earthworms live in and consume surface organic matter, anecic earthworms burrow vertical galleries to feed on a mixture of surface and soil organic matter, and endogeic earthworms burrow horizontal galleries to feed on soil organic matter (Bouché and Kretzschmar, 1974; Bouché, 1977; Jégou *et al.*, 1998). Additionally, within the anecic earthworms, epi-anecic species feed preferentially on fresh surface organic matter (*i.e.*, leaf litter) and differ from strict-anecic species that feed preferentially on aged organic matter already incorporated into the soil (Jégou *et al.*, 1998; Larsen *et al.*, 2016; Hoeffner *et al.*, 2019). Depending on their ecological categories and the associated feeding and burrowing behavior, earthworms contribute to important ecosystem services provided by the soil, such as nutrient cycling, water and climate regulation, and primary production (Blouin *et al.*, 2013; Bertrand *et al.*, 2015). For example, van Groenigen *et al.* (2014) reported in a meta-analysis that an increase in crop production was observed in the presence of earthworms. This increase varied from 18% in the presence of epigeic species to 32% in the presence of anecic species.

Earthworm communities are governed by different environmental filters, including biogeographical history, soil properties, land use and management, as well as species interactions within the community, *e.g.*, competition or facilitation (Lavelle, 1983; Curry, 2004; Decaëns *et al.*, 2008). Previous studies focusing on the impact of soil properties on earthworm communities highlighted the key role played by soil pH, soil organic matter content, and soil texture

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(Lee, 1985; Joschko *et al.*, 2006; Decaëns *et al.*, 2008). Other studies focused on the impact of land use on these earthworm communities (Boag *et al.*, 1997; Decaëns *et al.*, 2003, 2008; Cluzeau *et al.*, 2012). For example, Ponge *et al.* (2013) reported that grasslands exhibited higher anecic earthworm abundance than croplands. In addition, Zaller and Arnone (1999) observed a positive correlation between the density and biomass of earthworm communities and the plant species richness of grasslands, and in particular for endogeic species. Concerning land management, previous studies reported that ploughing (Chan, 2001; Briones and Schmidt, 2017), pesticide application (Pelosi *et al.*, 2014), and low permanent cover (Vršic, 2011) negatively impact earthworm communities, with a response intensity that depends on the ecological category considered.

Other studies have been undertaken at a greater scale to evaluate the impact of landscape diversity on earthworm communities within croplands (Vanbergen *et al.*, 2007; Lüscher *et al.*, 2014; Frazüo *et al.*, 2017). For example, Flohre *et al.* (2011) observed that earthworm species richness in croplands decreased with increasing percentage of surrounding agricultural fields. Regulska and Kołaczkowska (2015) also reported that a cropland surrounded by a diverse landscape supported higher earthworm diversity, density, and biomass than the same type of cropland surrounded by a simpler landscape. However, the majority of the previous studies did not report the effect of landscape diversity on the earthworm communities of croplands and vineyards (Kovács-Hostyánszki *et al.*, 2013; Buchholz *et al.*, 2017; Frazão *et al.*, 2017). Moreover, it has been reported that the field margins of croplands exhibit higher abundance and diversity of earthworms than the croplands themselves, but, surprisingly, it has not been reported that these field margins favor earthworm populations of these croplands (Smith *et al.*, 2008; Roarty and Schmidt, 2013; Crittenden *et al.*, 2015). It remains unclear whether and how earthworms disperse within agricultural landscapes.

In recent decades, a strong research effort has been made to study the earthworm communities of croplands. Grasslands are the largest terrestrial ecosystem in the globe and produce many key ecosystem services, such as carbon storage, soil erosion mitigation, or support for pollinators (Costanza *et al.*, 1997; Conant and Paustian, 2002; Werling *et al.*, 2014). The main objective of the present study was to conjointly evaluate the effects of soil properties, grassland management, and landscape diversity on the assembly of grassland earthworm communities. Specifically, we hypothesized that the intensity of grassland management would negatively affect earthworm community parameters, while the landscape diversity surrounding the grasslands would increase these parameters. The second objective was to evaluate the effect of hedgerow on these earthworm communities. By increasing the number of available niches, we hypothesized that the presence of a hedgerow on the grassland edge would increase earthworm community parameters (Tews *et al.*, 2004). We conducted the study in an agricultural landscape of Brittany, France. Earthworms were sampled in 24 grasslands and, within three of them, they were oversampled near a hedgerow and near a ditch (control without hedgerow). Several parameters of the earthworm communities were evaluated, including i) total abundance, total biomass, species richness, and species diversity, and ii) abundance, biomass, and richness within each earthworm ecological category.

MATERIALS AND METHODS

Study site

The study site covers 10 km^2 and is a part of the Long Term Ecological Research (LTER) "Zone Atelier Armorique", located in Brittany, France (48°50' N, 1°58' W). The climate of the area is oceanic with a mean annual temperature of 11.7 °C, a mean annual rainfall of 815.0 mm, and a mean annual relative humidity of 80.9% (mean values over 2010–2016, data from Météo France). The main soil types encountered are Cambisols (FAO, 2015) with high bedrock heterogeneity (granite, soft schist, and aeolian loam). Moreover, the study area presents a substantial micro-topography, mainly due to a high variability of landscape structures (*e.g.*, hedges and ditches as field margins) with a hedge density ranging from 50 to 100 m ha^{-1} (Baudry *et al.*, 2000; Thomas *et al.*, 2016). Land use comprises mainly annual crops (corn, wheat, barley) and temporary or permanent grasslands, forests, and unmanaged areas.

We used ground-truth aerial photos, which have been taken every year since 1990, to construct a detailed land-use history for all grasslands, allowing us to precisely determine the age of each grassland. Based on this land-use history and after verification with grassland owners, we selected 24 grasslands ranging from 1 to 25 years since the last crop. Among them, three grasslands with an age gradient of 1-, 2-, and 7-year-old were selected and oversampled with a hedgerow and a ditch at their surroundings to take into account the specific effect of the hedgerow on soil properties (Marshall and Moonen, 2002; Walter *et al.*, 2003).

Earthworm sampling and laboratory analyses

Earthworms were sampled in 2016 within the 24 grasslands at a distance of 30 m from any grassland edge, and then in the three selected grasslands near a ditch and near a hedgerow. For the three selected grasslands, we standardized the sampling with three sampling points in order to consider three replicates with a hedgerow (at 1, 5, and 10 m from the hedgerow) and three replicates without a hedgerow (at 1, 5, and 10 m from the ditch).

Earthworm sampling followed the normalized protocol ISO 23 611-1, that was modified and validated during the RMQS BioDiv program (Cluzeau *et al*., 2012), combining chemical and physical extractions. Briefly, each earthworm sampling was characterized by a mean of three sub-sampling spaced by 10 m in line. Earthworm sub-sampling consisted of three waterings of 10 L with a gradient concentration of formaldehyde (0.25%, 0.25%, and 0.4%) on 1 m^2. After each watering, earthworms were collected for 15 min. Afterwards, a block of soil (25 cm $\times$ 25 cm $\times$ 20 cm, length $\times$ width $\times$ depth) was excavated within each sub-sampling area and earthworms were hand-sorted. The number of hand-sorted earthworms (HS) was multiplied by 16 to obtain an estimation per square meter. This number was then added to the number of earthworms counted with the formaldehyde extraction (FE) to obtain the total number of earthworms per square meter (FH): $\text{FH} = \text{FE} + 16 \times \text{HS}$. Earthworms were fixed and preserved in a formaldehyde solution (4%).

In the laboratory, each earthworm individual was counted, weighed, assigned to a stage of development (juvenile, sub-adult, and adult), identified at the sub-species level, and assigned to its ecological category: epigeic, anecic, or endogeic (Bouché, 1972, 1977). Additionally, in the case of anecic earthworms, we distinguished the epi-anecic (genus *Lumbricus*) from the strict-anecic earthworms (genus *Aporrectodea*) (Ferrière, 1980; Jègou *et al*., 1998). For juvenile individuals, the identification was first limited to the genus, and thereafter they were attributed a species name according to the proportions of sub-adults and adults of the same genus present on each square meter. Earthworm diversity was analyzed through three levels: total species richness, Shannon diversity index, and species evenness index.

Environmental filters

We selected three types of environmental filters to explain earthworm community parameters: soil properties, grassland management, and landscape diversity. Soil properties characterized were soil texture, organic matter content, and pH (water). Ten soil samples were randomly collected at 3 m around the earthworm sub-samplings, using a cylindrical soil corer (5 cm diameter $\times$ 20 cm depth) in each grassland. These 10 soil samples were pooled and homogenized in order to consider one composite soil sample per grassland and sent to the analytical laboratory of LABOCEA (Combourg, France). Briefly, clay content ranged from 95 to 197 g kg^{-1}, sand content from 133 to 689 g kg^{-1}, organic matter content from 18 to 52 g kg^{-1}, and soil pH from 5.5 to 6.7 (Table SI, see Supplementary Material for Table SI).

Grassland management was assessed by interviewing farmers (Table SI, see Supplementary Material for Table SI) and using ground-truth aerial photos. The grassland age ranged from 1 to 25 years since the last row-cropping with quite similar species (*Lolium perenne* and *Trifolium repens* or *Trifolium pratensis*). In addition, livestock unit per hectare varied from 0 to 4.3. Since the fertilization rate was declarative, we used only the distinction between organic and mineral input.

Landscape structure within 100 m radius around the sampled fields was classified into nine habitats based on aerial photos (forest, grassland, crop, hedge, water, building, garden, asphalt area, and road). The radius of 100 m was chosen to reflect the overall low mobility of earthworms (Bardgett *et al*., 2005; Eijsackers, 2010, 2011). Landscape diversity was characterized by two indexes: landscape richness (total richness of habitats within the radius) and the Shannon diversity index of habitats (hereafter called SHDI) and plants (hereafter called plant diversity) and plants. Mapping and analysis were done using the QGis 2.8.1 and FRAGSTATS 4.296 softwares. In addition, we characterized the plant community of the 24 grasslands in the spring of 2015 using 10 quadrats (1 m $\times$ 1 m) evenly distributed in each grassland and determining for each species its covering percentage. Among the 24 grasslands selected, landscape richness varied from 1 to 7 habitats (maximum number of habitats has never been observed), SHDI from 0.1 to 1.6, and plant diversity within grasslands from 1.2 to 3.2 (Table SI).

Statistical analysis

We used multiple linear regression models to test the effects of soil properties (clay, sand and organic matter contents and pH), grassland management (grassland age, livestock unit, and fertilization), and landscape diversity (landscape richness, SHDI, and plant diversity) on all earthworm community parameters (*i.e.*, total abundance and biomass, total diversity indexes, and ecological categories abundance and biomass). We constructed a full model comprising all environmental filters, and then we selected the significant environmental filters using a backward stepwise selection procedure that selects the best model based on the AIC criterion (Crawley, 2013; stepAIC function of the MASS package). We also evaluated the variance inflation factor (VIF) of each variable selected by the previous procedure to test for multicollinearity among environmental filters. We removed all environmental filters that showed a $\text{VIF} > 5$, even if significant for the model. Data met the conditions of normality and homoscedasticity.

Second, within each of the three selected grasslands (*i.e.*, 1-, 2- and 7-year-old), we compared earthworm communities with and without a hedgerow (ditch) using the three sampling points per plot as replicates. We used separated t-tests within the three selected grasslands to assess the differences in earthworm abundance, earthworm biomass, and species richness according to the presence or absence of a hedgerow. Statistical analyses were performed with the R software 3.2.3 (R Core Team, 2017). Significance was evaluated in all cases at $P < 0.05$.

RESULTS

Impacts of soil properties, grassland management, and landscape diversity on earthworm communities

In the 24 grasslands sampled, the average earthworm abundance and biomass were 517.0 ± 57 individuals m^{-2} and 219.4 ± 20 g m^{-2}, respectively. The mean earthworm species richness was 10.8 ± 0.3. Eighteen species belonging to the three ecological categories were identified (Table SII, see Supplementary Material for Table SII). *Allolobophora chlorotica* and *Aporrectodea caliginosa* were the most abundant species, whereas *Eisenia tetraedra*, *Dendrobaena rubida*, and *Octalasium lacteum* were present in a single grassland (Table SII).

Higher soil organic matter content increased the total earthworm abundance (F = 5.3, P = 0.033) (Table I), the endogeic species abundance (F = 5.7, P = 0.028) (Table SIII, see Supplementary Material for Table SIII), and the endogeic species richness (F = 5.4, P = 0.031) (Table SIV, see Supplementary Material for Table SIV), while the abundance of endogeic species was negatively correlated with the sand content (F = 6.9, P = 0.017) (Table SIII). In addition, the total earthworm abundance and biomass increased when soil pH was more alkaline (F = 5.0 and 6.8, respectively, P < 0.05) (Fig. 1, Table I), but no category-specific impact was observed with respect to pH variation.

The increase in livestock unit decreased the total earthworm biomass (F = 5.7, P = 0.028) (Table I) and in particular the biomass of anecic species (F = 9.6, P = 0.005) (Fig. 2a, Table SV, see Supplementary Material for Table SV). However, this negative effect was only confirmed for the biomass of epi-anecic species (F = 4.4, P = 0.049) (Fig. 2b, Table SV). The increase in the livestock unit also decreased the earthworm species richness, the Shannon diversity index and the species evenness (F = 2.8 to 9.6, P < 0.05) (Fig. 2c, Table I), and in particular the endogeic species richness (F = 9.5, P = 0.006) (Table SIV). Mineral fertilization enhanced the epigeic species abundance and biomass compared to organic fertilization (F = 6.6 and 8.6, respectively, P < 0.02) (Tables SIII and SV).

Landscape richness decreased the biomass of epigeic species (F = 4.9, P = 0.041) (Table SIV), but enhanced the epi-anecic species richness (F = 6.6, P = 0.019) (Table SIV). The increase in SHDI decreased the total earthworm

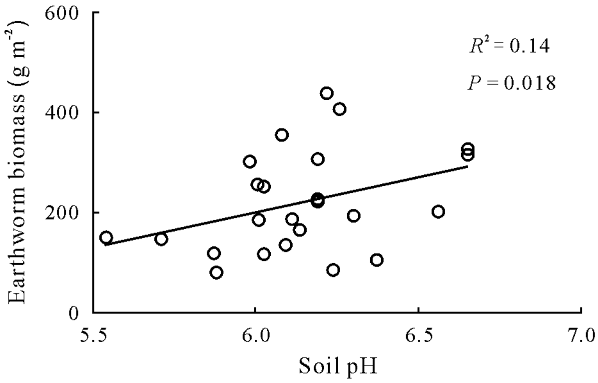

Fig. 1 Relationship between total earthworm biomass and soil pH in 24 temperate grasslands located in Brittany, France.

TABLE I

Analysis of variance (ANOVA) results of multiple linear models testing for the effects of three types of environmental filters, soil properties, grassland management, and landscape diversity, on total abundance, total biomass, species richness, Shannon diversity index, and evenness index (when variance inflation factor < 5) of earthworms in 24 temperate grasslands located in Brittany, France

Environmental filter	Total abundance				Total biomass				Species richness				Shannon diversity index				Evenness index			
	df[a]	PSS[a]	F	P	df	PSS	F	P	df	PSS	F	P	df	PSS	F	P	df	PSS	F	P
		%				%				%				%				%		
Soil properties																				
Clay content	1	5.9	2.2	0.157	–[b]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sand content	1	5.2	1.9	0.182	1	0.4	0.1	0.711	1	0.1	0.0	0.841	–	–	–	–	–	–	–	–
Organic matter content	1	14.4	5.3	0.033	1	6.0	2.1	0.164	–	–	–	–	–	–	–	–	–	–	–	–
pH	1	13.4	5.0	0.039	1	19.4	6.8	0.018	–	–	–	–	–	–	–	–	–	–	–	–
Grassland management																				
Grassland age	–	–	–	–	–	–	–	–	–	–	–	–	1	7.5	9.9	0.107	1	7.3	2.4	0.141
Livestock unit	–	–	–	–	1	16.3	5.7	0.028	1	31.3	9.6	0.005	1	26.2	2.8	0.005	1	15.1	4.8	0.040
Fertilization	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Landscape diversity																				
Landscape richness	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SHDI[c]	1	12.3	4.6	0.047	1	6.4	2.2	0.153	–	–	–	–	–	–	–	–	–	–	–	–
Plant diversity[d]	–	–	–	–	–	–	–	–	–	–	–	–	1	13.2	5.0	0.037	1	15.1	4.8	0.040
Residuals	18	48.7	–	–	18	51.5	–	–	21	68.6	–	–	20	53.1	–	–	20	62.5	–	–

[a] df = degree of freedom; PSS = percentage of sum of square.

[b] Not applicable.

[c] Shannon diversity index of habitats.

[d] Shannon diversity index of plants.

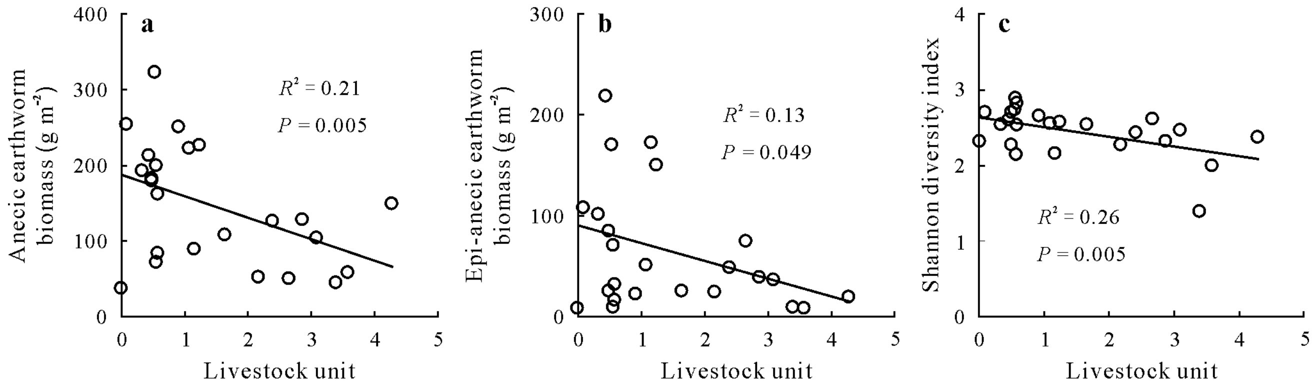

Fig. 2 Relationships between livestock unit and anecic earthworm biomass (a), epi-anecic earthworm biomass (b), and Shannon diversity index of earthworms (c) in 24 temperate grasslands located in Brittany, France.

abundance (F = 4.6, P = 0.047) (Table I). Moreover, the increase in plant diversity was positively correlated with the Shannon diversity index and species evenness (F = 5.0 and 4.8, respectively, $P < 0.04$) (Table I). Interestingly, the abundance of strict-anecic species, as well as their biomass and richness were not affected by any of the environmental filters measured (Tables SIII, SIV, and SV).

Impact of hedgerow presence on earthworm communities

Over the three grasslands oversampled, earthworm abundance was higher in the 2-year-old grassland (834 ± 76 individuals m^{-2}) than in the 1-year-old (306 ± 32 individuals m^{-2}) and 7-year-old ones (385 ± 32 individuals m^{-2}). Earthworm species richness was higher in the 2- and 7-year-old grasslands (11.0 ± 0.4 and 10.2 ± 0.3, respectively) than in the 1-year-old one (7.9 ± 0.4). Earthworm species composition was also greatly different between these three grasslands. For example, the presence of *Eisenia tetraedra* occurred only in the 2-year-old grassland and the presence of *Aporrectodea caliginosa meridionalis* occurred only in the 7-year-old grassland.

In the grasslands of 1- and 2-year-old, the earthworm species richness was 21.0% and 23.2% higher, respectively, with the presence of a hedgerow than with the presence of a ditch (t = 5.8 and 13.9, $P < 0.03$) (Fig. 3a, b). However, this variable was not affected in the 7-year-old grassland (t = 0.0, P = 0.85) (Fig. 3c). The abundance of earthworms was not affected by the presence of hedgerows in the three selected grasslands (t = 0.0–0.03, $P > 0.865$).

Overall, except for *Allolobophora icterica* and *Aporrectodea nocturna* that were more abundant with the presence of a hedgerow, the strict-anecic and endogeic species were evenly distributed between the plots with and without a hedgerow. The distribution of epi-anecic earthworm species was heterogeneous, but *Lumbricus rubellus rubellus* and *Lumbricus terrestris* were more often observed in the presence of a hedgerow. The distribution of epigeic earthworm was species-dependent since *Dendrobaena mammalis* occurrence was higher in the presence of a hedgerow, while *Eisenia tetraedra* was observed in the presence of a hedgerow only in the 2-year-old grassland. *Lumbricus castaneus* and *Lumbricus rubellus castanoïdes* occurrences were overall similar between the plots, regardless of the presence of a hedgerow.

DISCUSSION

In the present study, we clearly demonstrated that soil properties, grassland management, and landscape diversity conjointly affected the selected parameters of earthworm communities. Our findings contrast with those of Frazão *et al*. (2017), who reported that the earthworm communities of croplands were impacted only by agricultural practices, but neither by soil properties nor landscape diversity.

Contrary to previous studies that observed an effect of soil properties at the regional scale (Decaëns *et al*., 2003; Vanbergen *et al*., 2007; Decaëns *et al*., 2008), in the present study, taking into account the ecological categories of earthworms, we evidenced that soil properties impact the abundance, biomass and richness of earthworm ecological categories at a finer scale (*i.e.*, 10 km^2). This result might be due to the strong spatial heterogeneity of soil properties in the studied region (Jamagne, 2011). In accordance with previous studies, we observed that a higher soil sand content decreased the total abundance of earthworms (Hendrix *et al*., 1992; Lapied *et al*., 2009), which could be due to the low capacity of sandy soils to hold water, leading to an unfavorable habitat for earthworms (Lee, 1985). In addition, the increase in soil pH was positively correlated to both the earthworm species richness (Joschko *et al*., 2006) and the total abundance (Ma *et al*., 1990; McCallum *et al*., 2016). Nonetheless, several reviews observed that earthworm preference for soil pH was species-dependent due to their synecology (Bouché, 1972; Edwards and Lofty, 1977; Lee, 1985), but the underlying mechanisms for pH preference are

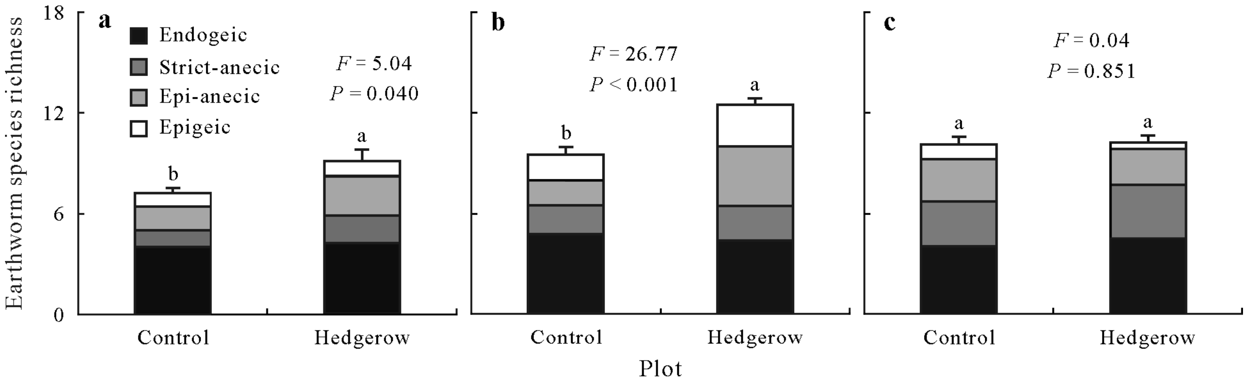

Fig. 3 Earthworm species richness in plots with a hedgerow or with a ditch (*i.e.*, control plot without a hedgerow) for grasslands of 1 year old (a), 2 years old (b), and 7 years old (c) located in Brittany, France. Bars represent standard deviations of the means ($n = 3$). Different letters denote significant difference between the two plots based on *post-hoc* Tukey's test.

not fully understood yet. In line with their feeding behaviour, which consists in consuming mainly aged organic matter, endogeic earthworm communities were more abundant and diversified in the grasslands with high contents of soil organic matter (Bouché, 1977; Piearce, 1978; Ferrière, 1980).

Regarding grassland management, the increase in the livestock unit was the most damaging factor for earthworm communities as the total biomass, the species richness, the Shannon diversity index, and the species evenness decreased. This strong negative effect could be associated with trampling at high stocking levels that damages soil structure and, therefore, adversely affects earthworm communities and burrows (Cluzeau *et al*., 1992; Pietola *et al*., 2005; Chan and Barchia, 2007). Interestingly, earthworms' response to the livestock unit was almost entirely confined to the largest epi-anecic and endogeic species, and only the earthworm biomass was affected, contrary to their abundance, suggesting a decrease in the mean body size rather than in the number of individuals. Surprisingly, mineral fertilization enhanced the abundance and biomass of epigeic species. Nonetheless, this finding is consistent with some previous studies that reported an increase in the earthworm abundance with nitrogen mineral fertilization (Muldowney *et al*., 2003; King and Hutchinson, 2007; Curry *et al*., 2008). Mineral fertilization would probably allow a better primary production that would lead to higher leaf litter inputs that constitute a source of refuge and food for earthworms. Further studies on grasslands are necessary to elucidate the different impacts of manure *versus* mineral fertilization on earthworms. Overall, we observed that within grasslands, grazing pressure led to smaller and less-diversified earthworm communities.

We observed a negative effect of the increase in landscape diversity (richness and Shannon Index) on the total abundance of earthworms and, to our knowledge, for the first time, the biomass of epigeic earthworms in grasslands. A negative correlation between the total abundance of earthworms and landscape diversity was also observed by Flohre *et al*. (2011) in croplands. These authors hypothesized that landscape diversity increases the number of earthworm predators. Indeed, several studies highlighted that landscape diversity enhance the abundances of invertebrates, mammals, and birds (Marshall and Moonen, 2002; Maudsley *et al*., 2002; Vickery *et al*., 2009) that are potential predators of earthworms (Granval and Aliaga, 1988; O'Brien *et al*., 2016). We can also hypothesize that the capacity of epigeic species to disperse is hindered by physical barriers (*i.e.*, hedge or ditch) and different soil properties (shelter and litter availability) in neighboring habitats that constitute landscape diversity. In contrast, the species richness of epi-anecic earthworm was enhanced by the landscape diversity. As epi-anecic earthworm species have a great mobility that varies from 1.5 to 14 m year^{-1} (Hoogerkamp *et al*., 1983; Eijsackers, 2011; Nuutinen *et al*., 2014) and the ability to burrow into the soil to protect themselves, a higher landscape diversity around grasslands could enhance their areas of emigration. Endogeic earthworm species were not impacted by landscape diversity and were highly abundant in each grassland, as previously reported (Lavelle, 1983; Decaëns *et al*., 2008). Overall, it is possible that low agricultural practices in grasslands, compared to croplands or vineyards, could increase the effect of the surrounding landscape diversity on earthworm communities (Roarty and Schmidt, 2013; Buchholz *et al*., 2017; Frazão *et al*., 2017).

In addition to the effect of landscape diversity, we highlighted the importance of hedgerows surrounding grasslands. Hedgerows especially acted on young grasslands (*i.e.*, 1- and 2-year-old grasslands), which is probably due to the increase in earthworm species aggregation with the age of the grasslands (Richard *et al*., 2012). It is well known that hedgerows locally modify soil properties (*i.e.*, soil moisture, temperature, or organic matter content (Marshall and Moonen, 2002)), and especially the amount and type of litter deposited on the soil surface (Walter *et al*., 2003). This litter input is a key factor for the development of earthworm communities (Lee, 1985; Edwards, 2004), and in particular for epigeic and epi-anecic species that have a diet mainly composed of fresh leaf litter (Bouché and Kretzschmar, 1974; Piearce, 1978; Ferrière, 1980). In the field, earthworm communities that live in grasslands surrounded by a hedgerow were richer in earthworm species than those in grasslands surrounded by a ditch, especially for epigeic and epi-anecic earthworm species. Thus, the presence of hedgerow could promote earthworm diversity in grasslands. The increase in the epi-anecic earthworm diversity in grasslands landscape could have consequences on ecosystem services provided by these species. Hoeffner *et al*. (2018) observed that burrows' fungal communities were regulated by epi-anecic species identity, which could increase the diversity of the drilospheric microbiota and improve soil functioning. In addition, as it is difficult to monitor the earthworm diversity response to global change drivers, earthworm databases often concern surveys carried out at regional or national scales (Rutgers *et al*., 2009; Cluzeau *et al*., 2012; Cameron *et al*., 2016). The first predictive model on the abundance and diversity of earthworms was created by Rutgers *et al*. (2016), taking into account soil occupation and properties. Therefore, future predictive models could consider the landscape as an additional factor that regulates these earthworm communities.

CONCLUSIONS

The earthworm communities in grasslands of our study were clearly affected by the three environmental filters considered: soil properties, grassland management, and landscape diversity. Soil properties were the main environmental filter that controls earthworm communities. However, we also highlighted the important effect of grassland management, for instance, a strong decrease in the abundance of earthworms with the increase in the livestock unit. We observed various effects of landscape diversity, such as a surprising overall decrease in the earthworm abundance or a higher epi-anecic richness in diverse landscapes. Therefore, our findings demonstrated the conjoint effects of various environmental filters as drivers of earthworm communities. Overall, our results suggest a strong context dependency in the assembly rules of earthworm communities, despite the fact that these communities are well known to be ubiquitous and resilient.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

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