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Original article

Earthworm populations and diversity under annual and perennial wheat in a North to South gradient in Western Europe

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ABSTRACT

The challenge to sustain food security while halting the loss of biodiversity and soil quality might be achieved by a transformation in agriculture from high-input management of annual crops to a more nature-based solution introducing perennial cropping systems. This study analysed earthworm communities (numbers, biomass, ecological categories) and diversity over two years, from annual wheat and perennial intermediate wheatgrass (IWG, *Thinopyrum intermedium*, Kernza®) within the EU-Biodiversa project NAPERDIV from Southern to Northern Europe. Study sites in France, Belgium and Sweden represented diverse soil, climatic and plant growth conditions. In total, 16 species were identified with IWG in France having the highest (13) and annual wheat in Belgium and Sweden the lowest (7) species numbers. Improved biodiversity under perennial wheat was indicated by alpha-diversity indices (Simpson index, Shannon-Weaver index, Evenness). Earthworm abundance and biomass were generally significantly higher in IWG across the three sites (GLMM model). The overall mean earthworm number under IWG was 424.7 No. m^{-2} compared to 164.7 No. m^{-2} for annual wheat. Mean earthworm biomass under IWG was 83.7 g m^{-2} relative to 45.9 g m^{-2} under annual wheat, respectively. Remarkably, mean number of juvenile earthworms was several times higher on IWG sites relative to the annual comparatives. Moreover, endogeic and epigeic earthworms were supported on the IWG plots. Beta diversity (Sorensen coefficient) emphasised highest similarity between Belgium and Sweden and lowest between France and Sweden, indicating a possible South to North distribution within Western Europe. The Canonical Correspondence Analysis showed discrete clusters for study sites and species distribution (including the subtypes of *Allolobophora chlorotica*) in relation to soil parameters (pH, soil texture, TOC, TN, WHC, C–N ratio). The CCA additionally discriminated between annual and perennial plots in France. In summary, earthworm communities were more diverse under IWG and seemed to follow a South to North gradient.

1. Introduction

Looking at the current state of agriculture, two main challenges arise: maintaining food security and halting the ongoing loss of biodiversity and soil fertility, both against the background of global change. The Sustainable Development Goal 2 joins these challenges together into one main drive towards a resilient and future-proof agriculture [1]. Two factors are vital for that implementation: a better inclusion and consideration of soil organisms and a transition from the current high intensity crop management to a more sustainable one to protect soil health.

Soil organisms and soil processes which are governed by them are essential drivers of soil functioning and for the implementation of nature-based solutions [2,3]. Earthworms, as one of the most vital ecosystem engineers [4], play a significant role in the soil ecosystem, soil formation, nutrient turnover and litter transformation [5].

The reasons for different earthworm assemblages and abundances in soils are the change in the physical and chemical characteristics of the

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soil, caused by the intensity of mechanical agricultural practices and fertiliser use, respectively [6,7]. According to Bouché [8], earthworms are divided into three ecological categories: anecic (deep burrowing), endogeic (horizontal burrowing) and epigeic (litter inhabitants). Additionally, they can be divided into more specific subcategories like for example epi-anecic [9] to better represent their vertical and lateral position in the soil.

As already indicated by a vast array of research activities, a shift from annual to perennial cropping practices brings several benefits to the ecosystem [10,11] and counteracts the negative aspects of the former by focusing more on improved environmental conservation and sustainability [12]. It is therefore recommended to switch the current high intensity agricultural management to a more nature-based alternative, such as the use of perennial crops to improve soil functioning and support climate change mitigation. An annual management system of maize (*Zea mays*) and wheat (*Triticum aestivum*) is characterised by high yields through selective breeding [13], which benefits food security but also impacts the soil they are cultivated on: higher risk of erosion and nutrient loss, resulting in potential water pollution, and increased greenhouse gas emissions are just some of the most prominent downsides [14–16]. This could be improved by the cultivation of perennial crops [17–20].

As previously described, nature-based solutions for arable crops are already in development. The threat of food security however also requires the maintenance of the current food production, while simultaneously reducing soil degradation. The implementation of perennial grain crops offers an opportunity to extend this possibility for more sustainability to the food and cash crop sector. The perennial intermediate wheatgrass (IWG, *Thinopyrum intermedium*), commercialized as Kernza®, originates from domestication developed by the Land Institute in Kansas. It all started in 1983 when the Rodale Institute chose the intermediate wheatgrass as a potential perennial grain candidate for dual use of grain and forage under low input conditions [21]. After several cycles of domestication to enhance grain yield, potential for human or animal uses, and resistance to diseases and pests, IWG has been recognized and used in the food sector in the US [21]. IWG tends to improve soil properties by permanent cover and through a deep and active rooting system [22]. As a result, the threat of erosion and nutrient runoff was diminished [11,20,23]. Additionally, the sequestered soil carbon in the roots helps mitigating CO_2 emissions [24]. Besides, the subsoil accumulation of carbon stimulates microbial activity [25], benefitting overall soil quality and health [23].

Perennial crops like Miscanthus have been already well understood and show great potential through its CO_2 mitigation potential and higher microbial and faunal diversity [26,27]. Besides, an increase in for example soil organic carbon, especially in the subsoil was reported [28,29]. Additionally, perennial crops support the soil fauna (below- and aboveground) [10,30] by providing a greater food source and habitat due to the year-round soil coverage [31]. Earthworms are found in higher numbers under energy crops like Szarvazi or under wild flower mixtures, due to their perennial nature and low disruption of the soil [32]. Miscanthus as well supports earthworm communities [33,34], same holds true for *Silphium perfoliatum* (cup-plant), whose cultivation increases earthworm abundance and diversity in comparison to maize [35] due to its higher resistance to environmental factors like droughts and frost [36].

However, the impact of IWG on soil fauna is less documented, since perennial systems are still under development [37]. We hereby hypothesise that earthworms will be promoted by IWG in comparison to annual grain crops, mainly due to the following characteristics: 1) no disruption of their habitat due to the no-till management, 2) consequently, an increased soil coverage and thereby an increased food source on the surface, 3) the improvement of soil functioning, e.g. through a more extensive root system to improve nutrient and water retention. This field study was part of an EU-Biodiversa-Project called NAPERDIV, whose objective is to establish the base for novel field management guidelines and techniques to improve the usage of perennial grain crops. Within the project soil microbes (bacteria, fungi) and above- and belowground soil fauna have been studied by the project partners, among other focus areas, in three different study sites (France, Belgium and Sweden) in order to analyse the effect of perennial cropping systems under different basic conditions (including climate and soil). This range of framework conditions allows for a good transferability of our results onto other study sites. The objective of this paper was to analyse differences in earthworm communities and link them to soil chemical and physical parameters, as well as to agro-climatic conditions and to show that the beneficial aspects of IWG are unaffected by changing soil or climate conditions, resulting in great transferability of IWG across study sites. This management system (IWG) might become an innovative grain management system in the future due to the benefits explained above. To our knowledge, this is the first systematic investigation of earthworm communities in perennial grain cultivation systems in a North to South gradient across Western Europe. Likewise, this field study was the first to acknowledge the *Allolobophora chlorotica* subtypes related to soil parameters.

2. Materials and methods

2.1. Study sites

In 2021 and 2022, sampling was conducted at three locations: Saint Marcel Bel Accueil (France), Gembloux (Belgium) and Lönnstorp (Sweden) (Table 1). This created a large diversity of agro-ecological conditions. Additionally, the sites cover a wide range of soil properties, including physical (e.g. soil type and texture) as well as chemical (pH, organic carbon content, etc.) parameters. At each site, IWG has been compared with annual wheat, with the latter having different years of establishments (Table 1). Detailed information about the mean annual precipitation and temperature as well as site specific details like soil type or year of establishment were provided by Meteorological Services [38] and the field owners (Table 1).

Within the NAPERDIV Project, each study site consisted of an annual wheat and perennial intermediate wheat grass (IWG, *Thinopyrum intermedium*, Kernza®) field. All fields were part of a nested sampling approach (NSA), which meant that all partners of the project within the EU-Biodiversa consortium took their samples from the same soil at a pedon scale. The fields had the following dimensions (length x width): France: 40 x 18 m, Sweden: 50 x 24 m and Belgium: 7 x 2 m. For each country there were four discrete equal subareas (field replicates) for sampling for two succeeding years with 3 sampling campaigns. The detailed description of the pseudo replicates within those field replicates for soil and earthworm sampling can be taken from section 2.2 and 2.3, respectively.

2.2. Sampling and analysis of soil properties

Soil samples were taken at each of the three sampling campaigns mentioned in section 2.1. A split tube sampler (Royal Eijkelkamp, Giesbeek, Netherlands; length: 45 cm; diameter: 5.3 cm) was used for the soil cores. Each field replicate consisted of 3 soil cores, resulting in 216 soil cores in total (3 countries x 3 sampling campaigns x 2 management systems x 4 discrete field replicates x 3 soil cores). To create a mixed sample, the three soil cores were then pooled for each treatment. Additionally, each soil core was split into two depths: 5–15 cm and 25–35 cm. Within the NAPERDIV project, various groups of soil organisms, including bacteria, fungi, nematodes, earthworms and spiders have been investigated. Since the entire consortium took their soil samples from the same soil core, the upper 5 cm were used by another partner for the analysis of soil microbes (bacteria, fungi).

Sieved (<2 mm) and air-dried soil was mixed with 0.01 M $CaCl_2$ and measured with a glass electrode (pH3310, WTW GmbH, Weilheim, Germany) to obtain the soil pH. Amounts of total carbon and nitrogen

Table 1
Soil properties and characteristics of the three study sites in France, Belgium and Sweden.

Location	Climate [a]	Soil type	Crop	Year of establishment	Soil coverage [b] [%]	Depth [cm]	Soil texture	pH [−]	TOC [g/kg]	TN [g/kg]	C–N ratio [−]	WHC [c] [Vol.-%]
France Saint Marcel Bel Accueil 45° … “N 5° … “E	Temperate climate Rainfall 842.60 mm Temperature 11.64 °C	Gleyic Eutric Fluvisol (Loamic, Humic)	Annual	–	2.61%	5–15	Clayey loam	7.5	52.16	4.28	19.25	66
						25–35	Clayey loam	7.5	46.96	3.82	19.57	74
		Gleyic Eutric Fluvisol (Loamic, Humic)	Perennial	2018	76.68%	5–15	Silty loam	7.4	31.76	2.93	12.29	74
						25–35	Clayey loam	7.5	25.54	2.13	16.05	66
Belgium Gembloux 50° …”N 4° … “E	Temperate maritime climate Rainfall 898.90 mm Temperature 10.67 °C	Haplic Luvisol (Loamic, Humic)	Annual	–	20.73%	5–15	Silty loam	6.9	9.97	1.14	9.26	64
						25–35	Silty loam	7.0	7.18	0.89	8.58	63
		Haplic Luvisol (Loamic, Humic)	Perennial	2019	82.71%	5–15	Silty loam	6.8	13.64	1.26	10.98	74
						25–35	Silty loam	6.8	10.25	1.01	10.14	65
Sweden Lönnstorp 55° … “N 13° … “E	Temperate climate Rainfall 653.66 mm Temperature 3.18 °C	Eutric Stagnic Cambisol (Loamic, Humic)	Annual	–	68.66%	5–15	Clayey loam	5.8	16.41	1.84	9.06	66
						25–35	Clayey loam	6.2	7.78	1.32	8.18	62
		Eutric Stagnic Cambisol (Loamic, Humic)	Perennial	2016	84.84%	5–15	Clayey loam	5.7	16.42	1.72	9.55	66
						25–35	Clayey loam	6.4	10.19	1.08	8.54	66

TOC = total organic carbon, TN = total nitrogen, C–N ratio = carbon-to-nitrogen ratio, WHC = water holding capacity.

[a] Rainfall and temperature provided by [38].

[b] Soil coverage percentage calculated during the last campaign of 2022 for an area of 60 × 60 cm by the image processing program ImageJ [39] from a binary image of the soil surface (including litter and remaining straw and stubble).

[c] Water holding capacity (corresponding to field capacity at pF 1.8) of the respective soil depth derived from soil texture, bulk density and amount of soil organic matter in accordance with the German manual of soil mapping (5th edition, KA5) [40].

were measured after combustion at 1100 °C with an elemental analyser (Vario EL Cube, Elementar GmbH, Langenselbold, Germany). The total organic carbon (TOC) was deduced from the difference of the total carbon (TC) and the inorganic carbon (IC) content, the latter which was measured via carbonate destruction after the addition of phosphoric acid (IC Kit with Elemental Analyser EA3000 Series, HekaTech GmbH, Wegberg, Germany). The C–N ratio was calculated by the TOC divided by the total nitrogen content (TN). The values of the maximum water holding capacity (WHC) correspond to the values of the field capacity at pF 1.8 derived from soil texture, bulk density and amounts of soil organic matter (SOM) in accordance with the German manual of soil mapping (5th edition, KA5) [40]. Additionally, soil coverage was calculated using the image processing program ImageJ [39] for the last sampling campaign in Autumn 2022 after harvest. For this, an image of the soil surface (length x width; 60 x 60 cm) was imported into the program and converted into binary to calculate the percentage of soil coverage (including litter and remaining straw and stubble) from the known area of 3600 cm^2.

2.3. Earthworm sampling

The earthworms were sampled using a combination of hand-sorting the topsoil and AITC (allyl isothiocyanate) extraction in autumn (October) 2021, spring (April) 2022 and autumn (October/November) 2022, according to the current version of DIN EN ISO 23611 [41]. According to the limited size of NSA, two excavations of 50 x 50 x 20 cm (length x width x depth) were done for each field replicate in Sweden and France. Due to much smaller field sizes in Belgium, only one excavation was done per field replicate. The earthworms were first stored in Formaldehyde (3.7%) and then transferred into Ethanol (70%) in the laboratory. To sample the deep-burrowing earthworms, the AITC solution was diluted in 10 L of tap water and then poured into the excavation hole. The hole was covered by a plastic bag to block out sunlight and checked periodically for minimum 30 minutes. Earthworm abundance and biomass were combined from the extractions and extrapolated onto a sampling area of 1 m^2.

2.4. Earthworm identification

The keys of Graff [42], Sims and Gerard [43] and Bouché [44] were used for earthworm identification; the species names were then confirmed by Blakemore [45]. Additionally, an unpublished interactive earthworm identification key by Mickaël Hedde [personal communication, October 11, 2022] was exclusively used for the identification of *Aporrectodea giardi* and *Aporrectodea longa ripicola*. The identified individuals were split into adults (with clitellum) and subadults (first signs of adulthood by either first development of clitellum or tubercula pubertatis). Individuals with no indication of such were labelled as juveniles. It is important to note that the endogeic species *Aporrectodea caliginosa* also contains the species *Aporrectodea trapezoides*, since they have not been identified as two different species or close relatives yet [46,47]. Finally, the individuals were split into the three ecological categories after Bouché [44] (epigeic, anecic and endogeic) and then cross-referenced with Bottinelli et al. [9], further assigning them into subcategories (epi-anecic, endo-anecic and intermediate). Earthworms without clear assignment by Bottinelli et al. [9] were deduced from their morphological characteristics.

2.5. Statistical methods

Statistical analysis was conducted using RStudio version 2023.3.0.386 [48]. A generalised linear mixed model (GLMM) was used to evaluate the relationship between species and environmental data using the package "lme4" [49]. The random effect was the sampling campaign (date of sampling), while the cropping system and study site (country) were considered fixed effects. They were tested against the references (the first levels in the dataset), with annual being set as a reference for the cropping system, while Gembloux (Belgium) was the reference for the country data. The significance for the combined boxplots were taken from the Wilcoxon rank sum test in the package "stats" [50]. The combined boxplot was produced using "ggplot2" [51].

The software PC-ORD 7 [52] was chosen for the ordination analysis (Canonical Correspondence Analysis) of earthworm species and study sites along environmental gradients of soil properties, the latter were taken from Table 1. Species names were abbreviated with their genus and the first three letters of the species name, followed by "s" for subadult or "j" for juvenile, if applicable (e.g. A ros s = *Aporrectodea rosea* subadult). The sampling sites were abbreviated according to the country, the management type, the month of the sampling campaign and the corresponding number (e.g. FR A A 2 = France Annual, April, 2nd campaign).

The alpha diversity of the adult earthworms was calculated with the Simpson index, Shannon-Weaver index and Evenness. The beta diversity is represented by the Sorensen Coefficient. Furthermore, the relative frequency and abundance was calculated.

3. Results

3.1. Earthworm abundance and biomass

Between three sampling campaigns across three countries over a span of two years, 16 earthworm species were determined (Table 2). Some were present at all sites (e.g. *Aporrectodea caliginosa*), while others preferred only selected habitats, e.g. *Lumbricus castaneus* in IWG (Table 3).

From the 16 species found in this study, earthworm abundance was significantly higher in IWG compared to annual wheat with a mean abundance of 424.7 No. m^{-2} and 164.7 No. m^{-2}, respectively, including adults, subadults and juvenile individuals (Table S1). Earthworm biomass followed the same trend, resulting in a 1.8-fold increase.

Adult earthworm abundance and biomass was highest on the IWG sites in Belgium and lowest in annual wheat in France (Table 2). Fig. 1

Table 2

Mean abundance [A; No. m-^{2}] and biomass [BM; g m-^{2}] of adults, consisting of the sum of subadult and adult earthworms.

Species	Ecological category		Annual			Perennial		
			France	Belgium	Sweden	France	Belgium	Sweden
Lumbricus terrestris	epi-anecic	**A.**	3.92 ± 0.21	5.33 ± 0.67	1.42 ± 0.13	9.25 ± 0.21	10.33 ± 0.83	2.58 ± 0 63
		BM.	8.07 ± 0.55	19.94 ± 4.40	1.84 ± 0.03	19.64 ± 1.49	27.01 ± 4.37	3.97 ± 1.18
Lumbricus friendi	epi-anecic	**A.**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.04	0.33 ± 0.17	0.00 ± 0.00
		BM.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.05	0.86 ± 0.43	0.00 ± 0.00
Lumbricus castaneus	epigeic	**A.**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.13	4.67 ± 0.00	3.17 ± 0.33
		BM.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.02	0.57 ± 0.05	0.33 ± 0.06
Aporrectodea longa	endo-anecic	**A.**	0.33 ± 0.08	1.67 ± 0.50	10.00 ± 2.25	0.92 ± 0.04	2.33 ± 0.17	20.33 ± 4.92
		BM.	0.56 ± 0.21	3.00 ± 1.08	15.74 ± 4.79	1.36 ± 0.15	2.12 ± 0.34	23.33 ± 4.34
Aporrectodea longa ripicola	endogeic	**A.**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.11	0.00 ± 0.00	0.00 ± 0.00
		BM.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.07	0.00 ± 0.00	0.00 ± 0.00
Aporrectodea caliginosa	endogeic	**A.**	4.42 ± 0.54	39.33 ± 10.67	13.75 ± 1.54	7.08 ± 0.79	41.33 ± 5.00	24.00 ± 4.33
		BM.	1.80 ± 0.35	13.81 ± 4.30	8.68 ± 1.70	2.21 ± 0.22	9.31 ± 1.11	14.05 ± 1.78
Aporrectodea nocturna	endo-anecic	**A.**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.08	0.00 ± 0.00	0.00 ± 0.00
		BM.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Aporrectodea rosea	endogeic	**A.**	0.00 ± 0.00	4.67 ± 2.00	4.75 ± 1.63	1.50 ± 0.67	5.33 ± 1.00	9.67 ± 1.50
		BM.	0.00 ± 0.00	0.80 ± 0.35	0.98 ± 0.37	0.30 ± 0.14	0.77 ± 0.20	1.57 ± 0.26
Aporrectodea icterica	endogeic	**A.**	8.58 ± 1.54	0.00 ± 0.00	0.00 ± 0.00	6.42 ± 0.96	0.67 ± 0.00	0.00 ± 0.00
		BM.	3.75 ± 0.83	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 0.52	0.32 ± 0.04	0.00 ± 0.00
Aporrectodea giardi	endo-anecic	**A.**	1.25 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.42 ± 0.21	0.00 ± 0.00	0.00 ± 0.00
		BM.	1.77 ± 0.35	0.00 ± 0.00	0.00 ± 0.00	0.78 ± 0.39	0.00 ± 0.00	0.00 ± 0.00
Allolobophora chlorotica chlorotica	intermediate	**A.**	5.25 ± 0.29	7.67 ± 3.50	2.25 ± 0.13	14.67 ± 1.83	28.33 ± 2.17	7.00 ± 2.33
		BM.	0.95 ± 0.12	1.35 ± 0.65	0.40 ± 0.04	2.52 ± 0.47	3.76 ± 0.03	0.96 ± 0.32
Allolobophora chlorotica postepheba	intermediate	**A.**	11.67 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	7.58 ± 0.79	0.00 ± 0.00	0.00 ± 0.00
		BM.	2.73 ± 0.14	0.00 ± 0.00	0.00 ± 0.00	1.98 ± 0.11	0.00 ± 0.00	0.00 ± 0.00
Allolobophora chlorotica waldensis	intermediate	**A.**	4.58 ± 0.29	4.33 ± 0.83	0.25 ± 0.04	12.42 ± 0.71	1.00 ± 0.17	0.08 ± 0.04
		BM.	0.78 ± 0.03	0.74 ± 0.18	0.04 ± 0.01	2.54 ± 0.28	0.10 ± 0.02	0.01 ± 0.01
Allolobophora limicola	endogeic	**A.**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.08	0.00 ± 0.00
		BM.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.01	0.00 ± 0.00
Allolobophora antipae	endogeic	**A.**	0.00 ± 0.00	0.33 ± 0.08	0.08 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		BM.	0.00 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Helodrilus oculatus	endogeic	**A.**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.04
		BM.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.04
Abundance adults			40.00	63.33	32.50	61.25	94.67	66.92
Biomass adults			20.41	39.68	27.70	35.31	44.89	44.29
Abundance juveniles			101.25	183.33	73.75	234.83	590.00	226.33
Biomass juveniles			12.09	25.76	12.37	27.62	51.47	47.60
Total abundance			141.25	246.67	106.25	296.08	684.67	293.25
Total biomass			32.49	65.44	40.06	62.93	96.36	91.89
Mean species number			8	7	7	13	10	8
Abundance adults	epigeic			0.00 ± 0.00			2.69 ± 0.00	
Abundance adults	epi-anecic			3.56 ± 1.78			7.53 ± 3.63	
Abundance adults	endo-anecic			4.42 ± 1.27			8.11 ± 2.58	
Abundance adults	endogeic			25.31 ± 2.65			32.25 ± 3.35	
Abundance adults	intermediate			12.00 ± 0.58			23.69 ± 4.42	

Table 3

Relative frequency [%] of earthworm species between the three study sites and their relative abundance [%] in relation to the cropping system.

Species	France		Belgium		Sweden		Relative abundance	
	Annual	Perennial	Annual	Perennial	Annual	Perennial	Annual	Perennial
Lumbricus terrestris	9.8	15.1	8.4	10.9	4.4	3.9	7.9	10.0
Lumbricus friendi	0.0	0.1	0.0	0.4	0.0	0.0	0.0	0.2
Lumbricus castaneus	0.0	0.4	0.0	4.9	0.0	4.7	0.0	3.6
Aporrectodea longa	0.8	1.5	2.6	2.5	30.8	30.4	8.8	10.6
Aporrectodea longa ripicola	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.2
Aporrectodea caliginosa	11.0	11.6	62.1	43.7	42.3	35.9	42.3	32.5
Aporrectodea nocturna	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.2
Aporrectodea rosea	0.0	2.5	7.4	5.6	14.6	14.5	6.9	7.4
Aporrectodea icterica	21.5	10.5	0.0	0.7	0.0	0.0	6.3	3.2
Aporrectodea giardi	3.1	0.7	0.0	0.0	0.0	0.0	0.9	0.2
Allolobophora chlorotica chlorotica	13.1	24.0	12.1	29.9	6.9	10.5	11.2	22.4
Allolobophora chlorotica postepheba	29.2	12.4	0.0	0.0	0.0	0.0	8.6	3.4
Allolobophora chlorotica waldensis	11.5	20.3	6.8	1.1	0.8	0.1	6.8	6.1
Allolobophora limicola	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.2
Allolobophora antipae	0.0	0.0	0.5	0.0	0.3	0.0	0.3	0.0
Helodrilus oculatus	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.04
Number of species	**8**	**13**	**7**	**10**	**7**	**8**	**10**	**15**

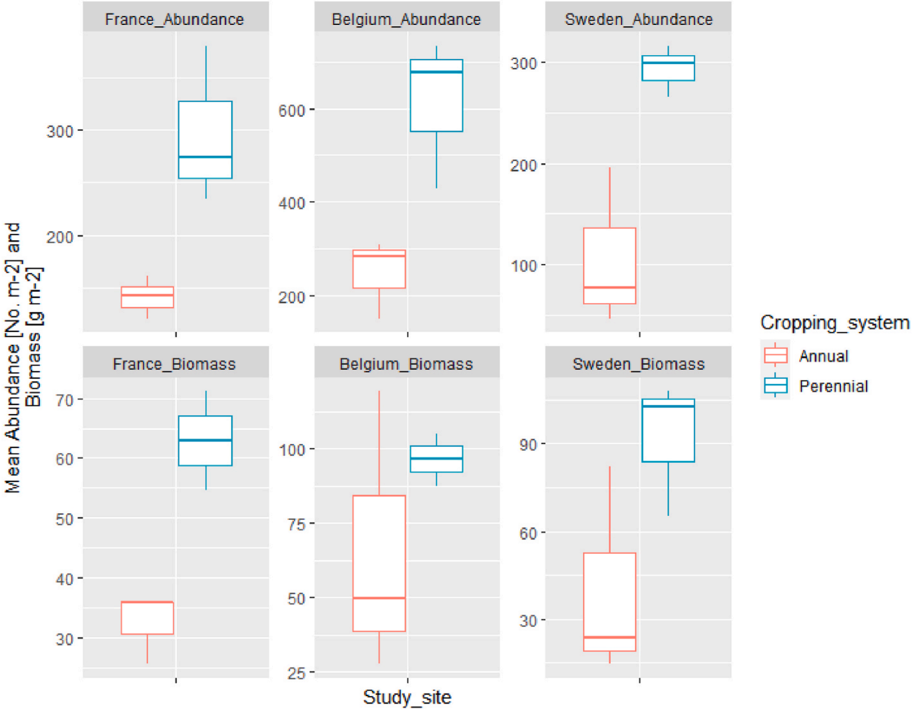

Fig. 1. Median values of earthworm abundance [No. m-^{2}] and biomass [g m-^{2}] at the three study sites, including adults, subadults and juveniles. All boxplots span the 0.25 and 0.75 quantile. Boxplot whiskers show the variability outside the upper and lower quantile. Testing for significance was done using the Wilcoxon rank sum test (p = 0.004 **).

shows a combined boxplot for all the campaigns with the mean abundance and biomass values, where IWG always achieved higher values than annual wheat. The exception was the total biomass in Belgium, which did not follow this trend due to the high occurrence of juveniles influencing the spread. The IWG plots in Belgium showed the highest ratio of 6.2:1 for the juvenile abundance and 1.2:1 for the biomass. In comparison, the sites in France had ratios of 3.8:1 for juvenile abundance under IWG.

Additionally, the GLMM revealed that the perennial crop always showed significantly higher earthworm abundance and biomass in comparison to the annual crop, except for the biomass in France (Table S2). Significant differences could be seen between the countries for the total abundance and total biomass as well, except for total abundance in Sweden.

3.2. *Earthworm species diversity*

The earthworm species diversity was higher under IWG than annual wheat. Combining the three sites resulted in 10 species for the annual plots, while 15 species were found in IWG (Table 3).

A. caliginosa was the most abundant species for both management systems with 42.3% for annual wheat and 32.5% for IWG. Other species were less dominant, and frequencies differed in annual and perennial plots (Table 3).

Species that only appeared on perennial sites were *L. friendi*, *L. castaneus*, *A. longa ripicola*, *A. nocturna* and *A. limicola*. The rest of the species appeared in both cropping systems with varying relative abundances (Table 3). Additionally, three species were country specific: *A. nocturna* (France), *A. limicola* (Belgium) and *H. oculatus* (Sweden). All three subspecies of *A. chlorotica* (*A. chlorotica chlorotica*, *A. chlorotica waldensis, A. chlorotica postepheba*) could only be found in France. For each country, the species numbers were higher under IWG than under annual wheat. (Table 2).

The Canonical Correspondence Analysis (CCA) offers the possibility of distributing earthworm species and study sites along environmental variables (Fig. 2). Concerning the distribution of the study sites, four clusters were separated. The Swedish sites, with the lowest pH, and the French IWG sites, with the highest pH values, are separated along axis 1. The same correlation can be seen with the WHC. However, there was no visible distinction by CCA between the perennial and annual treatments for the three countries, except for France. The annual plots showed the highest mean values of C–N ratio, TN and TOC, therefore being located at the bottom left of the CCA. Since the Belgian sites had the lowest mean values, they are on opposite ends of the France Annual plot. Looking at the species distribution, the clusters correlate with their occurrence at the different sites. For example, *H. oculatus* and *A. longa* subadult are located at the far right of the chart, same as the Swedish sites, since those species are only or mostly present there. A similar pattern was seen for *A. limicola* subadult and *L. friendi*, which correlate with the Belgium sites. Interestingly, the three subspecies of *A. chlorotica* are all located differently on the CCA (see rectangle shapes). They seem to follow the TOC and TN gradient from *A. chlorotica chlorotica* starting from the centre, followed by *A. chlorotica waldensis* and lastly *A. chlorotica postepheba.* It could therefore be hypothesised that the latter prefers sites, which are richer in carbon and nitrogen. The latter also correlates with the France annual sites most, same as *A. giardi* (see pentagon shape) and *A. icterica.* Soil coverage was not added into the CCA, since it had only been measured once at the last sampling campaign. The values can be taken from Table 1. In all cases, IWG had higher soil coverage than annual wheat.

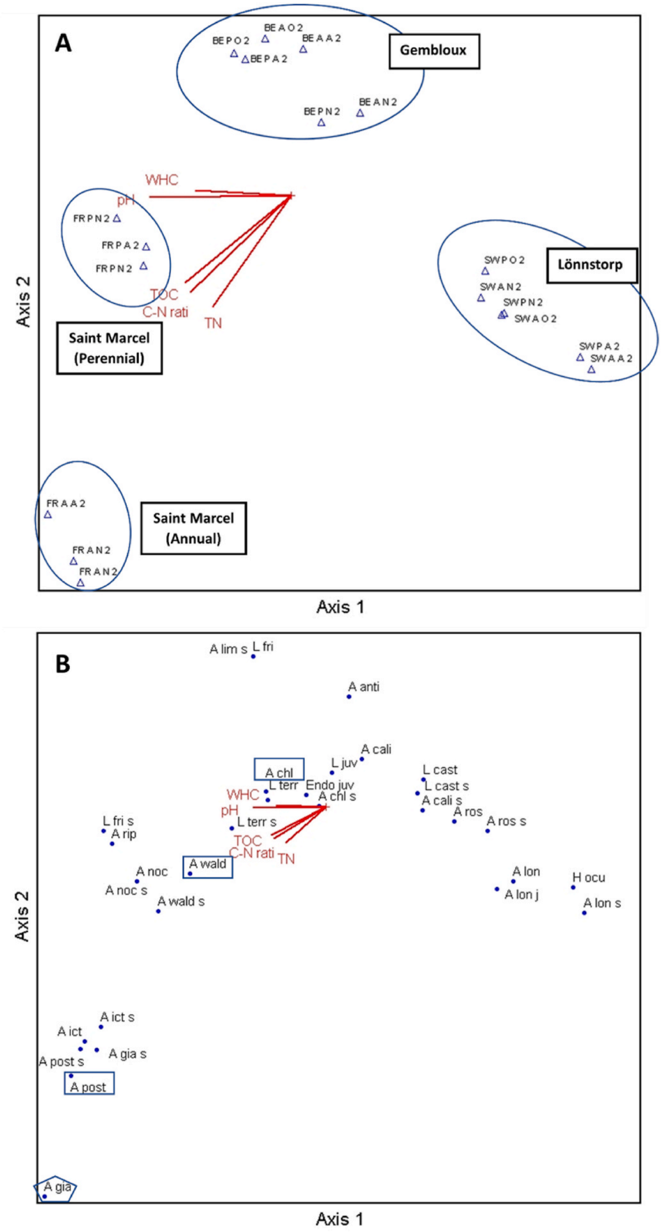

Fig. 2. Canonical Correspondence Analysis (CCA; Monte Carlo; p-value = 0.001***). **A:** CCA showing the relationship between soil parameters (red arrows; WHC = water holding capacity, TOC = total organic carbon, C–N rati = C–N ratio, TN = total nitrogen) and study sites (triangles). **B:** CCA showing the earthworm species (points) in relation to the soil parameters (red arrows). Abbreviations are explained in chapter 2.5.

The differentiation of the ecological categories of earthworms revealed an increase in their abundance on the perennial treatments. Endogeic species were supported by perennial cropping since their abundance increased by 0.8 in comparison to annual cropping system and nearly doubled when taking endo-anecic species into account as well (Table 2). Notably, epigeic species were only present in perennial treatments.

The Simpson index, Shannon-Weaver index and Evenness were chosen for the α-diversity (Table 4). The highest diversity was found in the autumn 2022 campaign for France IWG with a Simpson index of 6.16, a Shannon-Weaver index of 2.02 and a species number of 12. The highest Evenness was found in the spring 2022 campaign for Belgium IWG with 89.16%, indicating that the species are evenly represented within the community. Overall, diversity was higher for IWG, but there was no clear difference between the spring and autumn campaigns.

For β-diversity, the Sorensen's coefficient showed the highest similarity between Belgium and Sweden and the least between Sweden and France (0.80 and 0.64, respectively) (Table 5). This might indicate a South to North gradient. Split up into the management systems, the coefficients showed the highest similarity between the annual Belgium sites and the annual Sweden sites with a value of 1, showing total similarity. The least similar sites were the IWG France sites compared to the annual Belgium sites and to the annual Sweden sites.

4. Discussion

Recently, the goal is to double agricultural productivity by 2030 through the implementation of sustainable agriculture approaches, according to the Sustainable Development Goal 2 [1]. This should also result in an enhancement of biodiversity through the reduction of destructive agricultural practices. Evidence shows that a switch from the current high intensity agricultural management to a more nature-based alternative may support soil organisms, including earthworms, thus soil health and soil functioning [53], by creating a healthy and sustainable habitat due to the absence of tillage and an increase in soil coverage [12], among other factors.

4.1. *Perennial cropping systems promote earthworm communities*

Emmerling et al. [34] found twice as high earthworm abundance values under perennial crops, including cup plant and tall wheatgrass, compared with annual crops. This correlation was even higher for our data with an increase of 2.6 (Table 2). Other studies analysed the beneficial effects of Miscanthus specifically on contaminated soil and its biodiversity and found improvements as well [27,54], displaying the potential of using perennial crops on marginal lands in order to stimulate productivity on fields whose soil type or topography may limit annual crop cultivation [37]. Grasslands, with their "permanent" cultivation are oftentimes pioneers in biodiversity [10,55] and may promote

Table 4

α-diversity indices (Simpson index, Shannon-Weaver index, Evenness) and species richness (number of earthworm species) at the three study sites at various sampling campaigns.

	Simpson Index	Shannon-Weaver Index	Evenness [%]	Species richness
France Annual				
Autumn 21	4.87	1.69	86.85	7
Spring 22	5.54	1.81	87.03	8
Autumn 22	3.75	1.57	75.34	8
France Perennial				
Autumn 21	5.37	1.87	81.20	10
Spring 22	5.84	1.87	85.22	9
Autumn 22	6.16	2.02	81.47	12
Belgium Annual				
Autumn 21	2.47	1.21	67.59	6
Spring 22	1.85	0.89	64.00	4
Autumn 22	2.22	1.04	64.43	5
Belgium Perennial				
Autumn 21	2.59	1.16	59.83	7
Spring 22	5.75	1.85	89.16	8
Autumn 22	2.89	1.33	68.27	7
Sweden Annual				
Autumn 21	3.05	1.29	71.83	6
Spring 22	3.24	1.30	80.65	5
Autumn 22	3.88	1.54	85.90	6
Sweden Perennial				
Autumn 21	3.46	1.42	79.00	6
Spring 22	4.03	1.54	86.01	6
Autumn 22	3.96	1.55	74.72	8

earthworm abundance and diversity [56] with species like *A. longa*, *A. caliginosa* and *L. terrestris* being most abundant [57], similar to our data (Table 3). It is hypothesised that IWG, being similar to grasslands, may lead to an increase in biodiversity compared to annual wheat. Benefits for arthropod and fungal communities, as well as for earthworms, were related to the increase in the litter layer, among other factors [58,59]. Additionally, it supports soil fauna, including earthworms [58]. Lastly, perennial grains like IWG have a potential productive life span of 3–10 years [24], with the second year being the most profitable [20,60]. The deeper and denser root system fixes and stores nutrients [11,20,23] and indirectly promotes microbial and fungal communities through a shift in root litter [22,25]. The effect of IWG on soil fauna, more specifically earthworms, is not well investigated yet.

For our data, abundance and biomass of earthworms were less in annual wheat (Table 2) due to soil tillage (ploughing), which may impact soil organic matter content and endanger earthworms by exposing them to predators and UV-radiation (e.g. [57,61,62]). To analyse the reproduction rate of the earthworms, the ratio of juveniles to adults was calculated. It displayed higher ratios for IWG with the plots in Belgium having the largest ratio (6.2:1). Schmidt et al. [63] found an increased juvenile to adult ratio on fields with higher soil coverage, which was also true in our study as IWG had increased soil coverage compared to annual wheat (Table 1). This is likely due to the increase in food source [11,60] and decrease in destructive management practices, one of the most destructive being tillage (e.g. [64]), which leads to higher reproduction rates for the earthworms [65].

4.2. *Significance of the cropping systems*

The CCA distributes species and study sites along soil properties in the three study sites (Fig. 2). Earthworm abundance and biomass were significantly different between the cropping systems for all countries according to the GLMM (Table S2). Additionally, the CCA did not reveal a significant impact of soil properties on earthworm population between the cropping systems, except for France. This indicates that the differences primarily stem from the cropping system itself and not from the soil parameters. The low coefficient of determination of the earthworm biomass model in France (Table S2) further elaborates on this assumption, since a low coefficient could mean that other unknown (environmental) factors are more influential than the cropping systems, possibly referring to effects of historical land-use. For France, the annual sites had increased soil properties relative to IWG, especially regarding TOC (Table 1), which is why they are differentiated in the CCA (Fig. 2A). Additionally, there was no distinction between the sampling campaigns (Autumn or Spring).

4.3. *Earthworm species diversity along environmental parameters*

Each ecological category gets influenced differently by agricultural management practices, as pointed out by several studies. Epigeic species, like *L. castaneus*, are most exposed to land use change and harmful management practices (like tillage and herbicide application), since they live at the soil surface, which may explain their absence in our annual fields (e.g. [56,64]), as those sites had lower soil coverage

Table 5

β-diversity (Sorensen coefficient). I: Sorensen coefficient for the study sites and cropping systems (FR = France, BE = Belgium, SW = Sweden; A = annual, P = perennial). II: Sorensen coefficient for the three countries.

I	FR A	FR P	BE A	BE P	SW A	II	France	Belgium
FR P	0.76	1	–	–	–	**Belgium**	0.75	1
BE A	0.67	0.60	1	–	–	**Sweden**	0.64	0.80
BE P	0.67	0.78	0.71	1	–			
SW A	0.67	0.60	1.00	0.71	1			
SW P	0.63	0.67	0.80	0.78	0.80			

percentages than the IWG sites (Table 1). Endogeic species are often the dominant ecological category in arable land [66], since they live within the soil where they are mostly protected and therefore more resistant to disturbance in the environment, as for example in the case of *A. caliginosa* [67]. Emmerling et al. [34] and Kanianska et al. [56] found higher abundances of endogeic species under perennial energy crops and grassland, probably due to an increase in SOM, confirming the hypothesis that IWG supports endogeic species, as found in our field study (Table 2) due to its similarity to grassland, which also promotes endogeic species [68]. Reasons for that include the more extensive root system of IWG [60], which creates a better food source for endogeic earthworms since they feed on organic matter in the rhizosphere (e.g. [69,70]). Furthermore, anecic species, like *L. terrestris*, are adversely affected by annual cropping systems, since tillage decreases litter (their food source) and destroys their burrows [67], leading to lower numbers in annual or intensively managed sites [56,61] (Table 2).

The soil parameters in the CCA differentiated between earthworm species, especially concerning *A. chlorotica* and its subtypes (Fig. 2B). WHC correlated with earthworm species like *L. terrestris* and *A. chlorotica chlorotica*, similar to the findings from Hallam and Hodson [71] due to their burrows holding onto more water. The plots in France and the IWG plot in Belgium had the highest WHC. This could explain why the annual plots in France, despite being an annual wheat field with high disturbance, have comparable values to the IWG plots in terms of species diversity, since earthworm diversity is positively correlated with soil moisture [56]. To our knowledge, there is no indication in literature, how the subtypes of *A. chlorotica* depend on soil properties, resulting in different distributions in the CCA (Fig. 2B). Morphologically, *A. chlorotica chlorotica* can be distinguished by its green or red colour in comparison to the other two subtypes *A. chlorotica postepheba* and *A. chlorotica waldensis*. This could lead to the assumption that it is more endo-epigeic compared to the other two subtypes and therefore may prefer perennial cropping systems (Table 3) due to an increased litter layer and abundance of soil organic matter [67]. *A. chlorotica postepheba* and *A. chlorotica waldensis* are more greyish in colour, meaning they are not as protected from the UV radiations on the surface as *A. chlorotica chlorotica*. This could be why they are not as frequent in IWG (Table 3). The subtypes follow the TOC and C–N ratio gradient (Fig. 2B), with *A. chlorotica postepheba* preferring soils with higher values. Additionally, it had a more extreme distinction in abundance between annual and perennial cropping systems (Table 3). Since there is no indication about preferences for the subtypes of *A. chlorotica* in literature, it is hypothesised that it may be related to their carbon allocation in the burrows [72].

A. caliginosa appeared as the most dominant species (Table 3), which coincides with literature (e.g. [34,56,61]). The usage of mineral fertilisers and other adverse agricultural practices is not as damaging to the species in contrast to most other species, opening up its habitat to most land use types [67], including arable land and pastures [73]. Additionally, its abundance had a positive correlation with an increase in disturbance in the environment, since it can escape into its burrows [6,74], which explains the higher relative abundance for annual compared to IWG cropping. *A. longa* and *A. caliginosa* had the lowest relative frequency in the annual wheat plots in France due to the high nitrogen content at those sites, since there is only a narrow optimum for these species, meaning their abundance is less under extreme (low or high) nitrogen concentrations [75]. *A. limicola* was exclusively present in IWG due to its preference for grasslands [42]. *L. friendi* is often found under no-till crops, mostly in France and Spain [76], hence its only appearance on the France IWG plots. *A. giardi* was only present in France, preferably under annual wheat, which could correlate to the higher soil compaction under these cropping systems, since Capowiez et al. [77] found significant higher numbers of this earthworm species under compacted soil. *A. nocturna* is often absent in agricultural fields [57], which could be the reason why it was only present in IWG plots. The abundance of *A. longa* was highest in Sweden, while these plots had the lowest abundance of *L. terrestris* (Table 3). Reasons could be a potential competitive effect between the two species [78,79] due to their similar habitat, since they are both deep-burrowing species [44], which is probably why the abundance of *L. terrestris* was highest in France, where *A. longa* was not as abundant. Additionally, the study from Edwards and Lofty [79] analysed a competition between *A. chlorotica* and *A. caliginosa*, again due to their similar habitats, which could be partially seen in our data as well, since their abundances weakly negatively correlate (Table 3). *A. longa ripicola* was exclusively found in IWG in France. It is a rare species that prefers moist soils [44]. Since the WHC does not differentiate between our sites (Table 1), it remains unclear if the one occurrence on IWG in France is due to its soil properties or just shows the rarity of this species.

Within the scope of this study, we found more species in IWG than annual cropping systems with 8–13 species in the first and 7–8 in the latter (Table 2). The difference in species numbers for Sweden was not as prominent (7 species in annual wheat; 8 species in IWG) compared to the other countries (e.g. France with 8 species in annual wheat and 13 species in IWG) (Table 2), which could be due to their small difference in soil coverage between the cropping systems as compared to France (Table 1). The number of earthworm species is referred to be promoted by perennial crops, like for example bioenergy crops, including Miscanthus and cup plant (*Silphium perfoliatum*) (e.g. [58,80]). Cultivation of cup plant is defined by an increase in litter, which may promote abundance and diversity of earthworms up to 6-fold [81], despite having a higher C–N ratio than for example maize [82]. The cultivation aspects of no-till and increased surface cover seem to overrule these limitations.

Alpha-diversity (Simpson and Shannon-Weaver) and species richness increased under IWG and was lowest under annual wheat [34,61,83] (Table 4), meaning it decreased with increasing intensity in management of the agricultural system [6]. The trend for Evenness was not as clear, but in general, higher values were achieved under IWG as well, which leads to the assumption that these cropping systems create a balanced and more enriched earthworm community structure. Singh et al. [84] found lower diversity in June in comparison to October due to the change in soil moisture. This trend was partly visible in our data (Table 4) with the highest alpha-diversity (excluding Evenness) found in the autumn 2022 campaign on the IWG plots in France and the lowest in annual wheat in Belgium for the spring 2022 campaign. The beta-diversity showed a possible North to South gradient with Belgium and Sweden being most and Sweden and France least similar (Table 5) due to their geographical proximity within Europe.

5. Conclusion

This field study revealed that earthworms are supported under perennial wheat cropping systems, due to a more nature-based land-use management, excluding excessive use of tillage, disruption of their habitat and pesticides. Especially epigeic and endogeic species were promoted. IWG creates a better habitat for earthworms due to its more excessive root growth and an increased food source, for example. This leads to a more species-rich community with representatives of all ecological categories (epigeic, epi-anecic, endo-anecic, endogeic and intermediate). Additionally, earthworms had a higher reproduction rate under IWG, compared to annual wheat. As expected, a gradient from South to North for Western Europe became apparent, from 13 species in France to 7–8 in Belgium and Sweden. To our knowledge this was the first comprehensive study to acknowledge the distribution of *A. chlorotica* subtypes along selected soil parameters, especially TOC and TN. Since earthworm abundance and biomass were higher under IWG than annual wheat for all the study sites, it means that differences in soil and climate conditions do not interfere with the beneficial effects of IWG. This shows the great transferability of our study onto other study sites, possibly even to establish perennial crops on marginal lands as prospective research to create a future-proof alternative to the current high intensity agricultural management. These factors lead us to believe that IWG may become an innovative grain management system in the future, promoting soil health and functioning, which is the major driver for a more resilient agriculture in the future.

6. Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Frank Rasche reports financial support was provided by University of Hohenheim Institute of Agricultural Sciences in the Tropics.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2023.103561.

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