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Abstract

Mineral and organic fertilizers and amendments modify soil characteristics and impact beneficial soil organisms. However, conventional fertilizer experiments cannot separate impacts mediated through changes in crop productivity and through changes in soil chemical properties. We assessed populations of earthworms and enchytraeids (Oligochaeta, Annelida) in the surface horizons of a loess Luvisol of a world-unique long-term bare fallow experiment of INRAE (Versailles, France) to test the trophic and edaphic limits of existence for these soil taxa. Continuous annual applications since 1928 of 16 different treatments including nitrogen, phosphorus, potassium fertilizers but also basic (alkaline) and organic amendments, led to strongly diverging physical and chemical properties in the soil's surface layer. The feeding activity of soil organisms was also assessed using the bait lamina method, assuming that lower bait consumption rates would be observed in treatments where fewer terrestrial Oligochaeta persist. We showed that compared to conventionally managed cultivated soils, both taxa were much less abundant in these extreme soil treatments, but enchytraeids were relatively more abundant than earthworms, ascribed to the enchytraeids' higher tolerance to low pH soil conditions and a different feeding ecology. Most of the enchytraeid and earthworm individuals were found under horse manure inputs and basic amendments, suggesting that organic matter contents and/or pH would drive most of the Oligochaeta communities. Moreover, the bait-lamina feeding activity was significantly positively correlated with enchytraeid abundance. This study underlines the usefulness of historic long-term trials to improve knowledge on the ecology of soil organisms, and it presents an example of long-term soil biological resilience in highly degraded soils.

Keywords Potworms | fertilizers | physico-chemical soil properties | long-term field experiments

1. Introduction

In agricultural soils, the use of fertilizers, either mineral or organic, modify physical, chemical and biological soil properties (Riley et al. 2008). Fertilizers therefore constitute a major management tool for maintaining soil quality, in the sense given by Doran and Parkin (1996), i.e. the capacity of a soil to sustain biological productivity, maintain environmental quality, and promote plant and animal health. From a biological perspective, organic matter (OM) is of major interest in soils because it drives soil food webs encompassing microbes and invertebrate animals (Wardle et al. 2004), and greatly influences their overall properties and suitability as a habitat. Organic compounds act as linking agents with mineral particles i.e. clay, silt, and sand, thus controlling soil structure and stability (i.e. Tisdall & Oades 1982), and they represent a source of nutrients

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for plants after mineralization and a sink for organic and mineral pollutants (Mazzei & Piccolo 2015, Wood et al. 2018). Basically, OM exerts a major buffering role in soils with respect to hazardous anthropogenic pressures (Lal 2015). However, in OM-depleted agricultural soils with a diminished buffer capacity, impacts of fertilizing practices on soil properties become more clearly perceptible (van Oort et al. 2020). Long-term bare fallow experiments offer unique opportunities to study specific impacts of extreme soil conditions (e.g. soil OM depletion, acidification, alkalinization) and repeated fertilization on soil fauna.

Enchytraeids (potworms) and earthworms are soil fauna that fulfill key functions in natural and cultivated ecosystems. Both enchytraeids and earthworms are involved in the dynamics of soil OM (Parmelee et al. 1990, Liu et al. 2019) and soil structure (Zhang & Schrader 1993, Van Vliet et al. 1993) at different but complementary scales (Didden et al. 1994). Enchytraeids are part of the soil mesofauna that has a body diameter between 0.1 and 2 mm, while earthworms belong to the macrofauna, with a body diameter between 2 and 20 mm. Among terrestrial Oligochaeta, the use of earthworms as bioindicators is well documented (Paoletti 1999). Enchytraeids have been studied less but they are also recognized as bioindicators of soil quality (Jänsch et al. 2005). In general, enchytraeids are considered to be less sensitive to human activities (e.g., pesticide use e.g., Frampton et al.; 2006 tillage e.g., Nowak 2004) and environmental stressors (e.g. salinity, Owojori et al. 2009) than earthworms, being thus able to live in environments hostile to earthworms.

The use of mineral and organic amendments in agricultural fields influence earthworms and enchytraeids. While mineral fertilizers have contrasting effects on these soil organisms depending on applied nutrients, quantity, frequency and effects on soil pH (van der Wal et al. 2009, Lalthanzara & Ramanujam 2010), organic amendments are generally reported to promote terrestrial Oligochaeta (Ricci et al. 2015) because enchytraeid and earthworm populations, as many other organisms, are resource-controlled (Wardle et al. 2004). Earthworms are saprovorous and feed on decaying organic matter, spanning a gradient of decomposition (Lee, 1985; Curry & Schmidt 2007). Enchytraeids are both microbivorous and saprovorous, in a proportion that is still not well known (Gajda et al. 2017). For instance, Lagerlöf et al. (1989) considered that enchytraeids are 50 % saprovorous and 50 % microbivorous, while other authors (e.g., MacLean 1980) reported they were only 20 % saprovorous and 80 % microbivorous. Enchytraeids would be thus less dependent on younger decaying organic materials than most earthworms with the possible exception of strict endogeics.

The aim of this study was to assess earthworm and enchytraeid communities in soils that had not received organic amendments and had no vegetation for nine decades, and was hence strongly depleted in OM, but that had highly contrasting physico-chemical characteristics (in particular pH, aluminization, presence/absence of exchangeable calcium). These soils were compared to the same soils receiving horse manure amendments and untreated control soils. For that, earthworms and enchytraeids were sampled in a long-term trial that received 16 different types of fertilizers (including nitrogen, phosphorus, potassium fertilizers) and amendments (including basic and organic amendments) since 1928. We also measured the general feeding activity in the different treatments using the bait-lamina method. We hypothesized that the organic amendments would have the highest abundance, diversity and activity of soil annelids. Moreover, we hypothesized that, compared to conventional agricultural soils, the relative abundance of enchytraeids would be higher overall than that of earthworms in the trial, due to the higher tolerance of enchytraeids to stressed edaphic conditions and to their different feeding behaviour.

2. Material and methods

2.1 Experimental trial

The 42-plot experiment (Figure 1, Table 1) was initiated in 1928 at the 'Central Station of Agronomy', nowadays the 'French National Research Institute for Agriculture, Food and Environment' (INRAE), located in the gardens of the 'Chateau de Versailles". This experiment was originally designed as a long-term bare-fallow study to investigate the impacts of continuous inputs of fertilizers on soil composition and physical properties without the influence of plant inputs (Burgevin & Hénin 1939). The experiment is located on a Luvisol developed in aeolian loess, and according to its original design, it has always been kept free of vegetation, in order to exacerbate the action of the chemicals on soil constitution and properties. Haplic Luvisols are widespread in the Paris Basin and in large areas of northwestern Europe. Owing to their potential chemical and physical fertility, these soils are widely used for conventional agriculture. In 1929, soil properties were common in all the plots: 21.7 % sand (0.05–2 mm), 58.6 % silt (2–50 µm) and 19.7 % clay (<2 µm), a pH in water of 6.4 and a cation exchange capacity (cobaltihexammine method) of 15.3 cmol kg^{-1} (Pernes-Debuyser & Tessier 2002). The fertilizers used for 9 decades led to strongly diverging physical and chemical properties in the soil's surface layer (van Oort et al. 2018, 2020).

Table 1. Description of the treatments employed in the Versailles 42-plot long term bare fallow experiment.

Fertilizer/amendment	Equivalent rate	Plots	Type of treatment
None	-	9, 11, 30, 32, 34	Reference
Ammonium sulphate	150 kg N ha^{-1} yr^{-1}	2, 19	N
di-Ammonium phosphate	150 kg N ha^{-1} yr^{-1}	3, 14	N, P
Ammonium chloride	150 kg N ha^{-1} yr^{-1}	7, 15	N
Dried blood	150 kg N ha^{-1} yr^{-1}	8, 18	N
Ammonium nitrate	150 kg N ha^{-1} yr^{-1}	6, 20	N
Sodium nitrate	150 kg N ha^{-1} yr^{-1}	4, 17	N, Na
Calcium nitrate	150 kg N ha^{-1} yr^{-1}	5, 16	N, Ca
Manure	100 Mg ha^{-1} yr^{-1}	10, 12	N (Organic)
Natural phosphate	200 kg P_2O_5 ha^{-1} yr^{-1}	28, 33	P
Superphosphate	200 kg P_2O_5 ha^{-1} yr^{-1}	27, 38	P
Basic slag	200 kg P_2O_5 ha^{-1} yr^{-1}	24, 35	P, Ca
Calcium carbonate	1 Mg CaO ha^{-1} yr^{-1}	31, 39	Ca
Calcium oxide	1 Mg CaO ha^{-1} yr^{-1}	26, 40	Ca
Potassium chloride	250 kg K_2O ha^{-1} yr^{-1}	23, 37	K
Potassium sulphate	250 kg K_2O ha^{-1} yr^{-1}	25, 41	K
Sylvinite	250 kg K_2O ha^{-1} yr^{-1}	29, 36	K, Na

* Note that due to its properties Dried blood was assigned to Ammonium based fertilizers rather than Organic amendments.

Figure 1. Graphical presentation of the treatments and layout of the Versailles 42-plot long-term bare fallow experiment. Plots with a red cross were not sampled in the present study.

The experiment includes 42 plots of 2 m × 2.5 m each (Figure 1), representing 16 duplicate trials of continuous application of major types of N, P, and K-fertilizers as well basic and organic amendments, and additionally 10 reference plots (no inputs) (Table 1). The borders around all plots are 20 cm high, with 10 cm into the ground and 10 cm above the mean soil level, and since 2013, a 5 cm thick quartz gravel pavement separates the plots. Immigration into, and movement between plots by mobile earthworms can therefore not be excluded. The main annual experimental interventions were fixed rates of fertilizers and amendments (Table 1), spread by hand and incorporated in the upper 25 cm of the soil by digging by spade, twice a year (spring and autumn). Over time, the plots have been kept free of vegetation manually, currently along with sporadic use of a chemical weedkiller (i.e., glyphosate-based herbicide). Sampling of the 0–25 cm surface horizon occurred initially each year and since the early 1970s every 3 to 4 years. In this study, data were used from soil analyses on samples collected in 2017 (Table 2).

2.2. Sampling of Oligochaeta

The sampling was done in spring 2017 on 37 plots out of 42. Five control plots (numbered 1, 13, 21, 22, and 42) were not sampled in order to balance the number of plots for control and other treatments. For earthworms, conventional, destructive methods of sampling that combine a chemical extraction with hand-sorting of a soil core could not be used since soil chemical and physical properties in this unique, small-plot trial must be conserved. Earthworms were thus sampled by the non-destructive, electrical 'octet' method (Čoja et al. 2008) (model RWF1, Elektrotechnik Schuller, Germany). One sampling was done for each of the two treatment replicates. In each plot, 1 m² was sampled delineated by a plastic ring placed on the soil surface. The 16 steel electrodes placed around a circular template and another placed in the middle of the circle were pushed into the soil to at least 30 cm depth and connected to an AC generator, fed by a 12 V battery. During 20 minutes, the electric current and voltage were increased stepwise (every four minutes) from 0.2 A, 46 V to 1 A, 230 V and emerging earthworms were collected continuously. After being captured, earthworms were fixed and stored in a 4 % formaldehyde solution. Adult and sub-adult individuals were identified to species level (Sims and Gerard, 1999). Juveniles were also identified to the species level according to morphological characters of the adults and to the specific form they take in formalin in comparison with that of identified adults. For anecic species, a category '*A. giardi/longa* juveniles' was created when no discrimination was possible between juveniles of *Aporrectodea longa* and *Aporrectodea giardi*. All individuals were counted and weighed.

For enchytraeids, two samplings, randomly located, were done on each of the two replicates of each treatment. Sampling was conducted based on the the standard method (ISO, 2007). With a 5 cm diameter soil auger, two samples from under each other were taken at 0–5 cm and 5–10 cm depth because most enchytraeids generally stay in the uppermost soil layers (Healy 1980). Samples were transferred to the laboratory in plastic bags and kept at 4°C for a maximum of 15 days. Enchytraeids were extracted from soil cores using a modified wet-funnel method (O'Connor, 1962). After 3 hours, sediments containing enchytraeids were collected from the tube and filtered through a fine sieve (mesh size 100 μm). The sieve content was then placed in a Petri dish. To estimate abundances, enchytraeids were counted alive under a binocular microscope. Adult and sub-adult individuals were identified *in vivo* under a light microscope (Olympus BX53) according to Schmelz and Collado (2010).

2.3. Feeding activity

The general feeding activity of soil animals was measured using the bait lamina method (ISO 2014). PVC sticks (Terra Protecta GmbH, Berlin, Germany) containing 16 apertures (5 mm apart from each other), were filled with baits composed of cellulose powder (70 %), finely ground wheat bran (25 %), and activated carbon powder (5 %). In each plot, in spring 2017, a set of 16 sticks were inserted in a 4 × 4 grid within an area of about 30 cm × 30 cm. As a control, we regularly screened 10 additional strips inserted in the plot 12 (horse manure amendment) in order to identify the duration at which at least 30 % of the baits were fed on a stick. After 15 days, the sticks were removed from the soil. Removed, presumed fed apertures (i.e., disappearance of at least half of the organic bait material) or remaining, not fed apertures were directly counted. The overall rates and the vertical distribution of the feeding activity were calculated.

2.4. Soil analyses, moisture, and temperature

Analyses were performed at INRAE's national Soil Analyses Laboratory (LAS, Arras) according to international standard methods (for individual references for the cited standard methods please see AFNOR 2004): grain size distribution (NF X 31-107), pH in water (NF ISO 10390), the cation exchange capacity (CEC) and exchangeable cations by the cobaltihexammine method (NF ISO 23470). Total element concentrations of phosphorus (expressed as P_2O_5) was determined by ICP-AES or ICP-MS spectrometry (NF ISO 22036) on sub samples ground to 250 µm before complete dissolution by hydrofluoric and perchloric acid (NF X31-147). For total organic C and N analysis, the bulk soil samples were dried at 105°C for 48 h, milled to a homogeneous powder (<0.2 mm) and then stored in desiccators until analysis. Total organic C and N concentrations were measured by dry combustion in a CHN autoanalyser (Carlo Erba NA 1500).

Soil moisture and temperature were measured in spring 2017. One soil core per plot was sampled using a soil auger 3 cm in diameter at 0–10 cm depth. Soil samples were weighed fresh, placed in an oven for 72 h at 105°C, and weighed dry. Soil temperature at 10 cm depth was measured once in each plot when sampling Oligochaeta, using a probe.

2.5. Statistical analysis

For statistical tests, when conditions of normality and homoscedasticity of variances were not met, data were transformed by $f(x) = \ln(x + 1)$. After that, if conditions were met, ANOVA and Tukey tests were used to ascertain differences between treatments. When data did not meet the conditions, non-parametric tests were used, i.e. Kruskal-Wallis tests and post-hoc comparisons between treatments (R Development Core Team 2018). Sample data were converted to abundances per square meter for Figure 2.

To illustrate the relationships between enchytraeids communities and soil physico-chemical properties, we

Table 3. Mean number of enchytraeid genera and species, as well as overall bait-lamina feeding activity, according to fertilizer treatments. The same letter means that fertilizer treatments are not different at p = 0.05. One analysis (ANOVA) per column.

	Enchytraeid number of genera		Enchytraeid number of species		Feeding activity (%)	
Basic amendments	2.0	a	2.5	ab	23.7	c
Organic amendments	6.0.	ab	4.0	b	23.6	bc
Ammonium-based nitrogen fertilizers	0.2	a	0.4	a	1.5	a
Nitrate based nitrogen fertilizers	1.0	a	2.0	ab	9.8	abc
Phosphate fertilizers	1.0	a	1.7	ab	19.2	bc
Potassium fertilizers	1.3	a	2.0	ab	8.8	abc
Control (no amendments)	0.8	a	1.2	ab	7.5	abc

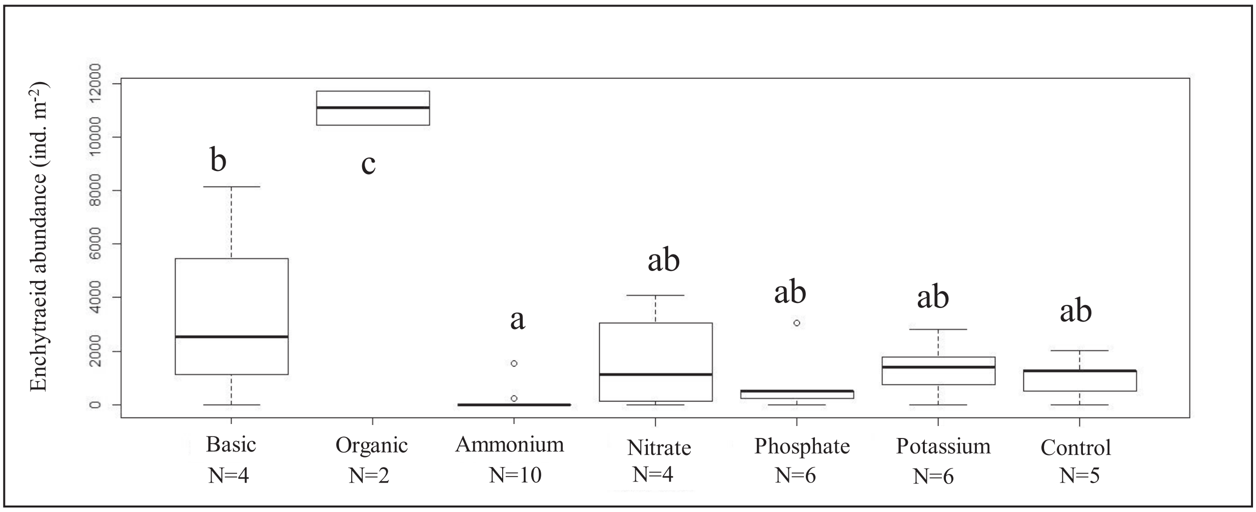

Figure 2. Boxplot representing the total abundance (expressed as individuals m-2) of enchytraeids according to fertilizers (from the left to the right): basic (alkaline) amendments, organic amendments (manure), ammonium-based nitrogen fertilizers, nitrate-based nitrogen fertilizers, phosphate fertilizers, potassium fertilizers, and control (without amendments). Fertilizer treatments with the same letter are not different at p = 0.05. N beneath treatment codes is the number of plots sampled per treatment group.

Table 2. Soil characteristics of the 37 sampled plots.

				Grain size distribution			Cation exchange capacity				Phosphorus	Organic matter		
					g.kg^{-1}			cmol^{+}.kg^{-1}			g.100g	mg.g^{-1}		
Plot n°	Treatment	Name in Fig. 1	pH	Clay	Silt	Sand	CEC	Ca_{exch}	Al_{exch}	K_{exch}	P_2O_5 tot	Org. C	N tot	C/N
2	$(NH_4)_2SO_4$	ammonium sulfate	3.62	189	594	217	8.18	0.12	6.48	0.12	0.12	11.41	0.90	12.72
3	$(NH_4)_2HPO_4$	di-ammonium phosphate	3.54	91	641	268	6.02	0.23	4.79	0.14	0.75	7.35	0.83	8.88
4	$NaNO_3$	sodium nitrate	6.72	162	596	242	10.02	6.89	0.09	0.15	0.10	13.22	0.72	18.32
5	$Ca(NO_3)_2$	calcium nitrate	5.16	185	586	229	8.78	5.17	1.82	0.54	0.12	7.04	0.65	10.92
6	NH_4NO_3	ammonium nitrate	4.03	179	582	239	8.48	0.16	6.59	0.17	0.11	6.68	0.83	8.03
7	NH_4Cl	ammonium chloride	4.07	182	588	230	8.12	0.26	5.86	0.16	0.12	8.92	0.95	9.34
8	blood	dried blood	3.96	192	592	216	8.74	0.20	6.87	0.19	0.12	7.79	1.00	7.75
9	control	reference	5.25	180	589	231	8.96	5.49	1.70	0.21	0.10	7.75	0.62	12.55
10	horse manure	horse manure	7.24	219	547	234	25.05	21.75	<0.02	1.82	0.31	50.91	3.61	14.10
11	control	reference	5.25	177	595	228	9.22	5.93	1.41	0.22	0.12	6.48	0.63	10.22
12	horse manure	horse manure	7.26	211	531	258	23.96	20.75	<0.02	1.67	0.30	42.07	3.25	12.95
14	$(NH_4)_2HPO_4$	di-ammonium phosphate	3.53	97	647	256	6.13	0.24	4.82	0.16	0.81	12.21	1.04	11.79
15	NH_4Cl	ammonium chloride	3.93	184	587	229	8.02	0.15	5.54	0.17	0.12	11.66	0.98	11.92
16	$Ca(NO_3)_2$	calcium nitrate	5.23	189	603	208	8.51	5.39	1.33	0.47	0.13	9.03	0.76	11.90
17	$NaNO_3$	sodium nitrate	6.14	165	612	223	8.51	4.98	0.71	0.18	0.10	6.83	0.57	11.89
18	blood	dried blood	3.92	191	594	215	8.90	0.23	6.87	0.20	0.12	10.65	1.08	9.87
19	$(NH_4)_2SO_4$	ammonium sulfate	3.72	197	588	215	8.18	0.30	6.42	0.17	0.14	10.02	0.98	10.22

20	NH_4NO_3	ammonium nitrate	4.15	193	585	222	8.48	0.39	6.63	0.19	0.12	15.69	0.97	16.17
23	KCl	potassium chloride	5.62	170	610	220	7.22	3.28	0.51	2.19	0.09	5.29	0.52	10.16
24	basic slag	basic slag	8.26	187	582	231	14.43	14.04	0.03	0.19	0.35	5.21	0.58	8.98
25	K_2SO_4	potassium sulfate	5.40	162	601	237	7.66	3.85	0.61	2.05	0.10	6.64	0.57	11.57
26	CaO	quick lime	8.51	178	588	234	14.41	14.58	0.03	0.19	0.09	5.16	0.61	8.42
27	super phosphate	super phosphate	6.41	175	596	229	11.43	10.21	0.10	0.18	0.22	4.88	0.57	8.61
28	natural phosphate	natural phosphate	6.23	160	588	252	11.14	9.10	0.14	0.20	0.45	6.39	0.62	10.34
29	(Na,K)Cl	sylvinite	5.82	139	617	244	6.90	3.90	0.31	0.92	0.09	6.44	0.53	12.09
30	control	reference	5.84	169	596	235	9.33	7.12	0.32	0.19	0.09	9.81	0.61	16.16
31	$CaCO_3$	calcium carbonate	8.33	181	577	242	14.82	15.36	0.04	0.22	0.11	6.61	0.72	9.23
32	control	reference	5.35	170	594	236	8.17	5.46	1.12	0.18	0.10	5.31	0.56	9.50
33	natural phosphate	natural phosphate	6.54	168	596	236	11.22	9.33	0.06	0.19	0.43	6.40	0.59	10.80
34	control	reference	5.57	186	586	228	10.01	7.40	0.68	0.20	0.09	5.63	0.57	9.90
35	basic slag	basic slag	8.18	174	565	261	14.57	14.07	<0.02	0.17	0.33	7.60	0.68	11.19
36	(Na,K)Cl	sylvinite	5.95	152	607	241	7.78	5.04	0.15	0.94	0.10	6.24	0.58	10.83
37	KCl	potassium chloride	5.38	173	601	226	7.34	2.99	0.80	2.28	0.10	5.68	0.58	9.85
38	super phosphate	super phosphate	5.94	168	595	237	11.17	9.51	0.30	0.17	0.26	5.98	0.58	10.26
39	$CaCO_3$	calcium carbonate	8.28	178	587	235	14.12	14.60	0.03	0.22	0.10	7.46	0.73	10.17
40	CaO	quick lime	8.57	182	579	239	15.42	15.65	0.03	0.20	0.09	6.86	0.69	9.90
41	K_2SO_4	potassium sulfate	**6.65**	**183**	**587**	**230**	**10.32**	**6.90**	**0.03**	**2.11**	**0.09**	**6.83**	**0.62**	**11.02**

tested the effects of soil pH, soil organic carbon content, and nitrogen content on enchytraeid abundance, number of genera, and number of species using Spearman correlation tests. The correlations between Oligochaeta parameters (i.e. abundance of enchytraeids, enchytraeids number of genera and species, feeding activity) and soil moisture and temperature conditions were computed using Spearman correlation tests.

3. Results

3.1. Earthworms

In total, 14 individuals were found in 9 plots out of 37 (i.e. plots 9, 10, 11, 12, 25, 26, 31, 33, and 35, see Figure 1). One earthworm individual was sampled in most of the plots where earthworms were found, except in horse manure (2 individuals in plot 12), quick-lime (4 individuals in plot 26), and natural phosphate (2 individuals in plot 33).

Earthworms belonged to 3 or 4 species: *Aporrectodea icterica*, *Allolobophora chlorotica*, and *Aporrectodea longa* or *Aporrectodea giardi*. The specimens of *A. longa* or *A. giardi* were juveniles and thus could not be identified to species level. The most abundant species was *A. chlorotica*, with 8 out of 14 extracted earthworms.

3.2. Enchytraeids

In total, 256 specimens were extracted, belonging to 13 species of 6 different genera. Of this total, 102 individuals (40 %) were in the upper 0–5 cm soil layer and 154 individuals (60 %) in the lower 5–10 cm layer; data from the two layers were pooled for subsequent analyses.

The most abundant genus was *Enchytraeus* (85 specimens), followed by *Achaeta* (68), *Buchholzia* (64), *Fridericia* (23), and lastly *Enchytronia* (2) and *Marionina* (2). About half (i.e., 49 %) of the sampled individuals were juveniles, with 26 % and 17 % being *Enchytraeus* and *Achaeta* juveniles, respectively.

The most abundant species was *Buchholzia appendiculata* (Buchholz, 1862) representing 24 % of the total abundance. However, most of them (35 individuals out of 57 in total) were found in the four plots with basic amendments, and 10 individuals were found in the two plots with horse manure. *Enchytraeus bulbosus* Nielsen and Christensen, 1963, was also represented in the trial and accounted for 7 % of the total abundance. The individuals of this species were present mainly in horse manure treatments (mean of 1273 individuals m^{-2} on average on the two plots), but they were also found in lime, natural phosphate, superphosphate treatments and control. We also found *Achaeta affinis* Nielsen and Christensen, 1959, to represent 5 % of the total abundance. However, this species was only found in horse manure treatments, with an equivalent density of 3056 individuals m^{-2} in plot 12. Other species represented less than 5 % of the total abundance: *Achaeta bohemica* (Vejdovský, 1879); *Achaeta eiseni* Vejdovský, 1878; *Achaeta pannonica* Graefe, 1989; *Buchholzia fallax* Michaelsen, 1887; *Enchytraeus buchholzi s.l.* Vejdovský, 1879; *Enchytronia parva* Nielsen and Christensen, 1959; *Fridericia bulboides* Nielsen and Christensen, 1959; *Fridericia isseli* Rota, 1994; *Fridericia perrieri* (Vejdovský, 1878) and *Marionina communis* Nielsen and Christensen, 1959.

The two plots with horse manure had significantly larger enchytraeid abundances than the other plots (Figure 2) with a mean of 11077 ± 4 individuals m^{-2}. Similarly, a higher number of genera was recorded in the two plots with organic amendments than in the other treatments (Table 3). The total abundance of enchytraeids was lower in the plots with ammonium-based nitrogen fertilizers than in the plots with basic and organic amendments (Figure 2). Ten times fewer enchytraeid genera and six times fewer species were found in the plots with ammonium-based nitrogen fertilizers than in the plots with basic amendments, although the differences were not significant (Table 3). The other four treatments (i.e. nitrate-based nitrogen fertilizers, phosphate fertilizers, potassium fertilizers, and control) were intermediate between ammonium-based nitrogen fertilizers and basic amendments for all the enchytraeid parameters (Figure 2, Table 3). In 13 plots out of 37, no enchytraeids were found (i.e. plots 2, 5, 6, 7, 8, 14, 15, 18, 20, 24, 29, 31, 32, see Figure 1). Eight plots without any enchytraeid had received ammonium-based nitrogen fertilizers (N = 10 in total, see Figure 1).

We found no significant correlation between the parameters describing enchytraeid communities (i.e. total abundance, number of genera and species) and the measured temperature and moisture in soils. Similarly, no correlation was found between soil organic carbon content, C/N ratio, or total nitrogen content and enchytraeid abundance or number of genera and species. However, soil pH was correlated positively with enchytraeid abundance (coef. rho 0.49; p = 0.002), number of genera (0.47; p = 0.004), and number of species (0.39; p = 0.02). Mean soil pH values spanned a wide range, from 3.79 (ammonium-based fertilizers) to 8.36 (basic amendments), with unamended controls (5.45) and manure (7.25) being intermediate (see Table 2 for full results). Organic carbon contents were very low in these soils, e.g. 7.0 mg g^{-1} in the five unamended control plots, with the notable exception of the two manure plots which had 46.5 mg g^{-1} (Table 2).

3.3. Feeding activity

The feeding activity was significantly higher in the plots that had received basic amendments, organic amendments and phosphate fertilizers than in the plots with ammonium-based nitrogen fertilizers (Table 3). In the plots with basic amendments, organic amendments and phosphorus fertilizers, 22 % of the baits were consumed (on average over the 12 plots) while only 9 % disappeared in the sticks left in the control and the treatments with nitrate-based nitrogen fertilizers or potassium fertilizers. In the three treatments with the highest feeding activity, most of the activity was located in the first two or three centimeters of soil, and below six centimeters depth (Figure 3). The lowest feeding activity was recorded for the ammonium-based nitrogen fertilizers (Table 3, Figure 3). The feeding activity was significantly negatively correlated to soil moisture (coef. rho -0.46; $p = 0.004$) and positively correlated with enchytraeid abundance (0.61; $p = 5.4\ 10^{-5}$).

4. Discussion

Our work demonstrates that after nearly 9 decades of complete absence of higher plants, fallow soils can still support true soil organisms such as terrestrial oligochaetes, albeit in depauperate communities. The low to very low abundances in this experimental trial, compared to more traditional cultivated agricultural soils (Lagerlöf et al. 1989; Leroy et al. 2008) were likely due to the lack of plant cover and the consequent low content of soil OM, although no correlation was found between soil organic carbon content and enchytraeid communities (probably due to the low number of plots with organic amendments). The organic carbon content in these soils was about two to three times lower than in the most intensive agricultural soils in temperate regions (e.g. Arrouays et al. 2002, Henneron et al. 2015, van Oort et al., 2020). Remarkably, some earthworms persisted, mainly of the endogeic, geophagous group that is known for its ability to access old soil carbon (Marhan and Scheu 2005, Curry and Schmidt 2007). However, it is worth underlining that enchytraeids displayed a relative higher abundance and species diversity: compared to conventional arable soils, population sizes observed in these extreme, bare, long-term soils were about two magnitudes smaller for earthworms in all treatments (Leroy et al. 2008), but only about one magnitude smaller (apart from the horse manure treatment) for Enchytraeidae (Lagerlöf et al. 1989). This

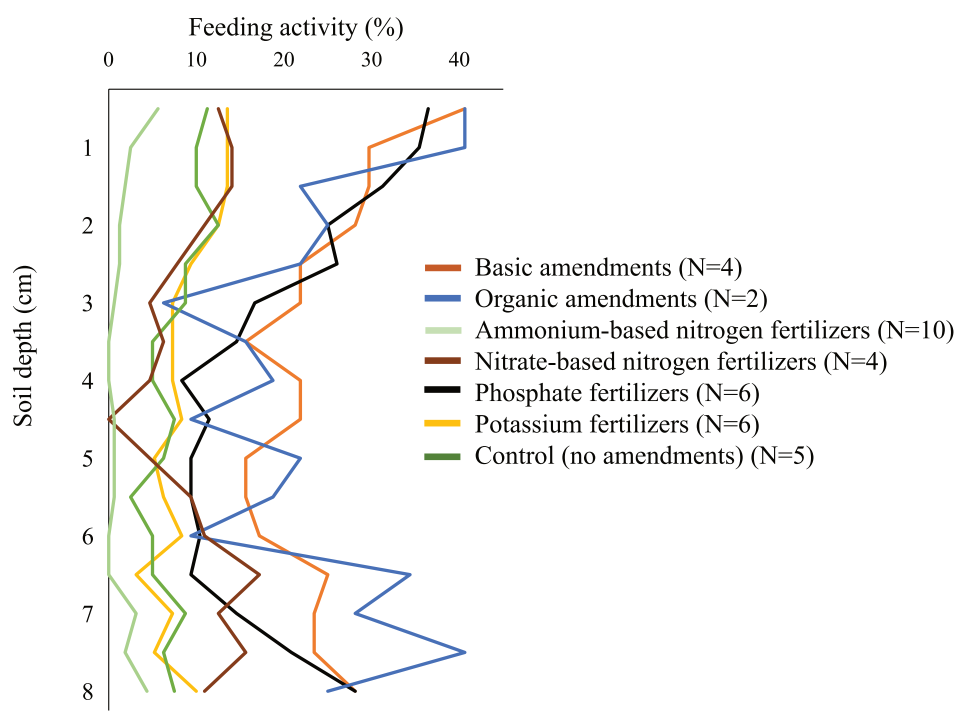

Figure 3. Feeding activity (mean of 16 bait-lamina sticks, N plots per treatment), in % of the total number of baits consumed.

suggests that enchytraeids can persist better in extreme soil conditions that earthworms can hardly bear such as low OM soil contents. These findings can be explained by their different feeding behaviour (see below), with enchytraeids being saprovorous but also microbivorous to a larger degree than lumbricids that need plant litter in their diets (Larsen et al. 2016). In addition to the food factor, the findings also reinforce previously published results about enchytraeids' higher tolerance to chemically stressed soil conditions compared to earthworms (e.g. Owojori et al. 2009; Bart et al. 2017), especially the fact that enchytraeids are much more able to thrive at low soil pH values (Didden 1993) than earthworms.

The higher diversity and abundance of enchytraeids and earthworms in the plots with horse manure can be explained by the known suitability of solid animal manure as a food resource for these organisms (Jensen et al. 2003, Leroy et al. 2008) and potentially by the physical addition of enchytraeid worms when the manure is applied. On the contrary, the low abundances of oligochaetes in the ammonium-based nitrogen fertilizer plots were probably due to the strongly acidic conditions (pH ranging from 3.5 to 4.1) that soil organisms can hardly bear. For instance, Kuperman et al. (2009) reported that adult survival and juvenile production by enchytraeids (i.e. *Enchytraeus albidus* and *Enchytraeus luxuriosus*) were inhibited in acidic soils with pH $\leq$ 5. Moreover, the enchytraeid abundance was found here to be positively correlated with the pH. Thus, pH and OM appear to be the main soil properties that drive Oligochaeta communities in the absence of plants.

From a purely trophic perspective, the current observation that terrestrial oligochaetes can survive in bare mineral soil, without higher plants and their inputs through litter and rhizodeposits, could be explained by carbon inputs through photo-autotrophic soil micro-organisms. Both conventional gut content analysis (Shtina et al. 1981) and ^{13}C stable isotope tracers (Schmidt et al. 2016) have provided evidence that soil algae are a food (and C) source for enchytraeids and earthworms. In fact, the study by Schmidt et al. (2016) showed that *A. chlorotica* (the most abundant lumbricid species in the present survey) derived about 3 % of its total body C from such photo-autotrophic soil surface microorganisms (cyanobacteria, algae) in just 7 days. Mineral fertilization treatments in the present plot experiment likely favoured the growth of such microbes, possibly explaining why soil invertebrates, and especially the smaller-bodied enchytraeids, can survive in such poor organic matter conditions.

Moreover, both natural abundance stable isotope measurements (Schmidt et al. 2004) and ^{14}C radiocarbon measurements (Briones & Ineson 2002, Briones et al. 2005) suggested that coexisting enchytraeid worms and endogeic earthworms assimilate similar soil C sources, with a trend for the use of older, more processed soil C by enchytraeids. However, the abundance findings in the present experiment suggest that the lack of food sources was more limiting for lumbricid worms than for enchytraeid worms. The latter also have to maintain a much smaller body mass than the former, and the degraded experimental soils likely represent the trophic limit of existence for large-bodied soil macrofauna. Finally, although the carbon in these soils is almost exclusively stable, with a turnover of several hundred years (Barré et al. 2010), small amounts of occasional labile carbon coming from atmospheric deposition, weeds and microbial activity (see above) could have been used better by enchytraeids to survive for so long.

Generally speaking, information on the distribution and ecology of enchytraeid species is scarcer and less comprehensive than for well-studied lumbricid species, but progess is being made on the former taxon (e.g Römbke et al. 2013). The most abundant enchytraeid species in the studied trial, *B. appendiculata* was reported to be common and widespread in neutral to slightly acidic soils (Schmelz & Collado 2010). Graefe & Schmelz (1999) described this species as an indicator of slightly acid to slightly alkaline conditions, never found in strongly acid soils. A soil is considered as strongly acidic if the pH is below 5.5. Only 7 % of the *B. appendiculata* individuals were found in plots where the pH was below this value, partly confirming the statement of Graefe & Schmelz (1999). *Achaeta affinis*, only found in horse manure treatments, were reported to be common and widespread in moderately acidic soils (Schmelz & Collado 2010). This means a pH of 5.6–6.0, while the pH in plots with horse manure reached 7.5. Interestingly, *E. bulbosus* that occurred in some mineral treatments was mostly associated with arable soils and low soil OM levels (<2 %) in the German dataset (Römbke et al. 2013). Although the low number of enchytraeid individuals makes it difficult to interpret the tolerance of the species to soil characteristics, it clearly underlines the need for more information on ecological aspects of enchytraeids, adding refinements to the previous works by considering available data. Also, this study is only based on one sampling date, which limits interpretations.

The continuous addition of a variety of fertilizers and amendments induced divergent soil chemical properties, that in turn were the drivers of persisting Oligochaeta communities through biotic and abiotic habitat conditions (e.g. nutrient content and availability, pH, microbial communities). Many studies such as Jastrzebska et al. (2020) found that fertilizers (in this case recycled phosphorus fertilizer and biofertilizer from sewage sludge ash and dried porcine blood) and superphosphate did not alter earthworm communities in the short-term (1–3 years). However, these authors explained that the consequences of fertilizer use can be invisible in the short term but may lead to significant environmental changes in the long term. They thus recommended long-term field research to address this topic. The present work also underlines the usefulness of long-term trials with initially identical soil properties for increasing our understanding of the ecology of soil organisms. In fact, this particular experiment has arguably created an extreme soil environment that offers novel insights into the factors controlling soil biodiversity (Wall & Virginia 1999).

Feeding activity on bait-lamina sticks has been proposed and used in field approaches since the beginning of the 1990s, mainly for ecotoxicological studies (von Törne 1990; Larink 1993; Paulus et al. 1999). This method was reported to be sensitive to soil moisture and authors generally found a positive relationship between the feeding activity and the soil moisture (Larink 1993, Filzek et al. 2004, Simpson et al. 2012). The opposite relationship found in this study might be explained by the low soil aggregation stability and the deterioration of soil structure (Paradelo et al. 2013) relative to the absence of OM and likely biological life. Consequently, after significant rain events, a part of the plots, particularly under sodic and/or potassic fertilizers, but also the reference plots are rapidly flooded, thus modifying soil oxygenation conditions that influence soil organisms and the feeding activity.

Notably, the consumption of the baits was found to be positively correlated with the abundance of enchytraeids. Enchytraeids are considered to be among the main consumers of the baits in the topsoil (Helling et al. 1998), and the feeding activity can here be considered as a good indicator of enchytraeid presence and activity. However, there were some exceptions, for example phosphate fertilizer treatments that had low enchytraeid abundances but high feeding activity (Fig. 2 and Table 3). This suggests that other soil invertebrates that are known to feed on bait-lamina such as micro-arthropods (von Törne 1990; Larink et al. 1993) may also be present and active in these soils, but this was not ascertained here.

In conclusion, it is remarkably that these experimental fallow soils still maintain depauperate invertebrate communities and some general feeding activity after almost 9 decades of the absence of plants. The finding that residual soil communities (including oligochaetes) can persist even in such severely degraded soils evinces the resilience of soils and soil food webs. By extension, this resilience bodes well for any soil rehabilitation initiatives because soil organisms my not have to be reintrocued at all but merely encouraged by appropriate managemement such as pH correction, vegetation and organic matter additions.

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