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Comparison of nematode communities in anecic earthworm casts and adjacent soil reveal a land use-independent trophic group signature

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Abstract

By ingesting soil and organic matter in different soil horizons and depositing casts on soil surface, anecic earthworms have large influence on soil ecological processes.

However, we still have a limited understanding of the consequences of earthworm casting activity on nematode communities, and the role played by the land use in this relationship. Therefore, the main objective of this study was to compare the effect of the anecic earthworm *Amynthas adexilis* (Thai, 1984) on the structure of nematode communities, in a woodland and meadow presenting different soil organic C content in northern Vietnam. Nematode population and physico-chemical properties of casts produced by the anecic earthworm *A. adexilis* and adjacent soil presenting no recent earthworm activity (0-10 cm deep) were characterized. *A. adexilis* incorporated organic matter into its casts compared to the adjacent bulk top soil horizon, reflected by a significant increase of the organic carbon and nitrogen contents (1.4 times more in the meadow and 1.8 times more in the woodland). Earthworm casts contained 2.6 and 1.7 times more nematodes than the adjacent top soil, in meadow and woodland, respectively. The effect of earthworm casting activity on nematode community structure was similar in both land uses. Compared to the soil, casts were significantly enriched in all trophic groups (between 1.9 and 11.6 times more in casts in the meadow, and between 1.6 and 23.7 times more in casts in the woodland, depending on the trophic group), except for obligate plant feeders that were under-represented (1.4 times less in casts for both land uses). The plant parasitic index decreased in the casts compared to the soil, indicating an environment less favourable for plant parasitic nematodes.

Key words: Meadow, Woodland, Vietnam, *Amynthas adexilis*

1. Introduction

Earthworms play an important role in the functioning of the soil due to their bioturbation activity (Medina-Sauza et al., 2019; Van Groenigen et al., 2019). Of the three main earthworm ecological categories (anecic, epigeic and endogeic), the anecic

earthworms are deep-burrowing subsoil dwellers, ingesting soil and fresh organic matter and depositing faecal material, or "casts", on the soil surface. Casts are usually enriched in bioavailable forms of carbon, nitrogen and organic and inorganic phosphorus in comparison with the bulk soil (Le Bayon and Milleret, 2009; Vidal et al., 2019). As a result, casts usually show enhanced microbial biomass and activity, thereby constituting hotspots where soil ecological processes are amplified (Aira et al., 2019). Earthworms also stabilise organic C by enhancing the formation of micro-aggregates (Bossuyt et al., 2005) and the association of organic matter to mineral particles (Bottinelli et al., 2020). Little is known on the effects of cast deposition by earthworms on nematodes, although the latter are among the most numerous multicellular animals in soil. Nematode communities impact multiple trophic levels of the soil food web as well as the cycling of nutrients, and some of them cause serious damages to most vascular plants (Costa et al., 2019; Ingham et al., 1985). The distribution, abundance and diversity of nematodes in soil has largely been documented and their sensitivity to environmental conditions made them useful indicators of environmental contamination, pollution and land use changes (Bongers and Ferris, 1999; Siebert et al., 2019; Yeates, 2003).

Earthworms can affect soil nematode populations by three main mechanisms: (1) comminution, passage within the guts and casting; (2) grazing; (3) dispersal (Brown, 1995). A positive impact of earthworms casting activity on nematodes can be expected if we consider that casts offer advantageous environmental conditions with higher nutrient contents, stable soil moisture and higher microbial activity, and possibly enhanced pore space (Neher et al., 1999; Yeates, 1981). Conversely, earthworms can also negatively impact nematode abundance and diversity by feeding

on them (i.e. direct trophic effects) (Dash et al., 1980). The influence of earthworm casting activity on soil nematode communities is highly variable and there is no consensus (Aira et al., 2003; Brown, 1995; Roessner, 1986; Tao et al., 2009) (Table 1).

Table 1. Summary of the field studies comparing earthworm casts and soil nematode content. BF-Bacterial feeders, FF-fungal feeders, Om-omnivores, (O)PF-(obligate)plant feeders.

Reference	**Land use**	**Earthworm species**	**Nematode population in casts (vs soil)**
Russom et al. (1993)	Fallow and cultivated land tropical rain forest (Nigeria)	*Agrotoreutus nyongi*	* Increase of total abundance * Increase all trophic groups
Tao et al. (2009)	Rice – wheat rotation (China)	*Metaphire guillelmi*	* Increase of total abundance * Increase BF and FF
Aira et al. (2003)	Pasture (Spain)	*Aporrectodea caliginosa*	* Decrease of total abundance * Decrease of BF, PF and Om
	Riverside (Spain)	*Aporrectodea molleri*	* Increase of the total abundance but not significant * Increase of BF but not significant
Roessner et al. (1986) in Brown (1995)	Cultivated land	*Aporrectodea caliginosa* *Aporrectodea longa*	* Decrease of OPF and BF
		Eisenia fetida *Lumbricus terrestris* *Lumbricus rubellus* *Lumbricus castaneus* *Octolasion cyaneum*	* Decrease of OPF * Increase of BF
Roessner et al. (1981) in Brown (1995)	Cultivated land	*Eisenia fetida*	* Reduced OPF abundance in soil and in casts

Variability might be attributed to the different behavior of earthworms. Only one study compared more than two earthworm species and results show difference between species in terms of impact on nematode trophic groups (Roessner, 1986). Another important parameter that might explain the variability would be the soil environment (soil properties and land use) that has been shown to influence

significantly nematode communities (Ferris et al., 2001). In order to disentangle the influence of the land use on the earthworm-nematode interactions, we compared the nematode community structure in casts and adjacent top soil horizon, and evaluated if the same pattern is observed in two contrasted land use. A meadow and a woodland from the same catchment were selected. In Vietnam, the anecic earthworm *Amynthas adexilis* (Thai, 1984) produces large, compact water stable casts (Jouquet et al., 2008) that influence a large number of ecological processes such as soil organic matter sequestration and microbial diversity (Bottinelli et al., 2020; Hong et al., 2011; Jouquet et al., 2011), and was chosen as earthworm model.

2. Material and methods

2.1 Study fields and sampling

This study was carried out at the M-Tropics long-term observatory catchment located in Dong Cao village in the North of Vietnam (20° 57'N, 105° 29'E). Surface earthworm activity in the catchment is high with the anecic earthworm *Amynthas adexilis*, identified as the only one species depositing large globular casts on the soil surface (Bottinelli, pers. com.). The climate is subtropical humid with annual mean temperature and rainfall of 20°C and 1,800 mm, respectively. The dominant soil is an Acrisol (WRB) (Podwojewski et al., 2008). We selected two contrasted land uses, i.e. a meadow and a woodland of approximately 1.5 ha each. The meadow is an area covered for more than 20 years by meadow mainly composed of short grass (*Paspalum* and *Stachytarpheta*). This area is subject to regular grazing by cattle and buffaloes for two thirds of the year. The woodland is an area formerly cultivated and then planted for 14 years with two woody evergreen species of *Annonaceae* with an average height of 10 m and with a planting space of 3 to 5 m in all directions. This

zone has low understory vegetation and is very little frequented by buffaloes during the year. The mass of casts found in meadow compared to those found in woodland was 2800 *versus* 4400 g (oven dry weight) / m^2. The soil (0-10-cm depth) in the meadow compared to those in the woodland had an organic carbon C content of 3.7 *vs* 2.5%, a pH (KCl) of 3.8 *vs* 3.9, a clay content of 58 *vs* 50% and sand content of 16 *vs* 17%. At both the meadow and the woodland, three 25-m^2 plots distant from each other by 5 m were delimited. All surface casts were removed from the six plots in December 2016. Three days after the clearance, one cast composite sample (composed of five casts) and one soil composite sample (topsoil surrounding the casts, 0-10 cm depth), were collected in each plot. In total, twelve samples were collected: two land uses × two sample types (cast vs. soil) × three plots.

2.2 Soil physico-chemical properties

Casts samples bulk density was measured with the paraffin-coated clod method after oven drying at 105°C for 24 h (Grossman and Reinsch, 2002). Soil bulk density was measured on samples collected using 250 cm^3 cores. The gravimetric moisture content of the bulk soil and casts was determined by oven drying at 105°C for 24 h. Soil and casts < 2 mm fraction was air-dried and ground at 200 µm for organic matter characterization. The carbon and nitrogen concentrations were measured using elemental analyser Flash 2000 HT. Medium infrared spectra of ground samples were collected with a Fourier Transform Spectrophotometer (FTIR 660 Agilent ex-Varian, Agilent Technologies, Inc., Santa Clara, USA). The diffuse reflectance measurements were made at 2 cm^{-1} intervals across 4,000-400 cm^{-1} and was converted to absorbance. The raw spectra were subjected to pre-processing, including Savitzky-Golay smoothing (first order polynomial filter with a 9-point

window) and baseline correction (polynomial fitting with degree 2). Data pre-processing was performed with "*spectacles*" and "*signal*" packages in R software. Based on prominent peaks representative of functional groups in the spectra we only selected three peaks and measured their absorbance heights with the software Origin. The organic functional groups of C–H bonds were identified at 2,925 cm^{-1} (asymmetric stretch vibration, C–Ha) and 2,850 cm^{-1} (symmetric stretch vibration, C–Hs), and C=C bonds at 1,640 cm^{-1}. Absorption intensities of C–Ha and C–Hs were measured as the vertical distance from a local baseline, and the sum of C–Ha and C–Hs bond heights were calculated as the height of C–H bonds. Absorption intensities of C=C bonds were measured as the vertical distance from the total baseline. As a measure of soil organic matter lability, the humification index (HI) was calculated as the ratio of absorbance intensities of C=C to C-H (Demyan et al., 2012; Inbar et al., 1989).

2.3 Extraction, identification and composition of nematodes

Nematodes were extracted from 150 g per casts or soil composite fresh samples using a modified elutriation system (Villenave et al., 2009). Nematodes were counted using a binocular microscope. After fixing in a formalin glycerol mixture and transferring to mass slides, the composition of soil nematofauna was determined at family or genus level through microscopic observation at 400x magnification. A total of 3,279 nematodes were identified with an average of 78 nematodes per mass slide. Nematode density was recorded as the total number of individuals per 100 g dry material. Each nematode was allocated to a colonizer-persister (cp) class according to Bongers (1990) and assigned to one of the five trophic groups defined by Yeates et al. (1993) : bacterial feeders (BF), fungal feeders (FF), predators (P = carnivores (Ca) +

omnivores (Om)), obligate plant feeders (OPF) and facultative plant feeders (FPF). While trophic groups provide information on the type of prey consumed by each taxon, the colonizer-persister scale categorizes the taxa according to life strategy and ranges from 1 to 5 (extreme r-to extreme K-strategists). The different trophic and c-p scale categories attributed to each family can be found in Table 3.

2.4 Calculations

Diversity indices (Shannon, richness), maturity indexes (for free-living taxa - MI, and for plant parasitic taxa - PPI), the Enrichment Index (EI) and Structure Index (SI) were calculated according to Bongers (1990) and Ferris et al. (2001). The MI is based on non-plant-feeding taxa and considered a measure of environmental disturbance; low MI values indicate a disturbed and/or enriched environment, high MI values a stable environment (Bongers, 1990). The Plant-Parasite Index, is comparable to the MI but computed only for the plant-feeding nematodes with the rationale that their abundance is determined by the vigour of their host plants which, in turn, is determined by system enrichment. The Enrichment Index and the Structure Index, both based on the indicator importance of functional guilds of nematodes, are descriptors of food web condition. Bacterial-feeding in c-p1 and fungal-feeding in c-p2 are indicators of enrichment while nematodes of all feeding habits in c-p3-5 are indicators of structure.

2.5 Statistical analysis

All statistical calculations and plots were carried out using the R software version 4.0.2 (R Core Team, 2020) using the packages car, stats¸ vegan (Oksanen et al., 2007), emmeans (Lenth, 2020), tidyverse (Wickham, 2019) and broom (Robinson

and Hayes, 2020). Generalized linear models (Poisson regression) were used to test the effect of sample type (cast and top soil) on the nematode trophic group and species abundances. One-way ANOVA was used to test the effect of sample type (cast and top soil) on physico-chemical properties and nematode indices. Prior to running ANOVA, data were tested for homogeneity of variances and normality and log-transformed if required. Differences among treatments were declared significant at the 0.05 probability level. A principal component analysis (PCA) was performed on the Hellinger-transformed nematode abundance matrix using the *rda* function in *vegan*. A post hoc explanation of PCA axis using environmental variables was done using the *envfit* function in *vegan*. *Envfit* finds vectors or factor averages of environmental variables. The projections of points onto vectors have maximum correlation with corresponding environmental variables, and the factors show the averages of factor levels. The result is an object containing coordinates of factor levels (points) or arrowheads (quantitative variables) that can be used to project these variables into the ordination diagram (Borcard et al., 2018).

3. Results

3.1 Soil and casts properties

In both land use, casts were enriched in N and C (1.4 and 1.8 times more in meadow and woodland, respectively) compared to the adjacent top soil (Table 2, all $p < .001$). They also presented a higher bulk density, and the lowest values of HI. The moisture content was similar between casts and soil samples in woodland (~41%) but higher in casts than in the soil samples in the meadow (65 vs 40%, respectively).

Table 2: Average values and standard deviations of physico-chemical characteristics and nematode indexes (n=3) for control soil (0-10 cm depth) presenting no recent earthworm activity and earthworm casts in meadow and woodland. Organic nitrogen (N); Organic carbon (C); C/N ratio (C/N); Humification index (HI); Gravimetric moisture content (Moisture); Bulk density (BD); the nematode Enrichment Index (EI) and Structure Index (SI); maturity indexes (for free-living taxa - MI, and for plant parasitic taxa - PPI); nematode diversity indices (Shannon, richness). Bold values indicated *p* values < .05.

Properties	Meadow			Woodland		
	Soil	Cast	*p* value	Soil	Cast	*p* value
N (%)	**0.27 ± 0.02**	**0.38 ± 0.01**	**<0.001**	**0.17 ± 0**	**0.3 ± 0.02**	**<0.001**
C (%)	**3.52 ± 0.23**	**5.03 ± 0.19**	**<0.001**	**2 ± 0.05**	**3.57 ± 0.1**	**<0.001**
C/N	13.2 ± 0.2	13.2 ± 0.2	0.995	11.8 ± 0.3	11.8 ± 0.6	0.920
HI	23 ± 6	14 ± 4	0.457	34 ± 16	19 ± 2	0.112
Moisture (%)	**39.5 ± 3.1**	**64.8 ± 5.1**	**<0.001**	41.8 ± 2.8	41.1 ± 7.0	0.873
BD (g cm^{-3})	**1 ± 0.1**	**1.6 ± 0.1**	**<0.001**	**1.1 ± 0.1**	**1.5 ± 0.1**	**<0.001**
EI	47.6 ± 11.6	48.3 ± 7.8	0.931	80.9 ± 18.2	59.2 ± 16.4	0.200
SI	80.0 ± 12.3	38.7 ± 24.7	0.061	89.9 ± 12.6	66.3 ± 17.8	0.135
PPI	**2.9 ± 0.1**	**2.3 ± 0.1**	**<0.001**	**3 ± 0.1**	**2.5 ± 0.1**	**0.006**
MI	2.9 ± 0.6	2.2 ± 0.2	0.110	3 ± 0.4	2.5 ± 0.4	0.209
Richness	17 ± 0.5	13.7 ± 2.3	0.067	11 ± 4	11.7 ± 1.5	0.801
Shannon	2.3 ± 0.2	2 ± 0.3	0.303	1.6 ± 0.6	2 ± 0.4	0.380

3.2 Nematode trophic groups and families

In both land uses, the total nematode abundance was higher in the casts than in the top soil (2.6 and 1.8 times more, in meadow and woodland, respectively, $p < .001$, Table 3). In both the meadow and woodland the casts presented a nematode trophic

group composition characterized by an enrichment in all trophic groups except in obligate plant feeders, less abundant in the casts (Fig. 1). In regards to the c-p scale categories, an increase in both land uses of bacterial feeders cp1 (Panagrolaimidae, $p < .001$ and = .016, respectively in meadow and woodland) and cp2 (Cephalobidae, $p < .001$ in meadow and woodland), but a decrease of cp3 (Prismatolaimidae, $p = .001$ and .041 in meadow and woodland, respectively, and Alaimidae, $p < .001$ in the meadow) was also observed in casts. Among the fungal feeders, only the cp2 were more abundant in the casts (Table 3): enrichment in Anguinidae and Aphelenchoididae in both sites ($p < .001$ in meadow; = .022 and < .001 in woodland, for Ditylenchus and Aphelenchoididae, respectively), and in Aphelenchidae in the meadow ($p < .001$). The Tylenchidae family was the most enriched in the casts, i.e. 12 and 24 times more, in meadow and woodland, respectively ($p < .001$, Table 3). The PPI was smaller in the casts than in the soil ($p < .001$ and = .006 in meadow and woodland, respectively). No significant difference of the other nematodes indexes was observed (Table 2), however, the SI and MI was markedly lower in the casts than in the soil in both land use. The PCA revealed that the changes in nematodes community structure in the casts compared to the soil was highly correlated with the modifications in physicochemical properties, i.e. organic matter (C and N) and moisture content, and bulk density (Fig. 2).

Table 3: Average values and standard deviation of nematode abundances (number of nematode per 100g of dry material, per family and in total) in the soil (0-10 cm deep) and earthworm casts in meadow and woodland (n=3). BF1-3= Bacterial feeder cp1-3, FF2-4 = Fungal feeder cp2-4, P4-5 = Predators (omnivores + carnivores) cp4-5, OPF3-5 = Obligatory plant feeders cp3-5, FPF2 = Facultative plant feeders cp2. Bold values indicated *p* values < .05.

		Meadow			**Woodland**		
Family		**Soil**	**Casts**	***p* value**	**Soil**	**Casts**	***p* value**
Panagrolaimidae	BF1	**1 ± 2**	**32 ± 39**	**<0.001**	**1 ± 1**	**3 ± 6**	**0.016**
Rhabditidae	BF1	6 ± 4	8 ± 8	0.273	6 ± 4	4 ± 4	0.465
Cephalobidae	BF2	**22 ± 8**	**188 ± 148**	**<0.001**	**1 ± 1**	**13 ± 6**	**<0.001**
Alaimidae	BF3	**19 ± 29**	**0 ± 0**	**<0.001**	1 ± 1	2 ± 3	0.249
Prismatolaimidae	BF3	**21 ± 4**	**10 ± 18**	**0.001**	**1 ± 2**	**0 ± 0**	**0.041**
Anguinidae	FF2	**5 ± 5**	**28 ± 18**	**<0.001**	**2 ± 3**	**5 ± 5**	**0.022**
Aphelenchidae	FF2	**5 ± 2**	**24 ± 26**	**<0.001**	2 ± 3	0 ± 0	0.068
Aphelenchoididae	FF2	**12 ± 16**	**113 ± 50**	**<0.001**	**0 ± 0**	**24 ± 9**	**<0.001**
Belondiridae	FF4	10 ± 9	13 ± 12	0.224	7 ± 6	9 ± 9	0.316
Leptonchidae	FF4	3 ± 4	3 ± 5	0.637	0 ± 0	5 ± 9	0.756
Dorylaimidae	P4	7 ± 6	6 ± 6	0.639	5 ± 4	6 ± 2	0.590
Qudsianematidae	P4	**4 ± 4**	**19 ± 11**	**<0.001**	1 ± 1	2 ± 2	0.148
Aporcelaimidae	P5	2 ± 2	2 ± 3	0.763	0 ± 0	0 ± 0	1.000
Criconematidae	OPF3	4 ± 3	2 ± 4	0.249	59 ± 51	51 ± 26	0.185
Hoplolaimidae	OPF3	46 ± 22	57 ± 44	0.061	**0 ± 0**	**8 ± 12**	**<0.001**
Meloidogynidae	OPF3	**7 ± 4**	**15 ± 8**	**0.002**	2 ± 2	3 ± 5	0.403
Pratylenchidae	OPF3	**87 ± 46**	**22 ± 29**	**<0.001**	**4 ± 4**	**0 ± 0**	**<0.001**
Rotylenchulidae	OPF3	0 ± 0	2 ± 3	0.076	**32 ± 36**	**8 ± 8**	**<0.001**
Longidoridae	OPF5	1 ± 2	0 ± 0	0.019	1 ± 1	1 ± 2	0.410
Tylenchidae	FPF2	**16 ± 17**	**185 ± 97**	**<0.001**	**3 ± 2**	**71 ± 22**	**<0.001**
Others		4 ± 4	5 ± 8	0.989	2 ± 2	4 ± 4	0.987
Total abundance		**284 ± 108**	**730 ± 447**	**<0.001**	**124 ± 73**	**218 ± 20**	**<0.001**

Figure 1: Total abundance per nematode trophic groups in earthworm casts and 0-10 cm soil (number of nematodes per 100g dry material) in woodland and meadow (n=3). Stars indicate a significant difference between casts and top soil within each land use (* = $p < .05$, ** = $p < .01$, *** = $p < .001$). The number next to the stars is the ratio (fold change) between casts and soil. BF = Bacterial feeders (cp1 and obligatory), FF = Fungal feeders, FPF = Facultative plant feeders, OPF = Obligatory plant feeders, P = Predators (omnivores + carnivores).

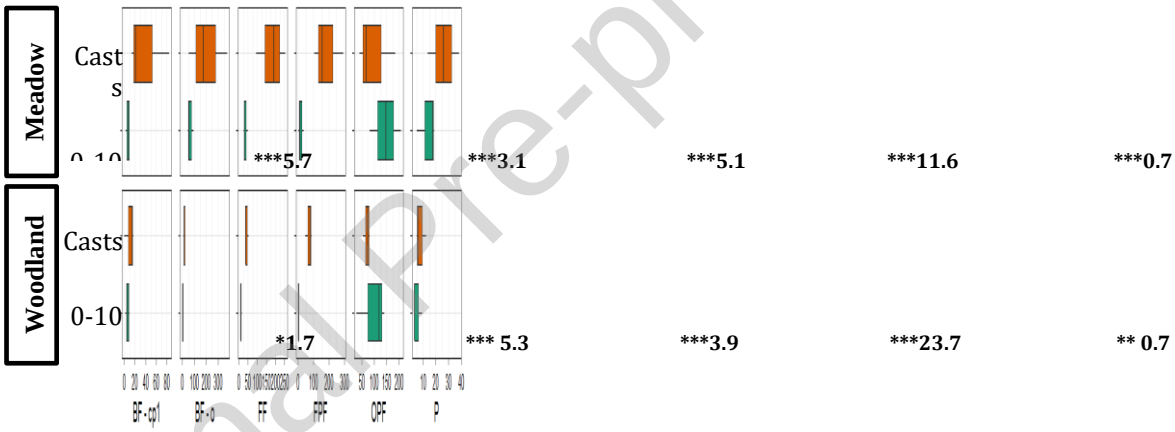
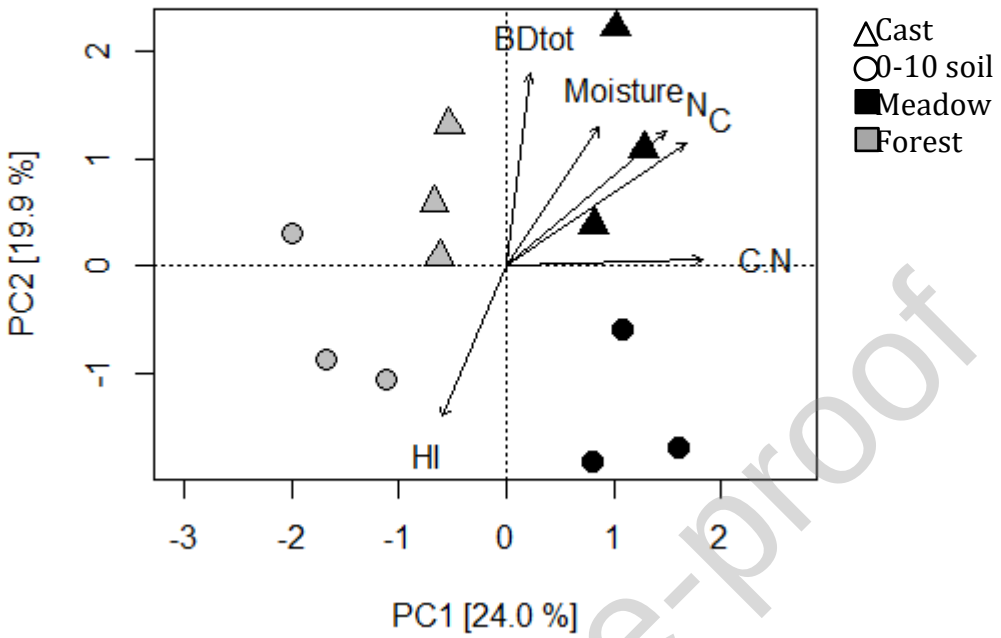


Figure 2: Principal component analysis (PCA) based on Hellinger-transformed nematode species abundance matrices, with projection of environmental variables. The variance explained by the axes (goodness of fit) is provided next to the axis headers. Meadow and woodland samples are color-coded in black and grey, respectively. Casts and top soil are represented with triangles and circles, respectively.



4. Discussion

At the heart of the bioturbation or soil engineering concept (*sensu* Lavelle et al. (1997)), is the ability of earthworms to influence the physical habitat of other subordinate organisms (Jouquet et al., 2006). Our study revealed an enrichment in nematodes, as well as a particular trophic-groups composition of nematodes in recently emitted casts compared to the adjacent soil in both land uses. Casts are enriched in all trophic groups, except the obligate plant feeders that are underrepresented (Fig.1). The strong inhibitory effect of the casts on obligate plant feeders is confirmed by the significant decrease of the PPI. Large and fragile nematodes (Alaimidae, Prismatolaimidae) are under-represented in the casts, which is confirmed by the decrease of the SI and MI in the casts. These differences might have different explanations. Firstly, the composition of the casts present an environment rich in labile and less processed organic matter (higher C and N contents, lower

humification index mainly due to higher proportions of aliphatic molecules) than the top soil. The enrichment in soil organic matter and more specifically in aliphatic compounds can easily be explained by the fact that *A. adexilis*, which belongs to the anecic trophic group, feed on a mixture of soil and organic matter from the litter (Jouquet et al., 2008). This environment is opportune for microorganisms and for nematodes that feed on them, i.e. bacterial and fungal feeders and facultative plant feeder (which feed on roots but also on fungi, lichens, algeas, etc). The PCA shows high correlation between the casts, nematode population and the C and N content, confirming the link between soil organic matter and the change of nematode community. Secondly, the passage of nematode through the earthworm's gut might play a role. A diversity of parameters (including $CaCO_3$, digestion enzymes, mucus and antimicrobial substances) influence the ability of ingested nematodes to survive (or not) to the passage through the earthworm gut, and their resultant capacity to recover and proliferate (or not) in earthworm casts (Brown, 1995). Nematodes belonging to different cp (coloniser-persister) classes might present contrasted sensitivities to the passage in the earthworms guts. Finally, modifications of the properties of nematode habitats (e.g. porosity, water content) was also likely to explain changes in nematode trophic-groups composition (Yeates, 1981).

5. Conclusion

Anecic earthworms *A. adexilis* incorporate organic matter in their casts, altering both the quantity and the chemical quality of the soil organic matter and the soil moisture. In meadow and woodland, casts were enriched in all nematode trophic groups, except for obligate plant feeders, that were under-represented. Earthworm casts thus present

a different environment to the surrounding soil and are either preferentially colonised by nematodes post excretion or allow for increased proliferation of nematodes that have survived passage through earthworms gut. The land use impacted the intensity, but not the direction of the nematode trophic group enrichment/depletion.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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