



## Original article

## Patterns of earthworm, enchytraeid and nematode diversity and community structure in urban soils of different ages



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## ABSTRACT

Annelids (Lumbricidae and Enchytraeidae) and nematodes are common soil organisms and play important roles in organic matter decomposition, nutrient cycling and creation of soil structure and porosity. However, these three groups have rarely been studied together and only few studies exist for urban soils. We studied the diversity and community composition of annelids and nematodes in soils spanning more than two centuries of urban soil development in Neuchâtel (Switzerland) and assessed the relationships 1) among these three groups and 2) between each group and environmental (physical, chemical and functional) characteristics of soils and soil age.

While the groups of environmental variables were correlated (Mantel tests) no correlation was found between pairs of soil fauna groups and between each soil fauna group and environmental variables. More specifically, redundancy analyses showed that earthworm assemblages were best correlated with soil bulk density and with soil depth, the latter being positively correlated with soil age. Enchytraeid assemblages and the proportion of enchytraeid *r*-strategists were respectively best correlated with soil carbonate content and negatively correlated with soil age. Nematodes assemblages were best correlated with soil water content. Moreover, relationships between pairs of soil biota groups, and between each group and environmental (physical, chemical and functional) variables, varied along the soil age gradient (moving window analysis).

This study provides new knowledge on urban soil biodiversity and how environmental conditions can influence soil diversity and community patterns in the urban context. The contrasted community patterns of earthworms, enchytraeids and nematodes in urban soils of different ages and their different ecological roles suggest that they represent potential complementary indicators of soil quality and functioning such as soil formation and organic matter dynamics.

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## 1. Introduction

Urban soils support mainly parks and gardens and contribute to local climate regulation, organic matter decomposition and primary production [1,2]. These processes are all controlled by soil

organisms, for which soils have a habitat function [3,4]. Soil fauna communities are useful indicators of changes in soil state or functioning [5–10]. However, they are still poorly studied in the urban context as compared to natural and agro-ecosystems and comparative studies of different groups are lacking. Our focus here is on the comparison of patterns of earthworm, enchytraeid and nematode diversity and community structure along a soil age gradient. Our aim was to assess to what extent these three

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contrasted groups of functionally important soil organisms could be used as indicators of soil ecological conditions in the urban context.

As soil engineers, earthworms modify environmental conditions for other organisms through their bioturbation activity [3,11]. They contribute to creating and maintaining the structure of soils by building pore networks and enhancing soil aggregation by mixing mineral and organic particle in their digestive tract [12–16]. Enchytraeids are commonly found in almost all soil types [17]. They are one of the most abundant groups of soil mesofauna in temperate soils [3,17]. Enchytraeids contribute significantly to litter fragmentation and organic matter decomposition [18]. They are also efficient at aerating the soil in the top centimetres [17–19]. Nematodes live in most terrestrial habitats that provide available organic carbon sources [6]. They belong to the microfauna (<0.2 mm in body diameter) and densities often reach millions of individuals per m<sup>2</sup> [3]. Nematodes are key components of soil food webs due to their various feeding habits (e.g. bacterivores, fungivores, herbivores and predators) and as food resources for other organisms [6,20]. Nematodes play various roles in the soil, especially regulation of microbial biomass and nutrient cycling [9,21]. Their community composition and life history indices are indicators of environmental disturbance [6,9,22]. The patterns of diversity and community structure of earthworms, enchytraeids and nematodes have been studied in natural and agro-ecosystems [23,24]. Earthworms, collembolans, nematodes and enchytraeids are amongst the most studied taxa in urban soils. They were studied for different purposes such as the effects of soil contaminants [25–29], land use or management [30–38] on soil fauna. However, the relationships among these groups remain poorly explored [39–41] and to our knowledge earthworms, enchytraeids and nematodes were never studied together in the urban context.

Although urban soils are strongly influenced by human activities and often very degraded, they are nevertheless highly diverse [42]. Urban soils are mainly characterized by high degrees of mixing, sealing, compaction and contamination [42,43]. The most affected part is often the topsoil, where most biological activity normally takes place [17]. Sealing and compaction reduce infiltration of water and air, organic matter transfer and turnover. The resulting low biological activity can feed back to compaction particularly in clay and wet soils, further inhibiting water movement and hindering root penetration [44,45]. As a consequence, available habitats for soil organisms are reduced [34,46]. This affects the overall soil quality and functioning.

While the diversity of above-ground organisms is reasonably well studied in urban areas, much less is known about the soil fauna. For example, it is unclear to what extent these organisms show similar patterns of diversity or community structure along environmental gradients or in response to disturbances in urban soils. Our aim was to study the patterns of diversity and community structure of earthworms, enchytraeids and nematodes in relation to soil conditions and functioning in an urban context and to assess if our observations matched those reported in agricultural or natural soils. As our study sites spanned more than two centuries of urban development, we especially focused on the diversity and community patterns in relation to soil age.

## 2. Material and methods

### 2.1. Study sites

The study was carried out in and around Neuchâtel, a thousand year old city in Switzerland (46° 59' 51" N; 6° 55' 86" E). Based on well-known periods of development of the city on surrounding ecosystems (forests, vineyards and lake) and preliminary soil

investigations, a series of eighteen study sites - spanning more than two centuries - were selected according to site history and land use (Table 1). We first investigated “native” and “near native” soils close to the city centre of Neuchâtel, and then explored “man-made” ones in the city and its suburbs (Table 1). At each site the soil was described and identified in 2011 and 2012 according to the 2006 World Reference Base for Soil Resources [47].

### 2.2. Soil analyses

At each site, we sampled the first horizon (top 8–12 cm) of the soil profile in 2011 and 2012. The soil samples were air dried, sieved at 2 mm in order to remove the coarse fraction and analysed for pH (H<sub>2</sub>O and KCl), particle-size distribution (% clay, % silt, % sand), loss on ignition (%), Allen method), organic carbon (C<sub>org</sub>, CHN method), total nitrogen (N<sub>tot</sub>, Kjeldahl method), available phosphorus (P<sub>bio</sub>, Olsen method), total phosphorus (P<sub>tot</sub>, Kjeldahl method), cation-exchange capacity (CEC, Cobaltihexamine method) and carbonate content (CaCO<sub>3</sub>, using a Bernard calcimeter according to Vatan's method, [48]). Water content and soil bulk density were measured on soil sampled using a metal cylinder, (5 cm height × 5 cm internal diameter) [49]. The C/N ratio was calculated. Four functional characteristics of the soil were measured: enzymatic activity (fluorecein diacetate hydrolysis/FDA, [50]), bacterial density (CyFlow<sup>®</sup> Space, [51,52]), ergosterol content [53,54], and soil respiration measured for 20 min (soil volume of 85.1 cm<sup>3</sup> at 40% of water content) at 20 °C in an acclimatized chamber (IRGA – LiCor 8100).

### 2.3. Soil annelids and nematodes

Annelids and nematodes were extracted from sites directly adjacent to the described soil profiles. Earthworms were collected from eight and ten sites in October 2011 and in October 2012, respectively. First, Lumbricidae were sampled using the hot mustard (2%) extraction method [55] in four squares of 0.25 m<sup>2</sup> surface (0.5 × 0.5 m) per site. A block of soil (20 × 20 × 20 cm, 8000 cm<sup>3</sup>) was then extracted in the same square in order to take into account the last individuals stuck in the roots. The combination of these two methods allowed us to estimate more precisely the density and the community patterns of earthworms. Earthworm numbers from the mustard extraction and the block of soil were multiplied by 4 and 25 respectively and expressed as density (ind.m<sup>-2</sup>). For each site, mean densities of earthworm species were calculated from the four samples. Earthworms were stored in formaldehyde (4% solution). They were identified at the species level [56–58] and counted. Juveniles were identified at the species level according to morphological characters as for adults. In cases where species-level identification was impossible (i.e. discrimination between pairs of species: *Octolasion tyrtaeum* and *O. cyaneum* and between *Lumbricus rubellus* and *Lumbricus castaneus*), individuals were allocated to species level using a pro rata distribution corresponding to adult and sub-adult proportions [59]. The species were classified according to three ecological categories (epigeic, endogeic and anecic) as defined by Bouché [60]; intermediate categories such as epi-anecic (*Lumbricus terrestris* Linnaeus, 1758) were grouped to the general category that best reflects the behaviour of the worm (for *L. terrestris*, anecic instead of epi-anecic).

Enchytraeids were collected twice, in autumn and in spring (October 2011 and March 2012 or October 2012 and March 2013). In each period, five soil samples were taken at each site with a split soil corer (diameter of 5.5 cm) to 10 cm depth. Each sample was transferred separately into a plastic bag in the field and stored at 4 °C. Soil samples were then vertically divided in two equal parts: one part was used for soil water content measurement (oven-dried

**Table 1**  
List of studied urban soils, soil type and land-use from Neuchâtel, Switzerland.

Code	Soil name (IUSS working group, 2007)	Soil origin	Land use
REFUFP	Luvic Hypocalcic Calcisol (Clayic)	Native	Oak forest
18thPD	Cambisol (Siltic)	Native	Lawn
19thGR	Cambisol (Siltic)	Native	Lawn
19thJA	Terric Anthrosol (Siltic)	Near native	Lawn
19thTU	Cambisol (Siltic)	Native	Meadow
19thTC	Terric Anthrosol (Siltic)	Near native	Meadow
20thFS	Cambisol (Clayic)	Native	Oak and maple forest
20thER	Terric Anthrosol (Siltic)	Near native	Lawn
1930VL	Terric Anthrosol (Siltic)	Near native	Lawn
1933PL	Terric Anthrosol (Siltic)	Near native	Lawn
1963WS	Terric Anthrosol (Clayic)	Near native	Lawn
1970JR	Urbic Garbic Technosol (Ruptic Calcaric Densic Siltic)	Man-made	Lawn
1995RP	Terric Hortic Anthrosol (Siltic)	Near native	Meadow
1995HR	Spolic Garbic Technosol (Ruptic Calcaric Siltic)	Man-made	Meadow
2005RU	Terric Anthrosol (Siltic)	Near native	Lawn
2005PB	Terric Anthrosol (Siltic)	Near native	Meadow
2010PR	Terric Hortic Technic Anthrosol (Siltic)	Man-made	Meadow
2010VM	Terric Anthrosol (Siltic)	Near native	Meadow

for 24 h at 105 °C) and the other part was used for enchytraeid extraction [61]. Enchytraeids were extracted using wet funnel extractors under light from incandescent light bulbs. Soil samples were heated up from 17 °C to 43 °C on their upper surface for 3 h [62,63]. Living individuals were kept in Petri dishes with tap water, counted and identified [64] under a light microscope (up to 400x magnification). For each site, the density ( $\text{ind.m}^{-2}$ ), the community patterns and the proportion of *r*-strategy type [8] of enchytraeids were calculated from the mean of both sampling periods.

Samples for nematodes were collected in October 2012. Five soil samples were taken for each site with a split soil corer (diameter of 4 cm) to 10 cm depth. Soil samples were then pooled and sieved at 5 mm in order to remove the coarse fraction and roots before nematode extraction and to maximize the representation of all genera [65,66]. Nematodes were extracted from 200 g of soil using a modified Beermann extraction method for 48 h [67]. They were then stored in a mixed solution of TriethanolAmine-Formalin (TAF) containing 2 ml of triethanolamine, 7 ml of formalin (40% formaldehyde solution) and 91 ml of deionized water. For each site, one hundred nematodes were sampled randomly and identified under a light microscope (up to 400x magnification) [68]. Individuals were identified at genus level except for two families, *Criconematidae* and *Diplogasteridae*, which were identified at the family level. The maturity index ( $MI_{1-5}$ ), enrichment index (EI) and structure index (SI) [20], were calculated from the proportion of each trophic group [69] and the life strategy of each family [70] using the NINJA software [71]. The maturity index is based on the proportion of colonizers and persisters (c-p) with lower values being indicative of disturbed soils [72]. The EI is calculated from the proportion of opportunistic bacterivores and fungivores. The SI derives from the proportion of carnivores and omnivores. Higher EI and SI values indicate, respectively, organic enrichment and soil food web complexity (interpreted as light to moderate disturbance or stress) [20].

#### 2.4. Numerical analyses

Soil age and its correlation with physicochemical and functional variables were tested in order to assess how soil properties change along the age gradient. Patterns of univariate metrics of soil faunal groups (density, species richness, Hill's numbers, Pielou's evenness, nematode indices, proportions of ecological categories for earthworms and *r*-strategist for enchytraeids) and their correlation with soil age, physicochemical and functional variables were tested

using Pearson or Kendall coefficient of correlation (respectively for normal and non-normal data). Given the high number of tests, Bonferroni's corrections to p-values were applied [73].

General relationships between earthworm, enchytraeid and nematode community patterns and between groups of environmental variables (physical, chemical and functional) were assessed using Mantel tests [74] on Bray–Curtis dissimilarity transformation matrices ( $p < 0.05$ , 999 permutations). After the selection of environmental variables using Pearson correlation tests, we then quantified the relationships between earthworm, enchytraeid (on Hellinger-transformed data) and nematode community data and environmental variables using redundancy analyses (RDA), and tested these relationships by Monte-Carlo permutation (999 iterations) [75].

Finally, we assessed, using the regression vector (RV) coefficients [76] of Multiple Factor Analyses (MFAs, on Hellinger-transformed data), if the relationships among datasets varied along the soil age gradient, using a « moving-windows » approach with a window width of six sites (i.e. starting with the six oldest sites and moving towards the six youngest ones) [77]. This number was a trade-off between having sufficient samples for calculation while limiting the calculation to a relatively short part of the age gradient.

All analyses were carried out with R statistical software [78] using the “vegan” [79] and “FactoMiner” [80] packages.

### 3. Results

#### 3.1. Site and soil characteristics

Three main soil types were found (Table 1). The oldest site, REFUFP, was a natural soil (classified as a Calcisol) located in an oak forest and sites 18thPD (lawn), 19thGR (lawn), 19thTU (meadow), and 20thFS (oak and maple forest) were near natural soils (assigned to Cambisols). Other soils located in lawns and meadows were strongly modified by human activities and were described as Anthrosols (19thJA, 19thTC, 20thER, 1930VL, 1933PL, 1963WS, 1995RP, 2005RU, 2005PB, 2010PR, and 2010VM) and Technosols (1970JR and 1995HR) [47].

The six oldest sites REFUFP, 18thPD, 19thGR, 19thJA, 19thTU, and 19thTC were up to 140 years old, while the six youngest sites 1995RP, 1995HR, 2005RU, 2005PB, 2010PR, and 2010VM were less than 18 years old. Soil age was positively correlated with soil depth and was negatively correlated with sand content and the

proportion of coarse fraction (Table 2). Soil depth was often higher in native soils compared to man-made soils (Tables 1 and 3). The characteristics of topsoils were most contrasted among sites for CaCO<sub>3</sub>, clay, phosphorous, and fungal biomass as assessed by ergosterol content (Table 3). Physical variables were correlated with functional variables (Mantel test, r = 0.475, p = 0.002) while chemical variables were neither correlated with physical nor with functional variables.

### 3.2. Earthworms

We identified 16 earthworm species at the 18 sites (average = 4.9 per site). Highest species richness was recorded at the old sites 18thPD and 20thFS and at the young sites 1995RP and 2010PR (7 species) (Table 4). Hill's numbers and evenness ranged from 0.18 (site 20thFS) to 0.97 (1933PL), and 0.1 (1933PL) to 1 (1930VL), respectively. Earthworm density ranged from 27 ind.m<sup>-2</sup> (site 1930VL) to 553 ind.m<sup>-2</sup> (site REFUFPP) and reached on average 220 ind.m<sup>-2</sup> (Table 5). Density and species richness were positively correlated (r = 0.435) (Supplementary table 1).

Community patterns and ecological categories of earthworms differed among sites (Tables 4 and 5). Epigeic earthworms were found at eight sites (REFUFPP, 18thPD, 20thFS, 1963WS, 1995RP, 2005RU, 2005PB and 2010PR) with highest densities recorded at the two oldest sites (REFUFPP and 18thPD) and at the second youngest site (2010PR) (115–148 ind.m<sup>-2</sup>, Table 5). *Dendrodrilus rubidus* (Savigny, 1926) was only found at the second youngest site (2010PR), *Lumbricus castaneus* (Savigny, 1826) was found only at four sites and, *Lumbricus rubellus* (Hoffmeister, 1843) and *Den-drobaena octaedra* (Savigny, 1826) were recorded only at five sites (Table 5). Endogeic earthworms were found at all sites except at 1930VL. Highest densities were recorded at the two oldest sites (REFUFPP and 18thPD), at 20thER and at the youngest site (2010VM) (277–411 ind.m<sup>-2</sup>, Table 4). *Octolasion tyrtaeum tyrtaeum* (Savigny, 1926) was identified at the two oldest sites (REFUFPP and 18thPD), whereas co-dominant endogeic species, *Allolobophora chlorotica*

**Table 2**  
Kendall correlations between soil age (non-normal data) and each environmental variable. Significant correlations (ANOVA, p < 0.05) are shown in bold and underlined.

Environmental variable	Soil age	
	r	p-value
<i>Physical variables</i>		
Depth	<b>0.358</b>	<b>0.045</b>
Bulk density	-0.172	0.324
Water content	0.059	0.765
Coarse fraction	<b>-0.503</b>	<b>0.003</b>
Clay	0.150	0.410
Silt	0.150	0.410
Sand	<b>-0.346</b>	<b>0.048</b>
<i>Chemical variables</i>		
pH <sub>KCl</sub>	-0.224	0.197
pH <sub>H2O</sub>	0.229	0.201
CaCO <sub>3</sub>	-0.006	1
CEC	0.294	0.096
P <sub>tot</sub>	0.124	0.501
P <sub>bio</sub>	-0.150	0.410
Loss on ignition	0.059	0.765
C <sub>org</sub>	-0.033	0.881
N <sub>tot</sub>	0.150	0.410
C/N	-0.046	0.823
<i>Functional variables</i>		
Enzymatic activity (FDA)	-0.046	0.823
Respiration	0.111	0.550
Bacterial density	-0.046	0.823
Ergosterol (fungal biomass)	0.216	0.229

**Table 3**  
Soil age, physicochemical and functional characteristics of the topsoils of urban soils from Neuchâtel, Switzerland.

Site code	Soil age [years]	Coarse fraction [%]	Silt [%]	Clay [%]	Sand loss on ignition [%]	pH	KCl H <sub>2</sub> O [%]	pH	CaCO <sub>3</sub> [%]	CEC <sup>a</sup> [cmol.c.kg <sup>-1</sup> ]	P <sub>tot</sub> [%]	P <sub>bio</sub> [%]	C <sub>org</sub> [%]	N <sub>tot</sub> [%]	C/N	Depth [cm]	Bulk density [g.cm <sup>-3</sup> ]	Water content [%]	FDA <sup>b</sup> [μg.g <sup>-1</sup> .h <sup>-1</sup> ]	Respiration [μmol.m <sup>-2</sup> .s <sup>-1</sup> ]	Bacterial density [nb.g <sup>-1</sup> dry soil]	Ergosterol [μg.g <sup>-1</sup> ]
REFUFPP	>250	3.2	49.5	31.8	18.7	16.5	6.8	7.4	0.3	38.5	0.74	0.031	7.6	0.56	13.6	40	0.33	10.4	114	2.50	1.42E+08	1.57
18thPD	250	10.5	21.6	26.6	51.3	8.4	7.2	7.8	23.7	19.4	1.65	0.051	3.6	0.36	9.9	61	0.62	8.4	107	0.57	1.03E+08	0.67
19thGR	200	14.4	5.6	51.0	43.4	8.8	7.2	8.0	28.8	27.3	1.83	0.073	4.1	0.38	10.8	60	0.49	7.2	119	0.44	7.96E+07	0.41
19thJA	148	14.4	19.4	32.4	48.2	7.7	7.3	7.8	24.2	15.2	1.64	0.084	3.7	0.32	11.6	45	0.59	6.9	128	0.51	1.23E+08	0.91
19thTU	143	14.0	35.2	27.4	37.4	7.2	7.2	7.9	26.5	19.5	0.87	0.013	3.7	0.34	11.1	60	0.81	5.9	31	0.46	1.55E+08	1.67
19thTC	140	50.0	2.3	40.2	57.6	6.9	7.3	8.0	29.5	17.1	0.88	0.048	4.1	0.24	16.8	60	0.90	3.8	97	0.24	1.31E+08	3.25
20thFS	107	18.0	40.3	27.0	32.6	15.0	7.2	7.6	20.1	36.8	0.55	0.019	9.7	0.56	17.2	57	0.45	8.3	137	1.29	1.41E+08	3.44
20thER	100	20.4	18.1	39.7	42.2	11.0	7.2	7.9	15.0	27.5	0.87	0.014	6.0	0.48	12.6	42	0.77	7.6	151	0.49	1.09E+08	1.66
1930VL	83	20.8	38.7	26.3	35.0	13.4	7.6	7.2	34.7	26.2	0.84	0.138	5.8	0.44	13.3	46	0.30	8.3	120	0.86	1.66E+08	0.70
1933PL	80	25.0	22.2	39.0	38.4	8.7	7.2	7.9	34.5	19.3	1.09	0.032	4.1	0.31	13.4	40	0.68	4.0	116	0.44	1.72E+08	1.96
1963WS	59	27.0	41.1	24.8	34.0	9.1	7.7	7.1	22.0	21.0	0.92	0.032	4.7	0.42	11.3	48	0.55	5.7	117	0.58	2.59E+08	0.07
1970JR	43	12.5	7.2	50.6	42.2	17.4	6.8	7.6	15.2	34.4	17.3	0.493	9.2	0.88	10.6	45	0.75	8.1	181	0.53	9.98E+07	2.16
1995RP	18	28.7	20.7	34.6	44.6	25.4	5.9	6.1	14.1	13.3	0.87	0.130	2.2	0.44	13.2	47	0.73	11.7	81	1.22	1.55E+08	0.84
1995HR	18	21.1	16.2	32.1	51.7	13.9	7.1	7.7	34.6	24.4	1.54	0.173	8.0	0.74	10.7	40	0.43	7.4	172	0.59	8.63E+07	1.06
2005RU	8	25.2	26.3	17.0	56.7	6.1	7.7	7.3	34.0	14.8	1.06	0.094	4.6	0.29	16.2	60	0.80	7.1	88	0.35	1.16E+08	0.13
2005PB	8	20.5	17.0	28.0	55.1	4.5	7.6	7.3	16.5	11.1	0.61	0.031	1.7	0.22	7.7	40	0.81	7.7	69	0.47	1.06E+08	0.69
2010PR	3	45.4	12.0	28.0	59.9	14.7	7.4	8.0	31.2	25.3	1.17	0.120	8.2	0.40	20.6	35	0.30	9.8	180	0.77	9.14E+07	0.14
2010VM	3	22.7	19.6	31.7	48.7	5.4	7.9	7.3	4.2	13.4	0.47	0.015	2.6	0.21	12.0	45	0.90	5.6	102	0.24	1.77E+08	0.57
Min	3	3.2	2.3	17.0	18.7	4.5	5.9	6.1	0.3	11.1	0.47	0.013	1.7	0.21	7.7	35	0.3	3.8	30.9	0.24	7.96E+07	0.07
Average	83	21.9	23.0	32.7	44.3	11.1	7.2	7.6	22.7	22.5	1.94	0.088	5.2	0.42	12.9	48.4	0.6	7.4	117.2	0.70	1.34E+08	1.22
Max	250	50.0	49.5	51.0	59.9	25.4	7.9	8.0	34.7	38.5	17.3	0.493	9.7	0.88	20.6	61	0.9	11.7	180.7	2.50	2.59E+08	3.44
Max/Min	83.3	15.6	21.5	3.0	3.2	5.6	1.3	1.3	115.7	3.5	36.8	37.9	5.7	4.2	2.7	1.7	3.0	3.1	5.8	10.4	3.3	49.1

<sup>a</sup> Cation Exchange Capacity.  
<sup>b</sup> Fluorecein DiAcetate hydrolysis.

**Table 4**  
Earthworm density [ind.m<sup>-2</sup>] per species and ecological categories (Bouché, 1977) in urban soils of Neuchâtel, Switzerland. Nomenclature follows Bouché (1972), Sims and Gerard (1999), and Blakemore (2008).

Genus	Species	Authority	REFUFP	18thPD	19thGR	19thJA	19thTU	19thTC	20thFS	20thER	1930VL	1933PL	1963WS	1970JR	1995RP	1995HR	2005RU	2005PB	2010PR	2010VM	Mean density	Ecological category
<i>Dendrodrilus</i>	<i>rubidus</i>	Savigny, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0.6	epigeic
<i>Bimastos</i>	<i>eiseni</i>	Gerard, 1964	0	0	0	0	0	14	0	0	0	0	0	0	9	0	0	0	0	0	1.3	epigeic
<i>Lumbricus</i>	<i>rubellus</i>	Hoffmeister, 1843	89	0	0	0	0	16	0	0	0	0	0	0	8	0	0	18	107	0	13.2	epigeic
<i>Lumbricus</i>	<i>castaneus</i>	Savigny, 1826	26	25	0	0	0	28	0	0	0	0	0	0	0	0	0	0	29	0	6.0	epigeic
<i>Dendrobaena</i>	<i>octaedra</i>	Savigny, 1826	0	111	0	0	0	0	0	0	0	5	0	3	0	11	0	1	0	0	7.3	epigeic
<i>Aporrectodea</i>	<i>caliginosa</i>	Savigny, 1826	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.9	endogeic
<i>Octolasion</i>	<i>tyrtaeum lacteum</i>	Oerley, 1885	0	11	9	16	0	0	0	0	0	0	0	13	3	0	0	9	0	0	3.4	endogeic
<i>Octolasion</i>	<i>tyrtaeum tyrtaeum</i>	Savigny, 1826	19	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.6	endogeic
<i>Aporrectodea</i>	<i>rosea</i>	Savigny, 1826	28	116	25	0	0	4	15	278	0	0	7	104	15	134	28	91	0	32	48.7	endogeic
<i>Allolobophora</i>	<i>chlorotica chlorotica</i>	Savigny, 1826	364	0	25	53	19	0	2	90	0	72	91	25	102	57	46	32	51	244	70.7	endogeic
<i>Allolobophora</i>	<i>icterica</i>	Savigny, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0.2	endogeic
<i>Octolasion</i>	<i>cyaneum</i>	Savigny, 1826	0	144	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.0	endogeic
<i>Aporrectodea</i>	<i>longa ripicola</i>	Bouché, 1972	0	0	0	0	0	0	0	0	0	0	0	0	14	11	0	57	0	0	4.6	anecic
<i>Aporrectodea</i>	<i>longa longa</i>	Ude, 1885	26	0	64	8	31	2	13	51	14	0	23	36	0	74	170	82	12	3	33.8	anecic
<i>Aporrectodea</i>	<i>nocturna</i>	Evans, 1946	0	0	0	0	29	31	0	0	0	0	0	0	0	0	0	0	0	0	3.3	anecic
<i>Lumbricus</i>	<i>terrestris</i>	Linnaeus, 1758	0	10	67	126	3	2	20	1	13	1	4	0	0	46	10	0	0	0	16.8	epi-anecic
Total (ind.m <sup>-2</sup> )			552	426	205	203	82	39	108	420	27	73	130	165	164	325	265	283	220	280		

**Table 5**  
Annelid density, annelid and nematode diversity metrics and community composition from urban soils of Neuchâtel, Switzerland.

Site	Year of annelid sampling	Enchytraeids										Nematodes							
		Mean density [ind.m <sup>-2</sup> ] ± SD	Species richness	Hill's numbers	Evenness	Epigeic [ind.m <sup>-2</sup> ]	Endogeic [ind.m <sup>-2</sup> ]	Anecic [ind.m <sup>-2</sup> ]	Mean density [ind.m <sup>-2</sup> ] ± SD	Species richness	Hill's numbers	Evenness r-strategists (%)	Genus richness	Hill's numbers	Evenness MI <sub>1-5</sub> EI	SI			
REFUFP	2012	552 ± 147	6	0.50	0.63	115	411	26	19877 ± 1548	15	0.15	0.78	27.1	18	0.25	0.70	2.02	90.0	82.2
18thPD	2011	426 ± 39	7	0.26	0.77	136	280	10	20296 ± 5955	13	0.16	0.82	15.4	14	0.11	0.90	2.26	76.6	74.3
19thGR	2011	205 ± 189	6	0.24	0.88	0	74	131	38322 ± 27276	9	0.16	0.89	35.5	18	0.17	0.77	2.30	71.4	72.0
19thJA	2011	203 ± 48	4	0.32	0.70	0	69	134	11707 ± 7266	8	0.28	0.72	31.7	18	0.09	0.85	2.31	67.8	69.2
19thTU	2012	82 ± 43	4	0.75	0.86	0	19	63	4632 ± 7265	5	0.36	0.77	81.8	21	0.08	0.89	2.66	46.3	72.0
19thTC	2012	39 ± 26	4	0.46	0.52	0	4	35	10463 ± 2144	14	0.15	0.85	15.7	22	0.12	0.90	2.44	56.5	69.8
20thFS	2012	108 ± 63	7	0.18	0.93	58	17	33	25941 ± 12030	13	0.22	0.74	47.7	14	0.35	0.61	1.45	95.1	70.9
20thER	2011	420 ± 271	4	0.51	0.63	0	368	52	6317 ± 1069	6	0.28	0.82	29.3	12	0.19	0.78	1.78	90.0	73.5
1930VL	2012	27 ± 25	2	0.53	1.00	0	0	27	49018 ± 7028	10	0.36	0.57	48.1	15	0.18	0.79	1.94	92.3	84.5
1933PL	2011	73 ± 52	2	0.97	0.10	0	72	1	2694 ± 476	4	0.46	0.69	9.4	15	0.19	0.75	2.10	54.7	33.3
1963WS	2012	130 ± 98	5	0.57	0.59	5	98	27	50366 ± 953	12	0.21	0.76	41.0	20	0.09	0.89	2.46	53.0	68.0
1970JR	2011	165 ± 156	3	0.47	0.83	0	129	36	6064 ± 1549	7	0.18	0.90	55.5	13	0.23	0.73	1.45	92.0	53.3
1995RP	2012	164 ± 98	7	0.51	0.67	20	130	14	39417 ± 6313	11	0.24	0.71	61.8	12	0.20	0.79	1.84	76.5	47.9
1995HR	2011	325 ± 100	6	0.27	0.80	0	194	131	20046 ± 22631	7	0.78	0.27	95.0	12	0.17	0.81	2.14	72.1	62.2
2005RU	2012	265 ± 120	5	0.46	0.67	11	74	180	3959 ± 476	5	0.43	0.70	25.5	12	0.23	0.75	1.84	87.2	69.3
2005PB	2012	283 ± 69	6	0.26	0.85	18	126	139	24930 ± 3097	11	0.18	0.80	39.5	18	0.13	0.82	1.97	84.5	71.1
2010PR	2011	220 ± 110	7	0.76	0.73	148	60	12	17351 ± 1668	7	0.17	0.49	92.7	15	0.18	0.81	2.08	84.4	74.5
2010VM	2012	280 ± 60	4	0.31	0.31	0	277	3	10949 ± 3440	9	0.59	0.88	49.3	16	0.18	0.77	1.84	78.4	51.5
Min		27	2	0.18	0.10	0	0	1	2694	4	0.15	0.27	9.4	12	0.08	0.61	1.45	46.3	33.3
Average		220	4.9	0.46	0.69	28	133	59	20131	9.2	0.30	0.74	44.6	15.8	0.17	0.80	2.05	76.1	66.6
Max		553	7	0.97	1.00	148	411	180	50366	15	0.78	0.90	95.0	22	0.35	0.90	2.66	95.1	84.5
Max/Min		20.5	3.50	5.49	10	n.a.	n.a.	180	18.7	3.75	5.11	10.1	1.83	4.64	1.48	1.83	2.05	2.54	

(Savigny, 1826) and *Aporrectodea rosea* (Savigny, 1826), were recorded at most sites (Table 5). Anecic earthworms were identified at all sites with lowest density found at 1933 PL (1 ind.m<sup>-2</sup>) and highest densities recorded at 19thGR, 19thJA, 1995HR, 2005RU, and 2005PB (131–180 ind.m<sup>-2</sup>, Table 4). *Aporrectodea longa ripicola* (Bouché, 1972) was only found at three young sites (1995RP, 1995HR and 2005PB), while *Lumbricus terrestris* (Linnaeus, 1758) and *Aporrectodea longa longa* (Ude, 1885) were found at most sites along the soil age gradient (Table 5).

Earthworm evenness was positively correlated with water content (r = 0.490) (Supplementary table 2). The density of epigeic earthworm species was positively correlated with soil water content (r = 0.523) and with soil respiration (r = 0.423). Densities of endogeics and anecics were negatively correlated with pH<sub>H2O</sub> (r = -0.485) and with the bacterial density (r = -0.356) respectively (Supplementary table 2).

### 3.3. Enchytraeids

We identified 34 enchytraeid species at the 18 sites (average = 9.2 per site). Highest and lowest species richness were recorded at the oldest site REFUFP (15 species) and at 1933PL (4 species) (Table 6), respectively. Enchytraeid density varied from 2694 ind.m<sup>-2</sup> (1933PL) to 50366 ind.m<sup>-2</sup> (1963WS) (average = 20131 ind.m<sup>-2</sup>) (Table 5). Hill's numbers and evenness ranged from 0.15 (REFUFP and 19thTC) to 0.78 (1995HR), and from 0.27 (1995HR) to 0.90 (1970JR), respectively. Density and species richness were positively correlated (r = 0.482, Supplementary table 1). The proportion of r-strategists varied from 11% (1933PL) to 86% (2010PR) with an overall average of 42% (Table 5).

Enchytraeid community structure varied among sites (Table 6). The dominant and fragmenting r-strategist species, *Buchholzia appendiculata* (Buchholz, 1962), was found at all sites except 1970JR. Higher densities were found at 1930VL, 1963WS, 1995RP, 1995HR, and 2010PR (13139–18024 ind.m<sup>-2</sup>). By contrast, species of *Fridericia* and *Achaeta* (K-strategists) were found at a limited number (1–11) of sites. For example, *Achaeta bohemica* (Vejdovský, 1879) and *Achaeta unibulba* (Graefe, Dózsa-Farkas & Christensen, 2005) were only recorded at three of the oldest sites (18thPD, 19thGR and 19thTC), whereas other species, such as *Achaeta eiseni* (Vejdovský, 1878) and *Achaeta iberica* (Graefe, 1989) – the latter considered rare in Europe [64] – were found at several sites along the soil age gradient (Table 6).

Enchytraeid evenness was negatively correlated with the coarse fraction (r = -0.407), the carbonate content (r = -0.354), and C/N ratio (r = -0.380). The proportion of enchytraeid r-strategists was correlated negatively with soil age (r = -0.380) and positively with loss of ignition (r = 0.381) and C<sub>org</sub> (r = 0.337; Supplementary table 3).

### 3.4. Nematodes

We identified 43 nematode genera at the 18 sites (average = 15.8 per site) with highest and lowest genera richness respectively recorded at site 19thTU (22 genera) and at sites 20thER, 1995RP, 1995HR, and 2005RU (12 genera) (Table 7). Hill's numbers ranged from 0.08 (19thTU) to 0.35 (20thFS). SI varied from 33.3 (1933PL) to 84.5 (1930VL) and EI from 46.3 (19thTU) to 95.1 (20thFS) (Table 5). Almost all sites were positioned in the upper right quadrant of the food web diagnostic except sites 19thTU, 1933PL, and 1995RP (Fig. 1).

Nematode community structure varied among sites (Table 7). The dominant genus *Rhabditis* (bacterivorous with a short life cycle and high reproduction rate, c-p 1) was found at all sites, while other genera were found in few sites, such as *Aporcelaimellus* (predator

**Table 6**Enchytraeid density [ind.m<sup>-2</sup>] per species in urban soils of Neuchâtel, Switzerland. Nomenclature follows Schmelz and Collado (2010). Categorization as r-strategists follows Graefe and Schmelz (1999).

Genus	Species	Authority	REFUPP	18thPD	19thGR	19thJA	19thTU	19thTC	20thFS	20thER	1930VL	1933PL	1963WS	1970JR	1995RP	1995HR	2005RU	2005PB	2010PR	2010VM	Mean density	r-strategists
<i>Achaeta</i>	<i>bohemica</i>	Vejdovský, 1879	0	674	674	0	0	1095	0	0	0	0	0	0	0	0	0	0	0	0	136	
<i>Achaeta</i>	<i>danica</i>	Nielsen & Christensen, 1959	84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	
<i>Achaeta</i>	<i>eiseni</i>	Vejdovsky, 1878	3874	2021	4632	4801	0	926	2021	0	1516	0	3201	0	0	0	2442	1263	0	168	1493	
<i>Achaeta</i>	<i>iberica</i>	Graefe, 1989	0	168	0	1600	0	253	0	0	0	0	1432	0	0	0	0	3959	0	0	412	
<i>Achaeta</i>	<i>aberrans</i>	Nielsen & Christensen, 1961	0	0	0	0	0	337	0	0	0	0	0	0	0	0	0	0	0	0	19	
<i>Achaeta</i>	<i>unibulba</i>	Graefe, Dozsa-Farkas & Christensen, 2005	0	1600	2190	0	0	842	0	0	0	0	0	0	0	0	0	0	0	0	257	
<i>Buchholzia</i>	<i>appendiculata</i>	Buchholz, 1962	2527	2274	7201	3285	2527	463	9349	253	18024	84	17519	0	15918	17687	758	1600	13139	926	6307	X
<i>Enchytraeus</i>	<i>buchholzi</i>	Vejdovský, 1879	1600	337	590	0	674	421	1937	337	1853	0	1937	926	5053	1011	253	3032	1011	842	1212	X
<i>Enchytraeus</i>	<i>bulbosus</i>	Nielsen & Christensen, 1963	421	505	5832	168	84	421	590	505	926	168	674	1179	926	84	0	84	1263	926	820	X
<i>Enchytraeus</i>	<i>bigeminus</i>	Nielsen & Christensen, 1963	0	0	0	0	0	0	0	0	0	0	0	0	0	253	0	0	674	0	52	X
<i>Enchytraeus</i>	<i>christenseni</i>	Dózsa-Farkas, 1992	842	0	0	253	505	337	505	0	2779	0	1011	1263	2442	0	0	5138	0	2695	987	X
<i>Enchytraeus</i>	<i>lacteus</i>	Nielsen & Christensen, 1961	0	0	0	0	0	0	0	758	0	0	0	0	0	0	0	0	0	0	42	X
<i>Buchholzia</i>	<i>fallax</i>	Michaelsen, 1887	505	2779	4064	168	0	84	1516	0	590	0	253	84	505	0	337	0	0	0	605	
<i>Enchytronia</i>	<i>parva</i>	Nielsen & Christensen, 1959	253	0	0	1263	842	842	1263	0	22740	1600	8507	253	253	253	0	7327	0	421	2545	
<i>Fridericia</i>	<i>galba</i>	Hoffmeister, 1843	3959	0	3516	0	0	0	758	0	0	0	0	0	8591	0	0	0	0	421	958	
<i>Fridericia</i>	<i>discifera</i>	Healy, 1975	0	5559	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	309	
<i>Fridericia</i>	<i>paroniana</i>	Issel, 1904	0	0	9623	0	0	3453	6654	2106	84	0	2358	1011	337	674	0	253	674	1937	1620	
<i>Fridericia</i>	<i>vixdiverticulata</i>	Sesma & Dózsa-Farkas, 1993	0	0	0	0	0	821	0	0	0	0	0	0	0	0	0	0	0	0	46	
<i>Fridericia</i>	<i>bulboides</i>	Nielsen & Christensen, 1959	0	0	0	0	0	0	0	2358	0	0	0	0	0	0	0	0	0	0	131	
<i>Fridericia</i>	<i>ratzeli</i>	Eisen, 1872	253	0	0	0	0	0	0	0	0	842	1011	0	4380	0	0	0	0	0	360	
<i>Fridericia</i>	<i>perrieri</i>	Vedovský, 1878	421	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	
<i>Fridericia</i>	<i>christeri</i>	Rota & Healy, 1999	0	0	0	168	0	0	0	0	84	0	11033	1348	0	0	168	1095	0	0	772	
<i>Fridericia</i>	<i>sp1</i>	–	0	3537	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	197	
<i>Fridericia</i>	<i>sp2</i>	–	0	337	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	
<i>Fridericia</i>	<i>rendsinata</i>	Dózsa-Farkas, 1972	4380	0	0	0	0	0	84	0	0	0	0	0	0	0	0	0	0	0	2611	393
<i>Fridericia</i>	<i>isseli</i>	Rota, 1994	0	0	0	0	0	0	0	0	0	0	0	0	926	0	0	0	0	0	51	
<i>Fridericia</i>	<i>nemoralis</i>	Nurminen, 1970	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	168	0	0	9	
<i>Fridericia</i>	<i>connata</i>	Bretscher, 1902	253	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	
<i>Fridericia</i>	<i>sylvatica</i>	Healy, 1975	421	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	
<i>Henlea</i>	<i>nasuta</i>	Eisen, 1878	0	0	0	0	0	0	253	0	0	0	0	0	0	0	0	0	0	253	0	28
<i>Henlea</i>	<i>ventriculosa</i>	Udekem, 1854	0	421	0	0	0	168	0	0	0	0	1432	0	0	0	0	0	337	0	131	
<i>Henlea</i>	<i>perpusilla</i>	Friend, 1911	84	0	0	0	0	0	421	0	421	0	0	0	84	0	0	0	0	0	56	
<i>Marionina</i>	<i>argentea</i>	Michaelsen, 1889	0	84	0	0	0	0	590	0	0	0	0	0	0	0	0	0	0	0	37	
<i>Marionina</i>	<i>communis</i>	Nielsen & Christensen, 1959	0	0	0	0	0	0	0	0	0	0	0	0	0	84	0	1011	0	0	61	
Total (ind.m <sup>-2</sup> )			19877	20296	38322	11707	4632	10463	25941	6317	49018	2694	50366	6064	39417	20046	3959	24930	17351	10949		

**Table 7**  
Nematode proportion [%] per genera, life strategy trait (c-p groups) and trophic group in urban soils of Neuchâtel, Switzerland.

Genus	REFUFP	18thPD	19thGR	19thJA	19thTU	19thTC	20thFS	20thER	1930VL	1933PL	1963WS	1970JR	1995RP	1995HR	2005RU	2005PB	2010PR	2010VM	Mean proportion	c-p group	Trophic group
<i>Alaimus</i>	1	0	0	3	2	0	1	0	3	2	1	0	1	0	0	2	3	3	1.2	4	Ba
<i>Anaplectus</i>	2	0	0	1	0	0	0	3	0	0	0	3	9	0	0	2	0	0	1.1	2	Ba
<i>Anatonchus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	3	0.4	4	Ca
<i>Aphelenchoides</i>	0	0	0	0	5	0	0	1	0	16	3	0	0	19	0	0	0	0	2.4	2	Fu
<i>Aphelenchus</i>	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0.2	2	Fu
<i>Aporcelaimellus</i>	2	3	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0.5	5	Ca
<i>Axonchium</i>	0	7	1	3	0	2	0	0	8	5	2	2	0	0	3	2	0	2	2.1	5	PP
<i>Bitylenchus</i>	0	0	0	4	0	2	0	14	1	0	0	0	0	0	1	2	0	0	1.3	3	PP
<i>Cephalobus</i>	3	3	4	6	5	5	1	8	3	2	7	0	0	2	0	2	4	3	3.2	3	Ba
<i>Cricematidae</i>	0	1	1	1	1	0	0	0	0	1	1	0	0	0	3	0	0	0	0.5	3	PP
<i>Clarkus</i>	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0.2	4	Ca
<i>Coomansus</i>	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0.2	4	Ca
<i>Diphtherophora</i>	1	4	0	0	3	2	1	0	6	0	4	0	0	0	2	8	0	1	1.8	3	Fu
<i>Diplogasteridae</i>	0	0	0	0	0	0	57	0	0	0	0	0	0	0	0	0	0	0	3.2	1	Ba
<i>Dolichorhynchus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0.1	3	PP
<i>Eucephalobus</i>	3	2	6	11	0	2	5	0	2	2	3	5	0	1	0	1	5	1	2.7	3	Ba
<i>Eudorylaimus</i>	11	0	2	0	2	3	3	2	7	1	6	2	0	6	0	1	7	3	3.1	4	Ca
<i>Filenchus</i>	8	7	2	7	15	10	6	0	0	7	1	9	1	7	8	4	7	7	5.5	2	PP
<i>Helicotylenchus</i>	0	11	36	26	17	10	2	25	11	28	19	21	2	0	12	15	5	2	13.4	3	PP
<i>Heterocephalobus</i>	0	3	2	0	0	1	0	0	4	0	5	1	9	5	1	0	3	0	1.9	3	Ba
<i>Longidorus</i>	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3	5	PP
<i>Loofia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0.3	3	PP
<i>Malenchus</i>	0	0	0	5	0	2	0	0	0	7	1	0	2	0	0	0	0	3	1.1	2	PP
<i>Merlinius</i>	0	0	1	0	0	8	0	0	0	0	5	5	0	0	0	3	0	0	1.2	3	PP
<i>Mesodorylaimus</i>	2	0	0	0	0	0	0	6	0	0	5	2	3	10	0	0	0	0	1.6	4	Om
<i>Metateratocephalus</i>	0	0	2	3	8	0	0	0	0	0	2	0	0	0	0	0	2	0	0.9	3	Ba
<i>Miconchus</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0.2	4	Ca
<i>Mylonchulus</i>	0	10	10	8	2	0	0	0	2	0	0	0	0	0	0	2	5	0	2.2	4	Ca
<i>Paraphelenchus</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	12	0	0.7	2	Fu
<i>Plectus</i>	5	14	7	3	4	5	2	1	1	2	5	4	19	19	17	9	3	25	8.1	2	Ba
<i>Prionchulus</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	6	0	0.5	4	Ca
<i>Prismatolaimus</i>	2	0	0	0	2	5	1	0	0	0	2	0	5	0	0	0	0	0	0.9	3	Ba
<i>Prodorylaimus</i>	0	4	0	3	1	5	0	0	1	0	0	0	0	0	9	2	0	0	1.4	4	Om
<i>Pseudhalenchus</i>	1	0	0	0	7	0	1	0	0	0	0	0	0	0	0	0	0	0	0.5	2	Fu
<i>Pungentus</i>	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	2	0.3	4	PP
<i>Rhabditis</i>	47	22	13	13	1	7	14	29	36	3	6	39	36	28	41	28	38	31	24.0	1	Ba
<i>Rotylenchus</i>	2	9	6	1	11	16	0	9	13	27	15	14	0	0	0	9	2	8	7.9	3	PP
<i>Teratocephalus</i>	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	3	Ba
<i>Trichodorus</i>	0	0	1	1	0	0	0	1	0	2	0	1	0	0	0	0	0	0	0.3	4	PP
<i>Tripyla</i>	6	0	0	0	2	5	0	0	0	0	0	0	0	0	0	0	1	0	0.8	3	Ca
<i>Tylencholaimus</i>	1	0	0	0	3	0	4	0	0	0	0	0	0	0	2	0	0	0	0.6	4	Fu
<i>Tylenchorhynchus</i>	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	1	0.3	3	PP
<i>Wilsonema</i>	0	0	0	0	0	6	0	0	2	1	0	0	1	4	0	0	0	0	0.8	2	Ba

Life strategy trait (c-p groups) according to Yeates et al., (1993); trophic group (PP = plant parasite, Fu = fungivore, Ba = bacterivore, Ca = carnivore and Om = omnivore, according to Bongers & Bongers (1998).

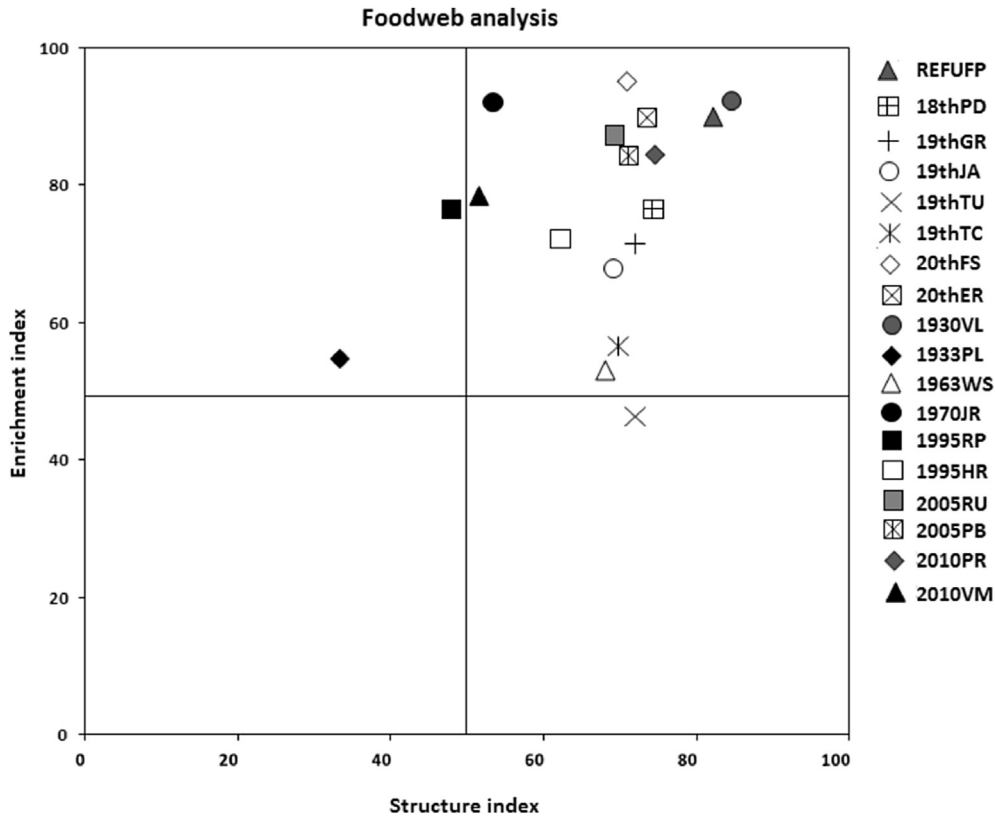


Fig. 1. Food web analysis using Enrichment and Structure indices for nematodes from urban soils in Neuchâtel, Switzerland.

with a long life cycle and low reproduction rate, c-p 5), which was only recorded at four of the oldest sites (REFUFP, 18thPD, 19thTU, and 19thTC). When adding the genera *Rhabditis* and *Diplogasteridae*, the proportions of *r*-strategists (c-p 1) were highest (36–71%) in forest soils (REFUFP and 20thFS) and at sites 1930VL, 1970JR, 1995RP, 2005RU, and 2010PR (lawns and meadows).

Hill's numbers were positively correlated with loss on ignition ( $r = 0.479$ ), CEC ( $r = 0.612$ ) and  $C_{org}$  ( $r = 0.615$ ) (Supplementary table 4).  $M_{1-5}$  was negatively correlated with  $C_{org}$  ( $r = -0.456$ ) while SI and EI were positively correlated with water content ( $r = 0.362$  and  $r = 0.454$  respectively) (Supplementary table 4).

### 3.5. Community patterns and community–environment relationships

The RDAs on environmental (soil age, physical, chemical and functional) variables revealed significant correlations with each soil fauna group: (1) between earthworms and soil depth, and between earthworms and soil bulk density (total explained variance = 22.6%; model p-value = 0.013; AIC = -12.10;  $r^2_{adj} = 0.123$ ), (2) between enchytraeids and calcium carbonate content (10.2%; 0.034; -11.21; 0.046), and (3) between nematodes and soil water content (13.5%; 0.010; -16.93; 0.081). Furthermore, the Mantel tests did not reveal any significant relationship between pairs of soil fauna groups (earthworms vs enchytraeids, earthworms vs nematodes, and enchytraeids vs nematodes) or between each individual soil fauna group and either one of the three groups of environmental (physical, chemical and functional) variables.

Correlations between earthworms and enchytraeid species assemblages and between earthworms and nematodes increased with soil age as shown by the higher RV coefficients in the moving window MFA at the oldest sites (N° 1 to 7, sites 1–12, Fig. 2) and

lower values at the youngest sites (N° 8 to 13, sites 8–18). Conversely, correlations between nematodes and enchytraeid assemblages decreased with soil age (Fig. 2). For each pair of soil fauna assemblages, linear regression tests showed significant

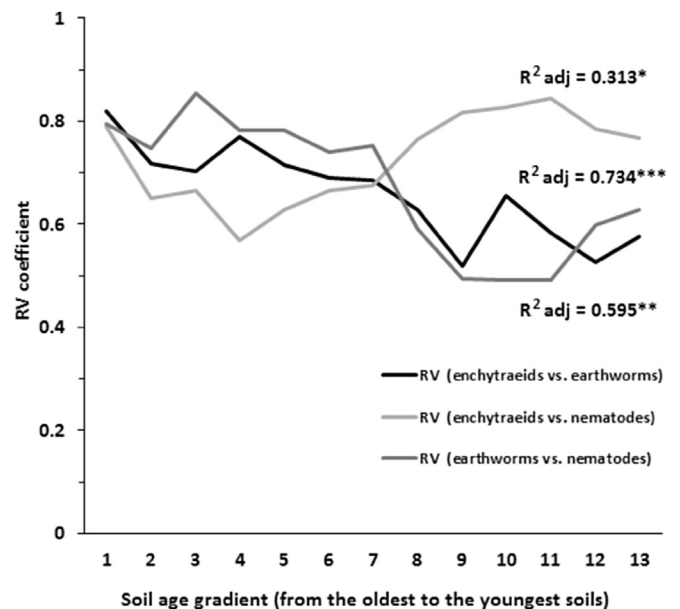


Fig. 2. Moving window analysis of correlations (RV scores from Multiple Factor Analyses) between pairs of community data (enchytraeids, earthworms and nematodes) along the age gradient of 18 urban soils in Neuchâtel, Switzerland. Window width = 6 sites; 1 = oldest sites; 13 = youngest sites. Significances of linear regression tests are  $p < 0.05^*$ ,  $p < 0.01^{**}$  and  $p < 0.001^{***}$ .

relations between RV coefficients and the soil age gradient. Correlations between each animal group and environmental (physical, chemical and functional) variables varied along the soil age gradient (Fig. 3). There was no clear pattern for all three groups vs. physical variables, and for enchytraeids and earthworms vs. functional variables. A general decline in correlation was observed from older to younger sites, especially for nematodes vs. chemical or functional variables, for which the highest overall RV scores were recorded at the oldest sites. However in the latter two cases the correlation again increased at the youngest sites. By contrast, RV-coefficients calculated from enchytraeid assemblages and chemical variables tended to increase with soil age.

#### 4. Discussion

##### 4.1. Ecological patterns of soil fauna communities in urban soils of different ages

Soil invertebrates are generally considered as useful tools to estimate the degree to which soils have been affected by human activities [81–83]. Our general goal was to study the diversity and community structure of earthworms, enchytraeids and nematodes as well as their relationships to environmental factors as a first step towards assessing their potential as bioindicators of urban soil quality and functioning.

The patterns of earthworms, enchytraeids and nematodes observed in urban soils partly matched the soil age gradient. Earthworm communities were most correlated with soil bulk density and with soil depth, the latter being positively correlated with soil age. Our results are in line with previous studies in alluvial soils [59,84] showing that earthworm community composition was most strongly correlated with soil depth, mainly because of the low aptitude of anecics to live in shallow soils [56,59,60]. Soil bulk density was also considered as one of the main factors of earthworm distribution in urban and agro-ecosystems (i.e. compacted soils) [36,85]. However, earthworm density, diversity and community structure were often reported to be correlated either with soil texture or with organic matter content in natural and agro-ecosystems [86–89]. The fact that we did not observe such a pattern - except for the correlation between the soil texture and soil age (Table 2) - suggests that this relationship was hidden by other (unmeasured) factors, such as soil compaction [34] or

contamination [90].

We showed that enchytraeid community patterns were significantly correlated with soil carbonate content, while nematode community patterns were significantly correlated with soil water content. No relation was found between these two physicochemical variables and soil age (Table 2). This suggests that enchytraeid and nematode community patterns are not correlated with the soil age gradient but may instead be more influenced by soil management such as irrigation [91,92], organic matter, nitrogen or carbonate inputs [10,40,92–94]. By contrast, the proportion of enchytraeid *r*-strategists, which indicates unstable soil conditions, was correlated negatively with soil age and positively with the coarse fraction and sand content, the latter being negatively correlated with soil age. These results, including the variations of *r*-strategist (*c*-p 1) proportions and nematode maturity index among sites, agree with the idea that land use (forests, lawns or meadows) and soil management can modify enchytraeid and nematode community composition.

##### 4.2. Annelid and nematode assemblages' relationships and their ecological roles

A high diversity of soil fauna is generally expected to increase soil functional diversity, resilience and stability [95,96]. In the urban context, functional diversity can be expected to increase with soil age [36,97]. However this relationship also depends on the identity of the species [98] and our data illustrate this well. Species richness of enchytraeids and nematodes indeed tended to increase with soil age but this trend was not observed for earthworms. Similar earthworm species richness was found in young (1995RP and 2010PR) and old (18thPD and 20thFS) soils. However, densities of epigeic, endogeic and anecic earthworms varied among sites and this can indicate differences in terms of soil functioning as observed for other taxa such as collembolans [28,32]. For example, high density of epigeic and low density of anecic earthworms were found at sites 18thPD and 2010PR, while the opposite was observed at sites 2005RU and 2005PB (Table 5). This indicates differences in terms of soil functioning as epigeics are mainly involved in litter comminution and early decomposition (pioneer species) whereas anecics are the main actors of soil aggregation and soil organic matter integration [56,60].

Enchytraeids are decomposers of organic matter in the topsoil

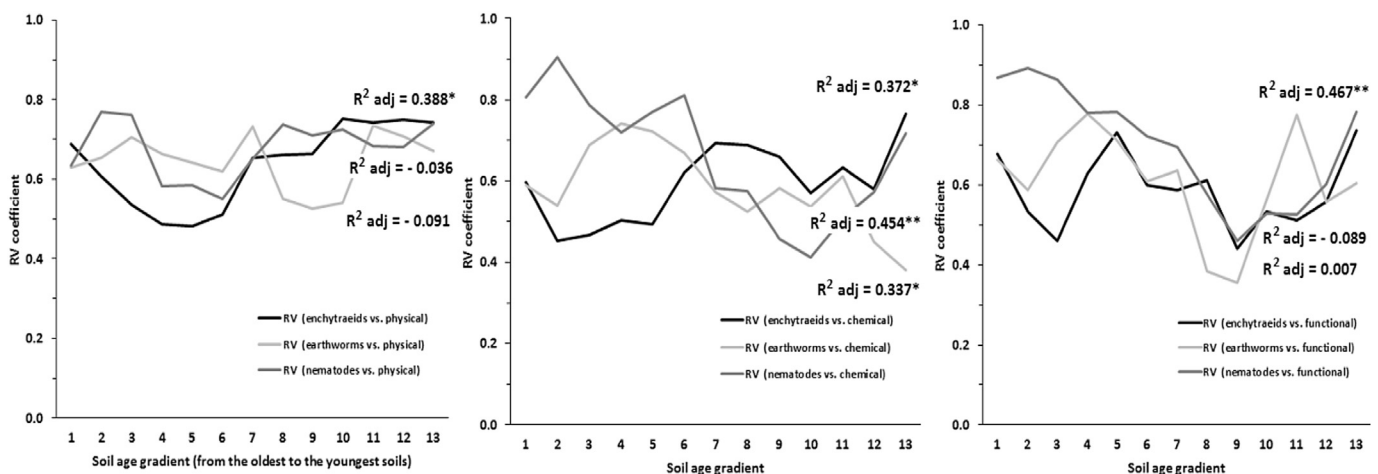


Fig. 3. Moving window analysis of the correlations (RV scores from Multiple Factor Analyses) among soil physical, chemical and functional variables, enchytraeid, earthworm and nematode community patterns from urban soils in Neuchâtel, Switzerland, in relation to soil age (window width = 6 sites; 1 = oldest sites). Significances of linear regression tests are  $p < 0.05^*$ ,  $p < 0.01^{**}$  and  $p < 0.001^{***}$ .

[94]. Our data suggest that the proportion of enchytraeid *r*-strategists [8] may be an indicator of soil age. Highest percentages were recorded in younger sites and lower percentages were observed in the oldest. Schlaghamerský and Pižl [37], found higher percentage of *Buchholzia* and *Enchytraeus* (mostly *r*-strategist species) in highly perturbed urban soils. Thus the proportion of *r*-strategists could also indicate the level of soil disturbance in urban soils.

The increasing correlation between nematode community patterns and chemical and functional variables along the soil age sequence, and the correlations between nematode assemblages, SI and EI indices and soil water content, are in line with the idea that nematodes are indicators of soil conditions and functioning [20]. However, the food web analysis showed high values of SI and EI in most sites (upper right quadrant, Fig. 1) indicating light to moderate soil disturbance and the stability of nematode community structure [20]. Soil moisture was correlated with soil organic matter content ( $r = 0.753$ ) and therefore enrichment, which suggests that *r*-strategists - mainly bacterial feeders such as *Rhabditis* - were probably favoured and decreased nematode evenness and diversity (Supplementary table 4) in fertilised urban soils.

Knowledge about the relationships among earthworms, enchytraeids and nematodes remains limited, especially in the urban context where more is known about each individual group, and bioindication tools for assessing soil quality are still being developed [29,99,100]. The effect that each of these groups has on the others or on interactions with other groups such as collembolans has been studied in forest and agricultural soils, especially showing effects of earthworms on smaller soil organisms [40,41,101–104]. In our study, no significant correlation was found between earthworms and enchytraeid and/or nematode assemblages, suggesting that these three groups represent potential complementary indicators of soil conditions and functioning in urban soils.

## 5. Conclusion

With the ever-increasing spread of urban areas and the general intensive use of soils, soil quality assessment has been identified as a priority for policy-making and ecosystem management in Switzerland and elsewhere [105]. In the urban context, the comparative analysis of earthworm, enchytraeid and nematode diversity metrics and community structure and their relationships with soil age and physicochemical and functional characteristics of soils revealed contrasting patterns among groups and in relation to soil age. The three groups therefore provide complementary information on soil properties and functioning. This study is a first step towards the potential development of usable bioindication tools. To reach this longer-term goal, more comparative observational studies are needed, ideally across longer ecological gradients, as well as experimental studies to further explore the relationships among these faunal groups and how they respond to the different ecological gradients, stress and perturbation (e.g. drought, eutrophication) that characterise the urban environment. It would also be desirable to include other soil fauna groups such as microarthropods in future studies.

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## Appendix A. Supplementary tables

Supplementary table related to this article can be found at <http://dx.doi.org/10.1016/j.ejsobi.2016.01.004>.

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