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10 **Patterns of earthworm, enchytraeid and nematode diversity and**  
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12

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28

29

30 **Abstract**

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

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

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

52 **Keywords:** soil fauna, community ecology, biodiversity, soil ecology, urban ecology, bioindication

## 53 **Introduction**

54 Urban soils support mainly parks and gardens and contribute to local climate regulation, organic  
55 matter decomposition and primary production [1, 2]. These processes are all controlled by soil  
56 organisms, for which soils have a habitat function [3, 4]. Soil fauna communities are useful indicators  
57 of changes in soil state or functioning [5-10]. However, they are still poorly studied in the urban  
58 context as compared to natural and agro-ecosystems and comparative studies of different groups are  
59 lacking. Our focus here is on the comparison of patterns of earthworm, enchytraeid and nematode  
60 diversity and community structure along a soil age gradient. Our aim was to assess to what extent  
61 these three contrasted groups of functionally important soil organisms could be used as indicators of  
62 soil ecological conditions in the urban context.

63         As soil engineers, earthworms modify environmental conditions for other organisms through  
64 their bioturbation activity [3, 11]. They contribute to creating and maintaining the structure of soils  
65 by building pore networks and enhancing soil aggregation by mixing mineral and organic particle in  
66 their digestive tract [12-16]. Enchytraeids are commonly found in almost all soil types [17]. They are  
67 one of the most abundant groups of soil mesofauna in temperate soils [3, 17]. Enchytraeids  
68 contribute significantly to litter fragmentation and organic matter decomposition [18]. They are also  
69 efficient at aerating the soil in the top centimetres [17-19]. Nematodes live in most terrestrial  
70 habitats that provide available organic carbon sources [6]. They belong to the microfauna (< 0.2 mm  
71 in body diameter) and densities often reach millions of individuals per m<sup>2</sup> [3]. Nematodes are key  
72 components of soil food webs due to their various feeding habits (e.g. bacterivores, fungivores,  
73 herbivores and predators) and as food resources for other organisms [6, 20]. Nematodes play various  
74 roles in the soil, especially regulation of microbial biomass and nutrient cycling [9, 21]. Their  
75 community composition and life history indices are indicators of environmental disturbance [6, 9,  
76 22]. The patterns of diversity and community structure of earthworms, enchytraeids and nematodes  
77 have been studied in natural and agro-ecosystems [23, 24]. Earthworms, collembolans, nematodes  
78 and enchytraeids are amongst the most studied taxa in urban soils. They were studied for different

79 purposes such as the effects of soil contaminants [25-29], land use or management [30-38] on soil  
80 fauna. However, the relationships among these groups remain poorly explored [39-41] and to our  
81 knowledge earthworms, enchytraeids and nematodes were never studied together in the urban  
82 context.

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

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

99

## 100 **Material and methods**

### 101 *Study sites*

102 The study was carried out in and around Neuchâtel, a thousand year old city in Switzerland (46° 59'  
103 51'' N; 6° 55' 86'' E). Based on well-known periods of development of the city on surrounding

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

110

### 111 *Soil analyses*

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

124

### 125 *Soil annelids and nematodes*

126 Annelids and nematodes were extracted from sites directly adjacent to the described soil profiles.  
127 Earthworms were collected from eight and ten sites in October 2011 and in October 2012,  
128 respectively. First, Lumbricidae were sampled using the hot mustard (2%) extraction method [55] in

129 four squares of 0.25 m<sup>2</sup> surface (0.5 x 0.5 m) per site. A block of soil (20 x 20 x 20 cm, 8 000 cm<sup>3</sup>) was  
130 then extracted in the same square in order to take into account the last individuals stuck in the roots.  
131 The combination of these two methods allowed us to estimate more precisely the density and the  
132 community patterns of earthworms. Earthworm numbers from the mustard extraction and the block  
133 of soil were multiplied by 4 and 25 respectively and expressed as density (ind.m<sup>-2</sup>). For each site,  
134 mean densities of earthworm species were calculated from the four samples. Earthworms were  
135 stored in formaldehyde (4% solution). They were identified at the species level [56-58] and counted.  
136 Juveniles were identified at the species level according to morphological characters as for adults. In  
137 cases where species-level identification was impossible (i.e. discrimination between pairs of species:  
138 *Octolasion tyrtaeum* and *O. cyaneum* and between *Lumbricus rubellus* and *L. castaneus*), individuals  
139 were allocated to species level using a pro rata distribution corresponding to adult and sub-adult  
140 proportions [59]. The species were classified according to three ecological categories (epigeic,  
141 endogeic and anecic) as defined by Bouché [60]; intermediate categories such as epi-anecics  
142 (*Lumbricus terrestris* Linnaeus, 1758) were grouped to the general category that best reflects the  
143 behaviour of the worm (for *L. terrestris*, anecic instead of epi-anecic).

144 Enchytraeids were collected twice, in autumn and in spring (October 2011 and March 2012 or  
145 October 2012 and March 2013). In each period, five soil samples were taken at each site with a split  
146 soil corer (diameter of 5.5 cm) to 10 cm depth. Each sample was transferred separately into a plastic  
147 bag in the field and stored at 4 °C. Soil samples were then vertically divided in two equal parts: one  
148 part was used for soil water content measurement (oven-dried for 24 h at 105 °C) and the other part  
149 was used for enchytraeid extraction [61]. Enchytraeids were extracted using wet funnel extractors  
150 under light from incandescent light bulbs. Soil samples were heated up from 17 °C to 43 °C on their  
151 upper surface for three hours [62, 63]. Living individuals were kept in Petri dishes with tap water,  
152 counted and identified [64] under a light microscope (up to 400x magnification). For each site, the  
153 density (ind.m<sup>-2</sup>), the community patterns and the proportion of *r*-strategy type [8] of enchytraeids  
154 were calculated from the mean of both sampling periods.

155 Samples for nematodes were collected in October 2012. Five soil samples were taken for  
156 each site with a split soil corer (diameter of 4 cm) to 10 cm depth. Soil samples were then pooled and  
157 sieved at 5 mm in order to remove the coarse fraction and roots before nematode extraction and to  
158 maximize the representation of all genera [65, 66]. Nematodes were extracted from 200 g of soil  
159 using a modified Bearmann extraction method for 48 h [67]. They were then stored in a mixed  
160 solution of TriethanolAmine-Formalin (TAF) containing 2 ml of triethanolamine, 7 ml of formalin (40%  
161 formaldehyde solution) and 91 ml of deionized water. For each site, one hundred nematodes were  
162 sampled randomly and identified under a light microscope (up to 400x magnification) [68].  
163 Individuals were identified at genus level except for two families, *Criconeematidae* and  
164 *Diplogasteridae*, which were identified at the family level. The maturity index ( $MI_{1-5}$ ), enrichment  
165 index (EI) and structure index (SI) [20], were calculated from the proportion of each trophic group  
166 [69] and the life strategy of each family [70] using the NINJA software [71]. The maturity index is  
167 based on the proportion of colonizers and persisters (c-p) with lower values being indicative of  
168 disturbed soils [72]. The EI is calculated from the proportion of opportunistic bacterivores and  
169 fungivores. The SI derives from the proportion of carnivores and omnivores. Higher EI and SI values  
170 indicate, respectively, organic enrichment and soil food web complexity (interpreted as light to  
171 moderate disturbance or stress) [20].

172

### 173 *Numerical analyses*

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

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

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

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

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

196

## 197 **Results**

### 198 *Site and soil characteristics*

199 Three main soil types were found (Table 1). The oldest site, REFUFP, was a natural soil (classified as a  
200 Calcisol) located in an oak forest and sites 18thPD (lawn), 19thGR (lawn), 19thTU (meadow), and  
201 20thFS (oak and maple forest) were near natural soils (assigned to Cambisols). Other soils located in  
202 lawns and meadows were strongly modified by human activities and were described as Anthrosols

203 (19thJA, 19thTC, 20thER, 1930VL, 1933PL, 1963WS, 1995RP, 2005RU, 2005PB, 2010PR, and 2010VM)  
204 and Technosols (1970JR and 1995HR) [47].

205 The six oldest sites REFUFP, 18thPD, 19thGR, 19thJA, 19thTU, and 19thTC were up to 140  
206 years old, while the six youngest sites 1995RP, 1995HR, 2005RU, 2005PB, 2010PR, and 2010VM were  
207 less than 18 years old. Soil age was positively correlated with soil depth and was negatively  
208 correlated with sand content and the proportion of coarse fraction (Table 2). Soil depth was often  
209 higher in native soils compared to man-made soils (Tables 1 and 3). The characteristics of topsoils  
210 were most contrasted among sites for CaCO<sub>3</sub>, clay, phosphorous, and fungal biomass as assessed by  
211 ergosterol content (Table 3). Physical variables were correlated with functional variables (Mantel  
212 test,  $r = 0.475$ ,  $p = 0.002$ ) while chemical variables were neither correlated with physical nor with  
213 functional variables.

214

### 215 *Earthworms*

216 We identified 16 earthworm species at the 18 sites (average = 4.9 per site). Highest species richness  
217 was recorded at the old sites 18thPD and 20thFS and at the young sites 1995RP and 2010PR (7  
218 species) (Table 4). Hill's numbers and evenness ranged from 0.18 (site 20thFS) to 0.97 (1933PL), and  
219 0.1 (1933PL) to 1 (1930VL), respectively. Earthworm density ranged from 27 ind.m<sup>-2</sup> (site 1930VL) to  
220 553 ind.m<sup>-2</sup> (site REFUFP) and reached on average 220 ind.m<sup>-2</sup> (Table 5). Density and species richness  
221 were positively correlated ( $r = 0.435$ ) (Supplementary table 1).

222 Community patterns and ecological categories of earthworms differed among sites (Tables 4  
223 and 5). Epigeic earthworms were found at eight sites (REFUFP, 18thPD, 20thFS, 1963WS, 1995RP,  
224 2005RU, 2005PB and 2010PR) with highest densities recorded at the two oldest sites (REFUFP and  
225 18thPD) and at the second youngest site (2010PR) (115-148 ind.m<sup>-2</sup>, Table 5). *Dendrodrius rubidus*  
226 (Savigny, 1926) was only found at the second youngest site (2010PR), *Lumbricus castaneus* (Savigny,  
227 1826) was found only at four sites and, *Lumbricus rubellus* (Hoffmeister, 1843) and *Dendrobaena*

228 *octaedra* (Savigny, 1826) were recorded only at five sites (Table 5). Endogeic earthworms were found  
229 at all sites except at 1930VL. Highest densities were recorded at the two oldest sites (REFUFP and  
230 18thPD), at 20thER and at the youngest site (2010VM) (277-411 ind.m<sup>-2</sup>, Table 4). *Octolasion*  
231 *tyrtaeum tyrtaeum* (Savigny, 1926) was identified at the two oldest sites (REFUFP and 18thPD),  
232 whereas co-dominant endogeic species, *Allolobophora chlorotica* (Savigny, 1826) and *Aporrectodea*  
233 *rosea* (Savigny, 1826), were recorded at most sites (Table 5). Anecic earthworms were identified at all  
234 sites with lowest density found at 1933 PL (1 ind.m<sup>-2</sup>) and highest densities recorded at 19thGR,  
235 19thJA, 1995HR, 2005RU, and 2005PB (131–180 ind.m<sup>-2</sup>, Table 4). *Aporrectodea longa ripicola*  
236 (Bouché, 1972) was only found at three young sites (1995RP, 1995HR and 2005PB), while *Lumbricus*  
237 *terrestris* (Linnaeus, 1758) and *Aporrectodea longa longa* (Ude, 1885) were found at most sites along  
238 the soil age gradient (Table 5).

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

244

#### 245 *Enchytraeids*

246 We identified 34 enchytraeid species at the 18 sites (average = 9.2 per site). Highest and lowest  
247 species richness were recorded at the oldest site REFUFP (15 species) and at 1933PL (4 species)  
248 (Table 6), respectively. Enchytraeid density varied from 2694 ind.m<sup>-2</sup> (1933PL) to 50366 ind.m<sup>-2</sup>  
249 (1963WS) (average = 20131 ind.m<sup>-2</sup>) (Table 5). Hill's numbers and evenness ranged from 0.15  
250 (REFUFP and 19thTC) to 0.78 (1995HR), and from 0.27 (1995HR) to 0.90 (1970JR), respectively.  
251 Density and species richness were positively correlated ( $r = 0.482$ , Supplementary table 1). The

252 proportion of *r*-strategists varied from 11% (1933PL) to 86% (2010PR) with an overall average of 42%  
253 (Table 5).

254 Enchytraeid community structure varied among sites (Table 6). The dominant and  
255 fragmenting *r*-strategist species, *Buchholzia appendiculata* (Buchholz, 1962), was found at all sites  
256 except 1970JR. Higher densities were found at 1930VL, 1963WS, 1995RP, 1995HR, and 2010PR  
257 (13139-18024 ind.m<sup>-2</sup>). By contrast, species of *Fridericia* and *Achaeta* (*K*-strategists) were found at a  
258 limited number (1-11) of sites. For example, *Achaeta bohemica* (Vejdovský, 1879) and *Achaeta*  
259 *unibulba* (Graefe, Dózsa-Farkas & Christensen, 2005) were only recorded at three of the oldest sites  
260 (18thPD, 19thGR and 19thTC), whereas other species, such as *Achaeta eiseni* (Vejdovský, 1878) and  
261 *Achaeta iberica* (Graefe, 1989) – the latter considered rare in Europe [64] – were found at several  
262 sites along the soil age gradient (Table 6).

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

267

## 268 *Nematodes*

269 We identified 43 nematode genera at the 18 sites (average = 15.8 per site) with highest and lowest  
270 genera richness respectively recorded at site 19thTU (22 genera) and at sites 20thER, 1995RP,  
271 1995HR, and 2005RU (12 genera) (Table 7). Hill's numbers ranged from 0.08 (19thTU) to 0.35  
272 (20thFS). SI varied from 33.3 (1933PL) to 84.5 (1930VL) and EI from 46.3 (19thTU) to 95.1 (20thFS)  
273 (Table 5). Almost all sites were positioned in the upper right quadrant of the food web diagnostic  
274 except sites 19thTU, 1933PL, and 1995RP (Figure 1).

275 Nematode community structure varied among sites (Table 7). The dominant genus *Rhabditis*  
276 (bacterivorous with a short life cycle and high reproduction rate, c-p 1) was found at all sites, while

277 other genera were found in few sites, such as *Aporcelaimellus* (predator with a long life cycle and low  
278 reproduction rate, c-p 5), which was only recorded at four of the oldest sites (REFUFP, 18thPD,  
279 19thTU, and 19thTC). When adding the genera *Rhabditis* and *Diplogasteridae*, the proportions of *r*-  
280 strategists (c-p 1) were highest (36-71%) in forest soils (REFUFP and 20thFS) and at sites 1930VL,  
281 1970JR, 1995RP, 2005RU, and 2010PR (lawns and meadows).

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

286

#### 287 *Community patterns and community-environment relationships*

288 The RDAs on environmental (soil age, physical, chemical and functional) variables revealed significant  
289 correlations with each soil fauna group: (1) between earthworms and soil depth, and between  
290 earthworms and soil bulk density (total explained variance = 22.6 %; model p-value = 0.013; AIC = -  
291 12.10;  $r^2_{adj} = 0.123$ ), (2) between enchytraeids and calcium carbonate content (10.2 %; 0.034; -11.21;  
292 0.046), and (3) between nematodes and soil water content (13.5%; 0.010; -16.93; 0.081).  
293 Furthermore, the Mantel tests did not reveal any significant relationship between pairs of soil fauna  
294 groups (earthworms vs enchytraeids, earthworms vs nematodes, and enchytraeids vs nematodes) or  
295 between each individual soil fauna group and either one of the three groups of environmental  
296 (physical, chemical and functional) variables.

297 Correlations between earthworms and enchytraeid species assemblages and between  
298 earthworms and nematodes increased with soil age as shown by the higher RV coefficients in the  
299 moving window MFA at the oldest sites (N° 1 to 7, sites 1-12, Figure 2) and lower values at the  
300 youngest sites (N° 8 to 13, sites 8-18). Conversely, correlations between nematodes and enchytraeid  
301 assemblages decreased with soil age (Figure 2). For each pair of soil fauna assemblages, linear

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

311

## 312 **Discussion**

### 313 *Ecological patterns of soil fauna communities in urban soils of different ages*

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

319         The patterns of earthworms, enchytraeids and nematodes observed in urban soils partly  
320 matched the soil age gradient. Earthworm communities were most correlated with soil bulk density  
321 and with soil depth, the latter being positively correlated with soil age. Our results are in line with  
322 previous studies in alluvial soils [59, 84] showing that earthworm community composition was most  
323 strongly correlated with soil depth, mainly because of the low aptitude of anecics to live in shallow  
324 soils [56, 59, 60]. Soil bulk density was also considered as one of the main factors of earthworm  
325 distribution in urban and agro-ecosystems (i.e. compacted soils) [36, 85]. However, earthworm  
326 density, diversity and community structure were often reported to be correlated either with soil

327 texture or with organic matter content in natural and agro-ecosystems [86-89]. The fact that we did  
328 not observe such a pattern - except for the correlation between the soil texture and soil age (Table 2)  
329 - suggests that this relationship was hidden by other (unmeasured) factors, such as soil compaction  
330 [34] or contamination [90].

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

342

#### 343 *Annelid and nematode assemblages' relationships and their ecological roles*

344 A high diversity of soil fauna is generally expected to increase soil functional diversity, resilience and  
345 stability [95, 96]. In the urban context, functional diversity can be expected to increase with soil age  
346 [36, 97]. However this relationship also depends on the identity of the species [98] and our data  
347 illustrate this well. Species richness of enchytraeids and nematodes indeed tended to increase with  
348 soil age but this trend was not observed for earthworms. Similar earthworm species richness was  
349 found in young (1995RP and 2010PR) and old (18thPD and 20thFS) soils. However, densities of  
350 epigeic, endogeic and anecic earthworms varied among sites and this can indicate differences in  
351 terms of soil functioning as observed for other taxa such as collembolans [28, 32]. For example, high

352 density of epigeic and low density of anecic earthworms were found at sites 18thPD and 2010PR,  
353 while the opposite was observed at sites 2005RU and 2005PB (Table 5). This indicates differences in  
354 terms of soil functioning as epigeics are mainly involved in litter comminution and early  
355 decomposition (pioneer species) whereas anecics are the main actors of soil aggregation and soil  
356 organic matter integration [56, 60].

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

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

372 Knowledge about the relationships among earthworms, enchytraeids and nematodes  
373 remains limited, especially in the urban context where more is known also about each individual  
374 group, and bioindication tools for assessing soil quality are still being developed [29, 99, 100]. The  
375 effect that each of these groups has on the others or on interactions with other groups such as  
376 collembolans has been studied in forest and agricultural soils, especially showing effects of

377 earthworms on smaller soil organisms [40, 41, 101-104]. In our study, no significant correlation was  
378 found between earthworms and enchytraeid and/or nematode assemblages, suggesting that these  
379 three groups represent potential complementary indicators of soil conditions and functioning in  
380 urban soils.

381

## 382 **Conclusion**

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

396

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406

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