8

9

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

12

13 Joël Amossé[1,2,3], Klára Dózsa-Farkas[4], Gergely Boros[5], Guy Rochat[2], Gauthier Sandoz[2], Bertrand

14 Fournier[2,6], Edward A.D. Mitchell[2,7] and Renée-Claire Le Bayon[1]

15 [1] Laboratory of Soil and Vegetation, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel,

16 Switzerland

17 [2] Laboratory of Soil Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel,

18 Switzerland

19 [3] Institute of Earth Surface Dynamics, University of Lausanne, Quartier UNIL-Mouline, Bâtiment

20 Géopolis, 1015 Lausanne, Switzerland

21 [4] Department of Systematic Zoology and Ecology, Eötvös Loránd University, Pázmány Pétersétány

22 1/C, 1117 Budapest, Hungary

23 [5] Institute of Ecology & Botany, Hungarian Academy of Sciences, Alkotmány 2-42163 Vácrátót,

24 Hungary

25 [6] Laboratoire Chrono-Environnement, UMR 6249 CNRS, Université de Bourgogne Franche-Comté, 16

26 route de Gray, 25030 Besançon Cedex, France

27 [7] Botanical Garden of Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000 Neuchâtel, Switzerland

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

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 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^2 [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

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_2O and KCl), particle-size distribution (% clay, % silt, % sand), loss on ignition (%, Allen method),

115 organic carbon (C_{org}, CHN method), total nitrogen (N_{tot}, Kjeldahl method), available phosphorus (P_{bio},

116 Olsen method), total phosphorus (P_{tot}, Kjeldahl method), cation-exchange capacity (CEC,

117 Cobaltihexamine method) and carbonate content ($CaCO_3$, 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® Space, [51, 52]), ergosterol content [53, 54], and soil

122 respiration measured for 20 minutes (soil volume of 85.1 cm^3 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^2 surface (0.5 x 0.5 m) per site. A block of soil (20 x 20 x 20 cm, 8 000 cm^3) 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^{-2}). 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^{-2}), 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, *Criconematidae* 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 $CaCO_3$, 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^{-2} (site 1930VL) to

220 553 ind.m^{-2} (site REFUFP) and reached on average 220 ind.m^{-2} (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^{-2}, Table 5). *Dendrodrilus 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^{-2}, 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^{-2}) and highest densities recorded at 19thGR,

235   19thJA, 1995HR, 2005RU, and 2005PB (131–180 ind.m^{-2}, 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_{H2O} (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^{-2} (1933PL) to 50366 ind.m^{-2}

249   (1963WS) (average = 20131 ind.m^{-2}) (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^{-2}). 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_{org} (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

References

408 [1] B.G. Rawlins, J. Harris, S. Price, M. Bartlett, A review of climate change impacts on urban soil
409 functions with examples and policy insights from England, Soil Use Manage., (2013)
410 doi: 10.1111/sum.12079.
411 [2] A. Lehmann, K. Stahr, The potential of soil functions and planner-oriented soil evaluation to
412 achieve sustainable land use, J. Soils Sediments, 10 (2010) 1092-1102.
413 [3] J.-M. Gobat, M. Aragno, W. Matthey, The Living Soil, Fundamentals of Soil Science and Soil
414 Biology, Science Publishers, Enfield, NH, 2004.
415 [4] P. Lavelle, T. Decaens, M. Aubert, S. Barot, M. Blouin, F. Bureau, P. Margerie, P. Mora, J.P. Rossi,
416 Soil invertebrates and ecosystem services, Eur. J. Soil Biol., 42 (2006) S3-S15.
417 [5] P.J. Bohlen, C.A. Edwards, Biology and Ecology of Earthworms, Third ed., Chapman & Hall, London
418 etc., 1996.
419 [6] T. Bongers, H. Ferris, Nematode community structure as a bioindicator in environmental
420 monitoring, Trends Ecol. Evol., 14 (1999) 224-228.
421 [7] M.G. Paoletti, The role of earthworms for assessment of sustainability and as bioindicators, Agric.
422 Ecosyst. Environ., 74 (1999) 137-155.
423 [8] U. Graefe, R.M. Schmelz, Indicator values, strategy types and life forms of terrestrial
424 Enchytraeidae and other microannelids, 3th International Symposium on Enchytraeidae, R. Schmelz
425 and K. Sühlo (eds.), Universitätsverlag Rasch, Osnabrück, Newsletter on Enchytraeidae, 6 (1999) 59-
426 67.
427 [9] H. Ferris, T. Bongers, Nematode indicators of organic enrichment, J. Nematol., 38 (2006) 3-12.
428 [10] J. Römbke, S. Jänch, H. Höfer, F. Horak, M. Ross-Nickoll, D. Russell, A. Toschki, State of
429 knowledge of enchytraeid communities in German soils as a basis for biological soil quality
430 assessment, Soil Organisms, 85 (2) (2013): 123-146.
431 [11] C.G. Jones, J.H. Lawton, M. Shachak, Organisms as ecosystem engineers, Oikos, 69 (1994) 373-
432 386.
433 [12] P. Lavelle, A.V. Spain, Soil Ecology, Kluwer Academic Publishers, Dordrecht, 2001.
434 [13] C.A. Edwards, Earthworm Ecology, 2nd ed., CRC, Boca Raton, Fla., 2004.
435 [14] P. Jouquet, J. Dauber, J. Lagerlof, P. Lavelle, M. Lepage, Soil invertebrates as ecosystem
436 engineers: Intended and accidental effects on soil and feedback loops, Appl. Soil Ecol., 32 (2006) 153-
437 164.
438 [15] G. Bullinger-Weber, R.-C. Le Bayon, C. Guenat, J.-M. Gobat, Influence of some physicochemical
439 and biological parameters on soil structure formation in alluvial soils, Eur. J. Soil Biol., 43 (2007) 57-
440 70.
441 [16] R. Milleret, R.C. Le Bayon, J.M. Gobat, Root, mycorrhiza and earthworm interactions: their
442 effects on soil structuring processes, plant and soil nutrient concentration and plant biomass, Plant
443 Soil, 316 (2009) 1-12.
444 [17] W.A.M. Didden, H.C. Fründ, U. Graefe, Fauna in Soil Ecosystems, Marcel Dekker Verlag, New
445 York, 1997.

446 [18] L. Cole, R.D. Bardgett, P. Ineson, P.J. Hobbs, Enchytraeid worm (Oligochaeta) influences on
447 microbial community structure, nutrient dynamics and plant growth in blanket peat subjected to
448 warming, Soil Biol. Biochem., 34 (2002) 83-92.
449 [19] S. Topoliantz, J.F. Ponge, P. Viaux, Earthworm and enchytraeid activity under different arable
450 farming systems, as exemplified by biogenic structures, Plant Soil, 225 (2000) 39-51.
451 [20] H. Ferris, T. Bongers, R.G.M. de Goede, A framework for soil food web diagnostics: extension of
452 the nematode faunal analysis concept, Appl. Soil Ecol., 18 (2001) 13-29.
453 [21] C. Villenave, K. Ekschmitt, S. Nazaret, T. Bongers, Interactions between nematodes and microbial
454 communities in a tropical soil following manipulation of the soil food web, Soil Biol. Biochem., 36
455 (2004) 2033-2043.
456 [22] M. Liu, X. Chen, B.S. Griffiths, Q. Huang, H. Li, F. Hu, Dynamics of nematode assemblages and soil
457 function in adjacent restored and degraded soils following disturbance, Eur. J. Soil Biol., 49 (2012) 37-
458 46.
459 [23] C. van Capelle, S. Schrader, J. Brunotte, Tillage-induced changes in the functional diversity of soil
460 biota - A review with a focus on German data, Eur. J. Soil Biol., 50 (2012) 165-181.
461 [24] M.B. Postma-Blaauw, R.G.M. de Goede, J. Bloem, J.H. Faber, L. Brussaard, Agricultural
462 intensification and de-intensification differentially affect taxonomic diversity of predatory mites,
463 earthworms, enchytraeids, nematodes and bacteria, Appl. Soil Ecol., 57 (2012) 39-49.
464 [25] J. Cortet, A. De Vaufleury, N. Poinsotbalaguer, L. Gomot, C. Texier, D. Cluzeau, The use of
465 invertebrate soil fauna in monitoring pollutant effects, Eur. J. Soil Biol., 35 (1999) 115-134.
466 [26] K. Lock, C.R. Janssen, Comparative toxicity of a zinc salt, zinc powder and zinc oxide to *Eisenia*
467 *fetida*, *Enchytraeus albidus* and *Folsomia candida*. Chemosphere, 53 (2003) 851-856.
468 [27] C. Fiera, Biodiversity of Collembola in urban soils and their use as bioindicators for pollution,
469 Pesquisa Agropecuaria Brasileira, 44 (2009) 868-873.
470 [28] L. Santorufo, C.A.M. Van Gestel, A. Rocco, G. Maisto, Soil invertebrates as bioindicators of urban
471 soil quality, Environ. Pollut., 161 (2012) 57-63.
472 [29] P. Salamun, M. Renco, E. Kucanova, T. Brazova, I. Papajova, D. Miklisova, V. Hanzelova,
473 Nematodes as bioindicators of soil degradation due to heavy metals, Ecotoxicology, 21 (2012) 2319-
474 2330.
475 [30] W. Schulte, H.-Ch. Fründ, M. Söntgen, U. Graefe, B. Ruszkowski, V. Voggenreiter, N. Weritz, Zur
476 Biologie Städtischer Böden, Beispielraum Bonn-Bad Godesberg. Schriftenreihe für Landschaftspflege
477 und Naturschutz, Bundesforschungsanstalt für Naturschutz und Landschaftsökologie, KILDA-Verlag F.
478 Peking, Greven, 1989.
479 [31] D.A. Steinberg, R. V. Pouyat, R. W. Parmelee, P. M. Groffman, Earthworm abundance and
480 nitrogen mineralization rates along an urban-rural land use gradient, Soil Biol. Biochem., 29 (1997)
481 427–430.
482 [32] N.E. McIntyre, J. Rango, W.F. Fagan, S.H. Faeth, Ground arthropod communities structure in a
483 heterogeneous urban environment, Landsc. Urban Plan., 52(2001) 257-274.
484 [33] I. Eitminaviciute, Microarthropod communities in anthropogenic urban soils, 1. Structure of
485 microarthropod complexes in soils of roadside lawns, Entomol. Rev., 86 (2006) S128-S135.
486 [34] V. Pižl, J. Schlaghamerský, The impact of pedestrian activity on soil annelids in urban greens, Eur.
487 J. Soil Biol., 43 (2007) S68-S71.
488 [35] J. Schlaghamerský, A. Šídová, V. Pižl, From mowing to grazing: Does the change in grassland
489 management affect soil annelid assemblages? Eur. J. Soil Biol., 43 (2007) 72–78.
490 [36] K.M. Smetak, J.L. Johnson-Maynard, J.E. Lloyd, Earthworm population density and diversity in
491 different-aged urban systems, Appl. Soil Ecol., 37 (2007) 161-168.
492 [37] J. Schlaghamerský, V. Pižl, Enchytraeids and earthworms (Annelida : Clitellata : Enchytraeidae,
493 Lumbricidae) of parks in the city of Brno, Czech Republic, Soil Organisms, 81 (2) (2009) 145-173.
494 [38] J. Schlaghamerský, Short note on enchytraeid occurrence in deep layers of urban soils, Soil
495 Organisms, 87 (2) (2015) 85-89.
496 [39] R. Hyvonen, S. Andersson, M. Clarholm, T. Persson, Effects of lumbricids and enchytraeids on
497 nematodes in limed and unlimed coniferous mor humus, Biol. Fert. Soils, 17 (1994) 201-205.

498 [40] M. Raty, V. Huhta, Earthworms and pH affect communities of nematodes and enchytraeids in
499 forest soil, Biol. Fert. Soils, 38 (2003) 52-58.

500 [41] J. Tao, Y. Xu, B.S. Griffiths, F. Hu, X. Chen, J. Jiao, H. Li, Earthworms reduce the abundance of
501 nematodes and enchytraeids in a soil mesocosm experiment despite abundant food resources, Soil
502 Sci. Soc. Am. J., 75 (2011) 1774-1778.

503 [42] C.R. De Kimpe, J.L. Morel, Urban soil management: A growing concern, Soil Sci., 165 (2000) 31-
504 40.

505 [43] A. Lehmann, K. Stahr, Nature and significance of anthropogenic urban soils, J. Soils Sediments, 7
506 (2007) 247-260.

507 [44] M.L. McKinney, Urbanization, biodiversity, and conservation, BioScience, 52 (2002) 883-890.

508 [45] P. Sikorski, I. Szumacher, D. Sikorska, M. Kozak, M. Wierzba, Effects of visitor pressure on
509 understory vegetation in Warsaw forested parks (Poland), Environ. Monit. Assess., 185 (2013) 5823-
510 5836.

511 [46] O. Roithmeier, S. Pieper, Influence of Enchytraeidae (*Enchytraeus albidus*) and compaction on
512 nutrient mobilization in an urban soil, Pedobiologia, 53 (2009) 29-40.

513 [47] Food and Agriculture Organization of the United Nations FAO, World Reference Base for Soil
514 Resources 2006, A Framework for International Classification, 2006 edition ed., Rome, 2006.

515 [48] M. Vatan, Manuel de Sédimentologie, Technip, Paris, 1967.

516 [49] M.R. Carter, E.G. Gregorich, Soil Sampling and Methods of Analysis, Second Edition, Boca Raton,
517 2007.

518 [50] J. Schnurer, T. Rosswall, Fluorescein diacetate hydrolysis ass a measure of total microbial activity
519 in soil and litter, Appl. Environ. Microb., 43 (1982) 1256-1261.

520 [51] K.G. Porter, Y.S. Feig, The use of DAPI for identifying and counting aquatic microflora, Limnol.
521 Oceanogr., 25 (1980) 943-948.

522 [52] H. Christensen, L.K. Poulsen, Detection of *Pseudomonas* in soil by rRNA targeted in-situ
523 hybridization technique, Soil Biol. Biochem., 26 (1994) 1093-1096.

524 [53] J.C. Young, Microwave-assisted extraction of the fungal metabolite ergosterol and total fatty-
525 acids, J. Agri. Food Chem., 43 (1995) 2904-2910.

526 [54] T. Larsen, J. Axelsen, H.W. Ravn, Simplified and rapid method for extraction of ergosterol from
527 natural samples and detection with quantitative and semi-quantitative methods using thin-layer
528 chromatography, J. Chromatogr. A, 1026 (2004) 301-304.

529 [55] A.P. Lawrence, M.A. Bowers, A test of the 'hot' mustard extraction method of sampling
530 earthworms, Soil Biol. Biochem., 34 (2002) 549-552.

531 [56] M.B. Bouché, Lombriciens de France, Ecologie et Systématique, Annales de Zoologie Ecologie
532 Animale, INRA Editions, Paris, 1972.

533 [57] R.W. Sims, B.M. Gerard, Earthworms: Notes for the Identification of British Species, Revised ed.,
534 Field Studies Council, Shrewsbury, 1999.

535 [58] R.J. Blakemore, An Updated List of Valid, Invalid and Synonym Names of Criodrioidea
536 (Criodrilidae) and Lumbricoidea (Annelida: Oligochaeta: Sparganophilidae, Ailoscolecidae,
537 Hormogastridae, Lumbricidae, and Lutodrilidae), Yokohama National University, 2008.

538 [59] C. Salomé, C. Guenat, G. Bullinger-Weber, J.-M. Gobat, R.-C. Le Bayon, Earthworm communities
539 in alluvial forests: Influence of altitude, vegetation stages and soil parameters, Pedobiologia, 54
540 (2011) S89-S98.

541 [60] M.B. Bouché, Stratégies lombriciennes, Soil Organisms as Components of Ecosystems, U. Lohm
542 and T. Persson (eds.), Stockholm, 1977.

543 [61] M. Prendergast-Miller, V. Standen, I.D. Leith, L.J. Sheppard, Response of enchytraeid worm
544 populations to different forms of nitrogen (ammonia, ammonium, and nitrate), Soil Organisms, 81
545 (2) (2009): 225-236.

546 [62] F.B. O'Connor, Extraction of enchytraeid worms from a coniferous forest soil, Nature, 175 (1955)
547 815-816.

548 [63] K. Kobetičová, J. Schlaghamerský, On the efficiency of three schemes of enchytraeid wet funnel
549 extraction, 5th International Symposium on Enchytraeidae, Newsletter on Enchytraeidae, 8 (2003) 25-
550 31.
551 [64] R.M. Schmelz, R. Collado, A guide to European terrestrial and freshwater species of
552 Enchytraeidae (Oligochaeta), Soil Organisms, 82 (1) (2010) 1-176.
553 [65] A. Treonis, K. Sutton, B. Kavanaugh, A. Narla, T. McLlarky, J. Felder, C. O'Leary, M. Riley, A. Pikus,
554 S. Thomas, Soil nematodes and their prokaryotic prey along an elevation gradient in the Mojave
555 desert (Death valley national park, California, USA), Diversity, 4 (2012) 363-374.
556 [66] C. Villenave, A. Jimenez, M. Guernion, G. Pérès, D. Cluzeau, T. Mateille, B. Martiny, M. Fargette,
557 J. Tavoillot, Nematodes for soil quality monitoring: results from the RMQS BioDiv programme, Open
558 J. Soil Sci., 3 (2013) 30-45.
559 [67] D.J.F. Brown, B. Boag, An examination of methods used to extract virus-vector nematodes
560 (Nematoda: Longidoridae and Trichodoridae) from soil samples, Nematol. Mediterr., 16 (1988) 93-99.
561 [68] T. Bongers, De Nematoden van Nederland: Een Identificatietabel voor de in Nederland
562 Aangetroffen Zoetwater - en Bodembewonende Nematoden, 2nd ed., Stichting Uitg. KNNV, Utrecht,
563 1994.
564 [69] G.W. Yeates, T. Bongers, R.G.M. Degoede, D.W. Freckman, S.S. Georgieva, Feeding-habits in soil
565 nematode families and genera - an outline for soil ecologists, J. Nematol., 25 (1993) 315-331.
566 [70] T. Bongers, M. Bongers, Functional diversity of nematodes, Appl. Soil Ecol., 10 (1998) 239-251.
567 [71] B. Sieriebriennikov, H. Ferris, R.G.M. de Goede, NINJA: An automated calculation system for
568 nematode-based biological monitoring, Eur. J. Soil Biol., 61 (2014) 90-93.
569 [72] T. Bongers, The maturity index: an ecological measure of environmental disturbance based on
570 nematode species composition, Oecologia, 83 (1990) 14-19.
571 [73] J.M. Bland, D.G. Altman, Multiple significance tests - the Bonferroni method, Brit Med J., 310
572 (1995) 170-170.
573 [74] N. Mantel, Detection of disease clustering and a generalized regression approach, Cancer Res.,
574 27 (1967) 209-220.
575 [75] P. Legendre, E.D. Gallagher, Ecologically meaningful transformations for ordination of species
576 data, Oecologia, 129 (2001) 271-280.
577 [76] P. Robert, Y. Escofier, Unifying tool for linear multivariate statistical methods- RV-coefficient, J.
578 R. Stat. Soc. Ser. C Appl. Stat., 25 (1976) 257–265.
579 [77] M.L. Carlson, L.A. Flagstad, F. Gillet, E.A.D. Mitchell, Community development along a proglacial
580 chronosequence: are above-ground and below-ground community structure controlled more by
581 biotic than abiotic factors?, J. Ecol., 98 (2010) 1084-1095.
582 [78] R Development Core Team, R: A language and environment for statistical computing. R
583 Foundation for Statistical Computing, Vienna, Austria, 2015.
584 [79] J. Oksanen, F.G. Blanchet, R. Kindt, P. Legendre, R.G. O'Hara, G.L. Simpson, P. Solymos, M.H.H.
585 Stevens, H. Wagner, vegan: Community Ecology Package, R package version 2.3-0, 2015.
586 [80] S. Le, J. Josse, F. Husson, FactoMineR: An R package for multivariate analysis, J. Stat. Softw., 25
587 (2008) 1-18.
588 [81] P. Andres, E. Mateos, Soil mesofaunal responses to post-mining restoration treatments, Appl.
589 Soil Ecol., 33 (2006) 67-78.
590 [82] B.S. Griffiths, L. Philippot, Insights into the resistance and resilience of the soil microbial
591 community, FEMS. Microb. Rev., 37 (2013) 112-129.
592 [83] I.D. Hodkinson, Terrestrial and aquatic invertebrates as bioindicators for environmental
593 monitoring, with particular reference to mountain ecosystems, Environ. Manage., 35 (2005) 649-666.
594 [84] B. Fournier, E. Samaritani, J. Shrestha, E.A.D. Mitchell, R.-C. Le Bayon, Patterns of earthworm
595 communities and species traits in relation to the perturbation gradient of a restored floodplain, Appl.
596 Soil Ecol., 59 (2012) 87-95.
597 [85] K.Y. Chan, I.M. Barchia, Soil compaction controls the abundance, biomass and distribution of
598 earthworms in a single dairy farm in south-eastern Australia, Soil Till. Res. , 94 (2007) 75-82.

599 [86] E. Lapied, J. Nahmani, G.X. Rousseau, Influence of texture and amendments on soil properties
600 and earthworm communities, Appl. Soil Ecol., 43 (2009) 241-249.
601 [87] D.H. Wall, R.D. Bardgett, V. Behan-Pelletier, J.E. Herrick, H.T. Jones, K. Ritz, J. Six, D.R. Strong,
602 W.H. van der Putten, Soil Ecology and Ecosystem Services, Oxford University Press, 2012.
603 [88] E. Huerta, D. Brunet, E. Velazquez, P. Lavelle, Identifying earthworm's organic matter signatures
604 by near infrared spectroscopy in different land-use systems in Tabasco, Mexico, Appl. Soil Ecol., 69
605 (2013) 49-55.
606 [89] P.S. Chaudhuri, S. Bhattacharjee, A. Dey, S. Chattopadhyay, D. Bhattacharya, Impact of age of
607 rubber (*Hevea brasiliensis*) plantation on earthworm communities of West Tripura (India), J. Environ.
608 Biol., 34 (2013) 59-65.
609 [90] J. Nahmani, M.E. Hodson, S. Black, Effects of metals on life cycle parameters on the earthworm
610 Eisenia fetida exposed to fiels-contaminated, metal-polluted soils. Environ. Pollut., 149 (2007) 44-59.
611 [91] G.W. Yeates, T. Bongers, Nematode diversity in agroecosystems, Agric. Ecosyst. Environ., 74
612 (1999) 113-135.
613 [92] W.-B. Ruan, Y. Sang, Q. Chen, X. Zhu, S. Lin, Y.-B. Gao, The response of soil nematode community
614 to nitrogen, water, and grazing history in the inner mongolian steppe, China, Ecosystems, 15 (2012)
615 1121-1133.
616 [93] C.M. Ugarte, E.R. Zaborski, M.M. Wander, Nematode indicators as integrative measures of soil
617 condition in organic cropping systems, Soil Biol. Biochem., 64 (2013) 103-113.
618 [94] L.S. Koutika, W.A.M. Didden, J.C.Y. Marinissen, Soil organic matter distribution as influenced by
619 enchytraeid and earthworm activity, Biol. Fert. Soils, 33 (2001) 294-300.
620 [95] A.R. Ives, K. Gross, J.L. Klug, Stability and variability in competitive communities, Science, 286
621 (1999) 542-544.
622 [96] D.A. Wardle, Communities and Ecosystems Linking the Aboveground and Belowground
623 Components, Princeton University Press, Princeton, N.J., 2002.
624 [97] X.-F. Wang, Y.-Z. Su, R. Yang, Characteristics of soil nematode community along an age sequence
625 of sandy desert soil cultivation in a marginal oasis of middle reaches of Heihe River, Appl. Soil Ecol.,
626 21 (2010) 2125-2131.
627 [98] J. Bengtsson, Which species? What kind of diversity? Which ecosystem function? Some problems
628 in studies of relations between biodiversity and ecosystem function, Appl. Soil Ecol., 10 (1998) 191-
629 199.
630 [99] G.W. Luck, R. Harrington, P.A. Harrison, C. Kremen, P.M. Berry, R. Bugter, T.P. Dawson, F. de
631 Bello, S. Diaz, C.K. Feld, J.R. Haslett, D. Hering, A. Kontogianni, S. Lavorel, M. Rounsevell, M.J.
632 Samways, L. Sandin, J. Settele, M.T. Sykes, S. van den Hove, M. Vandewalle, M. Zobel, Quantifying the
633 contribution of organisms to the provision of ecosystem services, BioScience, 59 (2009) 223-235.
634 [100] M. Blouin, N. Sery, D. Cluzeau, J.-J. Brun, A. Bedecarrats, Balkanized research in ecological
635 engineering revealed by a bibliometric analysis of earthworms and ecosystem services, Environ.
636 Manage., 52 (2013) 309-320.
637 [101] S. Salmon, The impact of earthworms on the abundance of Collembola: improvement of food
638 resources or of habitat? Biol. Fertil. Soils, 40 (2004) 323-333.
639 [102] S. Wurst, Effects of earthworms on above- and belowground herbivores, Appl. Soil Ecol., 45 (3)
640 (2010) 123-130.
641 [103] K. Karaban, A.V. Uvarov, Non-trophic effects of earthworms on enchytraeids: an experimental
642 investigation, Soil Biol. Biochem., 73 (2014) 87-92.
643 [104] J. Schlaghamerský, N. Eisenhauer, L.E. Frelich, Earthworm invasion alters enchytraeid
644 community composition and individual biomass in northern hardwood forests of Nort America, Appl.
645 Soil Ecol., 83 (2014) 159-169.
646 [105] C. Gardi, F. Jeffery, A. Saltelli, An estimate of potential threats levels to soil biodiversity in EU,
647 Glob. Chang. Biol., 19 (2013) 1538–1548.

648