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1 **Title:** Response of temperate anecic earthworm individual biomass to species interactions

2

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12 **Keywords:** soil fauna, *Aporrectodea*, *Lumbricus*, earthworm mass gain, species assemblages,

13 temperate grasslands

14

15 **Abstract**

16 Earthworms contribute to a wide range of ecosystem services provided by the soil.

17 Nevertheless, synecology of these organisms is still not properly elucidated especially in terms of

18 species interactions. The aim of the present study was to determine the effects of anecic earthworm

19 species interactions on their individual biomass. These effects were measured using three epi-

20 anecic species, *Lumbricus rubellus rubellus* (Hoffmeister, 1843), *Lumbricus centralis* (Bouché,

21 1972), *Lumbricus terrestris* (Linné 1758), and three strict-anecic species, *Aporrectodea caliginosa*

22 *meridionalis* (Bouché 1972), *Aporrectodea nocturna* (Evans, 1946), *Aporrectodea giardi*

23 (Savigny, 1826). Twenty-one pairs of individuals were established following five assemblages:
24 monospecific pairwise assemblages of epi- and strict-aneic earthworms (2×3 treatments),
25 bispecific pairwise assemblages within epi- and within strict-aneic earthworms (2×3 treatments)
26 and bispecific pairwise assemblages with one epi- and one strict-aneic earthworm (3×3
27 treatments). Treatments were maintained in mesocosms for 30 days under controlled conditions
28 (food provided at the soil surface at the beginning of the experiment) and changes in the earthworm
29 individual biomass were measured. Strict-aneic earthworms in monospecific or bispecific
30 assemblages maintained their initial biomass. In contrast, epi-aneic earthworms exhibited an
31 increase of 12.4% and 23.7% of their biomass in monospecific and bispecific assemblages,
32 respectively. In bispecific assemblages combining one epi- and one strict-aneic earthworm, epi-
33 aneic earthworms solely gained biomass leading to a total increase of a 6.9%. Surprisingly, the
34 biomass' changes were not homogenous within the two sub-categories as the six earthworm species
35 exhibited species-specific responses. The greatest increases in individual biomass were recorded
36 for epi-aneic earthworms in the bispecific assemblages. This study provides further evidence for
37 the distinction between the two aneic sub-categories, as it indicates that species interactions is
38 positive only for epi-aneic earthworm biomass.

39

40 **1. Introduction**

41 Earthworms are widespread organisms and constitute the most important animal biomass
42 in European soil under temperate climates (Hole, 1981; Curry, 1994; Bar-On et al., 2018).
43 According to their physiology, morphology and behaviour, earthworms are classified into three
44 ecological categories: epigeic, endogeic and aneic species (Bouché, 1972, 1977). Nevertheless,

45 several studies highlighted that within anecic earthworms two ecological sub-categories can be
46 identified based mainly on their feeding (Ferlian et al., 2014; Andriuzzi et al., 2016; Larsen et al.,
47 2016) and burrowing (Bastardie et al., 2003) behaviours. Briefly, epi-anecic earthworms
48 (*Lumbricus* sp.) feed preferentially on surface organic matter, mainly composed of leaf litter, which
49 they can bury into their main permanent burrows. In contrast, strict-anecic earthworms
50 (*Aporrectodea* sp.) feed on humified organic matter already incorporated into the soil with a slight
51 proportion of leaf litter and they establish a denser burrow network than epi-anecic earthworms.
52 Consequently, ecosystem services provided by earthworms belonging to these two anecic sub-
53 categories are expected to be different.

54 In this context, improving our understanding of the assembly rules of anecic earthworm
55 communities is important since (i) from one to six different anecic earthworm species can coexist
56 in European soils under temperate climates (Poier and Richter, 1992; Murchie et al., 2015) and (ii)
57 inoculations of anecic earthworms are frequently realised in order to enhance soil functions (Butt,
58 2008; Forey et al., 2018). Previous studies reported that interactions within anecic earthworms
59 could either delay or increase their growth rate depending on the degree of niche overlap between
60 species (Butt, 1998; Lowe and Butt, 1999, 2002; Uvarov, 2009). However, our knowledge about
61 these interactions within and between epi- and strict-anecic earthworms is still limited.

62 The present study aimed to determine the effects of anecic earthworm interactions on
63 individual biomass as a specific proxy of earthworm fitness (Butt, 1991; Butt et al., 1994). We
64 measured changes in earthworm individual biomass after 30 days of experimentation with leaf litter
65 placed at the soil surface to mimic natural leaf litter deposition. First, we hypothesized a loss of
66 biomass in mono- and bispecific mixtures within each ecological sub-category due to resource

67 competition. Second, we hypothesized no change in individual biomass when combining one epi-
68 and one strict-anecic species due to their different feeding behavior (no niche overlap expected).

69

70 **2. Materials and methods**

71 We studied three strict-anecic earthworm species, *Aporrectodea caliginosa meridionalis*
72 (Bouché, 1972), *Aporrectodea nocturna* (Evans, 1946), *Aporrectodea giardi* (Savigny, 1826) and
73 three epi-anecic earthworm species, *Lumbricus rubellus rubellus* (Hoffmeister, 1843), *Lumbricus*
74 *centralis* (Bouché, 1972), *Lumbricus terrestris*, (Linné, 1758). We collected the soil (5-20 cm
75 depth), fresh leaf litter of *Lolium perenne* (Linné, 1753; a typical grassland species), and the six
76 anecic earthworm species from temporary grasslands in the Long Term Ecological Research
77 (LTER) site “Zone Atelier Armorique” (48°50’ N, -1°58’ W), Brittany, France. We determined
78 soil and leaf litter characteristics according to the protocols described in Hoeffner et al. (2018). We
79 identified the soil as a brown soil with 48.2% sand, 37.5% silt and 14.3% clay, characterized by
80 2.9% organic matter, a C:N ratio of 9.7 and a pH of 6.4. The leaf litter was characterized by a C:N
81 ratio of 13.9, a phenolic concentration of 3.4%, a water holding capacity of 392.6% and a specific
82 leaf area of 330.2 cm².g⁻¹.

83 Two weeks before the beginning of the experiment, we selected adult earthworms from the
84 six species, we grouped them in monospecific boxes containing the soil previously hand-sieved at
85 4-mm and fed them with air-dried leaves of *L. perenne*. Thirty-six hours before the start of the
86 experiment, we placed each earthworm on a moist sponge in a plastic box to void its gut content
87 (depuration).

88 We filled mesocosms (PVC cylinder, 30 cm high, 10 cm diameter) with 4.9 kg of fresh
89 sieved soil, placed 3.5 g of air-dried leaves of *L. perenne* at the soil surface to mimic the natural
90 leaf litter deposition and re-humidified them with deionized water. Then, we determined the
91 individual biomass of adult earthworms (i.e. presence of a turgid clitellum, to ensure taxonomic
92 identity) and placed them in the corresponding mesocosm. The six earthworm species presented an
93 initial mean biomass gradient varying from 0.62 g for *L. rubellus* to 4.72 g for *L. terrestris*
94 (Supplementary Fig. S1). We placed the mesocosms in a climatic chamber at 12 °C, with a relative
95 humidity of 85% and a 12 h: 12 h light: dark regime. From the six anecic earthworm species, we
96 established 21 pairs of individuals in five replicates following five assemblages (Supplementary
97 Fig. S2): monospecific pairwise assemblages of strict- and epi-anecic earthworms (2 sub-ecological
98 categories × 3 earthworm species treatments), bispecific pairwise assemblages within strict- and
99 within epi-anecic earthworms (2 sub-ecological categories × 3 earthworm species treatments) and
100 bispecific pairwise assemblages with one strict- and one epi-anecic earthworm (3 epi-anecic
101 species × 3 strict anecic species treatments). We maintained soil moisture by spraying deionized
102 water on the soil surface twice a week. Given the large number of mesocosms, we established
103 replicates of each treatment one day apart leading to five blocks of 21 mesocosms.

104 After 30 days, leaf litter was visually still available at the soil surface and we collected
105 earthworm individuals by destroying the mesocosms. We determined each individual earthworm
106 biomass after depuration, as previously described. We calculated the percentage of change in
107 earthworm individuals' biomass following the formula: $((\text{final fresh biomass} - \text{initial fresh}$
108 $\text{biomass}) / \text{initial fresh biomass}) \times 100\%$. For monospecific assemblages, we assigned the biomass

109 of the lightest earthworm at the end of the experiment to the lightest one at the beginning of the
110 experiment.

111 We performed statistical analyses with the R software 3.2.3 (R. Core Team, 2017). We
112 evaluated significance in all cases at $P < 0.05$. Data met the conditions of normality and
113 homoscedasticity. We excluded four soil mesocosms that contained dead earthworms (from
114 random species and random assemblages) from the data analysis. First, we used a one-way
115 ANOVA, followed by a Tukey HSD test for post hoc pairwise comparisons, to assess differences
116 in initial biomass between the six earthworm species. Second, within the two ecological categories,
117 we used two-way ANOVAs to test for the effects of species assemblage and block on earthworm
118 biomass change. Third, we used two-way ANOVAs to test for the effects of species combinations
119 and block on the biomass change of the 6 studied species. Finally, we used separated one-sample
120 t -tests with adjusted P -values due to multiple comparisons to test whether (i) earthworm species
121 and (ii) earthworm assemblages significantly lost or gained biomass during the experiment.

122

123 **3. Results**

124 During the experiment, four earthworms over the 210 died. Whatever the treatment, the
125 surviving earthworms remained adults (with a turgid clitellum) until the end of the experiment.
126 Overall, strict anecic earthworms in mono- and bispecific pairwise assemblages within their sub-
127 category maintained their initial biomass during the experiment (Fig. 1A). In contrast, the biomass
128 of epi-anecic earthworms in mono- and bispecific pairwise assemblages within their sub-category
129 increased (Fig. 1B), and this increase was twice higher in bispecific compared to monospecific
130 assemblages (+23.7% vs. +12.4%, respectively, Fig. 1B). The biomass of earthworms in bispecific

131 assemblages combining one epi- and one strict-aneic was 6.9% higher than at the beginning of
132 the experiment, but this increase was solely due to the biomass gained by epi-aneic earthworms
133 (+17.0%, Fig. 1B).

134 The biomass' changes were not homogenous within the two aneic sub-categories as the
135 six earthworm species exhibited species-specific responses (Fig. 2). Contrary to the two other
136 strict-aneic species, *A. giardi* lost biomass in monospecific assemblage (-9.2%) as well as in
137 bispecific assemblages with *A. nocturna* (-7.3%), *L. rubellus* (-7.5%) and *L. terrestris* (-13.6%,
138 Fig. 2). While the biomass of *A. caliginosa meridionalis* and *A. nocturna* did not vary significantly
139 in both mono- and bispecific assemblages with an epi-aneic species, these two strict-aneic
140 species gained biomass in presence of *A. giardi* (+6.8% and +9.0% for *A. caliginosa meridionalis*
141 and *A. nocturna*, respectively, Fig. 2). Within the epi-aneic species, the biomass of *L. centralis*
142 only increased in presence of *A. caliginosa meridionalis* (Fig. 2), while *L. terrestris* gained biomass
143 whatever the pairwise assemblage considered (Fig. 2). Finally, the biomass of *L. rubellus* increased
144 in monospecific assemblage (+12.0%) as well as in bispecific assemblages with *L. centralis*
145 (+29.8), *A. nocturna* (+21.2%) and *A. caliginosa meridionalis* (+12.3%, Fig. 2).

146

147 **4. Discussion**

148 In contrast to our first hypothesis of a negative effect of species interaction on biomass
149 change in pairwise assemblages within each aneic ecological sub-category (Butt, 1998; Lowe and
150 Butt, 1999, 2002; Frazão et al., 2018), we did not observe any change in the biomass of strict-
151 aneic earthworms but a gain of biomass of epi-aneic earthworms. However, the organic resources
152 and the experimental time used in previous studies differed to those applied in the present study

153 (i.e. *Lolium perenne* and 30 days). For example, Lowe and Butt (1999) used separated cattle solids
154 during 84 days and Frazão et al. (2018) a mixture of *Triticum aestivum* and *Raphanus sativus*
155 during 61 days. Postma-Blaauw et al. (2006) observed that the assemblage of *L. rubellus* with *L.*
156 *terrestris* fed with *Solanum tuberosum* enhanced the bacterial biomass in their burrows. In addition,
157 Hoeffner et al. (2018) observed specific fungal communities within *L. rubellus*, *L. centralis* and *L.*
158 *terrestris* burrows fed with *L. perenne*. These previous observations of positive effects of epi-
159 anecic species on microbial communities could lead to enhanced leaf litter decomposition and then
160 to higher organic matter assimilation by epi-anecic earthworms (Hoeffner et al., 2018). To confirm
161 that these observed effects are due to the ecological category of earthworms and not the genus they
162 belong to, it would be interesting to study other genus within anecic earthworms such as
163 *Scherotheca*, *Octodrilus* or *Fitzingeria*.

164 Overall, the effect of the interaction between one strict- and one epi-anecic earthworm was
165 mainly additive confirming our second hypothesis of reduced niche overlap. Nevertheless, we
166 observed some gain or loss of biomass depending on the earthworm assemblages. It may be
167 assumed that other mechanisms, such as facilitation or competition for resource access and gallery
168 formation, could explain these differences and some additional experiments are required.

169 Within strict-anecic earthworms, *A. caliginosa meridionalis* and *A. nocturna* in
170 monospecific assemblages maintained their initial biomass while *A. giardi* lost biomass. These
171 findings are in agreement with previous studies that also reported no change in *A. caliginosa*
172 *meridionalis* biomass after 31 days when fed with *Castanea sativa* (Cortez and Bouché, 2001) and
173 a 38% loss of biomass of *A. giardi* after 31 days when fed with *Triticum aestivum* (Cortez et al.,
174 1989). Overall, epi-anecic earthworms in monospecific assemblages gain biomass (Shipitalo et al.,

175 1988; Binet and Trehen, 1992; Hoeffner et al., 2018). For example, Shipitalo et al. (1988) reported
176 a 100% and a 35% increase in biomass of *L. rubellus* and *L. terrestris* when fed with *Medicago*
177 *sativa* litter after 32 and 36 days of experimentation, respectively. Thus, the present study confirms
178 the two distinct behaviours to process the leaf litter of strict- and epi-anecic earthworms and
179 provides support to this sub-category distinction. In fact, feeding on leaf litter at the surface by epi-
180 anecic earthworms (Larsen et al., 2016; Hoeffner et al., 2018, 2019) allowed them to increase their
181 biomass with our experimental conditions. In contrast, strict-anecic earthworms by feeding mainly
182 on soil organic matter had restricted access to organic matter (2.9% of soil content). Additionally,
183 we also cannot exclude that soil organic matter consumption by strict-anecic earthworms was
184 restricted due to the low soil volume of 1571 cm³ in the experimental mesocosms.

185 Finally, the positive species interaction between epi-anecic species highlighted in our lab
186 experiment could partly explain field species assemblages reported in previous studies (Decaëns et
187 al., 2008; Murchie et al., 2015). For example, Murchie et al., (2015) observed three epi-anecic
188 species and only one strict-anecic species in a same temperate grassland suggesting facilitation
189 within epi-anecic species. Furthermore, Decaëns et al. (2008) observed a higher co-occurrence rate
190 between epi-anecic species than between strict anecic species in twenty temperate grasslands.

191

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197

198 **References**

- 199 Andriuzzi, W.S., Ngo, P.-T., Geisen, S., Keith, A.M., Dumack, K., Bolger, T., Bonkowski, M.,
200 Brussaard, L., Faber, J.H., Chabbi, A., Rumpel, C., Schmidt, O., 2016. Organic matter composition
201 and the protist and nematode communities around anecic earthworm burrows. *Biol. Fertil. Soils*
202 52, 91–100.
- 203 Bar-On, Y.M., Phillips, R., Milo, R., 2018. The biomass distribution on Earth. *Proc. Natl. Acad.*
204 *Sci.* 115, 6506–6511.
- 205 Bastardie, F., Capowiez, Y., de Dreuzy, J.-R., Cluzeau, D., 2003. X-ray tomographic and hydraulic
206 characterization of burrowing by three earthworm species in repacked soil cores. *Appl. Soil Ecol.*
207 24, 3–16.
- 208 Binet, F., Trehen, P., 1992. Experimental microcosm study of the role of *Lumbricus terrestris*
209 (Oligochaeta : Lumbricidae) on nitrogen dynamics in cultivated soils. *Soil Biol. Biochem.* 24,
210 1501–1506.
- 211 Bouché, M.B., 1972. *Lombriciens de France: écologie et systématique*, INRA-Annales de Zoologie
212 *Ecologie Animale*. ed. INRA, France.
- 213 Bouché, M.B., 1977. Strategies lombriciennes. *Ecol. Bull., Soil Organisms as Components of*
214 *Ecosystems* 25, 122–132.
- 215 Butt, K.R., 1991. The effects of temperature on the intensive production of *Lumbricus terrestris*
216 (oligochaeta, Lumbricidae). *Pedobiologia* 35, 257–264.
- 217 Butt, K.R., Frederickson, J., Morris, R., 1994. The life cycle of the earthworm *Lumbricus terrestris*
218 L. (Oligochaeta, Lumbricidae) in culture. *Eur. J. Soil Biol.* 30, 49–54.

219 Butt, K.R., 1998. Interactions between selected earthworm species: a preliminary, laboratory-based
220 study. *Appl. Soil Ecol.* 9, 75–79.

221 Butt, K.R., 2008. Earthworms in soil restoration: lessons learned from United Kingdom case
222 studies of land reclamation. *Restor. Ecol.* 16, 637–641.

223 Cortez, J., Bouché, M., 2001. Decomposition of mediterranean leaf litters by *Nicodrilus*
224 *meridionalis* (Lumbricidae) in laboratory and field experiments. *Soil Biol. Biochem.* 33, 2023–
225 2035.

226 Cortez, J., Hameed, R., Bouché, M.B., 1989. C and N transfer in soil with or without earthworms
227 fed with ¹⁴C- and ¹⁵N-labelled wheat straw. *Soil Biol. Biochem.* 21, 491–497.

228 Curry, J.P., 1994. *Grassland invertebrates: ecology, influence on soil fertility and effects on plant*
229 *growth*, Chapman & Hall. ed. Springer, London, UK.

230 Decaëns, T., Margerie, P., Aubert, M., Hedde, M., Bureau, F., 2008. Assembly rules within
231 earthworm communities in North-Western France - a regional analysis. *Appl. Soil Ecol.* 39, 321–
232 335.

233 Ferlian, O., Cesarz, S., Marhan, S., Scheu, S., 2014. Carbon food resources of earthworms of
234 different ecological groups as indicated by ¹³C compound-specific stable isotope analysis. *Soil*
235 *Biol. Biochem.* 77, 22–30.

236 Forey, E., Chauvat, M., Coulibaly, S.F.M., Langlois, E., Barot, S., Clause, J., 2018. Inoculation of
237 an ecosystem engineer (Earthworm: *Lumbricus terrestris*) during experimental grassland
238 restoration: consequences for above and belowground soil compartments. *Appl. Soil Ecol.* 125,
239 148–155.

240 Frazão, J., de Goede, R.G.M., Capowiez, Y., Pulleman, M.M., 2018. Soil structure formation and
241 organic matter distribution as affected by earthworm species interactions and crop residue
242 placement. *Geoderma* 338, 453–463.

243 Hoeffner, K., Monard, C., Santonja, M., Cluzeau, D., 2018. Feeding behaviour of epi-aneic
244 earthworm species and their impacts on soil microbial communities. *Soil Biol. Biochem.* 125, 1–
245 9.

246 Hoeffner, K., Santonja, M., Cluzeau, D., Monard, C., 2019. Epi-aneic rather than strict-aneic
247 earthworms enhance soil enzymatic activities. *Soil Biol. Biochem.* 132, 93–100.

248 Hole, F.D., 1981. Effects of animals on soil. *Geoderma* 25, 75–112.

249 Larsen, T., Pollierer, M.M., Holmstrup, M., D’Annibale, A., Maraldo, K., Andersen, N., Eriksen,
250 J., 2016. Substantial nutritional contribution of bacterial amino acids to earthworms and
251 enchytraeids: a case study from organic grasslands. *Soil Biol. Biochem.* 99, 21–27.

252 Lowe, C.N., Butt, K.R., 1999. Interspecific interactions between earthworms: a laboratory-based
253 investigation. *Pedobiologia* 43, 808–817.

254 Lowe, C.N., Butt, K.R., 2002. Growth of hatchling earthworms in the presence of adults:
255 interactions in laboratory culture. *Biol. Fertil. Soils* 35, 204–209.

256 Murchie, A.K., Blackshaw, R.P., Gordon, A.W., Christie, P., 2015. Responses of earthworm
257 species to long-term applications of slurry. *Appl. Soil Ecol.* 96, 60–67.

258 Poier, K.R., Richter, J., 1992. Spatial distribution of earthworms and soil properties in an arable
259 loess soil. *Soil Biol. Biochem.* 24, 1601–1608.

260 Postma-Blaauw, M.B., Bloem, J., Faber, J.H., van Groenigen, J.W., de Goede, R.G.M., Brussaard,
261 L., 2006. Earthworm species composition affects the soil bacterial community and net nitrogen
262 mineralization. *Pedobiologia* 50, 243–256.

263 R. Core Team, 2017. R: a language and environment for statistical computing. R foundation for
264 statistical computing, Vienna, Austria.

265 Shipitalo, M.J., Protz, R., Tomlin, A.D., 1988. Effect of diet on the feeding and casting activity of
266 *Lumbricus terrestris* and *L. rubellus* in laboratory culture. *Soil Biol. Biochem.* 20, 233–237.

267 Uvarov, A.V., 2009. Inter- and intraspecific interactions in lumbricid earthworms: their role for
268 earthworm performance and ecosystem functioning. *Pedobiologia* 53, 1–27.

269

270 **Figure legends**

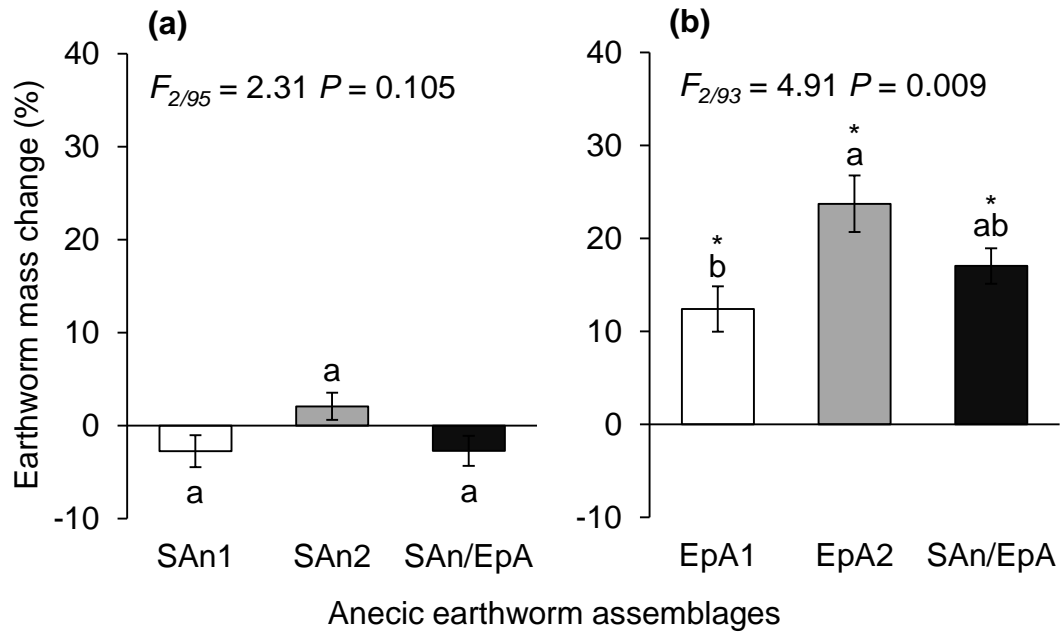
271 **Fig. 1.** Earthworm biomass changes according to anecic earthworm assemblages of (a) strict- and
272 (b) epi-anecic earthworms. Values are means \pm SD. Different letters denote significant differences
273 among earthworm assemblages with $a > b$ (post hoc Tukey test results). Biomass changes
274 significantly different from 0 are indicated with a star. SAn1 = Monospecific assemblages of strict-
275 anecic, n= 28; SAn2 = Bispecific assemblages of strict-anecic, n= 30; SAn/EpA = Bispecific
276 assemblages with one strict- and one epi-anecic, n= 44; EpA1 = Monospecific assemblages of epi-
277 anecic, n= 30; and EpA2 = Bispecific assemblages of epi-anecic, n= 26.

278

279

280 **Fig. 2.** Biomass changes of earthworms in mono- and bispecific pairs of AM, AN, AG, LR, LC
281 and LT. Values are means \pm SD, n = 4 to 10. Biomass changes significantly different from 0 are
282 indicated with a star. AM = *A. caliginosa meridionalis*; AN = *A. nocturna*; AG = *A. giardi*; LR =
283 *L. rubellus rubellus*; LC = *L. centralis*; LT = *L. terrestris*.

284 **Figure 1**



285

