Response of temperate anecic earthworm individual biomass to species interactions

Kevin Hoeffner, Cécile Monard, Daniel Cluzeau, Mathieu Santonja

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Authors: Kevin Hoeffner[1*], Cécile Monard[1], Daniel Cluzeau[1], Mathieu Santonja[1,2]

Addresses:

1. Univ Rennes, CNRS, ECOBIO UMR 6553, F-35000 Rennes, France.

2. Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France.

*** Corresponding author**

E-mail: kevin.hoeffner@gmail.com

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

Abstract

Earthworms contribute to a wide range of ecosystem services provided by the soil. Nevertheless, synecology of these organisms is still not properly elucidated especially in terms of species interactions. The aim of the present study was to determine the effects of anecic earthworm species interactions on their individual biomass. These effects were measured using three epi-anecic species, *Lumbricus rubellus rubellus* (Hoffmeister, 1843), *Lumbricus centralis* (Bouché, 1972), *Lumbricus terrestris* (Linné 1758), and three strict-anecic species, *Aporrectodea caliginosa meridionalis* (Bouché 1972), *Aporrectodea nocturna* (Evans, 1946), *Aporrectodea giardi*

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

1. Introduction

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

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

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

The present study aimed to determine the effects of anecic earthworm interactions on individual biomass as a specific proxy of earthworm fitness (Butt, 1991; Butt et al., 1994). We measured changes in earthworm individual biomass after 30 days of experimentation with leaf litter placed at the soil surface to mimic natural leaf litter deposition. First, we hypothesized a loss of 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 $cm^2.g^{-1}$.
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).

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

After 30 days, leaf litter was visually still available at the soil surface and we collected earthworm individuals by destroying the mesocosms. We determined each individual earthworm biomass after depuration, as previously described. We calculated the percentage of change in earthworm individuals' biomass following the formula: ((final fresh biomass – initial fresh biomass) / initial fresh biomass) × 100%. For monospecific assemblages, we assigned the biomass

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

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

3. Results

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

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

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

4. Discussion

In contrast to our first hypothesis of a negative effect of species interaction on biomass change in pairwise assemblages within each anecic ecological sub-category (Butt, 1998; Lowe and Butt, 1999, 2002; Frazão et al., 2018), we did not observe any change in the biomass of strict-anecic earthworms but a gain of biomass of epi-anecic earthworms. However, the organic resources 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.,

1988; Binet and Trehen, 1992; Hoeffner et al., 2018). For example, Shipitalo et al. (1988) reported a 100% and a 35% increase in biomass of *L. rubellus* and *L. terrestris* when fed with *Medicago sativa* litter after 32 and 36 days of experimentation, respectively. Thus, the present study confirms the two distinct behaviours to process the leaf litter of strict- and epi-anecic earthworms and provides support to this sub-category distinction. In fact, feeding on leaf litter at the surface by epi-anecic earthworms (Larsen et al., 2016; Hoeffner et al., 2018, 2019) allowed them to increase their biomass with our experimental conditions. In contrast, strict-anecic earthworms by feeding mainly on soil organic matter had restricted access to organic matter (2.9% of soil content). Additionally, we also cannot exclude that soil organic matter consumption by strict-anecic earthworms was restricted due to the low soil volume of 1571 cm^{-3} in the experimental mesocosms.

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

Acknowledgements

We thank the Institut National de la Recherche Agronomique (Arras, France) for mineral soil analyses. We also thank Clément De Graaf, Clément Bertran, Albin Fertil, Alan Schneider and Anaïs Rancon for their technical assistance in the laboratory. Kevin Hoeffner was supported by a PhD grant from the French Ministère de la Recherche.

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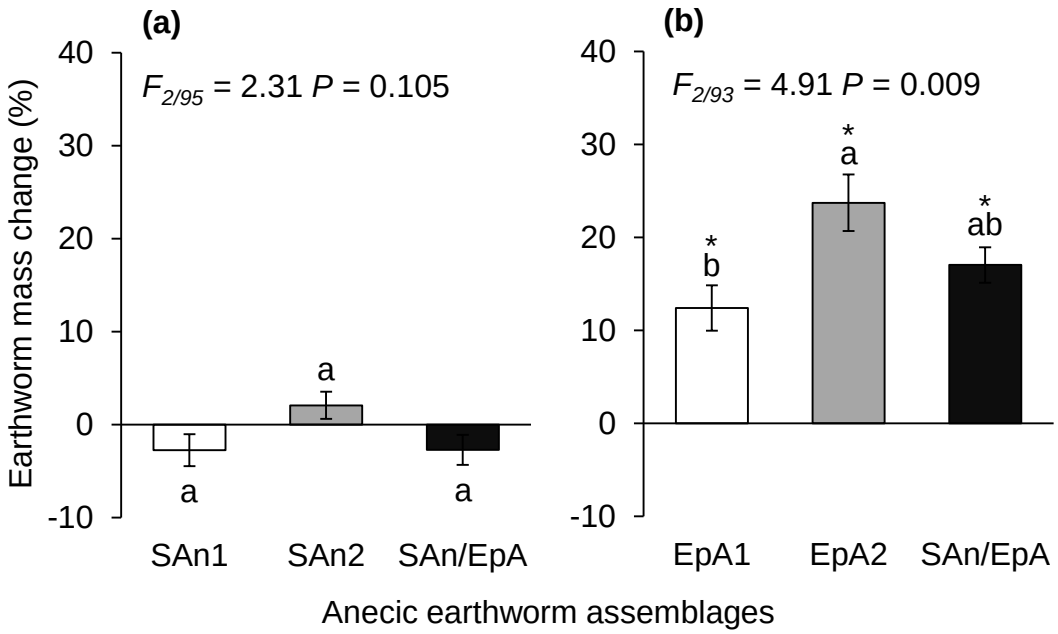
Figure legends

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

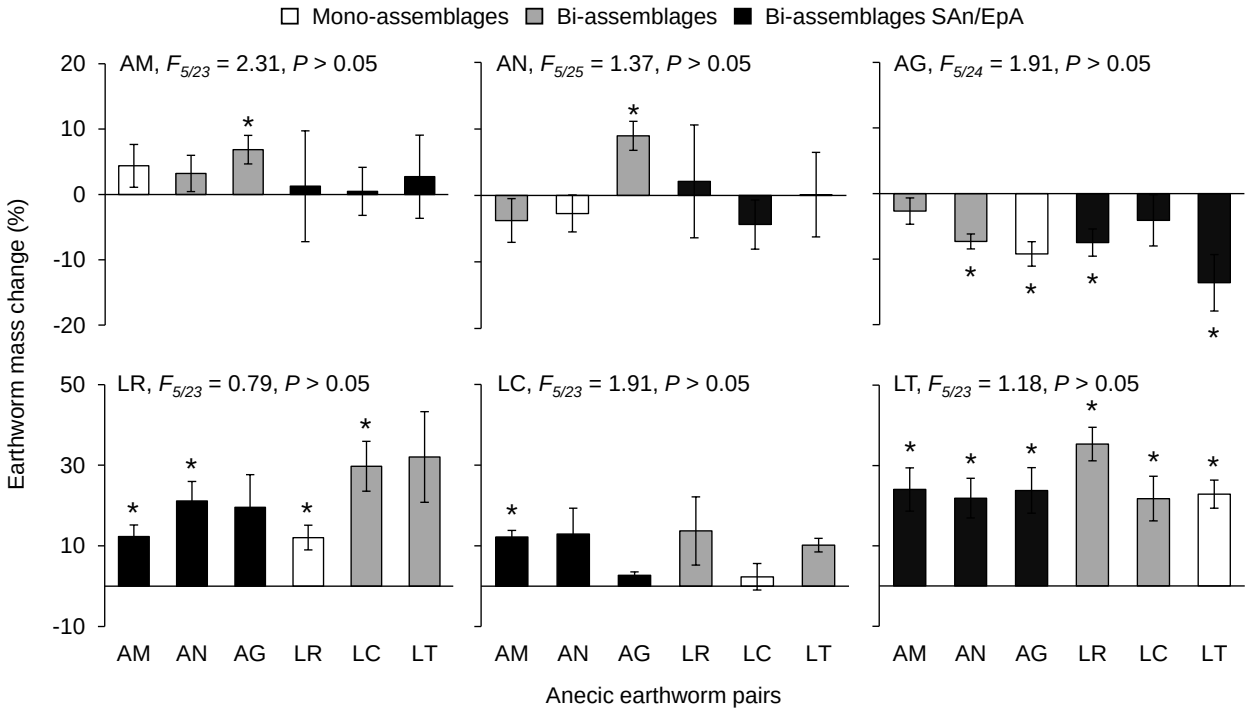
Fig. 2. Biomass changes of earthworms in mono- and bispecific pairs of AM, AN, AG, LR, LC and LT. Values are means ± SD, n = 4 to 10. Biomass changes significantly different from 0 are indicated with a star. AM = *A. caliginosa meridionalis*; AN = *A. nocturna*; AG = *A. giardi*; LR = *L. rubellus rubellus*;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

284 **Figure 1**



285

Figure 2


Mono-assemblages
Bi-assemblages
Bi-assemblages SAn/EpA
AM, $F_{5/23}$ = 2.31, P > 0.05
AN, $F_{5/25}$ = 1.37, P > 0.05
AG, $F_{5/24}$ = 1.91, P > 0.05
LR, $F_{5/23}$ = 0.79, P > 0.05
LC, $F_{5/23}$ = 1.91, P > 0.05
LT, $F_{5/23}$ = 1.18, P > 0.05
Earthworm mass change (%)
20
10
0
-10
-20
50
30
10
-10
AM AN AG LR LC LT
Anecic earthworm pairs