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1 **Decreased burrowing activity of endogeic earthworms and effects on water infil-**  
2 **tration in response to an increase in soil bulk density**

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24 **Abstract**

25 Endogeic earthworms live and burrow in the soil to find their food. They burrow by pushing the soil  
26 aside or ingesting it and are thus sensitive to soil compaction. However there is a scarcity of data  
27 regarding the effects of soil bulk density on the burrowing behavior and activity of endogeic earth-  
28 worms. We carried out laboratory experiments using repacked soil cores with various levels of bulk  
29 density (from 1.18 to 1.38 g cm<sup>-3</sup>) in which individuals of *Aporrectodea caliginosa* or *Allolobophora*  
30 *chlorotica* were incubated for six weeks. The burrow systems inside the soil cores and the compac-  
31 tion around the burrows were then analyzed using X-ray tomography. Soil water infiltration meas-  
32 urements were also carried out. The increase in bulk density had a negative impact on all burrow  
33 system characteristics (length, volume, diameter, continuity, number of burrows). When bulk densi-  
34 ty increased from 1.18 to 1.38 g cm<sup>-3</sup>, volume, diameter, continuity and the number of burrows de-  
35 creased on average by 77%, 21%, 81% and 58%, respectively. The increase in density due to com-  
36 paction around the burrows was similar whatever the species and the bulk density. Increasing soil  
37 bulk density from 1.18 to 1.38 g cm<sup>-3</sup> also greatly decreased water infiltration (-89% for both species)  
38 and increased breakthrough time (10 and 25-fold for *A. chlorotica* and *A. caliginosa* respectively).  
39 However, compared to a control without earthworms, water infiltration in cores incubated with en-  
40 dogeic species was only increased significantly at 1.18 and 1.23 g cm<sup>-3</sup>. This illustrates that burrows  
41 made by endogeic earthworms moderately increase water infiltration and only when the soil bulk  
42 density is low. Data provided in this study could be used to refine simulation models of earthworm  
43 burrowing behavior where burrowing is assumed to be mainly governed by soil water content, tem-  
44 perature and soil bulk density.

45

46 *Keywords:* compaction; burrow system; *Aporrectodea caliginosa*; *Allolobophora chlorotica*; behav-  
47 iour

## 48 **Abbreviations**

49 BD: soil bulk density

50

## 51 **Introduction**

52 Compaction, partly due to an increase of engine loads, is a major threat to agricultural and forest  
53 soils and their resilience (Hansen 1996). Earthworms are particularly sensitive to soil compaction as  
54 they burrow their way in the soil either by pushing the soil aside or ingesting it (Beylich et al., 2010;  
55 Keller et al., 2017). Indeed, ingestion is the only possible way of moving in highly compacted soils  
56 (Dexter, 1978) and often results in higher quantities of excreted surface casts (Joschko et al., 1989).  
57 A high BD also means that more energy is spent burrowing (Ruiz et al., 2010). Although earth-  
58 worms generally avoid highly compacted zones (Stovold et al., 1994), epi-anecic such as *Lumbricus*  
59 *terrestris*, earthworms have been observed to burrow in them under semi-field conditions in the  
60 presence of a plough pan (Capowiez et al., 2009). Epi-anecics and other species have also been  
61 found to burrow in compacted zones below wheels tracks under field conditions (Capowiez et al.,  
62 2012).

63 Thus bulk density (BD) is one important factor indirectly governing earthworm abundance in  
64 croplands (Chan and Barchia, 2007; Ouellet et al., 2008; Capowiez et al., 2009), forests (Jordan et  
65 al., 1999; Bottinelli et al., 2014), tallgrass prairies (Althoff et al., 2009) and urban soils (Smetak et  
66 al., 2007). Among earthworms, anecic and epi-anecic earthworms (*sensu* Bouché, 1972) are thought  
67 to be more powerful axially (Keudel and Schrader, 1999) and thus less sensitive to compaction  
68 (Beylich et al., 2010). Paradoxically, much more information has been reported on the effects of soil  
69 compaction on anecic or epi-anecic earthworm behavior than on other ecological categories. Rush-  
70 ton (1986) and Joschko et al. (1989) demonstrated that burrow length was decreased when *L.*  
71 *terrestris* was challenged with increased BD. Stovold et al. (1994) made a similar observation for  
72 *Aporrectodea nocturna* in a choice experiment. Kretzschmar (1991) estimated that *Aporrectodea*

73 *longa* burrowing activity, as assessed by surface cast production, decreased for higher BD. This bias  
74 towards the anecic or epi-anecic species is explained by the fact that these species have a greater  
75 influence on water infiltration since their burrows are more vertical, more continuous (because less  
76 refilled) and have surface openings (Capowiez et al., 2015). In fact, compaction impacts not only  
77 burrow numbers but also burrow continuity (Schäffer et al., 2007; Capowiez et al., 2012) and this in  
78 turn can drastically reduce water infiltration (Kim et al., 2010). In comparison, information regard-  
79 ing the effects of compaction on endogeic earthworms is scarce. Langmaack et al (1999) studied *A.*  
80 *caliginosa* burrowing behavior in compacted vs non-compacted soils and concluded that in response  
81 to compaction this species increased burrow continuity but no clear trend was observed for burrow  
82 length. Dexter (1978) also found that high BD did not have a negative impact on *A. caliginosa* bur-  
83 rowing. In contrast, Söchtig and Larink (1992) determined that *A. caliginosa* burrow length de-  
84 creased linearly when pore volume decreased (and thus BD increased).

85 In most of these studies, the effects of high BD on earthworms were measured indirectly, i.e. either  
86 by assessing surface cast production (assuming most of the casts were produced at the soil surface)  
87 or the burrows on the outer borders of soil cores (assuming these burrows were representative of  
88 burrows in the cores). This latter case is due to the absence of non-invasive techniques for estimat-  
89 ing burrows in soil cores in past decades. Nevertheless the first application of X-ray tomography to  
90 analyze earthworm systems was in the early 90s (Joschko et al., 1991) and medical scanners are  
91 now readily available to solve this problem. Microtomography with an higher resolution can now  
92 also be used (Balseiro-Romano et al., 2020). Another point that sometimes limits the relevance of  
93 past studies is the fact that they were observed using choice experiments: earthworms were placed  
94 in mesocosms containing soils with different BD and their burrowing activity was estimated in the  
95 different situations (Stovold et al., 1994; Söchtig and Larink, 1992). This kind of study provides in-  
96 formation on earthworm preferences but not really on their ability to burrow and live in compacted  
97 soils. Furthermore, in most studies only two to three BD were tested (Joschko et al., 1989; Joschko

98 et al., 1993; Langmaack et al., 1999; Stovold et al., 1994; Jégou et al., 2002; Capowiez et al., 2012)  
99 whereas recent studies suggested that depending on the soil texture, earthworm responses in terms  
100 of burrowing are not always monotonic (see Figure 5 in Pöhlitz et al., 2020).  
101 Overall, there is an increasing demand for accurate estimates of the effects of BD on earthworm  
102 burrowing (Roeben et al., 2020) since this kind of information is required, for example, to simulate  
103 earthworm behavior and better assess their real exposure to pesticides. However, there is a scarcity  
104 of useable data in the literature, especially for endogeic species.  
105 Thus, in the present study, we carried out a lab experiment with soil cores compacted at five differ-  
106 ent BD currently observed in orchard soils close to Avignon, France. We then studied the resulting  
107 effects on the burrow system characteristics of two very common endogeic species (*Aporrectodea*  
108 *caliginosa* and *Allolobophora chlorotica*). We also determined how BD and earthworm species  
109 modified water infiltration. Furthermore, it is known that earthworms can compact the soil when  
110 they burrow (Rogasik et al., 2014; Capowiez et al., 2011; Milleret et al., 2009) and thus we assessed  
111 whether the lateral compaction around the burrows was influenced by soil BD for these species.

112

## 113 **Material and Methods**

### 114 **Soil and earthworms**

115 Soil (0-20 cm depth) and earthworms were sampled in an orchard abandoned since at least ten years  
116 in Avignon, in the south east of France. The soil has the following characteristics: pH = 8.2, clay =  
117 19.7%, silt = 50.8%, sand = 29.5%, OM = 3.4%, CEC = 8.48 cmol C kg<sup>-1</sup>. The soil was sieved at 3  
118 mm and kept at constant humidity (20% on a mass basis, i.e. about 80% of its water holding capaci-  
119 ty). In this orchard, we found high densities of earthworms (> 300 individuals m<sup>-2</sup>) and the most  
120 dominant species were two endogeic species: *Allolobophora chlorotica* (leucotypic form according  
121 to Bouché (1972) but often called pink morph) and *Aporrectodea caliginosa*. However, these two  
122 species presumably do not occupy exactly the same niche as these species were indeed described as

123 an ‘intermediate’ and ‘endogeic’ species by Bouché (1972), respectively. Recently, Bottinelli et al.  
124 (2020) demonstrated that each lumbricid earthworm species can be categorized by three percentages  
125 into the three main ecological categories defined by Bouché (1977). According to that study, *A.*  
126 *chlorotica* (leucotypic form) is 97% endogeic and 3% anecic, whereas *A. caliginosa* is 80%  
127 endogeic, 16% epigeic and 4% anecic.

128

### 129 **Mesocosms and experimental design**

130 Mesocosms were PVC cores (height: 30 cm, diameter:16 cm) filled with sieved soil. For each soil  
131 layer (600 g of soil at 80% WHC), the soil was pressed to the desired bulk density using a hydraulic  
132 press with increasing pressures. For this experiment, 50 cores were prepared with ten cores for each  
133 soil bulk density (BD): four with *A. caliginosa*, four with *A. chlorotica* and two control cores with-  
134 out earthworms. The five levels of BD were 1.18, 1.23, 1.29, 1.34 and 1.38 g cm<sup>-3</sup>. This range was  
135 chosen to encompass the BD observed in orchards, from tilled zones under the row to compacted  
136 zones below wheel tracks (personal observation). In each core, we introduced three individuals of  
137 either *A. caliginosa* or *A. chlorotica* (thus a density of 150 individuals m<sup>-2</sup>) or no earthworms. These  
138 endogeic densities can be found in most agricultural soils (excepted vineyards). Only adults were  
139 used in the experiment and the mean (+/- SD) biomass for each species was 0.57 (+/- 0.04) and 0.44  
140 (+/- 0.04) g for *A. caliginosa* and *A. chlorotica*, respectively. At the end of the experiment (5 weeks),  
141 5 ml of chloroform was poured into each core to kill earthworms and prevent further burrowing. In  
142 each core, surface casts were sampled, dried for 48 h at 110°C and weighed.

143

### 144 **X-ray computed tomography and burrow system characterization**

145 Macroporosity within the soil cores was analyzed by X-ray computed tomography using a medical  
146 scanner (BrightSpeed Exel 4, General Electric) in the INRA Nancy research centre. The scanner  
147 settings were 130 kV and 50 mA. DICOM images were transformed into 8-bit images using the fol-

148 lowing parameters  $WL = -1000$  and  $WW = 2000$  HU. The greylevel histograms of the images  
149 showed two well separated peaks, one for the soil matrix and one for the porosity. We decided to  
150 choose a binary threshold value set at 1/3 of the distance between the two peaks. Since the peak  
151 value is dependent on the density of the soil matrix, this ensures that differences in density between  
152 BD treatments did not influence the total macroporosity volume. Images were filtered using a mean  
153 filter of radius 2 to remove some noise. Burrow system 3D reconstructions and skeletonizations  
154 were obtained using Avizo software (ThermoScientific). Other computations were carried out using  
155 ImageJ (Schindelin et al., 2012) and the 'SoilJ' and 'BoneJ' plugins. We thus estimated burrow sys-  
156 tem volume. Mean burrow diameter was estimated by selecting the most circular pores ('circulari-  
157 ty' > 0.8 in ImageJ) on 2D images, assessing their area and computing an equivalent diameter. To  
158 assess burrow system continuity, as no standardized measure exists, we compute the number of bur-  
159 rows whose vertical extension was larger than 30% of the core length (i.e. 9 cm) was counted. We  
160 also computed the degree of anisotropy of the burrow system using BoneJ ; this algorithm assesses  
161 whether objects are oriented in the same direction (degree of anisotropy close to 0) or randomly in  
162 3D (degree of anisotropy close to 1). To assess whether the endogeic species compacted the soil  
163 around their respective burrows, we selected the 8 mm spatial zones around the burrows (as as-  
164 sessed visually on the images) and simply computed the mean greylevel in these zones. For cores  
165 without earthworms, we randomly selected zones of similar size in the images to compute the mean  
166 greylevel value.

167

## 168 **Water infiltration**

169 To study the effects of the presence of an earthworm burrow system and its characteristics on water  
170 infiltration, we used the same protocols in Capowiez et al. (2015). Soil cores were placed on a fun-  
171 nel to be able to observe water drainage below the cores. In brief, 150 ml of water was poured onto  
172 a soil core, the time was recorded until the water was completely absorbed and then a new volume



173 was added. We also noted the time at which water began to drain ('breakthrough time'). This proce-  
174 dure was repeated until a linear trend was observed (i.e. same time intervals between applied water  
175 volumes), which was typically after around eight volumes. This single-ring protocol allowed the  
176 steady-state infiltration rate to be estimated by linear regression for each core. The first data points  
177 of the relationship between time and the number of water volumes poured were removed.

178

## 179 **Statistical analysis**

180 Our experimental design was balanced for comparing the responses of the two species (if we ex-  
181 cluded cores without earthworms). For count data (burrow continuity), we used a GLM with a qua-  
182 si-poisson family. For other burrow system characteristics and cast production, we used ANOVA  
183 since no large deviations from normality (Shapiro-Wilk test: all p-values > 0.061) or  
184 homoscedasticity (Levene test: all p-values > 0.047) were detected. In both cases, we first ran the  
185 analyses with two factors (BD and earthworm species) and then if the interaction was not signifi-  
186 cant (the most common case), we ran two tests (GLM or ANOVA) for each species separately. For  
187 infiltration data (infiltration rate and breakthrough time), we applied the same analysis but we also  
188 computed the infiltration gain, i.e. the difference between infiltration of each species compared to  
189 the cores at the same BD but without earthworms. These gains were also analyzed using a two-way  
190 ANOVA and then two one-way ANOVA. Linear regressions were carried out for burrow volume  
191 with the 'lm' function. All analyses were made in R (R core team, 2019).

192

## 193 **Results**

### 194 **Effects of bulk density on the earthworm burrow system**

195 Examples of the 3D burrow systems are shown in Figure 1. The two-way ANOVA indicated that  
196 BD had a highly significant effect on burrow volume whereas species and the interaction between  
197 BD and species were not significant (Table S1). One-way ANOVA for each species separately

198 showed that burrow volumes were significantly larger at 1.18 g cm<sup>-3</sup> than at other BD for both spe-  
199 cies (Fig. 2). At 1.38 g cm<sup>-3</sup>, the burrow volume was significantly lower than at 1.18 and 1.23 g cm<sup>-3</sup>  
200 for both species. The decrease in burrow volume between the minimal (1.18 g cm<sup>-3</sup>) and maximal  
201 (1.38 g cm<sup>-3</sup>) tested BD was high with -75% and -80% for *A. caliginosa* and *A. chlorotica*, respec-  
202 tively. The linear regressions between BD and burrow volume were significant ( $p = 0.027$  and  
203  $0.0071$ ) and the  $R^2$  values were 0.79 and 0.91 and for *A. caliginosa* and *A. chlorotica* respectively,  
204 even if the linear trend was more obvious for the latter species. We observed very similar decreases  
205 and significant differences in burrow lengths (Table 1).

206 BD and species ( $p < 0.001$ ) also had a significant influence on burrow diameter, but no interaction  
207 was observed between these factors ( $p = 0.21$ ). For *A. caliginosa*, burrow diameter was only sig-  
208 nificantly different at the lowest BD compared to the highest BD. For *A. chlorotica* diameter values  
209 at the two lowest BD were significantly different from those at the other BD (Table 1).

210 BD also has a significant effect on burrow continuity, assessed as the number of burrows with a ver-  
211 tical elongation larger than 9 cm (Table S1). At the lowest BD, a mean of 6 and 7 such burrows  
212 were observed for *A. caliginosa* and *A. chlorotica* respectively. At the highest BD, only one such  
213 burrow was observed on average for both species (Fig. 3). Thus, BD had a highly significant effect  
214 on burrow continuity ( $p < 0.001$ ), whereas species and interaction did not ( $p = 0.17$  and  $0.16$ , re-  
215 spectively). For *A. caliginosa*, only the continuity for the lowest and the highest BD were different,  
216 whereas for *A. chlorotica*, continuity at the three highest BD was significantly different from that at  
217 the lowest BD.

218 The degree of anisotropy of the burrow systems increased with increasing BD whereas species and  
219 the interaction were not significant (Table S1 and Fig. 4). For both species, the degree of anisotropy  
220 for the two lowest BD was significantly lower than those observed at the highest BD which sug-  
221 gests that increased BD leads to less randomly orientated burrow systems. Finally BD or species

222 (results not shown) did not affect burrow system verticality (mean deviation from the vertical of the  
223 segments of the skeleton).

224 Surface cast production was low (less than 10 g of dry soil) and not influenced by BD for *A. chlo-*  
225 *rotica* (Table 1). In contrast, for *A. caliginosa*, cast production was higher (15-32 g of dry soil; Table  
226 1) and was the highest at a BD of 1.34 g cm<sup>-3</sup> and significantly different from the amount produced  
227 at the two lowest tested BD.

228

### 229 **Effects on water infiltration**

230 BD ( $p < 0.001$ ) had a significant impact on infiltration rates and breakthrough times, but the earth-  
231 worm factor (with three modalities: *A. caliginosa*, *A. chlorotica* and no earthworm) and the interac-  
232 tion were not significant ( $p = 0.36$  and  $0.55$  respectively for infiltration rate ;  $p = 0.30$  and  $0.21$  res-  
233 pectively for breakthrough time). When BD increased, infiltration rates decreased and breakthrough  
234 times increased (Table 2).

235 When infiltration was expressed relative to the control without earthworms, BD has a significant  
236 impact ( $p < 0.001$ ) but species (with two modalities: *A. caliginosa* and *A. chlorotica*) and the inter-  
237 action were not significant ( $p = 0.26$  and  $0.17$  respectively). Significant differences due to earth-  
238 worm's presence were only observed at the lowest BD for *A. caliginosa* and at the two lowest BD  
239 for *A. chlorotica* (Fig. 5). No significant effect was observed for breakthrough times expressed rela-  
240 tive to the control (data not shown).

241

### 242 **Effects on soil bulk density around the burrows**

243 The mean soil BD around the burrows increased when BD of the soil core increased (Fig. S2). Soil  
244 BD had a highly significant effect ( $p < 0.001$ ) as did the presence of earthworms ( $p < 0.001$ ), but  
245 there was no significant interaction between these two factors ( $p = 0.83$ ). For each BD, the differ-

246 ence in BD around the burrows appeared to be similar (around five greylevels corresponding to 59  
247 HU) in the presence or absence of earthworms.

248

## 249 **Discussion**

### 250 **Effects of bulk density on earthworm burrowing and casting behavior**

251 BD strongly and negatively influenced all the geometrical characteristics of the burrow systems of  
252 both endogeic species. First of all in terms of magnitude, the volumes (and lengths) of the burrow  
253 systems decreased almost linearly with increasing BD. Thus with our soil which lies between loam  
254 and silt loam soil, we did not observe a non-monotonic relationship between BD and soil  
255 macroporosity as reported by Pöhlitz et al. (2020) using the epi-anecic earthworm *L. terrestris*. This  
256 earthworm species has a mono-typical burrowing behavior with the creation of an often single ver-  
257 tical burrow, eventually with a few lateral branches close to the surface resulting in a Y-shaped bur-  
258 row system (Shipitalo and Butt, 1999). These features make this species less prone to drastically  
259 decreasing burrow length since their burrow is also a vital shelter.

260 Burrow diameter gradually decreased when BD increased for both species, but this change was  
261 more marked for *A. chlorotica* for which we already observed a significant difference between 1.18  
262 and 1.29 g cm<sup>-3</sup>. As the earthworms were of similar weight at the beginning of the experiment, this  
263 suggests that they may have created thinner burrows in denser soil, probably to save energy.

264 Other burrow system characteristics were also modified. The most drastic change was burrow sys-  
265 tem continuity. Its decrease with increasing BD was almost linear for *A. caliginosa*, but for *A. chlo-*  
266 *rotica* the most important changes occurred between 1.18 and 1.29 g cm<sup>-3</sup> with a sharp decrease of -  
267 60%. This suggests that the burrows of this species were less vertically continuous as soon as the  
268 BD increases. Moreover, the degree of anisotropy of the burrow systems increased significantly  
269 with BD for both species. This showed that the burrows had no preferential orientation at low BD  
270 but then a trend for a non-preferential orientation appeared for higher BD. Taken together, these ob-

271 servations further suggests that *A. chlorotica* burrows less vertically in soil with intermediate to  
272 higher BD values, even if we did not observe significant differences for verticality.

273

274 Overall, we did not observe large differences between the responses of the two species to the in-  
275 crease in BD. However, for most of the burrow system characteristics (except the degree of anisot-  
276 ropy) the decreases observed at 1.34 compared to 1.18 g cm<sup>-3</sup> were less marked for *A. caliginosa*  
277 than for *A. chlorotica*, even if the values observed for a BD of 1.38 g cm<sup>-3</sup> were similar for both spe-  
278 cies. Thus although a BD of 1.38 g cm<sup>-3</sup> was a strongly limiting factor for both species, *A. caliginosa*  
279 appeared to have a better resistance to lower but still high BD (1.34 g cm<sup>-3</sup>).

280

281 Another striking difference between the two species was the production of casts at the surface with  
282 *A. chlorotica* producing very few casts regardless of the BD. In contrast, *A. caliginosa* produced  
283 more casts and showed a tendency to cast more at the soil surface when BD increased (except for  
284 the highest BD but in that case the burrow volume was very limited). This corroborates previous  
285 findings (Rushton, 1986; Kretzschmar, 1991) showing that when the soil is compacted the earth-  
286 worms tend to produce more surface casts in order to increase the air-filled porosity and aeration  
287 within the soil. If we assume that the casts had the same BD than the soil from which they were  
288 created, we could compute the ratio of the volume of macroporosity (burrows) that was deposited at  
289 the soil surface as casts. In this case, the proportion of burrow volume increased with SBD for both  
290 species (Fig. S3) ; this increase was however higher for *A. caliginosa* with casts representing 100%  
291 of the burrow volume at the highest BD tested. The difference between the two species may partly  
292 be explained by the fact that *A. caliginosa* is epigeic at 16% (*A. chlorotica* at 0%) and thus is more  
293 prone to defecate at the soil surface.

294

295 **Consequences for water infiltration**

296 Breakthrough time characterizes the transient regime of infiltration while the infiltration rate is a  
297 measure of the stationary flow regime. In control samples without earthworms, infiltration rates de-  
298 creased by a factor of 4.4 and and breakthrough time increased by a factor of 7.6 when BD in-  
299 creases from 1.18 to 1.38 g cm<sup>-3</sup>. The occurrence of macropores (earthworm burrows) in the homo-  
300 geneous porous sample tended to mitigate the effect but only for the lowest BD. Infiltration rates  
301 and breakthrough times increased when BD increased. This finding was expected since an increased  
302 BD means lower macroporosity and generally a decreased continuity of such pores as illustrated in  
303 the present study with the decreased burrow continuity.

304 Endogeic burrows are often discontinuous, since they can be refilled by casts, and often less con-  
305 nected to the surface (Capowiez et al., 2014). Thus their involvement in increased water transfer  
306 efficiency is still open for debate. In the present study, we demonstrated that the effect of the activi-  
307 ty of endogeic species on infiltration was only significant for low BD (1.18 g cm<sup>-3</sup> for *A. caliginosa*  
308 and 1.18 and 1.23 g cm<sup>-3</sup> for *A. chlorotica*). This may appear to be counterintuitive: when the soil is  
309 compacted the burrows could have been preferential pathways for air and water transfer  
310 (Mossadeghi-Björklund et al., 2016). However these pathways are less vertical and only partial  
311 since the burrow continuity rapidly decreased when BD increased. In addition, the burrow systems  
312 were less extensive at moderate and high BD and thus their functional role was limited.

313 We observed a small but significant increase in the soil matrix greylevels surrounding earthworm  
314 burrows (for both species). Thus, regardless of the initial BD, earthworm burrowing appears to  
315 cause a constant increase in the BD around the burrows. This also limit the roles of earthworm bur-  
316 rows in water transfer (Pelisek et al., 2018) since the two species produced discontinuous burrows  
317 and thus water would need to enter and go out of several burrows to reach the bottom of the cores.  
318 Rogasik et al. (2014) previously showed that *L. terrestris* burrowing caused a lateral compaction of  
319 the soil around the burrows. We further demonstrate that this is true for the two studied endogeic

320 species. However, we were unable to detect an influence of BD on the intensity of this lateral com-  
321 paction even though the burrow diameter significantly decreased at the highest tested BD.

322

### 323 **Conclusions**

324 We demonstrated that increasing BD impaired the burrowing behavior of two common endogeic  
325 species, and that total burrow volume and length decreased linearly with increasing BD. Although  
326 the earthworms promoted the soil infiltration capacity compared to control samples without earth-  
327 worms, their effect was limited and only significant at low BD. These results were obtained using a  
328 single soil and only two species. The main findings could be compared with previous results ob-  
329 tained in other soils by computing an effective BD (Beylich et al., 2010) or by using a reference BD  
330 (Keller and Hakansson, 2010). Our results could be used in simulation models of earthworm behav-  
331 iour, such as the model recently presented by Roeben et al. (2020). In this model, an energy budget  
332 is applied for each earthworm and our data could help parameterize the energy costs of burrowing  
333 as a function of bulk density.

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439 **Figure legends:**

440 **Fig. 1** Examples of 3D burrow systems made by three individuals of either *A. chlorotica* or *A.*  
441 *caliginosa* for 6 weeks in soil cores with increasing bulk densities.

442 **Fig. 2** Volume of burrows (means + SE) created by three individuals of either *A. chlorotica* or *A.*  
443 *caliginosa* for 6 weeks in soil cores with increasing bulk densities. Bars bearing different letters are  
444 significantly different (each species was tested separately).

445 **Fig. 3** Continuity of the burrow systems (means + SE) made by three individuals of either *A. chlo-*  
446 *rotica* or *A. caliginosa* for 6 weeks in soil cores with increasing bulk densities. Bars bearing differ-  
447 ent letters are significantly different (each species was tested separately).

448 **Fig. 4** Degree of anisotropy of the burrow systems (means + SE) made by three individuals of either  
449 *A. chlorotica* or *A. caliginosa* for 6 weeks in soil cores with increasing bulk densities. Bars bearing  
450 different letters are significantly different (each species was tested separately).

451 **Fig. 5** Gain in water infiltration (means + SE) compared to control without earthworms, when three  
452 individuals of either *A. chlorotica* or *A. caliginosa* were incubated for 6 weeks in soil cores with  
453 increasing bulk densities. Stars indicate values significantly different from zero (each species was  
454 tested separately).

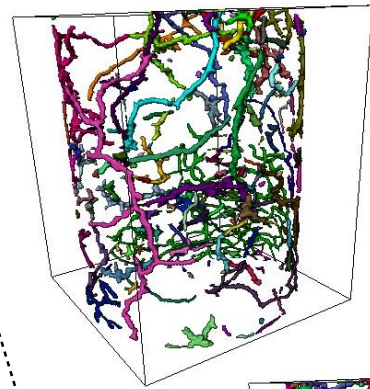
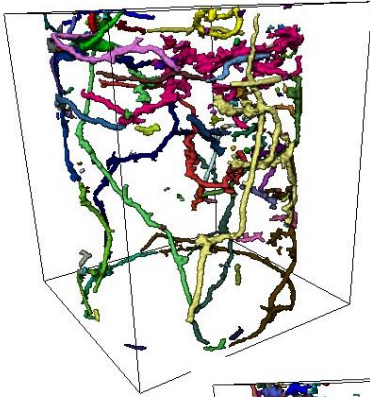
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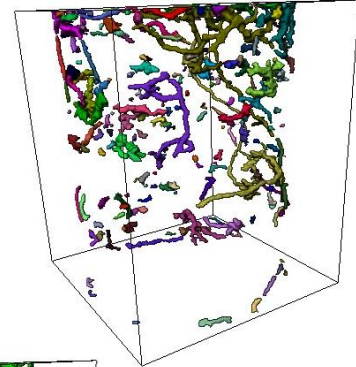
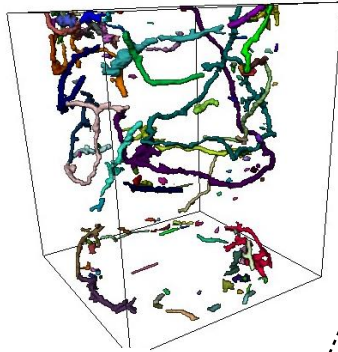
*A. caliginosa*

*A. chlorotica*

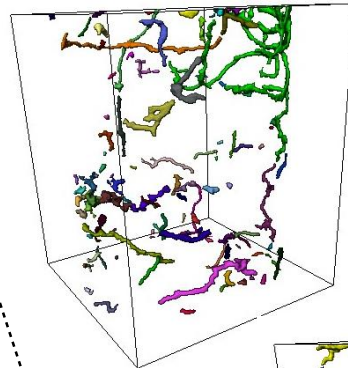
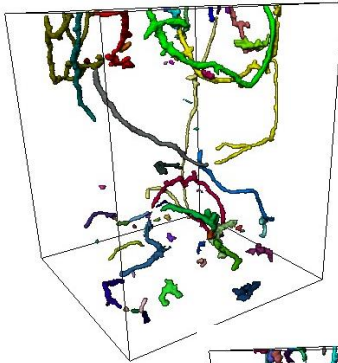
1.18 g cm<sup>-3</sup>



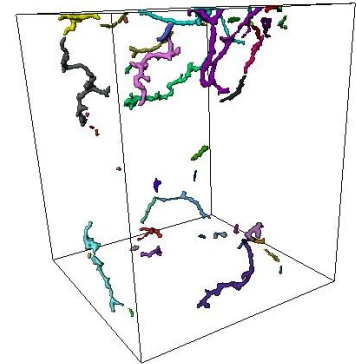
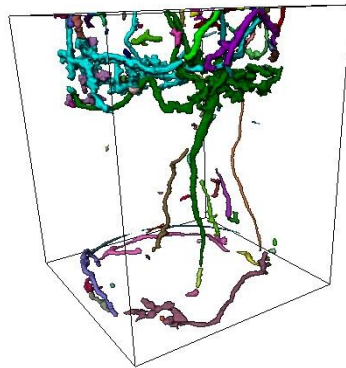
1.23 g cm<sup>-3</sup>



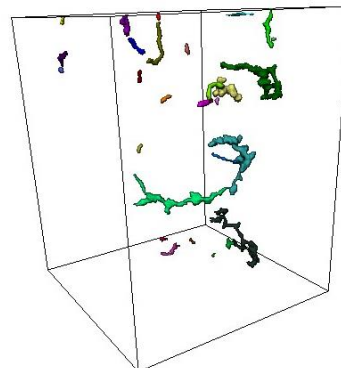
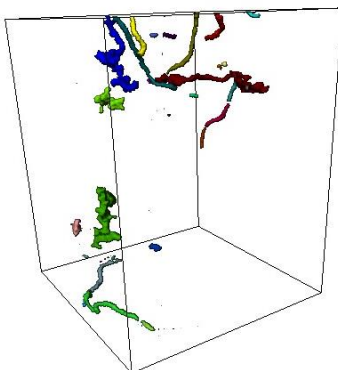
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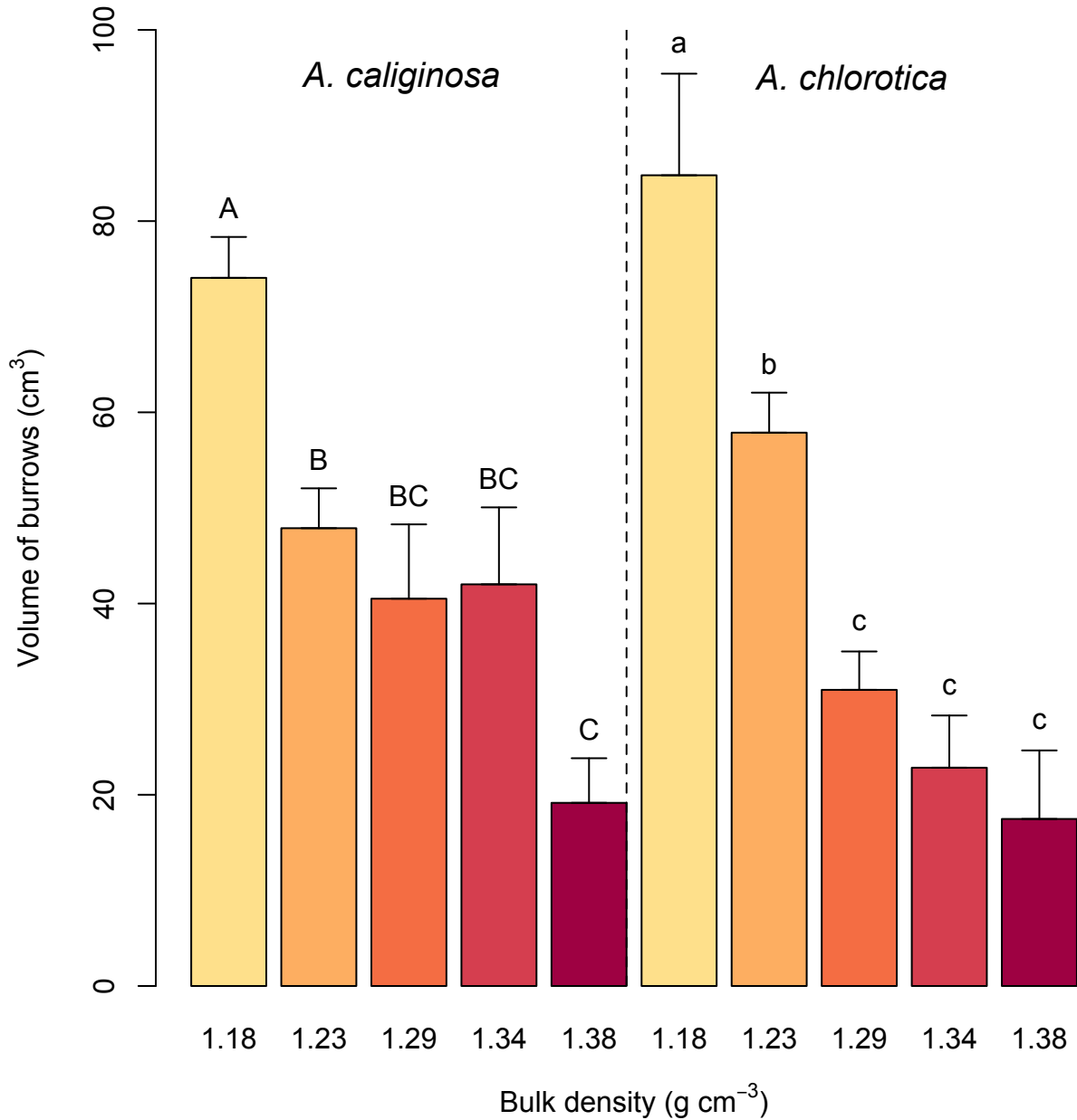


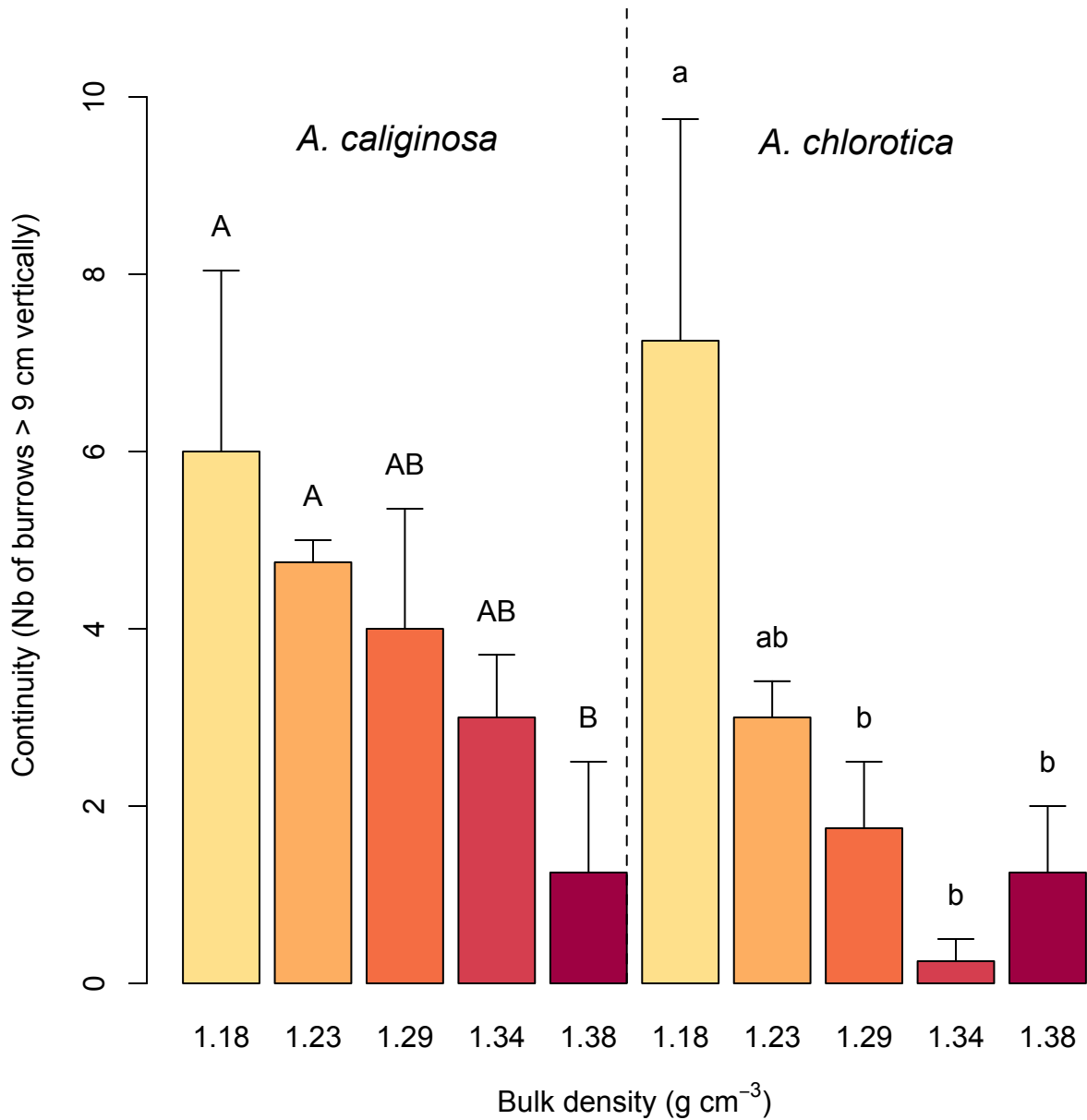
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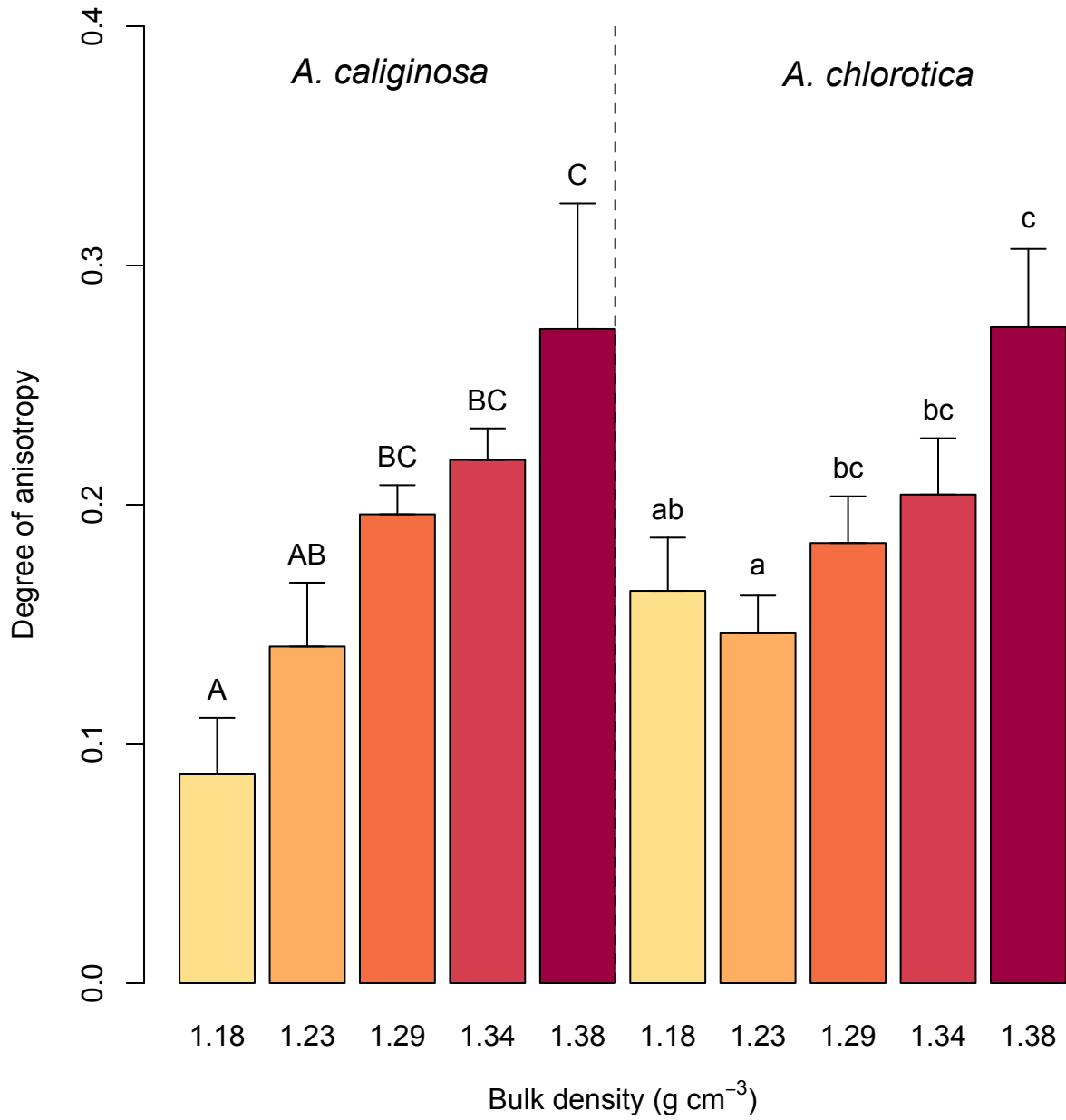


1.38 g cm<sup>-3</sup>

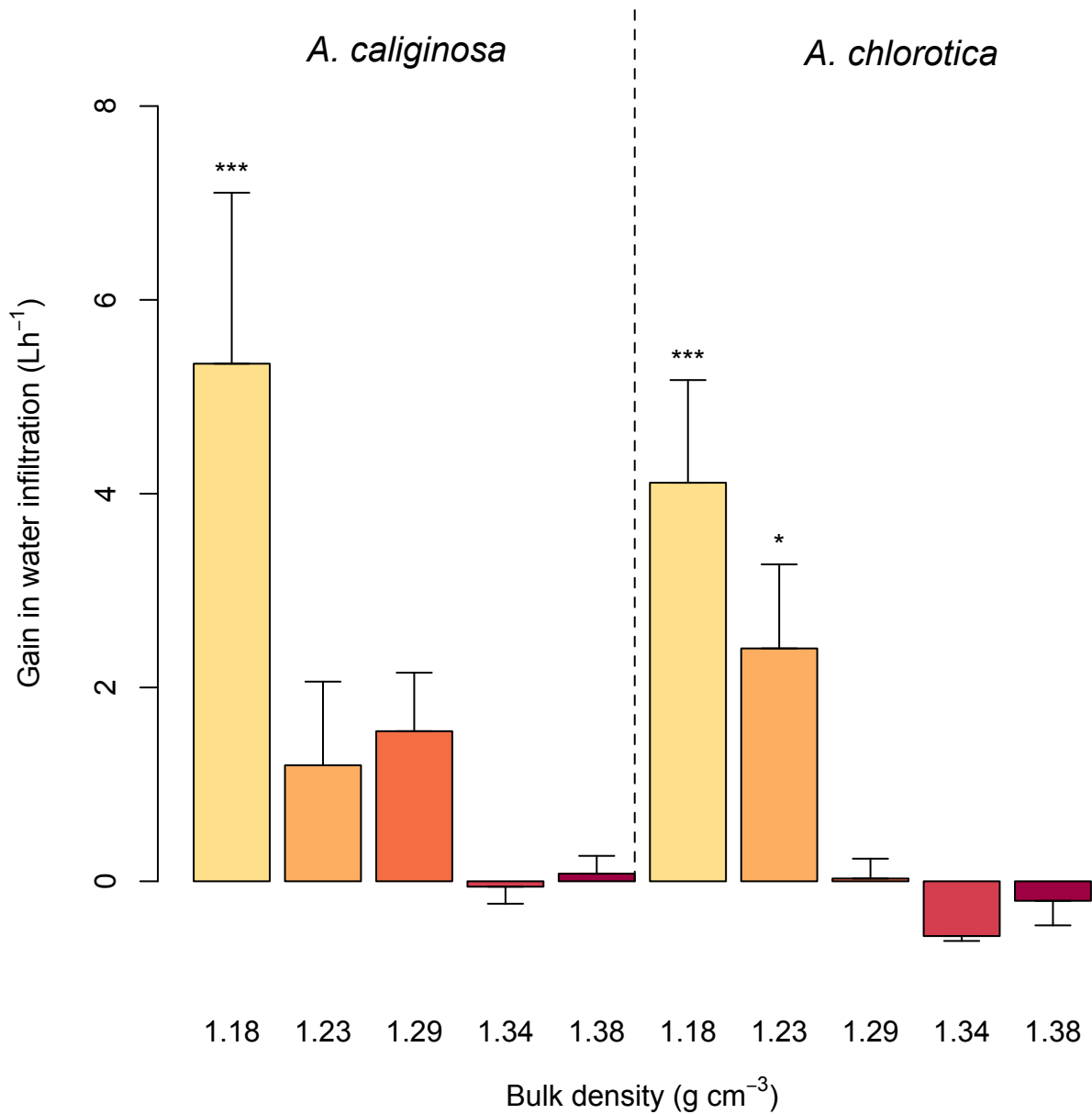












**Table 1.** Effects of an increase of soil bulk density on mean (SE) burrow diameter, total length and surface cast production in soil cores incubated with three individuals of either *Aporrectodea caliginosa* or *Allolobophora chlorotica*. ANOVA were carried out for each species separately.

Values bearing different letters are different at the 5% significance threshold.

	Soil bulk density (g cm <sup>-3</sup> )				
	1.18	1.23	1.29	1.34	1.38
<i>A. caliginosa</i>					
Burrow diameter (mm)	2.97 <sup>a</sup> (0.06)	2.96 <sup>ab</sup> (0.07)	2.87 <sup>ab</sup> (0.07)	2.82 <sup>ab</sup> (0.06)	2.65 <sup>b</sup> (0.14)
Burrow length (cm)	58.82 <sup>a</sup> (5.66)	38.52 <sup>ab</sup> (3.52)	34.74 <sup>b</sup> (6.73)	35.02 <sup>b</sup> (5.93)	15.73 <sup>c</sup> (3.95)
Surface cast production (g)	17.17 <sup>b</sup> (3.65)	15.17 <sup>b</sup> (1.84)	24.36 <sup>ab</sup> (4.97)	32.11 <sup>a</sup> (5.43)	24.85 <sup>ab</sup> (6.33)
<i>A. chlorotica</i>					
Burrow diameter (mm)	2.71 <sup>a</sup> (0.09)	2.62 <sup>a</sup> (0.09)	2.36 <sup>b</sup> (0.06)	2.30 <sup>b</sup> (0.06)	2.59 <sup>b</sup> (0.14)
Burrow length (cm)	73.89 <sup>a</sup> (9.85)	50.00 <sup>ab</sup> (4.48)	30.05 <sup>b</sup> (4.08)	21.50 <sup>b</sup> (4.52)	15.02 <sup>c</sup> (6.02)
Surface cast production (g)	11.12 (2.13)	10.42 (0.78)	8.49 (0.25)	11.59 (2.44)	6.03 (1.88)

**Table 2.** Mean (SE) infiltration rates and breakthrough times in the soil cores incubated with three individuals of either *A. caliginosa* or *A. chlorotica* or no earthworm (control) for six weeks. Values bearing different letters are statistically different (each species tested separately).

	Soil bulk density (g cm <sup>-3</sup> )				
	1.18	1.23	1.29	1.34	1.38
Infiltration rate (L h <sup>-1</sup> )					
<i>A. caliginosa</i>	9.95 <sup>a</sup> (1.58)	3.70 <sup>b</sup> (0.96)	4.00 <sup>b</sup> (1.00)	1.66 <sup>b</sup> (0.64)	1.12 <sup>b</sup> (0.52)
<i>A. chlorotica</i>	8.72 <sup>a</sup> (1.48)	4.91 <sup>ab</sup> (1.11)	2.48 <sup>b</sup> (0.79)	1.15 <sup>b</sup> (0.54)	0.84 <sup>b</sup> (0.46)
Control	4.61 (1.07)	2.50 (0.79)	2.45 (0.78)	1.71 (0.65)	1.04 (0.51)
Breakthrough times (mn)					
<i>A. caliginosa</i>	0.55 <sup>b</sup> (0.27)	6.89 <sup>a</sup> (1.31)	6.73 <sup>a</sup> (1.29)	9.08 <sup>a</sup> (1.51)	14.28 <sup>a</sup> (1.89)
<i>A. chlorotica</i>	1.93 <sup>b</sup> (0.69)	2.58 <sup>b</sup> (0.80)	9.60 <sup>ab</sup> (1.55)	18.80 <sup>a</sup> (2.17)	12.63 <sup>a</sup> (1.78)
Control	2.23 (0.75)	6.93 (1.32)	11.35 (1.68)	15.72 (1.98)	16.96 (2.06)