

1 **Interactions between organisms and parent materials of a**
2 **constructed Technosol shape its hydrostructural**
3 **properties**

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11

12 **Abstract**

13 There are no information on how organisms influence hydrostructural properties of
14 constructed Technosols and how such influence will be affected by the parent material
15 composition factor. In a laboratory experiment, Parent materials, which were excavated deep
16 horizons of soils and green waste compost (GWC), were mixed at six levels of GWC (from
17 0% to 50%). Each mixture was set up in the presence/absence of plants and/or earthworms, in
18 a full factorial design (n = 96). After 21 weeks, hydrostructural properties of constructed
19 Technosols were characterized by soil shrinkage curves. Organisms explained the variance of
20 hydrostructural characteristics (19%) a little better than parent-material composition (14%).
21 The interaction between the effects of organisms and parent-material composition explained
22 the variance far better (39%) than each single factor. To summarize, compost and plants
23 played a positive role in increasing available water in macropores and micropores; plants were
24 extending the positive effect of compost up to 40 and 50% GWC. Earthworms affected the
25 void ratio for mixtures from 0 to 30% GWC and available water in micropores, but not in
26 macropores. Earthworms also acted synergistically with plants by increasing their root
27 biomass resulting in positive effects on available water in macropores. Organisms and their
28 interaction with parent materials positively affected the hydro-structural properties of
29 constructed Technosols, with potential positive consequences on resistance to drought or

30 compaction. Considering organisms when creating Technosols could be a promising approach
31 to improve their fertility.

32 Keywords

33 Hydro-structural properties; Organic matter; Shrinkage curve; Plants; Earthworm; Interactions

34

35 **1 Introduction**

36 Pedogenesis results from the dynamic interaction between climate, parent rock and organisms.
37 The most important factor(s) have been debated for a long time (Wilkinson et al., 2009) and
38 studied independently (Jenny, 1941), but their interactions remain little understood
39 (Amundson et al., 2007; Paton, 1978). Understanding of the influence of bioturbation
40 (physical displacement by organisms): is not straightforward on soil formation (Amundson et
41 al., 2007; Wilkinson et al., 2009). Some authors consider biotic mixing agents as a secondary
42 cause of soil formation (Carson and Kirkby, 1972), while others argue that bioturbation plays
43 a major role in forming soil (James, 1998; Kemp, 1997; Paton, 1978; Wilkinson and
44 Humphreys, 2005).

45 Soils developed on non-traditional substrates and largely influenced by human activity are
46 now referenced as Technosols in the World Reference Base for Soil Resources. When
47 Technosols are technogenic materials or artifacts assembled deliberately to create soils, they
48 are called constructed Technosols (WRB 2014). Many urban planners and greenspace
49 enterprises are interested in constructed Technosols because these materials could be used as
50 an alternative to topsoil material removal from the countryside and the damage implied on the
51 collecting site which need ten thousand years at least for reconstruction. Also, transportation
52 costs and downsides could be avoided. Moreover, Technosols offer an opportunity to recycle
53 urban waste, such as excavated deep horizons/backfills from enterprises of the building
54 sector, sewage sludge from waste water plants or green waste from greenspaces enterprises or
55 local authorities. In this regard, Technosols offer another life to these materials, which
56 accumulation is urgent to cope with, due to health and environmental problems (Nemerow,
57 2009; Marshall and Farahbakhsh, 2013) while they could be used to improve urban ecosystem
58 services (Morel et al., 2014), and form a closed loop that reduces the impact of cities on the
59 environment. Constructed Technosols are different from other soils, because they are
60 designed assemblages of technogenic materials. Thus, the evolution of Technosols is different
61 compared to the pedogenesis of natural soils (soils that generally show genetic relationships

62 between the horizons they are composed of, and in which transitions among soils' types are
63 visible. Human does not influence their formation process (Lehmann and Stahr, 2007)).
64 However, Technosols exhibit some formation processes similar to those observed in natural
65 soil pedogenesis, such as decarbonization and aggregation (Séré et al., 2010).

66 The pedogenesis of constructed Technosol is particularly interesting. It begins with the
67 mixing of parent materials in a proportion chosen by the experimenter, whereas the initial
68 state of natural soils is never under the control of researchers. In this study, we will focus on
69 one specific aspect of Technosol pedogenesis: the physical structuration, by analyzing hydro-
70 structural properties.

71 Parent materials strongly influence the type of soil formed (Charman et al., 2000). Organo-
72 mineral composition of constructed Technosols determines several soil chemical and physical
73 properties (pH, cationic exchange capacity, texture, etc.) and affects their quality (Arocena et
74 al., 2010; Molineux et al., 2009; Olszewski et al., 2010; Rokia et al., 2014). The Influence of
75 organic matter and texture on compactability of Technosols (Paradelo and Barral, 2013) and
76 the formation of the organo-mineral complex in newly formed soil (Monserie et al., 2009)
77 have also been documented. However, hydro-structural properties have not yet been
78 investigated. This is of particular importance since constructed Technosols are often
79 influenced by compaction (Jangorzo et al., 2013). Moreover, they are expected to provide
80 water regulation services and to supply vegetation requirements. Therefore, we were
81 interested in determining influences of different functional groups of organisms on soil hydro-
82 structural properties. We focused on two kinds of organisms with different impacts on soil
83 physical structure. Earthworms make an important contribution to soil function by influencing
84 chemical, biological and physical soil processes (Edwards, 2004; Lavelle and Spain, 2001),
85 with consequences for ecosystem services (Blouin et al., 2013). Their major physical
86 contributions are due to their high consumption rates and burrowing activity that affect soil
87 structure, aggregation and aeration (Blanchart et al., 1997), which influence the hydric
88 properties of soil (Schrader and Zhang, 1997; Shipitalo and Butt, 1999). These modifications
89 of hydro-structural properties by earthworms have tremendous consequences for plant growth
90 (van Groenigen et al., 2014; Scheu, 2003; Eisenhauer et al., 2007). Plant roots and
91 rhizosphere inhabitants (microorganisms) also have a significant influence on aggregates and
92 their stability (Jastrow et al., 1998; Rillig et al., 2002), sometimes more significant than that
93 of earthworms (Blanchart et al., 2004). Roots penetrate the soil and create macropores which
94 guarantee the exchange of gases in the vadose zone (Beven and Germann, 1982). Roots also
95 create weak zones that fragment the soil and form aggregates, whose formation is

96 strengthened by wetting-drying cycles due to water uptake by the plant (Angers and Caron,
97 1998). In addition, plant root residues provide a food source for microorganisms and fauna,
98 which contribute to soil structure formation and stabilization (Innes et al., 2004). In return,
99 microorganism-mediated changes in soil structure affect plant growth, mostly by modifying
100 the root's physical environment (Dorioz et al., 1993).

101 In this study, we were interested in the effect of two soil-forming factors, i.e. parent materials
102 and organisms, on hydro-structural parameters via measurements of soil shrinkage curves
103 (SSC) which represents the concomitant decrease in soil volume and water mass during
104 drying (Haines, 1923). The influence of parent materials properties (especially clay content
105 and type) (Boivin et al., 2004), organic matter (Boivin et al., 2009) and organisms (Kohler-
106 Milleret et al., 2013; Milleret et al., 2009) on shrinkage properties has already been studied in
107 natural soils. This study addresses the question of materials-organisms interaction on the
108 hydrostructural properties of a constructed Technosols in a five-months microcosm
109 experiment with four "organism" treatments (control, plants, earthworms, plants and
110 earthworms) combined with six percentages of green waste compost/excavated deep horizons
111 under controlled climatic conditions.

112

113 **2 Materials and methods**

114 **2.1 Parent materials**

115 The mineral material excavated from deep horizons of soil (EDH) used in this study was
116 provided by the ECT Company (Villeneuve sous Dammartin, France). This material is
117 typically what is found when foundations are dug in the Ile-de-France. It is mainly the result
118 of the weathering of carbonated rock fragments of the Parisian Basin (France) from the
119 Eocene. For our study, we collected 500 kg of EDH at eight locations from the base of ECT's
120 landfill site, in order to have a composite sample representative of what may be used to
121 construct Technosols around Paris. EDH is classified as carbonated sandy soil (Nachtergaele,
122 2001). Our material was composed of 880 g kg⁻¹ sand, 100 g kg⁻¹ silt and 20 g kg⁻¹ clay after
123 carbonate (lime) removal, which represents 431 g kg⁻¹(W/W) of total dry mass. Without
124 carbonate removal, EDH was composed of 110 g kg⁻¹ particles < 2 µm in size, 300 g kg⁻¹
125 particles from 2--50 µm, and 590 g kg⁻¹ particles from 50 µm to 2 mm. X-ray diffraction
126 performed with a Siemens D500 diffractometer (Cu-Ka, 40 kV, 30 mA) identified quartz,
127 calcite and dolomite as major minerals. The concentrations of organic carbon and nitrogen

128 were measured by elemental analysis (Elementar Vario EL III). The green waste compost
129 (GWC) used in our experiment was composed of cuttings from urban areas. Table 1 shows
130 the main chemical properties of EDH and GWC.

131 **2.2 Experimental design and conditions**

132 EDH and GWC were mixed using a concrete mixer to prepare six different mixtures with
133 specific volumetric percentages of GWC at 0%, 10%, 20%, 30%, 40% and 50%. One liter of
134 each mixture was placed in a microcosm of 13×13×12.5 cm with maximum capacity of 1.2 L.
135 Water retention capacity of each mixture was measured at the beginning of the experiment by
136 using a pressure plate apparatus (Richards, 1948) with a water potential of -31 kPa. During
137 the experiment, microcosms were moistened two to three times a week with deionized water
138 to maintain soil moisture at 80% of field capacity for each mixture (Table S1).

139 Plants were sown 24 hours after watering the pots, and earthworms were introduced 24 hours
140 after sowing. Each percentage of GWC was combined with four treatments: a control without
141 organisms (C), a treatment with two individuals (0.5 ± 0.1 g each) of the endogeic earthworm
142 species *Aporrectodea caliginosa* (E), a treatment with *Lolium perenne* plants (50 seeds with a
143 80% germination rate scattered homogeneously on the microcosm surface) (P), and a
144 treatment with both earthworms and plants (EP). In total, 96 microcosms were divided into 24
145 treatments, each with four replicates.

146 Microcosms were kept 21 weeks in a climate chamber (S10H, Conviron, Canada) under the
147 following conditions: photoperiod of 12 h, luminosity of 500 ± 20 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$,
148 temperature at $22/20 \pm 0.2$ °C day/night respectively and 75 ± 2 % air humidity.

149 **2.3 Shrinkage analysis**

150 Technosol samples were collected from the surface of each microcosm at the end of the
151 experiment using a 5 cm high, 5 cm diameter cylinder and were placed on a wet porous plate
152 for saturation with deionized water according to the manual instructions of Eijkelkamp
153 (referee) for seven days by applying a water potential of 0 kPa at the base of the sample. The
154 shrinkage curve was continuously measured according to Braudeau et al. (1999) by using the
155 retractometer[®] apparatus. Water-saturated Technosol samples were placed in an oven at a
156 constant temperature (30°C) to provide continuous and rapid evaporation. An electronic scale
157 (0.01 g precision) ensured accurate measurement of water loss during drying. Each sample's

158 volume (diameter, height) was determined with laser beams and recorded along with its mass
159 every 10 minutes.

160 At the end of the measurement, samples were dried in an oven at 105°C for 48 h to measure
161 dry mass and bulk density. These data were converted into soil specific volume (V , $\text{cm}^3_{\text{soil}} \cdot \text{g}^{-1}$
162 $^{\text{dry soil}}$) and water content (W , $\text{g}_{\text{water}} \cdot \text{g}^{-1}_{\text{soil}}$). We then determined the soil shrinkage curve
163 (SSC) to describe hydro-structural properties, as proposed by Assi et al. (2014). The data
164 obtained by shrinkage measures were fitted according to the pedostructure model (Braudeau
165 et al., 2004). In this model, the SSC is subdivided into a maximum of four shrinkage phases
166 (interpedal/saturated (ip), structural (st), basic (bs) and residual (re) shrinkage phases) due to
167 the four types of water (W_{ip} , W_{st} , W_{bs} , W_{re}) (Fig. 1). The pedostructure is considered an
168 assembly of primary peds (aggregates formed by clay particles) that determines two nested
169 levels of organization: the macropore level (containing $W_{ma} = W_{ip} + W_{st}$) and the micropore
170 level (containing $W_{mi} = W_{re} + W_{bs}$). These levels do not refer to pore size by itself, but to water
171 pore behavior during soil drying. Based on this distinction, the two pore systems were called
172 plasma (micropores) and structural properties (macropores) (Boivin et al., 2004; Schäffer et
173 al., 2008).

174 The three transition points separating the four pseudo linear shrinkage phases (Fig. 1) are
175 points L, M and N, which are at the intersection of the tangent straight lines of the linear
176 phases. According to this model of SSC (Braudeau et al., 1999, 2004), the value of the water
177 content at each point is equal to the value of $\max(W_{st})$ for W_L , $\max(W_{mi}) = \max(W_{re}) +$
178 $\max(W_{bs})$ for W_M , and $\max(W_{re})$ for W_N . The other hydro-structural parameters are: slope of
179 the saturated phase (K_{ip}), slope of the structural phase (K_{st}), slope of the basic shrinkage phase
180 (K_{bs}), slope of the residual phase (K_{re}), and three parameters (k_L , k_M and k_N) related to the SSC
181 shape at points L, M and N, respectively. Finally, according to Braudeau et al., (2001):

$$182 \quad \text{Max} (W_{re}) = W_N \quad (1)$$

$$183 \quad \text{Max} (W_{bs}) = W_M - W_N \quad (2)$$

$$184 \quad \text{Max} (W_{st}) = W_L - W_M \quad (3)$$

185 Specific volume V as a function of the water content W obtained from the Braudeau model
186 was converted into a void ratio, (e , $\text{cm}^3_{\text{pore}} \cdot \text{cm}^{-3}_{\text{solid}}$) as a function of the moisture ratio
187 (v , $\text{cm}^3_{\text{water}} \cdot \text{cm}^{-3}_{\text{solid}}$). This step makes it easier to compare Technosols that have different
188 compositions and thus different particle densities. Considering Eqs. (4) and (5):

189 $v = (\rho_s/\rho_w) W$ (4)

190 $e = V \rho_s - 1$ (5)

191 With ρ_w the water density and ρ_s the particle density (g.cm^{-3}) calculated for all mixtures from
 192 measurements of GWC and EDH using a pycnometer on materials sieved at 2 mm (ISO
 193 17892-3:2004).

194 All hydro-structural parameters were transformed with Eqs. (4) and (5) and thus became the
 195 moisture ratio at macropore saturation (v_L), the moisture ratio at micropore saturation (v_M),
 196 the moisture ratio at the shrinkage limit (v_N), the four slopes (K_L , K_{st} , K_{bs} , K_{re}), parameters
 197 related to the SSC shape (k_L , k_M , k_N) and the void ratio at the end of the shrinkage period (e_0).

198 Considering these hydro-structural parameters (Braudeau et al., 2004), the ratio of the
 199 maximum available water for plants from macropores (v_{ma} , $\text{cm}^3_{\text{water.cm}^{-3}_{\text{solid}}}$) and the ratio of
 200 the maximum available water for plants from micropores (v_{mi} , $\text{cm}^3_{\text{water.cm}^{-3}_{\text{solid}}}$) can be
 201 calculated from the Eqs. (2) and (3) as follows:

202 $v_{ma} = v_L - v_M$ (6)

203 $v_{mi} = v_M - v_N$ (7)

204 The sum of both is the total moisture ratio (v_{Total} in $\text{cm}^3_{\text{water.cm}^{-3}_{\text{solid}}}$). Finally, volumetric
 205 water content (Θ , $\text{cm}^3_{\text{water.cm}^{-3}_{\text{soil}}}$) was calculated to compare available water reservoirs
 206 (holding capacities) for plants.

207 $\Theta = v \cdot (\rho_d/\rho_s) = v \cdot (\rho_d/\rho_s)$ (8)

208 With ρ_d the bulk density ($\text{g}_{\text{solid.cm}^{-3}_{\text{soil}}}$). Similarly, we calculated the volumetric water content
 209 from macropores (θ_{ma}) and micropores (θ_{mi}), by applying the following equations:

210 $\theta_{ma} = \theta_L - \theta_M$ (9)

211 $\theta_{mi} = \theta_M - \theta_N$ (10)

212 Eventually the sum of both known as the total volumetric water content for plants (θ_{Total}).

213 **2.4 Plant harvest and root size distribution**

214 Plants were cut at the soil surface 21 weeks after sowing. Fresh leaves were weighed, dried in
 215 an oven at 50°C for two days and weighed again. Root mass was estimated from one quarter

216 of the pot, since other quarters were used for physico-chemical and shrinkage analyses,
217 requiring non disturbed soil physical properties (i.e. root or earthworm sampling).

218 Dry root biomass distribution among diameter classes was determined according to the
219 method of Blouin et al. (2007). It is based on the granulometry method used to assess soil
220 texture: roots are dried, cut transversely with a mixer and placed on a column of sieves with
221 decreasing mesh size. During the shaking of the sieve column, root fragments with a section
222 diameter smaller than the mesh size pass through this mesh and stop on the first sieve with a
223 mesh size below that of the root section diameter. Biomass distribution is assessed by
224 weighing the biomass recovered in each sieve. Five diameter classes were chosen according
225 to sieve mesh size: 0-100, 100-200, 200-400, 400-800 and >800 μm .

226 **2.5 Data analysis**

227 We calculated means and standard errors of hydro-structural parameters for all treatments by
228 fitting the curves with the hydro-structural model (Table S2). The hydro-structural parameter
229 representing the slope of the interpedal K_{ip} phase, the k_M parameter related to the shape of the
230 soil shrinkage curves and K_{re} the slope of the residual phase were not included, since they
231 were constants for all mixtures ($K_{ip} = 1$), ($k_M = -53$) and ($K_{re} = 0$). Statistical analyses were
232 performed with the R 3.0.3 software (R Core Team 2014). To assess the correlation of each
233 factor's influence on the variance of the eight hydro-structural parameters, redundancy
234 analysis (RDA) was performed with the Vegan package (Jari Oksanen, F et al. 2013). Then
235 partial RDA was performed to decompose the variation of hydro-structural metrics according
236 to the combination of GWC, organisms and their interaction. Differences between treatments
237 were tested with Tukey's honest significance test. To identify which hydro-structural variables
238 separated the treatments, the MASS and ade4 packages were used for principal component
239 analysis (PCA) (Venables and Ripley, 2002) and for linear discriminant analysis (LDA) (Dray
240 and Dufour, 2007). Treatment separation based on hydro-structural variables was tested with
241 Wilks and Pillai tests. The influences of the presence/absence of earthworms and the
242 percentage of GWC were assessed with two-way or three-way ANOVA with GWC,
243 earthworms and plants taken separately. Independent variables were considered to have an
244 influence on dependent variables when the probability value was < 0.05 .

245

246 **3 Results**

247 **3.1 Plant growth and development**

248 Belowground biomass ranged from 1.7 g to 3.6 g and aboveground biomass from 2.9 g to 4.4
249 g, which amounted to a total biomass of 4.6 g to 8.1 g (Fig. 2). Two-way ANOVA showed
250 that both GWC percentage and the presence of earthworms had a positive effect on dry
251 belowground, aboveground, and total biomasses (Table 2). GWC percentage had almost no
252 influence from 0 to 30% on total biomass but increases plant production at 40% and 50%
253 (Fig. 2a, b, c). Earthworm presence had a positive effect on belowground biomass only at
254 50% GWC, whereas aboveground biomass was affected only in the 0-30% GWC range. As a
255 result, total biomass was always significantly higher in the presence of earthworms, except at
256 40% GWC. In average, earthworms increased total plant biomass of 21% (Fig. 2c). The best
257 treatment for plant growth was clearly the mixture of 50% GWC with earthworms, with a
258 total dried plant biomass of 8.1 g, which was significantly higher than all other mixtures,
259 except for 40% GWC with earthworms. There was no interaction between the effects of GWC
260 percentage and earthworms on plant biomasses, which means that these two effects are
261 additive. All parameters describing biomass allocation inside the plant, such as the root: shoot
262 ratio, the thick ($\geq 400\mu\text{m}$) and fine ($< 400\mu\text{m}$) root percentages, were not affected by the
263 presence of GWC percentage, earthworms or their interaction (Table 2); we thus concluded
264 that GWC percentage and presence of earthworms had a quantitative influence but not a
265 qualitative one, as growth was affected but not development.

266 **3.2 Specific influence of organisms and parent materials on the** 267 **hydrostructural parameters**

268 All our Technosols exhibited the classical sigmoid shape of the shrinkage curve reported for
269 most natural soils (Laurizen 1948; Braudeau et al. 1999; Peng and Horn 2005) (Fig. 3, 4);
270 thus, shrinkage phases (residual, basic, structural and the saturating shrinkage phase) were
271 easy to recognize. All the parameters deduced from SSC are given in Table S2.

272 High GWC percentage caused moisture ratio v and void ratio e to increase (Fig. 3). The
273 positive effect of GWC percentage was particularly important in treatments with plants at
274 50% GWC (Fig. 3c) and in treatment with earthworms and plants at 40 and 50% (Fig. 3d).
275 Earthworms showed a positive influence on the void ratio in the 0-30% GWC range, but this

276 positive effect disappeared at 40 and 50% GWC (Fig. 4). The influence of plants on void ratio
277 was positive for 10, 20, 30 and 50% GWC but not at 0 and 40% GWC (Fig. 4). The
278 simultaneous presence of plants and earthworms resulted in a positive effect on void ratio for
279 all mixtures (Fig. 4). For example, e_0 varied from 0.9-1.4, 1.0-1.4, 0.9-1.6, 1.2-1.9 $\text{cm}^3.\text{cm}^{-3}$,
280 for control, earthworms, plants and plants and earthworms respectively (Fig. 4). This was
281 corresponding to an increase of 59% in the presence of plants, 42% in the presence of
282 earthworms, and 77% in the presence of both plants and earthworms as compared with the
283 control, for the void ratio at macropore saturation (v_L) in the 50% GWC mixture. The
284 moisture ratio was also positively affected by the GWC percentage, for example when we
285 compared moisture ratio at macropore saturation we noticed an increase of 59% between
286 treatments 0% and 50% GWC in the control without organisms (Fig. 3a). SSC revealed that
287 the presence of organisms had a somewhat similar effect on hydrophysical properties of
288 Technosols than GWC percentage: for example, the aspect of shrinkage curves when GWC
289 was 0% in the presence of earthworms and plant seemed like the control treatment at 30%
290 GWC (Fig. 4): e_0 ($e_0 = 1.1$) and total moisture ratio ($\approx 1 \text{ cm}^3.\text{cm}^{-3}$) (Table S2). The slopes in
291 the structural phase (K_{st}) was steeper in the presence of plant. We noticed that the structural
292 phase in the presence of earthworms reveals to be shorter for 40 and 50 % GWC than in the 0-
293 30% GWC range (Fig. 4).

294 RDA performed on eight hydro-structural parameters of the Table S2) showed that the factors
295 "GWC percentage" and "organisms" had an influence on hydro-structural parameters. The
296 total percentage of variance explained by these factors was high: 72% ($P = 0.005$). The
297 influence of factors taken independently was not very high: the total percentage of variance
298 explained by the GWC percentage, regardless of the organisms, was 14% ($P = 0.005$), while
299 the total percentage of variance explained by the organisms, regardless of the GWC
300 percentage, was 19% ($P = 0.005$). Taken together, the single factors accounted thus for 33%
301 of explained variance, whereas their interaction (organisms x GWC percentage effect,
302 estimated from the subtraction of single factors effects from total variance) was responsible
303 for 39% of the variance (72% - 33%). This means that predicting variations in hydro-
304 structural parameters of our Technosols requires taking into account variation in parent
305 materials and organisms simultaneously.

306 The LDA explained 76% of hydro-structural properties observed variance ($P < 0.001$; Wilks
307 and Pillai tests) (Fig. 5). Axis 1, which explained 42% of the total variance, distinguished

308 treatment “earthworms” from treatment “earthworms and plants” whereas axis 2, which
309 explained 26% of the total variance, separated the “control” and the “plants” treatments. By
310 relating the correlation circle (Fig. 5a) to the factorial plan (Fig. 5b) we found that: (i) the
311 parameter related to the shape of shrinkage curves between interpedal and structural phases
312 (K_L) was higher for the control than for organism treatments; (ii) earthworms increased
313 moisture ratio at the shrinkage limit (v_N); (iii) plants increased the slope of the structural
314 phase (K_{st}); (iv) the simultaneous presence of plants and earthworms increased the moisture
315 ratio at saturated macropores (v_L), minimum void ratio (e_0), and a parameter related to the
316 shape of shrinkage curves (K_N).

317 Additional PCA were performed to characterize the effect of organisms on hydro-structural
318 properties for each GWC percentage. The effect of plants was not significant at 0%, 10%, and
319 20% GWC ($P > 0.05$, Monte Carlo test), while it was significant at 30%, 40% and 50% of
320 GWC ($P < 0.05$, Monte Carlo test). In contrast, combined influences of plants and
321 earthworms were always significant ($P < 0.05$, Monte Carlo test).

322 **3.3 Influence of organisms and parent materials on moisture ratio and** 323 **available water for plants**

324 The complete ANOVA model with GWC percentage, earthworms and plants had a significant
325 effect ($P < 0.001$) on micropore, macropore and total moisture ratios and available volumetric
326 water contents (Table 3). Considering single factors, increasing the GWC had a positive
327 influence on micropore, macropore (GWC < 40%) and total moisture ratios and available
328 volumetric water contents ($P < 0.001$). Plants had an influence on all of the previous
329 variables, except for micropore volumetric available water content. Earthworms affected
330 micropore and total moisture ratios, but not the macropores moisture ratio; they affected
331 micropore volumetric available water content (Table 3).

332 The presence of earthworms influenced the effect of GWC percentage on moisture ratio and
333 total volumetric available water contents at macropore and micropore. For example, in the
334 absence of earthworms, GWC percentage had a positive influence on moisture ratio at
335 macropore for 0-40% GWC, while in the presence of earthworms, moisture ratio at macropore
336 decreased at percentages of 30-50%. The presence of plants modified the influence of GWC
337 on moisture ratios at micropore and macropore, and total volumetric available water at
338 macropore and micropore. For example, in the absence of plants, the influence of GWC
339 percentage on moisture ratio at macropore was positive at percentages of 0-40% and became

340 negative at 50%, whereas in the presence of plants, the influence of GWC percentage was
341 positive regardless of its percentage (Fig. 4a). A similar influence was observed for the
342 interaction between plants and GWC percentage on macropore volumetric available water
343 (Fig. 6d). The interaction between earthworms and plants had a significant effect only for
344 moisture ratios in micropore and macropore but not for total moisture ratio, suggesting
345 opposite effect on micropores and macropores (Table 3). Indeed, v_{ma} was higher in the plants
346 and earthworms treatment as compared with the plant treatment and the earthworm treatment,
347 but v_{mi} was higher in the earthworm treatment or the plant treatment as compared with the
348 plants and earthworms treatment. The triple interaction had a significant influence on
349 moisture ratio and volumetric available water at macropore (Table 3). For example, in the
350 absence of plants, earthworms amplified the negative influence of high GWC percentages on
351 moisture ratio at macropore, whereas in the presence of plants, earthworms amplified the
352 positive influence of plants at high GWC percentages, giving a maximum moisture ratio at
353 macropore and total volumetric available water. (Fig. 6a and 6d).

354 **3.4 Relation between total plant biomass and available water**

355 Linear regressions between total plant biomass (g) and available volumetric water content
356 ($\text{cm}^3_{\text{water}} \cdot \text{cm}^{-3}_{\text{soil}}$) were performed using earthworm presence or absence as a categorical
357 independent variable (Fig. 6). Significant differences were found between total plant biomass
358 with or without earthworms ($P < 0.001$), and plant biomass was higher with earthworms than
359 without. In addition, total plant biomass increased with available water ($P < 0.001$). However
360 the difference in slope of the two linear regressions (Fig. 6) was not significant ($P = 0.569$).
361 The best equations summarizing the relation between total dried plant biomass (X, g) and
362 plant available water ($\theta_{\text{Total}}, \text{cm}^3_{\text{water}} \cdot \text{cm}^{-3}_{\text{soil}}$) were: $X = 8.97 \cdot \theta_{\text{Total}} + 4.07$ and $X = 8.97 \cdot \theta_{\text{Total}} +$
363 2.69 with and without earthworms, respectively ($P < 0.001$, adjusted $r^2 = 0.65$). Table S3
364 showed the results of both equations.

365

366 **4 Discussion**

367 Shrinkage analysis was initially developed to describe hydrostructural properties of natural
368 soils (Haines 1923; Milleret et al. 2009) and it was used by Kohler-Milleret et al. (2013) and
369 Milleret et al. (2009) to evaluate the influence of organisms in natural soils. However, the

370 effect of organisms on hydrostructural properties of constructed Technosols has never been
371 studied before. Our study shows that shrinkage curve analysis was relevant for describing
372 Technosol structure and water-holding capacities. In our case, parent materials exhibited
373 highly divergent behaviors: EDH showed a SSC with the typical sigmoid shape that reveals
374 two levels of organization (presence of both micropores and macropores). However, the green
375 waste compost shrinkage curve had a hyperbola shape (Deeb et al.,2015). Thus, the behaviour
376 of the mixtures was difficult to predict. Here, we showed two embedded levels of
377 organization in the mixtures, with a sigmoid shape even at the highest GWC percentage (50%,
378 V/V). Because this organization is often, but not always, observed in natural soils, we
379 conclude that after five months, mixtures of mineral and organic materials behave as many
380 natural soils from a hydro-structural viewpoint.

381 **4.1 Influence of green waste compost on hydro-structural properties**

382 Shrinkage curve analysis indicated a positive correlation between the amount of GWC
383 percentage and the quantity of macropores and micropores. This is likely due to organic
384 matter present in the GWC: an increase in total void ratio was also observed in natural soil
385 amended with organic matter (McCoy 1998; Marinari et al. 2000; Tejada and Gonzalez 2003)
386 and recently in Technosols (Paradelo and Barral 2013). The addition of GWC to EDH seems
387 a promising strategy to obtain useful hydric properties that match plant needs for water and
388 are similar to those observed in natural organic soils.

389 **4.2 Influence of earthworm *Aporrectodea caliginosa* on hydro-structural** 390 **properties**

391 Earthworms were responsible for a significant increase in total moisture ratio (Fig. 5c). This
392 was the result of an increase in moisture ratio at saturated micropore, not macropore (Fig. 5).
393 Through this mechanism, earthworms are likely to have a positive impact in climates with
394 occasional droughts. Earthworms might thus help plants to face a water deficit in drying
395 Technosols and effectively contribute to water regulation. This result was surprising:
396 earthworms are generally known to affect macroporosity through their galleries. Our results
397 differed from those obtained with *Allolobophora chlorotica*, an endogeic earthworm that
398 compact the soil and was responsible for a decrease in porosity, measured by shrinkage curves
399 (Kohler-Milleret et al., 2013; Milleret et al., 2009). These discrepancies between results could
400 be due to the endogeic earthworm influences on hydro-structural properties that are species-

401 specific, or to the parent materials used in the experiment. For example, when the percentage
402 of GWC was $> 30\%$, the soil was also slightly compacted by earthworm. On the contrary,
403 with GWC ratio $\leq 30\%$, earthworm tends to increase void ratio (Fig. 4). The absence of an
404 increase in macroporosity caused by earthworms could also be explained by a progressive
405 compaction of the soil throughout the experiment, with a decrease in macroporosity, as is
406 observed in Technosols (Jangorzo et al. 2013). This phenomenon could be particularly
407 common with experimental Technosols made of sieved parent materials, which have never
408 been subjected to previous shrinkage.

409 **4.3 Influence of *Lolium perenne* on hydro-structural properties**

410 The general influence of roots on soil structure was observed by Monroe and Kladvko
411 (1987), Angers and Caron (1998), and Kautz et al., 2013. This positive effect is mainly due to
412 plants' abilities to create macro-aggregates and macropores. Similar results have been
413 reported in other studies (Reid and Goss 1982; Caron et al. 1996). Moreover, the positive
414 influence of plants on moisture ratio at macropore increased with the presence of earthworms.
415 It was not due to the direct influence of earthworms, which improved moisture ratio at
416 saturated micropore (v_N and v_M) but had a null influence on moisture ratio at saturated
417 macropore. This synergistic effect between plants and earthworms was thus likely to be due to
418 an increase of the plant influence in the presence of earthworms (Fig. 6). Indeed, earthworms
419 were increasing plant root biomass (Fig. 2), and thus the positive effect of plant roots on
420 hydro-structural properties was improved. This result emphasizes the importance of
421 considering ecological interactions among functional groups such as plants and earthworms.
422 We also showed how plants and earthworms can help confront one of the main problems
423 encountered by Technosols: compaction. Technosols often tend to compact with time
424 (Jangorzo et al. 2013). Organisms such as plants or earthworms are responsible for
425 maintaining a high volume of voids and moisture per solid-volume unit (void and moisture
426 ratios, respectively). By introducing these organisms at the very beginning of Technosol
427 creation, i.e. before compaction, managers could initiate a virtuous cycle in which organisms
428 maintain loose soil structure. This favors the establishment of other organisms that maintain
429 their own habitats, which in turn could benefit from plants and earthworms by preventing later
430 compaction.

431 Because the influence of plants on hydro-structural properties was significant at 30-50%
432 GWC, one had to consider the initial composition of mixtures of materials to benefit from this
433 organismal positive feedback.

434 **4.4 Interactions between organisms and parent materials in Technosol** 435 **pedogenesis**

436 This study allows comparing the influence of the proportion of parent materials (0-
437 50% GWC) and the presence of organisms (presence/absence of plants and earthworms) on
438 pedogenesis. These situations are far from covering all kinds of parent materials and
439 organisms, but are a first attempt to compare the relative importance of soil-forming factors
440 under experimental conditions based on parent materials that never experienced the biological
441 activity of macro-organisms such as plants and earthworms. We found that variations in
442 Technosol hydro-structural properties were poorly explained by parent materials alone (14%
443 of explained variance) and by organisms alone (19% of variance), whereas
444 materials*organisms interaction explained more than the sum of their individual influences
445 (39% > 33%). This complexity brought about by ecological interaction between organisms
446 and their abiotic environment could partly explain the debate between those considering that
447 organisms play a negligible role in pedogenesis (Jenny 1941; Carson and Kirkby 1972) versus
448 those stressing their importance (Paton 1978; Wilkinson and Humphreys 2005). Indeed, if the
449 influence of organisms is particularly important in interaction with parent materials, its
450 observation may be random. Pedogenesis, and more particularly in the case of Technosol,
451 appears as an internal disciplinary field of study that needs to ecological aspects. We found
452 that biological activity improved Technosol properties by increasing aggregation, porosity and
453 water-retention capacity, with potential consequences on resistance to drought and erosion.
454 An original research perspective could be to investigate benefits of these changes caused by
455 plants and earthworms for their own survival and reproduction to determine if these biological
456 activities increase the fitness of these organisms and could thus be considered as niche
457 construction (Odling-Smee et al. 1996).

458

459 **5 Conclusions**

460 In a nutshell, we found that compost and plants play a positive role in macroporosity and
461 microporosity in Technosols, while earthworms affect only microporosity. GWC percentage
462 positively affected macroporosity up to a percentage of 30% and plants were responsible for
463 extending this positive influence at 40% and 50% GWC. The simultaneous presence of
464 earthworms and plants was responsible for a synergistic positive influence on macroporosity.
465 These observations highlighted the need to consider plants not only as an output indicating the
466 level of fertility, but also as an actor in Technosol construction, like earthworms. Organisms
467 that physically modify their environment by creating, destroying or maintaining ecological
468 niches have been called “ecosystem engineers” (Jones et al. 1994). These ecosystem
469 engineers can help restore ecosystems (Byers et al. 2006) and create new ecosystems such as
470 constructed Technosols by assisting managers, who could “sub-contract” one aspect of
471 management. Therefore, instead of increasing the amount of compost, which is usually
472 expensive, managers could avoid the difficult-to-explain negative influence of high
473 percentages of compost by favoring conservation, recolonization or inoculation of ecosystem
474 engineers such as plants and earthworms, especially in combination (Blouin et al. 2013).

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643

644 Table 1. Mean \pm 1 s.e. (n = 4), Main agronomic properties of technogenic materials used to make the constructed
645 Technosols. EDH: excavated deep horizons; GWC: green waste compost

Property	EDH	GWC
pH _{H2O}	8.3 \pm 0.0	7.9 \pm 0.1
pHKCL	8.1 \pm 0.1	7.5 \pm 0.1
Organic carbon (g kg ⁻¹)	0.38 \pm 0.0	210.41 \pm 4.2
Total nitrogen (g kg ⁻¹)	0.03 \pm 0.0	1.47 \pm 0.0
Particle density (g cm ⁻³)	2.75 \pm 0.2	2.06 \pm 0.1
Bulk density (g cm ⁻³)	1.33 \pm 0.0	0.61 \pm 0.0
The residual moisture content after air-drying (g kg ⁻¹)	65.8 \pm 4.0	87.9 \pm 2.3

646

647 Table 2 Two-ways ANOVA showing the effects of the presence/absence of earthworms (E) and the proportion
 648 of green waste compost (GWC) in the mixtures on plant dry biomasses, shoot: root ratio and root system
 649 structure (thick root $\geq 400\mu\text{m}$ and fine root $< 400\mu\text{m}$) (n = 48). The number in the table are the F-values,
 650 significance codes: *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$, ns: $P > 0.05$.

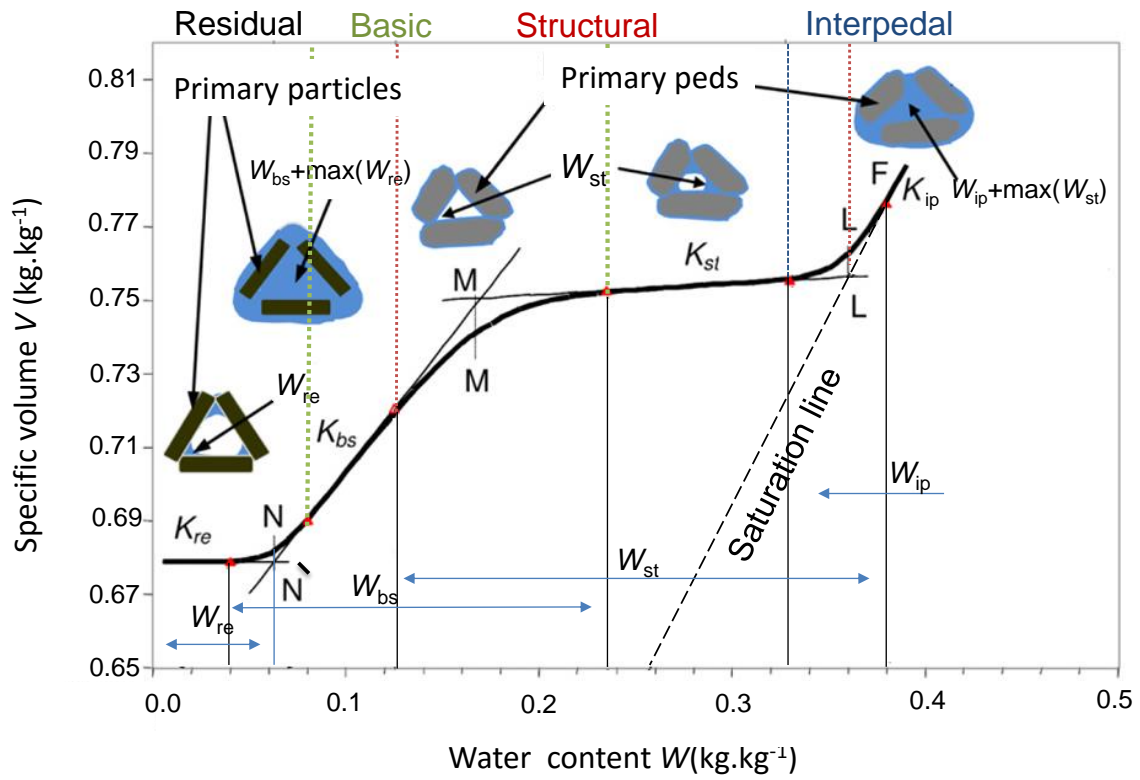
	d.f.	Aboveground biomass (g)	Belowground biomass (g)	Total biomass (g)	Shoot:root ratio	Thick root proportion	Fine root proportion
Complete model	11	11.29***	5.85***	13.33***	1.27 ^{ns}	0.78 ^{ns}	0.95 ^{ns}
GWC	5	10.27***	8.73***	16.22***	2.08 ^{ns}	0.49 ^{ns}	0.72 ^{ns}
E	1	65.65***	15.24***	60.12***	0.14 ^{ns}	0.62 ^{ns}	1.59 ^{ns}
GWC * E	5	1.43 ^{ns}	1.08 ^{ns}	0.39 ^{ns}	0.68 ^{ns}	0.56 ^{ns}	1.05 ^{ns}

651

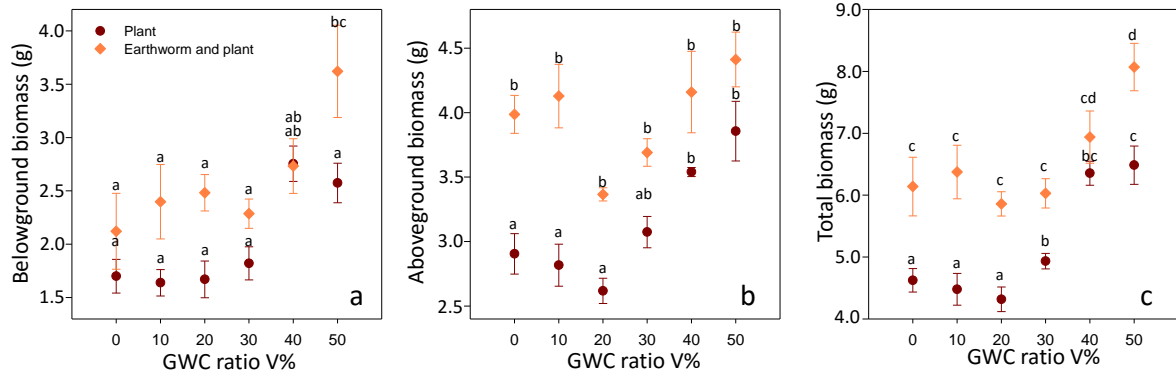
652 Table 3 Three ways ANOVA testing the effect of green waste compost (GWC), earthworms (E) and plants (P)
 653 on the maximum moisture ratio from macropores ($v_{ma} \text{ cm}^3_{\text{water}} \cdot \text{cm}^{-3}_{\text{solid}}$), maximum moisture from micropores
 654 ($v_{mi} \text{ cm}^3_{\text{water}} \cdot \text{cm}^{-3}_{\text{solid}}$), total moisture ratio ($v_{\text{Total}} \text{ cm}^3_{\text{water}} \cdot \text{cm}^{-3}_{\text{solid}}$), macro available water ($\theta_{ma} \text{ cm}^3_{\text{water}} \cdot \text{cm}^{-3}_{\text{soil}}$),
 655 micro available water ($\theta_{mi} \text{ cm}^3_{\text{water}} \cdot \text{cm}^{-3}_{\text{soil}}$) and finally total available water ($\theta_{\text{Total}} \text{ cm}^3_{\text{water}} \cdot \text{cm}^{-3}_{\text{soil}}$) (n = 96). The
 656 number in the table are the F-values, significance codes: *: $P \leq 0.05$, **: $P \leq 0.01$, *** $P \leq 0.001$, ns: $P > 0.05$.

	d.f.	v_{ma}	v_{mi}	v_{Total}	θ_{ma}	θ_{mi}	θ_{Total}
Complete model	23	13.68***	18.63***	34.91***	10.73***	26.77***	23.8***
GWC	5	34.35***	122.36***	124.30***	13.89***	103.01***	98.61***
P	1	66.16***	23.97***	43.06***	35.47***	0.07ns	16.88***
E	1	0.42ns	31.62***	19.59***	0.36ns	4.26*	1.51ns
P * E	1	5.63*	7.25**	1.88ns	2.28ns	3.09ns	0.23ns
GWC * P	5	27.64***	4.87***	1.46ns	17.97***	16.16***	2.64*
GWC * E	5	3.55**	0.96ns	1.78ns	2.41*	2.02ns	2.73*
GWC * P * E	5	11.47***	1.42ns	1.26ns	7.44***	0.45ns	1.80ns

657



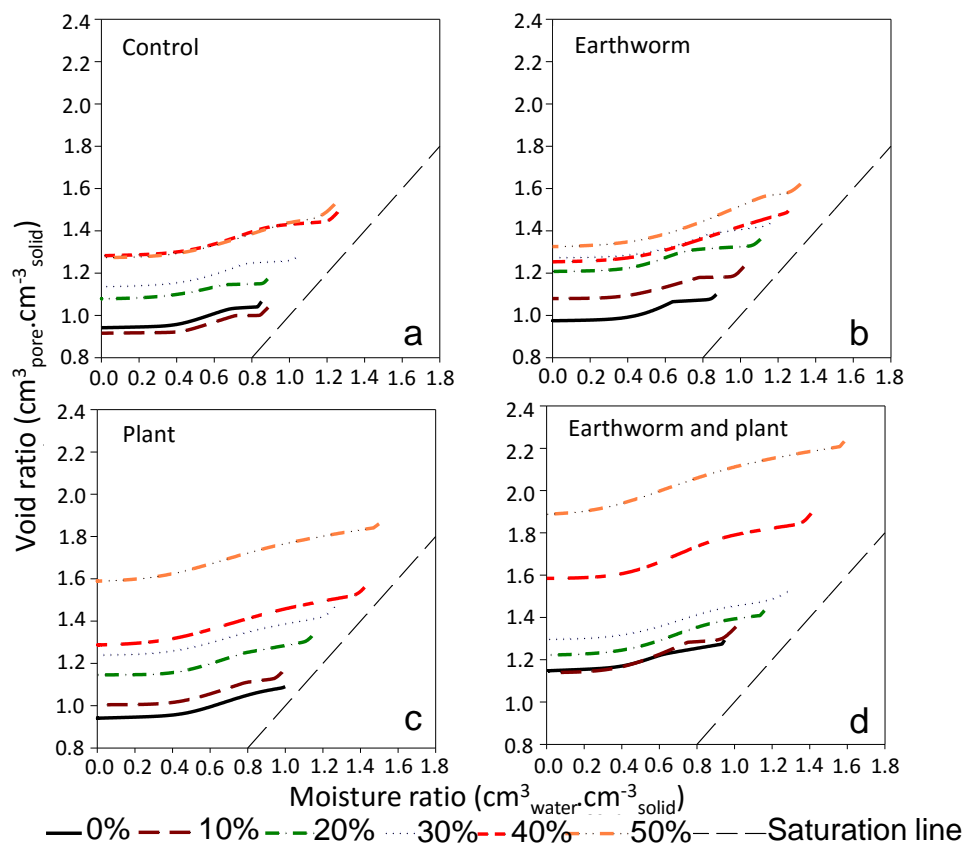
658
 659 Figure 1. Configurations of water partitioning in macropores and micropores related to the
 660 shrinkage phases of a standard shrinkage curve (water content W , specific volume V).
 661 (Adapted from Braudeau et al, 2004)



662

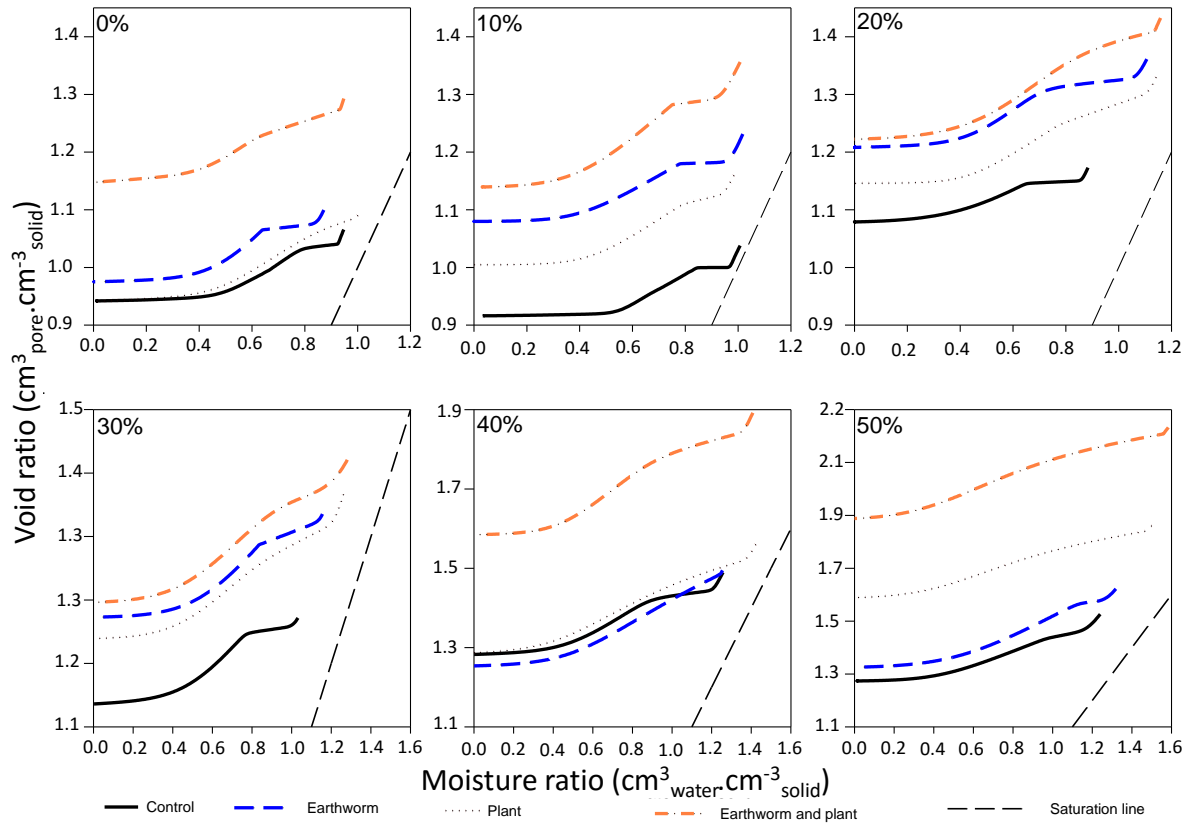
663 Figure 2. **a** Belowground, **b** aboveground and **c** total biomasses production of *Lolium perenne*
 664 according to different ratios of green waste compost in the presence/absence of the earthworm
 665 *Aporrectodea caliginosa*; mean \pm s.e., n = 4 per treatment. Tukey test, significant differences
 666 are indicated by different letters, $P < 0.05$.

667



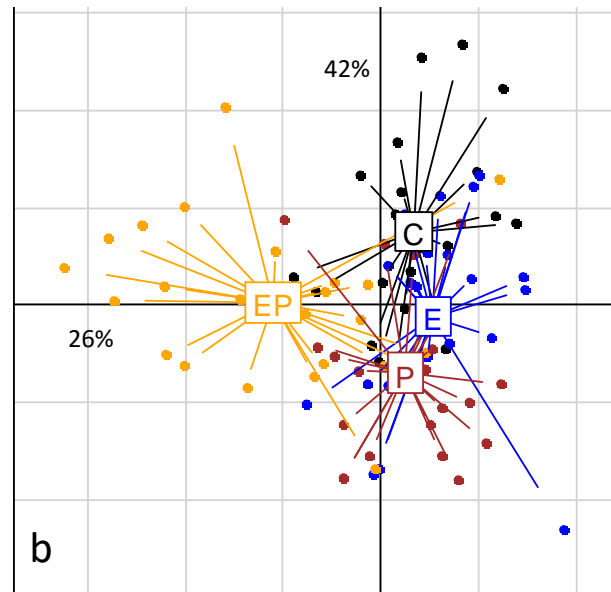
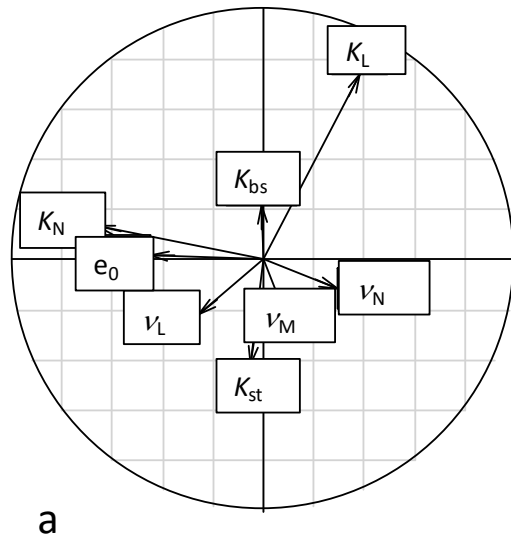
669

670 Figure 3. Averaged shrinkage curves ($n = 4$ per curve) for the six mixtures of green waste
 671 compost (GWC) and excavated deep horizons (0, 10, 20, 30, 40, 50% of GWC) reported as
 672 the void ratio as a function of the moisture ratio. Each panel represents one of the four
 673 treatments: (a) control, (b) earthworms, (c) plants, (d) earthworms and plants. The dashed line
 674 represents the saturation line.



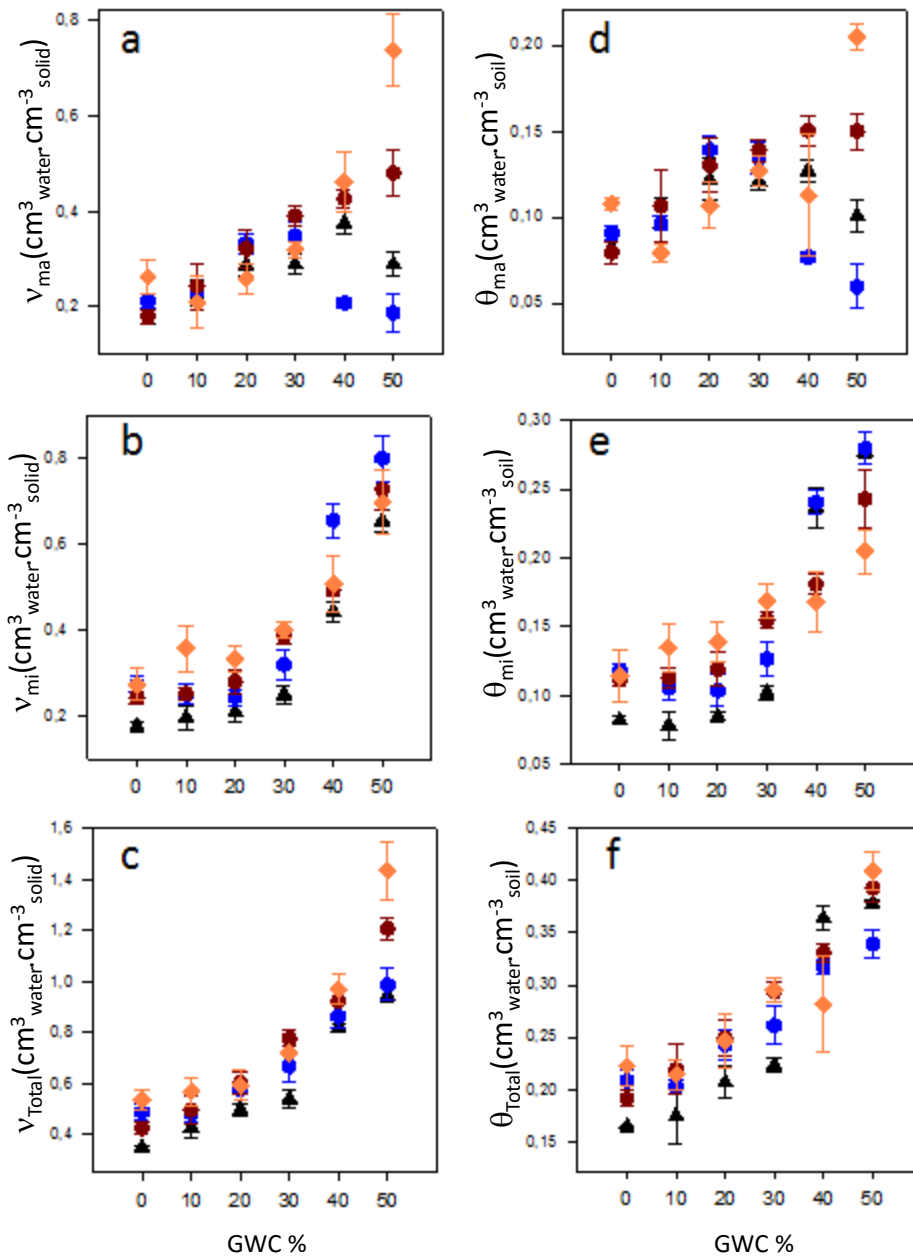
675

676 Figure 4. Averaged shrinkage curves ($n = 4$ per curve) for the four treatments (control,
 677 earthworms, plants, earthworms and plants) reported as the void ratio as a function of the
 678 moisture ratio. Each panel represents one mixture of green waste compost (GWC) and
 679 excavated deep horizons: (a) 0% GWC, (b) 10% GWC, (c) 20% GWC, (d) 30% GWC, (e)
 680 40% GWC, (f) 50% GWC. The dashed line represents the saturation line.



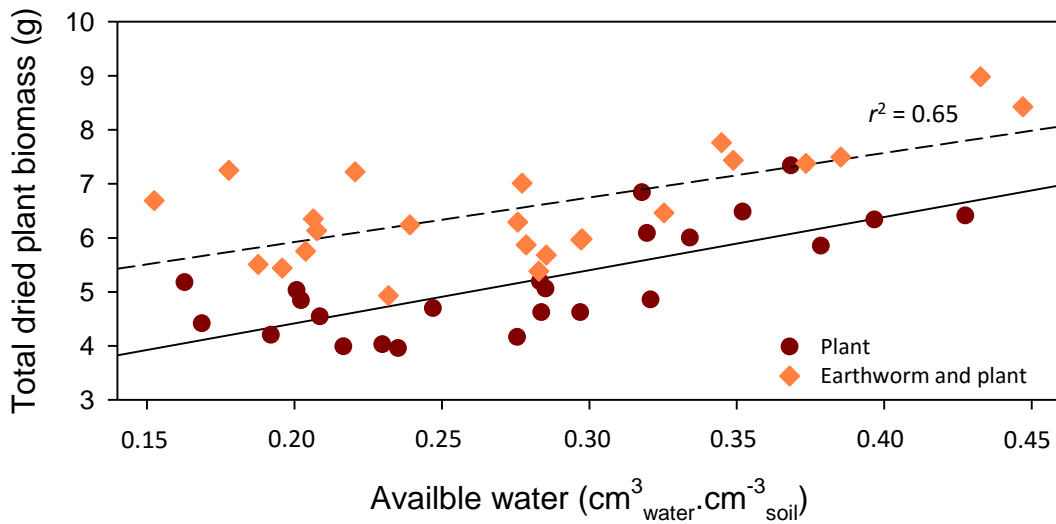
681

682 Figure 5. Linear Discriminant Analysis of the influence of control, earthworm, plant, and both
 683 earthworm and plant on hydro-structural parameters. The first and the second axes explained
 684 42% and 26% of the variance, respectively. v_L , moisture ratio at saturated macropores, v_M
 685 moisture ratio at saturated micropores, v_N limit of shrinkage, e_0 void ratio at the end of the
 686 shrinkage curve, K_{st} the slope of structural phase, K_{bs} the slope of the basic phase and K_L , K_N
 687 parameters related to shape form.



688

689 Figure 6. Moisture ratio at **a** maximum saturated macropores ($v_{ma} \text{ cm}^3 \text{water} \cdot \text{cm}^{-3} \text{solid}$), **b**
 690 maximum saturated micropores ($v_{mi} \text{ cm}^3 \text{water} \cdot \text{cm}^{-3} \text{solid}$), **c** total moisture ratio (v_{Total}
 691 $\text{cm}^3 \text{water} \cdot \text{cm}^{-3} \text{solid}$); available water of **d** macropores ($\theta_{ma} \text{ cm}^3 \text{water} \cdot \text{cm}^{-3} \text{soil}$), **e** micropores (θ_{mi}
 692 $\text{cm}^3 \text{water} \cdot \text{cm}^{-3} \text{soil}$), and **f** total available water ($\theta_{Total} \text{ cm}^3 \text{water} \cdot \text{cm}^{-3} \text{soil}$) according to the proportion
 693 of compost for the four organism treatments (presence/absence of earthworms and/or plants).
 694 Mean \pm s.e., n = 4 per treatment.



695

696 Figure 7. Linear regression between total dry plant biomass and available water (cm³_{water} · cm⁻³_{soil})
 697 ³_{soil}) with earthworm (dotted line) or without earthworm (plain line). Plant biomass was higher
 698 with earthworms than without ($P < 0.001$). Total plant biomass increased with available
 699 water, but the difference in slope of the two linear regressions was not significant. The best
 700 equations fitting the relation between total dried plant biomass (X, g) and plant available
 701 water (θ_{Total} , cm³_{water} · cm⁻³_{soil}) are: $X = 8.97 * \theta_{\text{Total}} + 4.07$ and $X = 8.97 * \theta_{\text{Total}} + 2.69$ with and
 702 without earthworms, respectively ($P < 0.001$, adjusted $r^2 = 0.65$).