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1 **Increasing the ability of a green roof to provide ecosystem services**  
2 **by adding organic matter and earthworms**

3

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22 Abstract:

23 The aim of this study was to increase the ecosystem services provided by a green roof by  
24 ameliorating the biological and chemical properties of a commercial green roof substrate  
25 through the addition of earthworms and compost. We conducted a mesocosm (120 x 80 x 33  
26 cm) experiment with a plant community comprising *Hylotelephium maximum*, *Centaurea*  
27 *jacea*, *Lotus corniculatus*, *Koeleria glauca*, and *Dianthus carthusianorum*. Two substrates  
28 were compared (i) a low complexity commercial green roof substrate with no alterations and  
29 (ii) a high complexity substrate with a layer of locally produced vermicompost and  
30 earthworms (*Lumbricus terrestris*) placed on top of the commercial substrate. The mesocosms  
31 were placed on the roof of a 20 m high building and at ground level.

32 Results show that the percent of vegetative cover on both substrates was lower in the roof  
33 mesocosms than on those placed at ground level. This is explained by climatic conditions, such  
34 as higher light intensity at roof level. The substrate with earthworms and vermicompost had  
35 significantly higher enzyme activity, microbial biomass, and metabolic activity. This resulted  
36 in more available nitrogen and phosphorus for plants, increased the plant biomass, floral  
37 nectar volume and sugar concentration which resulted in the flowers receiving more diverse  
38 and abundant insect pollinators. This is the first time that adding earthworms and compost to a  
39 green roof substrate has been shown to have a significant effect on plant growth and plant-  
40 pollinator interactions. These findings will help in designing green roof systems that are more  
41 efficient at preserving ecosystem services in urban areas.

42

43

44 Keywords:

45 Green roofs; Earthworms; Compost; Microbial activity; Ecosystem services.

## 46 **1. Introduction**

47 Under urbanization pressure and climate change, ecological services provided by urban  
48 ecosystems are endangered (McMichael et al., 2006). To counteract this tendency, green roofs  
49 are increasingly set up on top of buildings. This technology has become increasingly  
50 important over the last 20 years with thousands of green roofs being installed worldwide,  
51 especially in countries with temperate and subtropical climates (Brenneisen, 2006). Green  
52 roofs are created to provide a range of ecosystem services, from rainwater retention to thermal  
53 insulation. They also provide habitats for biodiversity among which invertebrates such as  
54 insects, spiders and soil arthropods, whose diversity is in relation with the composition of the  
55 plant community and growth substrate (MacIvor and Ksiazek, 2015). Indeed, studies  
56 sampling invertebrate communities on a variety of green roof types have found many  
57 hundreds of species (Brenneisen, 2006; Schindler et al., 2016; Ksiazek et al., 2012). Most of  
58 them colonize green roofs during their installation (as they are already present in the growing  
59 substrate) or by their own means. Many of these invertebrates are involved in ecosystem  
60 functions such as plant pollination and reproduction, contributions to food webs, and the  
61 maintenance and stabilization of soil through the degradation of organic matter  
62 (Vijayaraghavan, 2016; Wang et al., 2017; Zhang et al., 2019).

63 Green roofs are comprised of vegetation placed in a growth medium, called substrate  
64 (Oberndorfer et al., 2007) on top of building roofs. In addition to the harsh microclimate on  
65 rooftops, plant communities in green roof system are often subject to low nutrient availability  
66 of the substrate (Rowe et al., 2006). As the nature of the substrate directly influences plant  
67 growth and the performance of the green roofs (Young et al., 2014), choosing an appropriate  
68 substrate is, therefore, critical for the sustainability of a green roof. However, the substrates  
69 used for green roofs have depauperate biological activities because they are sterilized prior to  
70 installation or are derived mostly from dry mineral materials (John et al., 2014). More and

71 more research shows the critical relationships between plants and their microbial associates,  
72 called “holobionts” are essential for plants to deal with various environmental stressors  
73 (Vandenkoornhuysen et al., 2015). Moreover, both detritivores and microorganisms play an  
74 important role in soil fertility because they are responsible for the recycling of nutrients from  
75 organic matter (Madsen, 2005) and increased microbial activity facilitates the production of  
76 readily available forms of nutrients (Trasar-Cepeda et al., 2007). Improving biological  
77 activities in the substrate might thus improve growing conditions and therefore reinforce plant  
78 health (Molineux et al., 2014; Molineux et al., 2015a, 2015b).

79 Earthworms act as soil engineers (Lavelle, 2002a; Scheu et al., 2002; Brown et al., 2000)  
80 and they are known to enhance carbon and nitrogen mineralization through direct or indirect  
81 effect on the composition, distribution and activity of soil microorganisms (Scheu et al., 2002;  
82 Aira and Domínguez, 2011). Furthermore, earthworms can survive to harsh environmental  
83 conditions such as highly polluted soils (Jusselme et al., 2013) or extreme rainfall events  
84 (Andriuzzi et al., 2015), making them good candidates for the improvement of biological  
85 activities of green roofs substrates. Actually, earthworms are not commonly found on green  
86 roofs because of the shallow substrate depth in combination with frequent drought conditions.  
87 The use of earthworms in this context is thus dependent on the characteristics of the substrate,  
88 including its composition and depth.

89 In recent years, utilization of compost issued from green waste material produced in cities  
90 has emerged as a way of improving soil quality while protecting the environment. Weber et  
91 al. (2007) have shown that the application of compost improved soil fertility and increased  
92 cation exchange capacity in a sandy soil. This also improves water retention and soil structure  
93 by increasing the stability of soil aggregates (Celik et al., 2004). A recent study focused on  
94 using compost to increase the long-term stability of soil organic matter (Bass et al., 2016).  
95 Thus adding compost to green roof systems may enhance the substrate biological activities.

96 [Ondoño et al. \(2014\)](#) compared the mix of compost and brick to compost and soil media and  
97 showed the first-mentioned had far less microbial activity and fewer nutrients than the last-  
98 mentioned.

99 The aim of this study was to test if the addition of earthworms and compost may improve  
100 the biological and chemical properties of a standard green roof substrate. We conducted a  
101 mesocosm experiment using a plant community comprising five species grown on a  
102 commercial green roof substrate, with or without the addition of vermicompost and  
103 earthworms. The mesocosms were set up on a rooftop and at ground level. We recorded the  
104 weather conditions, the physiological characteristics of the plants, the activity of the microbial  
105 communities in the substrate and the pollinator abundance and visitation rates to the plant  
106 community. We tested three hypotheses: (i) the roof microclimate affects the green roof  
107 ecosystem, (ii) adding earthworms and compost stimulates microbial activities and improves  
108 plant development in the green roof system, and (iii) adding earthworms and compost  
109 supports ecosystem services such as pollination.

110

## 111 **2. Materials and methods**

### 112 2.1. Experimental design and sample collection

113 The experiment was conducted on the University of Paris Est Créteil (UPEC) campus, Val de  
114 Marne (48°47'17.9"N 2°26'36.0"E). To test the effect of roof microclimate on plant  
115 development, mesocosms were set at two heights: (i) on the roof, 20 m high (“R”) and (ii) at  
116 ground level (“G”) ([Fig. 1](#)).

117 The mesocosms (120 x 80 x 33 cm) were filled randomly with either: (i) a low complexity  
118 substrate (LC), comprising a 25 cm layer of a commercial green roof growing medium  
119 (Substrate Type E, Optigreen, Germany); (ii) a high complexity substrate (HC), with a 5 cm  
120 layer of vermicompost and earthworms on top of 20 cm of the same commercial medium.

121 Each combination of height and substrate was replicated five times. According to the  
122 manufacturer's data sheet, the composition of the substrate is variable according to the  
123 production site. The main constituents are expanded shale, expanded clay, stone, crushed  
124 bricks, perlite and compost from green waste. The main characteristics of the substrate are a  
125 pH comprised between 6 and 8, salt, organic substances content < 65 g/l, permeability to  
126 water  $\geq 0.6$  mm/min and total porosity comprised between 60 and 70 %. The vermicompost  
127 was obtained by adding earthworms *Eisenia foetida* and *Eisenia Andrei* to locally sourced  
128 green waste from the Val de Marne district. In addition to the vermicompost, each HC  
129 mesocosms was inoculated with 53 (about 170 g) adult earthworms, *Lumbricus terrestris*.  
130 This species digs temporary deep burrows and returns to the surface to feed, playing an  
131 important role in the incorporation of the vermicompost into the substrate. Worms were  
132 provided by the Lombri Carraz Company (France). The initial pH at a 1:5 soil-to-water ratio  
133 was 8.5 for the LC mesocosms and 6.0 for the HC mesocosms.

134 The plant community comprised five perennial species *Hylotelephium maximum* L.  
135 (Holub, Grand Orpine *Sedum telephium* var *maximum* L., *Crassulaceae*); *Centaurea jacea* L.  
136 (Brown knapweed, *Asteraceae*), *Lotus corniculatus* L. (Bird's-foot trefoil, *Fabaceae*),  
137 *Koeleria glauca* (Spreng.) DC. (Blue hair grass, *Poaceae*), *Dianthus carthusianorum* L.  
138 (Carthusian pink or German pink, *Caryophyllaceae*). The plants were selected for their  
139 suitability for green roofs in the Ile-de-France region as well as for their insect-pollination  
140 system (except *K. glauca* which is wind-pollinated). There were five plants of each species in  
141 each mesocosm. The plants within each plant mixture were randomly distributed in each  
142 mesocosm.

143

144 2.2. Weather conditions

145 The light level, the ultraviolet index, the air temperature, the relative humidity and the wind  
146 speed were measured with a Pro WMR86 weather station (Oregon Scientific®, France) at  
147 each height. The data were recorded during June, July and August 2015 to characterize  
148 microclimates on the roof and at ground level with a minimum of 27 measurements for each  
149 parameter, height and time of day (morning 9 am and afternoon 4 pm).

150

151 2.3. Plant parameters

152 During the experiment, the plant parameters (vegetative cover, above-ground biomass, *H.*  
153 *maximum*, *C. jacea*, and *L. corniculatus* leaf chlorophyll content, *H. maximum* nectar volume  
154 and nectar sugar concentration) were measured from June to August 2015.

155 All images were taken from 1 meter above the surface of the substrate. The vegetative  
156 cover was determined by processing photographs with Image J (the number of “green” pixels  
157 divided by the total number of pixels). The chlorophyll concentration ( $\mu\text{mol.m}^2$ ) was  
158 measured for three species of plants, *H. maximum*, *C. jacea*, and *L. corniculatus* using an  
159 SPAD 502 Plus chlorophyll meter (Minolta, Japan). In each mesocosm with 5 plants of each  
160 species, three measurements for each plant were taken on three randomly chosen leaves and in  
161 addition, at least one node from the growing tip. At the end of the experiment, the above-  
162 ground biomass per plant per mesocosm was collected by cutting at the level of the root  
163 collar, oven-dried at 70°C for 48h and weighed.

164 The nectar volume and the nectar sugar content were measured on *H. maximum* flowers  
165 following the protocol described in [Flacher et al. \(2015\)](#). Measurements were carried out over  
166 three successive weeks (one measurement per week) from 7<sup>th</sup> to 30<sup>th</sup> August 2015. The nectar  
167 volume was measured early in the morning on three flowers per plant and five plants per  
168 mesocosm. To prevent visits by insects, flowers were covered with mesh bags the evening



169 before the measurements. For each plant, three open flowers were collected at random. For  
170 each flower, the nectar was collected using microcapillary tubes (0.5 µl, end to end Minicaps,  
171 Hirschmann Laborgeräte, Germany) and the nectar volume was calculated by measuring the  
172 length of liquid in the microcapillary tube with a digital caliper. The nectar sugar  
173 concentration (estimated as a sucrose equivalent) was determined using a hand-held  
174 refractometer (Eclipse E-line 80, Bellingham Stanley Ltd., UK) which was calibrated using a  
175 30° brix sucrose solution. If necessary, the nectar was diluted in Milli-Q water before the  
176 measurement. The mean amount of sugar produced per flower, in each mesocosm, was  
177 estimated by multiplying the mean sugar concentration by the mean volume of nectar per  
178 flower.

179

## 180 2.4. Physical and chemical properties

181 The physical and chemical properties of the substrate were measured at the INRA Soil  
182 Analysis Laboratory (Arras, France) in accordance with ISO/IEC 17025.

183 The substrate samples were passed through a 2-mm sieve prior to analysis. The substrate  
184 pH was measured using a 1:5 soil-to-water ratio. The cation exchange capacity (CEC) was  
185 determined by ammonium acetate saturation. The total organic carbon content was  
186 determined by rapid titration.  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were extracted by shaking in 0.5M KCl and  
187  $\text{NO}_3^-$  and  $\text{NH}_4^+$  measured by colorimetry. The available phosphorus was determined by  
188 colorimetry using the phosphomolybdic blue reaction.

189

## 190 2.5. Microbial activity in substrates

### 191 2.5.1. Enzyme activity

192 The total microbial activity of the substrates was determined by fluorescein diacetate  
193 (FDA) hydrolysis (Schnurer and Rosswall, 1982). The mineralization of organic phosphorus

194 was evaluated by the measurement of alkaline phosphatase (ALP) and acid phosphatase  
195 (ACP) activities (Nelson, 1982). The assays were conducted in quintuplicate.

196 For FDA assay, the wells in a 96 well microplate were loaded with 100  $\mu\text{L}$  of substrate  
197 sample solution, 50  $\mu\text{L}$  of citrate phosphate buffer and 50  $\mu\text{L}$  of FDA solution ( $3\text{g L}^{-1}$ ). The  
198 microplates were incubated for 2 h at  $37^\circ\text{C}$ , shaken at 900 rpm. At the end of incubation the  
199 microplates were centrifuged at 1500 rpm for 10 min (Sigma 2-5, Fisher Bioblock, France).  
200 100  $\mu\text{L}$  of the supernatant was transferred to a new microplate with 100  $\mu\text{L}$  of citrate  
201 phosphate buffer at  $4^\circ\text{C}$ . The absorbance at 490 nm was measured using a BioTek EL800  
202 Universal plate reader (Bio-Tek Instruments, Winooski, VT). The FDA hydrolyzed was  
203 expressed as  $\mu\text{g}$  fluorescein  $\text{g}^{-1}$  substrate  $\text{h}^{-1}$ .

204 For alkaline and acid phosphatase activity assays, the wells in a 96 well microplate were  
205 loaded with 50  $\mu\text{L}$  substrate sample solution, 25  $\mu\text{L}$  borate buffer (pH 5 for ADP and pH 9 for  
206 ALP) and 0.5  $\mu\text{L}$  of *p*-nitrophenyl phosphate solution ( $10\text{g L}^{-1}$ ). The microplates were  
207 incubated for 2 h at  $37^\circ\text{C}$ , shaken at 150 rpm. 125  $\mu\text{L}$  2%  $\text{Na}_2\text{CO}_3$  solution was added to stop  
208 the reaction. The microplates were centrifuged at 1500 rpm for 10 min (Sigma 2-5, Fisher  
209 Bioblock, France). 50  $\mu\text{L}$  of the supernatant was transferred to a new microplate with 25  $\mu\text{L}$   
210 2%  $\text{Na}_2\text{CO}_3$  solution. The absorbance at 405 nm was measured using a BioTek EL800  
211 Universal plate reader (Bio-Tek Instruments, Winooski, VT). The ADP and ALP enzyme  
212 activities were expressed as  $\mu\text{g}$  *p*-nitrophenol  $\text{g}^{-1}$  substrate  $\text{h}^{-1}$ .

213

#### 214 2.5.2. Microbial respiration

215 The microbial respiration (MR) was measured as proposed by Garcia et al. (1994). We placed  
216 2 g of substrate in hermetically sealed flasks and incubated for 4 days at  $28^\circ\text{C}$  to reactivate the  
217 microorganisms. The flasks were then moistened to 70 % of water holding capacity (WHC)  
218 with  $9\text{ g L}^{-1}$  NaCl solution. Emitted  $\text{CO}_2$  was measured every 6 h by chromatography (490

219 Micro GC, Agilent, France). The cumulative CO<sub>2</sub> emitted was calculated and the respiration  
220 rate was expressed as mg CO<sub>2</sub>-C kg<sup>-1</sup> dry substrate h<sup>-1</sup>.

221

### 222 2.5.3. Microbial metabolic activity and diversity

223 96-well EcoPlates (AES Laboratory France) were used to assess the microbial metabolic  
224 activity of the substrates. Each Ecoplates<sup>TM</sup> had three replicates of 31 different  
225 environmentally significant carbon sources and three control wells (Garland, 1996). Each well  
226 of an EcoPlate (AES Laboratory France) was filled with 150 µL substrate sample solution and  
227 the plates were incubated in darkness on a shaker (150 rpm) at 20°C for 3 days. The color  
228 development was measured as absorbance at 595 nm using a Microplate spectrophotometer  
229 (Xenius, Safas SA, Monaco) every 24 h for one week. Data were normalized using the  
230 average wellcolor development (AWCD) as described by (Garland and Mills, 1991):

$$231 \quad AWCD = \Sigma (C - R) / n.$$

232 where *C* is the color developed within each well (optical density measurement), *R* is the color  
233 of the control well, and *n* is the number of substrates in each replicate (*n*=31). All negative  
234 values were considered to be zero in the subsequent data analyses. Diversity indexes  
235 (Shannon index and Richness) were calculated as suggested by John C. Zak (1994).

236

### 237 2.5. Floral visitation rates

238 Over 3 consecutive weeks during the flowering period in July and August 2015, we recorded  
239 the total number of pollinators that visited flowers. On days when weather conditions were  
240 favorable for pollination (i.e. wind speed < 15 km/h, without rain), pollinator visits were  
241 recorded for a period of 5 minutes per mesocosm, 3 days per week.

242

## 243 2.6. Statistical analyses

244 All data was analyzed using Statgraphics (Centurion XVI, Sigma Plus, France). Before testing  
245 for significant differences between the treatments, the normality of the data was tested using  
246 the Kolmogorov Smirnov test ( $P < 0.05$ ).

247 We used one-way ANOVA ( $\alpha = 0.05$ ) to analyze the effect of the height on the  
248 meteorological parameters and the effect of the substrate on pollinators.

249 We used two-way ANOVA ( $\alpha = 0.05$ ) to analyze the effects of the substrate, the height  
250 and their interaction on physical, chemical, biological, and plant parameters.

251 We used Pearson's product-moment coefficient ( $r$ ) to test whether the variations in  
252 microbial activity were correlated with the weather conditions and with the chemical  
253 properties of the substrate. We tested for linear dependences between local weather conditions  
254 (temperature, light intensity, air humidity and wind speed), substrate properties (pH, soil C/N  
255 ratio, organic matter, N and P concentrations, water content), microbial activities (enzyme  
256 activities, respiration, metabolic activities) and plant parameters (height, diameter, vegetative  
257 cover, chlorophyll, nectar volume and sugar concentration).

258

## 259 **3. Results**

### 260 3.1. Weather conditions

261 The mean temperatures were not significantly different between heights but differed  
262 significantly between the morning and afternoon with a slight increase in the afternoon for  
263 both heights (ground,  $F = 5.0$ ,  $P < 0.05$  and roof,  $F = 6.1$ ,  $P < 0.05$ ) (Table 1). The light  
264 intensity on the roof was significantly higher than at ground level in the afternoon only (Table  
265 1). The UV index on the roof was always higher than at ground level. The wind speed in the  
266 morning and afternoon was not significantly different between heights. However, the  
267 variation of wind speed on the roof was significant higher than at ground level ( $F = 10.6$ ,  $P <$

268 0.01, Table 1). The relative humidity was not significantly different between heights but was  
269 significant higher in the morning than in the afternoon ( $F = 14.9$ ,  $P < 0.001$ , Table 1).

270

### 271 3.2. Physical and chemical characteristics of the substrates at the end of the experiment

272 Whatever the location, the HC substrate had significantly higher availability of mineral  
273 nutrients (C, N and P), higher field capacity ( $F = 60.5$ ,  $P < 0.001$ ), higher water content ( $F =$   
274  $121.3$ ,  $P < 0.001$ ), and lower pH ( $F = 66.4$ ,  $P < 0.001$ ) for both heights (Table 2). The  
275 available  $N-NO_3^-$  was significantly higher on the roof ( $F = 14.1$ ,  $P < 0.05$ ) (Table2).

276 The HC substrate C content was 58% (G, ground) and 68% (R, roof) higher than for the  
277 LC substrate, while the HC substrate total N content was 56% (G) and 68% (R) higher. The  
278 HC substrate available  $N-NH_4^+$  was 69% (G) and 89% (R) higher and  $N-NO_3^-$  was 94% (G)  
279 and 96% (R) higher. The HC substrate field capacity was 23% (G) and 55% (R) higher than  
280 for the LC substrate (Table 2).

281

### 282 3.3. Plant parameters

283 All plants looked healthy throughout the experiment (i.e. no signs of pathogen or herbivore  
284 attacks).The HC substrates had 55% (R) and 67% (G) greater aboveground plant biomass,  
285 16% (R) and 7% (G) greater vegetative cover, and 53% (R) and 41% (G) more *H. maximum*  
286 buds than the LC substrates (Table 3).

287 For the HC substrate, the chlorophyll contents of *H. maximum*, *C. jacea* and *L.*  
288 *corniculatus* were significantly higher (30%) than for the LC substrates at both heights (Table  
289 3). The mean nectar volume per flower for *H. maximum* was 73% (R) and 68% (G) higher  
290 and the nectar sugar concentration was 65% (R) and 66% (G) higher (Table 3).

291 The HC substrates had significantly higher values for all plant parameters: above-ground  
292 biomass ( $F = 21.6$ ,  $P < 0.001$ ), vegetative cover ( $F = 63.7$ ,  $P < 0.001$ ), *H. maximum* buds

293 number ( $F = 65.8$ ,  $P < 0.001$ ), chlorophyll content of *H. maximum*, *C. jacea* and *L.*  
294 *corniculatus* ( $F = 100.5$ ,  $P < 0.001$ ;  $F = 29.2$ ,  $P < 0.001$ ;  $F = 84.4$ ,  $P < 0.001$ , respectively), *H.*  
295 *maximum* mean nectar volume ( $F = 32.8$ ,  $P < 0.001$ ) and nectar sugar concentration ( $F = 12.6$ ,  
296  $P < 0.05$ ). Height had a significant effect, for both substrates, on vegetative cover and *H.*  
297 *maximum* mean nectar concentration, with lower values at the ground level, and *H. maximum*  
298 chlorophyll content, with higher values at ground level (Table 3).

299

#### 300 3.4. Microbial activity and diversity

301 The flowering phase is a critical period in which plants produce many compounds that will  
302 shape the composition and activities within the microbial community. We therefore tested the  
303 differences between HC and LC substrates in both the vegetative and flowering phases  
304 separately.

305 The microbial respiration in the vegetative phase was 76 (R) and 92 (G) mg CO<sub>2</sub> kg<sup>-1</sup> DW  
306 h<sup>-1</sup> for the HC substrate, which was significantly higher than 39 (R) and 49 (G) mg CO<sub>2</sub> kg<sup>-1</sup>  
307 DW h<sup>-1</sup> for the LC substrate (Table 4). The results in the flowering phase were similar.

308 The HC substrates had a significantly higher metabolic activity, expressed as average well  
309 color development (AWCD), independently of the height ( $F = 16.0$ ,  $P < 0.001$ , Table 4). The  
310 Shannon diversity index ( $F = 7.6$ ,  $P < 0.01$ ) and richness ( $F = 8.8$ ,  $P < 0.01$ ) of the microbial  
311 community were significantly higher in the HC substrates, but there was no significant  
312 interaction between height and substrate.

313 Generally the HC substrates had significantly higher enzyme activities than the LC  
314 substrates in both time periods (Table 4). In the vegetative phase, the FDA measured in HC  
315 substrates were 2 (R) and 6 (G) U g<sup>-1</sup> DW which were significantly higher than in the LC  
316 substrates (~ 1 U g<sup>-1</sup> DW). In the flowering phase, the acid and alkaline phosphatase activities  
317 in HC were significantly higher than those in LC (Table 4). The substrate had a significant

318 effect ( $F = 24.2$ ,  $P < 0.001$  for FDA,  $F = 8.2$ ,  $P < 0.01$  for ACP, and  $F = 4.9$ ,  $P < 0.05$  for  
319 ALP).

320

### 321 3.5. Floral visitation by pollinators

322 Solitary bees, honey bees and bumblebees were the most common insect pollinators observed  
323 foraging on the mesocosms. The mean number of visits and pollinator abundances were  
324 significantly ( $F = 32.4$ ;  $P < 0.01$  and  $F = 15.7$ ;  $P < 0.05$ ) higher for HC substrates than for LC  
325 substrates (Fig. 2A, B).

326

### 327 3.6. Relationship between physical and chemical parameters, microbial activity and plant 328 parameters

329 ACP and ALP activity were positively correlated with available P. FDA activity was  
330 positively correlated with available N, P, and C content and total N. Microbial respiration and  
331 AWCD were positively correlated with all measured chemical properties. The plant  
332 parameters (biomass, cover, volume of nectar and nectar sugar concentration) were positively  
333 correlated with the total and available nutrient content (C, N and P) (Table 4).

334

## 335 4. Discussion

### 336 4.1. Effect of microclimate on the vegetation in the green roof system

337 Green roofs are known to be exposed to a variety of harsh abiotic factors such as intense solar  
338 radiation, temperature, humidity and wind speed (Fulthorpe et al., 2018); however there is  
339 limited knowledge on the effect of these microclimate parameters on plant growth on roofs. In  
340 our study, we observed that the vegetative cover and above-ground plant biomass on a roof  
341 were lower than those at ground level for either substrate. This can be explained by the higher  
342 light levels on the roof top than at ground level. Light is an absolute requirement for plants

343 growth and development, and different plants have different light requirements. An increase  
344 in light will increase the rate of photosynthesis; but excessive light intensity may scorch the  
345 leaves and reduce growth (Sargent, 1940; Thut and Loomis, 1944). Moreover, humidity  
346 seemed more important on the roof than on the ground, even though not significantly so, but  
347 this may have affected soil moisture and plants.

348 We also observed that *H. maximum* nectar sugar concentration was higher on the roof than  
349 at ground level, but there was no significant difference in mean nectar volume per flower. A  
350 higher nectar sugar concentration in *H. maximum* can be related to the abiotic factors. In fact,  
351 abiotic parameters such as light, water, temperature, and CO<sub>2</sub> concentration can have  
352 important impacts on plant physiology that can in turn affect the nectar quantity and quality  
353 (Gardener and Gillman, 2001a; Burkle and Irwin, 2009). In soil, the availability of nutrients  
354 can indirectly affect flower production and nectar (Smith and Read, 2008). Foliar dust  
355 deposition may also be more important on the roof than on the ground, which could supply  
356 supplementary nutrients for plants. Overall, the higher levels of plant nutrients (N, N-NO<sub>3</sub><sup>-</sup>  
357 and P, P-PO<sub>4</sub><sup>2-</sup>) on the roof may result in the higher concentration of sugar in the nectar of *H.*  
358 *maximum* flowers growing on the roof.

359

#### 360 4.2. Effect of adding vermicompost and earthworms on the green roof ecosystem

361 It is known that plants in green roofs encounter harsh meteorological factors and low nutrient  
362 availability in the substrate (Oberndorfer et al., 2007; Rowe, 2011). The idea of this study was  
363 to use the synergic interaction between earthworms and vermicompost in order to improve the  
364 physico-chemical and biological parameters of the substrate. Results show that the application  
365 of vermicompost and earthworms increased substrate field capacity by about 50% and  
366 humidity by about 80%. This is an important factor as it is directly involved in the cooling  
367 capacity of green roofs as well as in the development of the plant and animal communities in



368 the substrate (Getter et al., 2009; MacIvor and Lundholm, 2011). Our results also show that  
369 the addition of vermicompost and earthworms improved the quantities of nutrients and  
370 organic matter of the substrate with an increase by about 68 and 58% in organic matter, 68  
371 and 58% in organic C, as well as 68 and 56% in total nitrogen observed in the substrate at  
372 rooftop and ground levels, respectively. Moreover, as earthworms are known to affect the  
373 distribution of microorganisms and microbial biomass (Brown, 1995), we hypothesized that  
374 the effects of earthworms on the plant growth resulted in part from their impact on soil  
375 microorganisms. Indeed, our results show that inoculating the substrate with earthworms and  
376 vermicompost resulted in higher values for microbial respiration and enzyme activity in the  
377 substrate. Although we recorded increased microbial activity in the substrate in the presence  
378 of earthworms and vermicompost, the mechanisms involved are unclear. However, Lavelle,  
379 (2002b) assumed that microorganisms are largely dormant in the soil awaiting suitable  
380 conditions that earthworms can provide as they burrow and cast through soil, secreting  
381 substances such as mucus resulting in microbial reactivation and stimulation.

382 As well as more available N and P, supporting the hypothesis that higher microbial activity  
383 would lead to more available nutrients for plants. ACP and ALP activity were positively  
384 correlated with available P. Similar results have been reported for earthworm casts, but it is  
385 not known whether the increase in activity is due to earthworm-derived enzymes or to  
386 increased microbial activity (Satchell et al., 1984). Greater ALP activity in the presence of  
387 earthworms would increase the hydrolysis rate of organic P compounds, increasing the  
388 available P released (Aira et al., 2007). N and P are major nutrients for plant growth, and  
389 expanding availability of these nutrients should increase the above-ground plant biomass,  
390 vegetative cover, chlorophyll content, nectar volume and nectar sugar concentration (Flacher  
391 et al., 2015). We suggest that earthworms transformed this organic matter into plant-available  
392 nutrients (Villenave et al., 1999). This was confirmed by the positive correlations between the

393 plant parameters and the available nutrients (Table 5). Thus, using a combination of  
394 vermicompost and anecic earthworms favors plant-growth in a green roof ecosystem.

395 Nectar, the main source of energy for pollinators, plays a central role in the behavior and  
396 composition of pollinator communities (Baude et al., 2011). Many studies have investigated  
397 the role of below-ground communities on above-ground biota including pollinators (Barber  
398 and Gorden, 2013). Green roofs are known to provide habitats for pollinators, particularly,  
399 bees and butterflies (Ksiazek et al., 2012). Soil nutrient availability to plants is known to  
400 influence the quantity and quality of nectar and can lead to changes in pollinator visitation  
401 (Burkle and Irwin, 2009). However, less is known about the effect of compost and  
402 earthworms on nectar production and pollinator behavior in a green roof ecosystem. In our  
403 study, the *H. maximum* nectar sugar concentration was higher with added vermicompost and  
404 earthworms. This led to an increase in the abundance of flower visitors (solitary bees, honey  
405 bees and bumblebees) as well as frequency of pollinator's visits. Kudo and Harder, 2005  
406 suggested that nectar availability and floral display are the most important traits influencing  
407 attractiveness of plants to pollinators. Plants also interact with a diverse set of substrate biota  
408 and these interactions have the potential to affect nutrient uptake by plants (Smith and Read,  
409 2008). Consequently this can indirectly affect flower production (Burkle and Irwin, 2009),  
410 pollen (Lau and Stephenson, 1994) and nectar (Gardener and Gillman, 2001b; Becklin et al.,  
411 2011).

412 MacIvor and Ksiazek (2015) mentioned that despite their important role in soil,  
413 earthworms are rarely encountered on green roofs because of their limited ability to disperse  
414 and also due to lack of moisture at peak summer periods or freezing temperatures in winter.  
415 Furthermore, the shallow depth of extensive green roofs substrate (< 15 cm) restricts their  
416 ability to move to deeper soil during extreme weather conditions. In our case, the substrate  
417 depth was 25 cm including 5 cm of vermicompost which created favorable conditions for the

418 development of the earthworms, enabling them to move to deeper areas during dry or cold  
419 conditions, thus enhancing their survival probability and the degree to which they provide  
420 ecosystem services. In our experimental design, despite no direct estimates of earthworm  
421 survival, the presence of turricules at the surface of the substrate two years after the start of  
422 the experiment is a good indicator of the survival of the earthworms in the substrate.

423

#### 424 4.3. Practical implications

425 Composting is an easy and promising alternative for producing organic fertilizer from food  
426 and green waste. For many local authorities, implementing a food waste collection provides a  
427 realistic solution to meet regulatory targets for diverting biodegradable waste from landfill  
428 and increasing recycling and composting rates. There are several possibilities for food waste  
429 collections: the choice will depend on the current waste collection organization, the quantities  
430 required, budget, and proximity of treatment facilities, political acceptability and public  
431 support. Overall, utilizing food waste in composting is a promising approach which will not  
432 only help fulfill fertilizer demand but also provide sustainable waste management.

433 Although the addition of compost was shown to increase the quality of green roof  
434 substrates, there are other consequences leading to some practical recommendations. Firstly,  
435 compost is generally not stable over the medium and long-term: it must be regularly reapplied  
436 over extended periods in order to maintain organic matter levels (Bates et al., 2015).  
437 Secondly, home-composting systems may be a source of anthropogenic greenhouse gas  
438 emissions (Lou and Nair, 2009). Therefore, special attention must be paid to reduce  
439 greenhouse gas emissions during composting. Nevertheless, several studies (Li et al., 2010;  
440 Morau et al., 2017; Kuronuma et al., 2018) have shown that when compost is used on green  
441 roofs, this may lower CO<sub>2</sub> emission in the atmosphere.

442 The uses of compost are growing as the means of improving its chemical and physical  
443 qualities are increased. The combination substrate/compost/earthworms on green roofs can  
444 help reduce the risk of flooding in urban areas by increasing rainwater retention (Graceson et  
445 al., 2013), and green roofs seem to be a sink for anthropogenic gas emission (Li et al., 2010;  
446 Morau et al., 2017). The addition of earthworms may allow improving the distribution of the  
447 substrate and compost within the substrate and may also improve soil structure, fertility and  
448 microbial activity (Paz-Ferreiro et al., 2015). Overall, this may indirectly contribute to  
449 preserving biodiversity in cities.

450

## 451 5. Conclusions

452 This study showed that adding vermicompost and anecic earthworms to a commercial green  
453 roof substrate will (i) increase available N and P, (ii) increase alkaline and acid phosphatase  
454 and fluorescein diacetate hydrolysis activities, and (iii) increase microbial respiration and C  
455 source metabolic rates. The increased enzyme activity in the amended substrate increased the  
456 available N and P which are important nutrients for plant growth. Plant above-ground  
457 biomass, nectar volume and nectar sugar concentration were, therefore, increased, leading to  
458 higher pollinator visitation. To our knowledge, this is the first experiment to show that  
459 amending green roof substrates with earthworms and vermicompost can have a significant  
460 effect on green roof ecosystem function, through plant biomass production and plant-  
461 pollinator interactions. The results confirm our hypothesis that amending commercial green  
462 roof substrates with earthworms and compost can improve the biological quality of the  
463 substrate.

464

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650

651 Captions

652 Figure 1: Experimental design at the University of Paris Est Créteil, Val de Marne (Ile-de-  
653 France, south east of Paris).

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655 Figure 2: Number of pollinators visiting flowers (A) and number of floral visits by pollinators  
656 (B) at Rooftop and Ground locations during floral period. Difference between the treatments  
657 with (HC) and without (LC) vermicompost and earthworms at two different times (July and  
658 August) tested through one-way ANOVA models. (\*P < 0.05; mean ± SE, n = 27).

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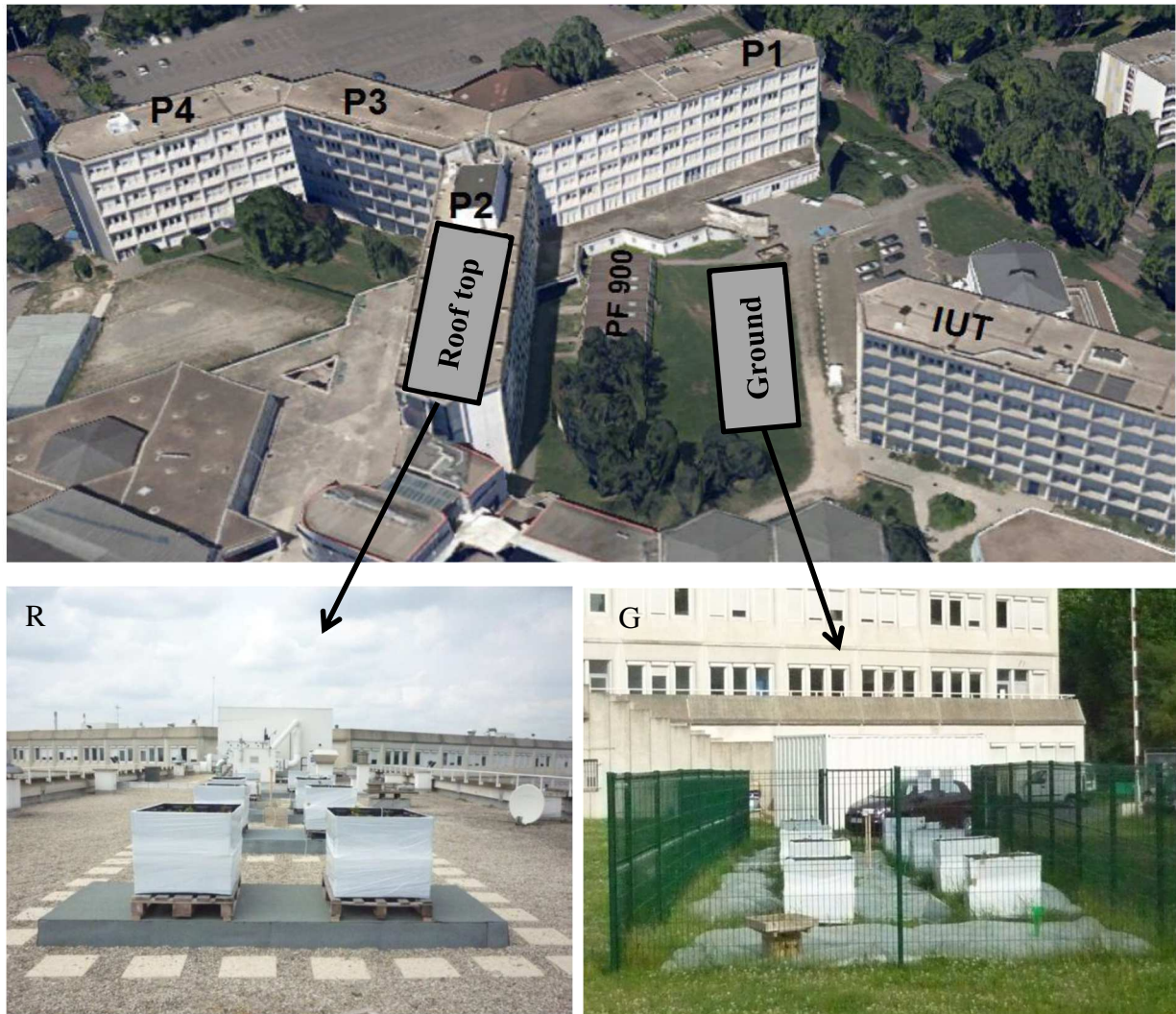


Figure 1: Experimental design at the University of Paris Est Créteil, Val de Marne (Ile-de-France, south east of Paris)

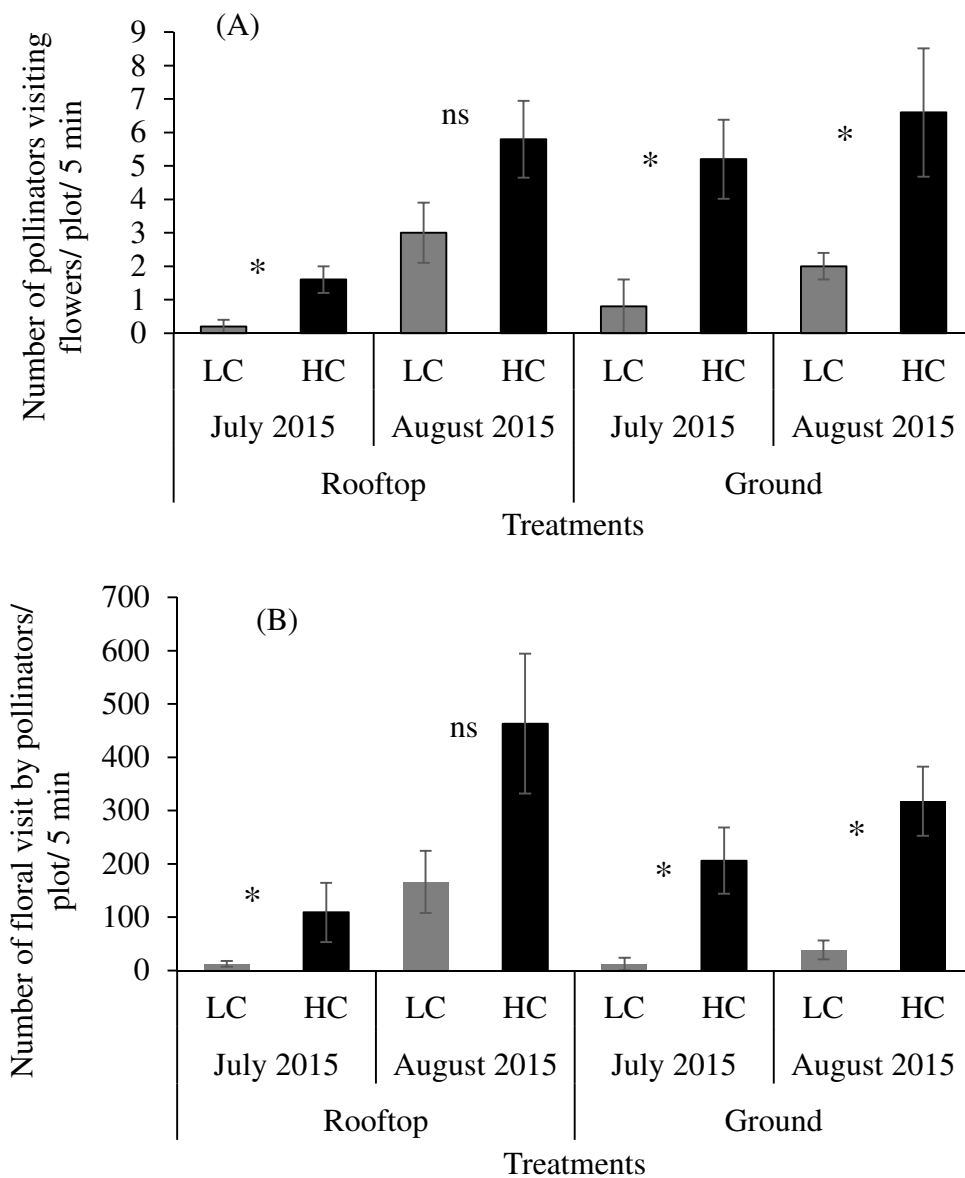


Figure 2: Number of pollinators visiting flowers (A) and number of floral visits by pollinators (B) at Rooftop and Ground locations during floral period. Difference between the treatments with (HC) and without (LC) vermicompost and earthworms at two different times (July and August) tested through one-way ANOVA models. (\* $P < 0.05$ ; mean  $\pm$  SE,  $n = 27$ ).

Table 1: Meteorological parameters of the Rooftop and Ground locations in the morning and afternoon.

Meteorological parameters	Morning		Afternoon	
	Ground	Rooftop	Ground	Rooftop
Mean temperature (°C)	30.7 ± 7.2a	29.2 ± 9.2a	36.6 ± 8.3b	34.8 ± 11.3b
Light intensity (lux)	23.8 ± 8.2a	20.8 ± 4.9a	57.2 ± 10.3b	70.1 ± 13.8c
UV index	2.0 ± 0.5a	3.5 ± 0.5b	4.5 ± 0.7c	6.2 ± 1.5d
Mean wind speed (m/s)	3.4 ± 1.8a	5.7 ± 2.1b	2.8 ± 0.7a	6.3 ± 1.2b
Variation in wind speed	7.1 ± 2.1a	10.3 ± 1.8b	6.8 ± 1.9a	11.9 ± 3.2b
Humidity (%)	54.3 ± 10.5b	50.5 ± 13.8b	35.6 ± 8.3a	37.7 ± 10.3a

In each column, numbers followed by different letters are significantly different ( $p < 0.05$ ) according to Fischer's PLSD-test. Mean values ( $n = 27$ ) ± standard error.



Table 2: Physical and chemical properties of the substrate in “LC”, low complexity, and “HC”, height complexity, treatments at two locations at the end of the experiment and F-values of a two-way Anova measuring the effects of earthworm and compost on plant parameters.

OM, organic matter; N: nitrogen; N-soil, total soil nitrogen; NO<sub>3</sub><sup>-</sup>, soil nitrate content; NH<sub>4</sub><sup>+</sup>, soil ammonium content; P<sub>2</sub>O<sub>5</sub>, soil phosphorus content.

Location	Treatment	pH	OM (g/kg)	Organic C (g/kg)	Total N (100mg/kg)	N-NH <sub>4</sub> <sup>+</sup> ( mg/kg)	N-NO <sub>3</sub> <sup>-</sup> (mg/kg)	P-P <sub>2</sub> O <sub>5</sub> (mg/kg)	Field capacity (g/kg)	Soil humidity (g/kg)
Rooftop	LC	8.1 ± 0.06b	79.7 ± 23.3a	46.1 ± 13.5a	30.7 ± 1.0a	41.6 ± 2.5a	31.2 ± 16.2a	108.3 ± 45.7a	21.7 ± 4.9a	17.2 ± 1.5a
Rooftop	HC	7.8 ± 0.02a	250.3 ± 66.6b	144.7 ± 13.8b	96.6 ± 3.4c	137.7 ± 21.2b	716.0 ± 60.3c	862.0 ± 40.9b	48.8 ± 2.3b	93.2 ± 4.0b
Ground	LC	8.1 ± 0.10b	87.9 ± 28.5a	50.7 ± 16.4a	33.3 ± 1.2a	38.1 ± 4.6a	19.6 ± 6.2a	135.0 ± 37.6a	24.4 ± 2.1a	9.6 ± 7.4a
Ground	HC	7.9 ± 0.03a	211.3 ± 35.9b	122.4 ± 20.1b	76.6 ± 2.1b	199.0 ± 81.3c	352.3 ± 81.3b	639.3 ± 161.6b	34.5 ± 5.3b	90.4 ± 14.9b
Location (A)		1.2ns	1.1ns	1.1ns	2.7ns	1.4ns	14.1**	6.9ns	4.6ns	3.1ns
Treatments (B)		66.4***	98.8***	99.7***	104.9***	28.0***	103.3***	407.1***	60.5***	121.3***
A x B		1.2ns	2.6ns	2.5ns	4.5ns	1.8ns	12.8**	16.0**	17.6**	1.8ns

In each column, numbers followed by different letters are significantly different ( $p < 0.05$ ) according to Fischer's PLSD-test. Mean values ( $n = 5$ ) ± standard error. F values and significance level: ns: not significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

Table 3: Plant parameters for “LC”, low complexity, and “HC”, high complexity, treatments at rooftop and on the ground at the end of the experiment and F-values of a two-way Anova measuring the effects of earthworm and compost on plant parameters.

Location	Treatment	Above-ground biomass plant (g)	Vegetative cover (%)	Number of bourgeons ( <i>Sedum</i> )	Chlorophyll content ( $\mu\text{mol.m}^2$ ) ( <i>Sedum</i> )	Chlorophyll content ( $\mu\text{mol.m}^2$ ) ( <i>Centaurea</i> )	Chlorophyll content ( $\mu\text{mol.m}^2$ ) ( <i>Lotier</i> )	Volume of nectar in flowers ( <i>Sedum</i> )	[sugar] in flowers ( <i>Sedum</i> )
Rooftop	LC	129.5 $\pm$ 28.7a	77.7 $\pm$ 5.2a	20.7 $\pm$ 2.6a	22.7 $\pm$ 5.3a	32.5 $\pm$ 2.3a	37.5 $\pm$ 6.7a	0.16 $\pm$ 0.06a	17.5 $\pm$ 5.0a
Rooftop	HC	289.2 $\pm$ 39.6b	92.9 $\pm$ 9.8b	44.4 $\pm$ 3.4b	34.4 $\pm$ 6.4 b	43.4 $\pm$ 4.9b	45.4 $\pm$ 7.9b	0.53 $\pm$ 0.07b	49.8 $\pm$ 4.8c
Ground	LC	120.2 $\pm$ 26.1a	88.5 $\pm$ 8.4a	24.1 $\pm$ 2.6a	38.5 $\pm$ 10.3b	32.3 $\pm$ 5.2a	39.9 $\pm$ 5.9a	0.17 $\pm$ 0.02a	12.0 $\pm$ 2.3a
Ground	HC	369.2 $\pm$ 43.9c	94.8 $\pm$ 9.2b	40.8 $\pm$ 2.1b	56.9 $\pm$ 9.7c	41.8 $\pm$ 4.5b	45.1 $\pm$ 10.4b	0.59 $\pm$ 0.06b	40.4 $\pm$ 3.0b
Location (A)		0.6ns	12.3**	0.1ns	163.4***	2.6ns	2.2ns	2.3ns	8.3*
Treatments (B)		21.6***	63.7***	65.8***	100.5***	29.2***	84.4***	32.8***	12.6**
A x B		1.4ns	10.9**	0.01ns	4.9*	12.6***	3.6ns	2.8ns	0.2ns

In each column, numbers followed by different letters are significantly different ( $p < 0.05$ ) according to Fischer's PLSD-test. Mean values ( $n=5$ )  $\pm$  standard error. F values and significance level: ns: not significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

Table 4: Microbial activities (respiration, Biolog<sup>TM</sup> and enzyme) of the “LC”, low complexity, and “HC”, height complexity, substrates at two locations at different growth stages.

Stage of plant	Localization	Treatment	Respiration	AWCD	Richness (R)	Shannon (H)	FDA	ACP	ALP
Vegetative	Rooftop	LC	39.3 ± 4.2a	0.28 ± 0.02b	11.8 ± 2.2b	1.21 ± 0.06c	0.69 ± 0.3a	7.6 ± 2.1b	12.1 ± 5.5d
	Rooftop	HC	75.9 ± 4.1d	0.34 ± 0.07c	15.4 ± 2.1c	1.41 ± 0.23d	3.32 ± 0.8c	14.5 ± 4.3c	43.8 ± 11.5f
	Ground	LC	48.6 ± 8.2ab	0.21 ± 0.04ab	10.8 ± 1.8ab	1.02 ± 0.13b	1.10 ± 0.8ab	8.8 ± 6.9b	20.6 ± 17.8e
	Ground	HC	92.0 ± 1.9e	0.35 ± 0.07c	16.2 ± 4.2c	1.48 ± 0.23d	7.00 ± 3.7e	20.7 ± 9.0d	51.5 ± 17.3f
Floral	Rooftop	LC	37.9 ± 2.1a	0.16 ± 0.05a	8.4 ± 2.7a	0.81 ± 0.15b	3.80 ± 0.6c	2.8 ± 1.9a	2.9 ± 2.6a
	Rooftop	HC	67.2 ± 2.4c	0.23 ± 0.09ab	9.2 ± 2.9ab	0.90 ± 0.27ab	4.94 ± 1.6c	2.8 ± 0.8a	6.1 ± 2.5b
	Ground	LC	44.0 ± 2.2ab	0.13 ± 0.09a	6.2 ± 3.8a	0.64 ± 0.29a	1.11 ± 1.3a	2.4 ± 1.3a	3.7 ± 1.7a
	Ground	HC	63.8 ± 3.9c	0.28 ± 0.10b	11.0 ± 3.8b	1.01 ± 0.39b	4.35 ± 0.2cd	8.5 ± 2.7b	8.8 ± 4.1c
Localization (A)			3.14ns	0.07ns	0.01ns	0.21ns	0.4ns	1.2ns	0.7ns
Treatments (B)			66.1***	16.0***	8.8**	7.6**	24.2***	8.2**	4.9*
(A x B)*Stage of plant			0.03ns	1.76ns	1.4ns	1.6ns	3.5ns	1.3ns	0.02ns

LC: low complexity; HC: high complexity; AWCD: Average well color development; FDA: Fluorescein diacetate hydrolysis; ADP: Acid phosphatase; ALP: Alkaline phosphatase.

In each column, numbers followed by different letters are significantly different ( $P < 0.05$ ) according to Fischer's PLSD-test.

Mean values ( $n = 5$ ) ± standard error.

F values and significance level: ns: not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

Table 5: Correlation matrix between parameters from different treatments with and without earthworms and compost at Ground and Rooftop levels.

	Organic matter	C content	Total N	Available N (NH <sub>4</sub> <sup>+</sup> )	Available N (NO <sub>3</sub> <sup>-</sup> )	Available P (PO <sub>4</sub> <sup>2-</sup> )
Acid phosphatase activity	0.73**	0.74**	0.73**	0.67*	0.63*	0.75**
Alkaline phosphatase activity	0.81**	0.81**	0.78**	0.91***	0.65*	0.78**
FDA activity	0.67*	0.67*	0.62*	0.88***	NS	0.62*
Microbial respiration	0.89***	0.89***	0.86***	0.89***	0.72**	0.87***
AWCD	0.67*	0.67*	0.63*	0.79**	0.57*	0.73**
Plant biomass	0.76**	0.76**	0.74**	0.75**	0.63*	0.79**
Plant cover	0.78**	0.78**	0.76**	0.70*	0.63*	0.74**
Nectar volume	0.89***	0.89***	0.89***	0.77**	0.84***	0.94***
Amount of sucrose in nectar	0.88***	0.88***	0.88***	0.70*	0.88***	0.93***

AWCD: Average well color development; FDA: Fluorescein diacetate hydrolysis; ADP: Acid phosphatase; ALP: Alkaline phosphatase; C: Carbon; N: Nitrogen; NO<sub>3</sub><sup>-</sup>, soil nitrate content; NH<sub>4</sub><sup>+</sup>, soil ammonium content; P<sub>2</sub>O<sub>5</sub>, soil available phosphorus content.

F values and significance level: ns: not significant; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001 according to Fischer's PLSD-test.