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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 ≥ 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 7th to 30th 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. NO_3^- and NH_4^+ were extracted by shaking in 0.5M KCl and
187 NO_3^- and 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 μL of substrate
197 sample solution, 50 μL of citrate phosphate buffer and 50 μL of FDA solution (3g L^{-1}). The
198 microplates were incubated for 2 h at 37°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 μL of the supernatant was transferred to a new microplate with 100 μL of citrate
201 phosphate buffer at 4°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 μg fluorescein g^{-1} substrate h^{-1} .

204 For alkaline and acid phosphatase activity assays, the wells in a 96 well microplate were
205 loaded with 50 μL substrate sample solution, 25 μL borate buffer (pH 5 for ADP and pH 9 for
206 ALP) and 0.5 μL of *p*-nitrophenyl phosphate solution (10g L^{-1}). The microplates were
207 incubated for 2 h at 37°C , shaken at 150 rpm. 125 μL 2% Na_2CO_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 μL of the supernatant was transferred to a new microplate with 25 μL
210 2% Na_2CO_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 μg *p*-nitrophenol g^{-1} substrate 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°C to reactivate the
217 microorganisms. The flasks were then moistened to 70 % of water holding capacity (WHC)
218 with 9 g L^{-1} NaCl solution. Emitted CO_2 was measured every 6 h by chromatography (490

219 Micro GC, Agilent, France). The cumulative CO₂ emitted was calculated and the respiration
220 rate was expressed as mg CO₂-C kg⁻¹ dry substrate h⁻¹.

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 EcoplatesTM 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):

$$231 \quad AWCD = \frac{\sum (C - R)}{n}$$

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 CO₂ kg⁻¹ DW
306 h⁻¹ for the HC substrate, which was significantly higher than 39 (R) and 49 (G) mg CO₂ kg⁻¹
307 DW h⁻¹ 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⁻¹ DW which were significantly higher than in the LC
316 substrates (~ 1 U g⁻¹ 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 CO₂ 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-NO₃⁻
357 and P, P-PO₄²⁻) 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 CO₂ 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).

654

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 \pm SE, $n = 27$).

659

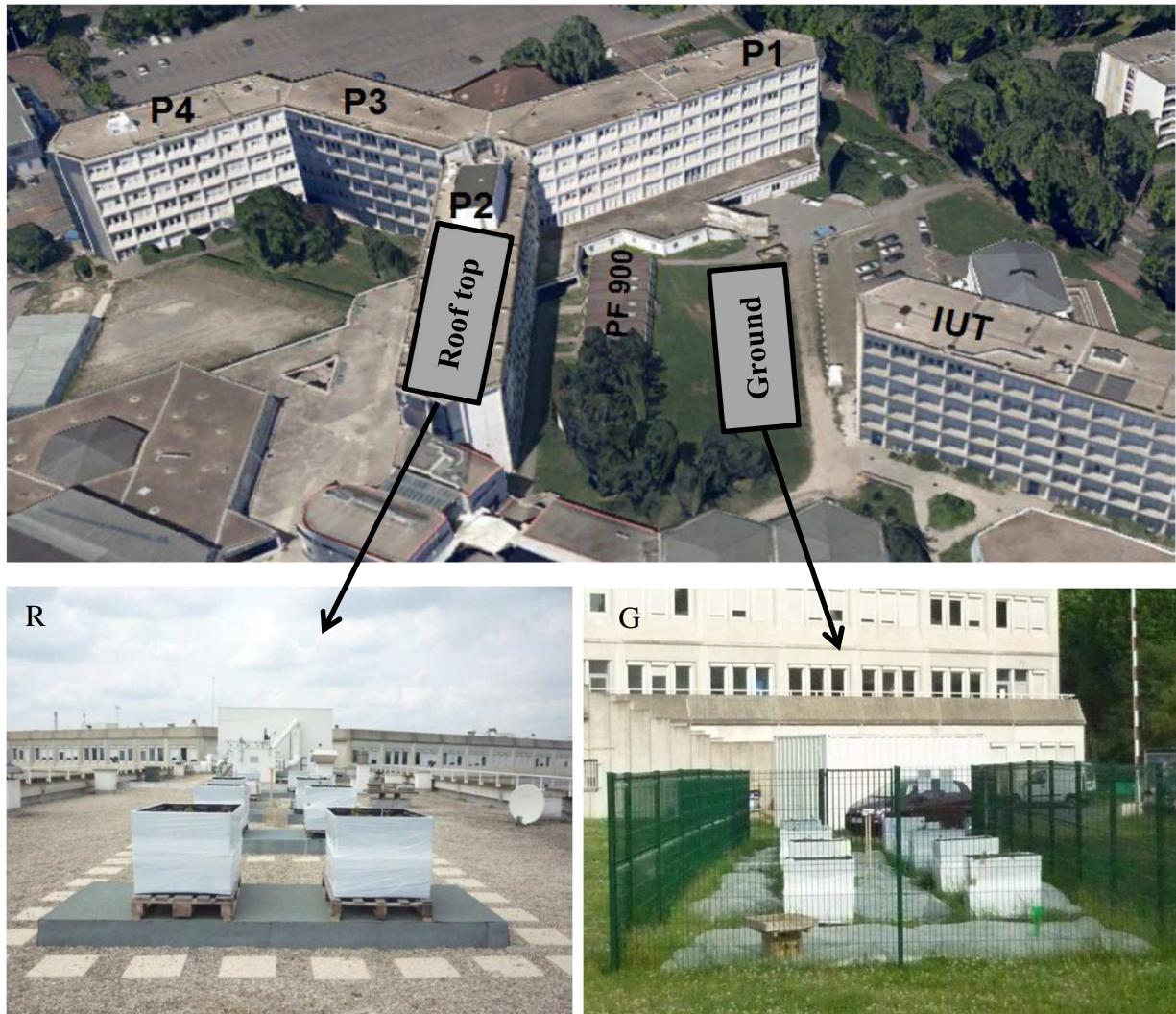


Figure 1: Experimental design at the University of Paris Est Créteil, Val de Marne (Ile-de-France, south east of Paris)

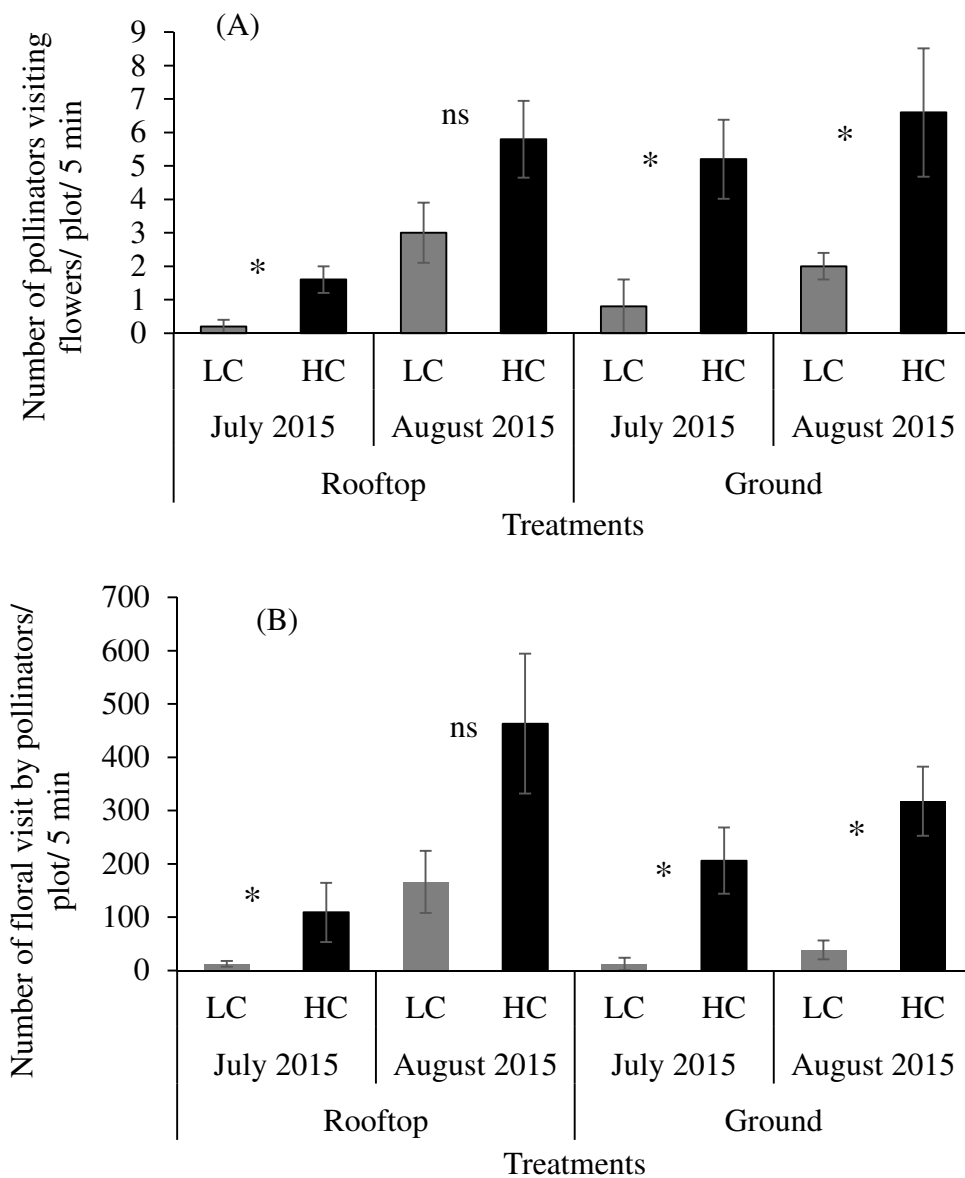


Figure 2: Number of pollinators visiting flowers (A) and number of floral visits by pollinators (B) at Rooftop and Ground locations during floral period. Difference between the treatments with (HC) and without (LC) vermicompost and earthworms at two different times (July and August) tested through one-way 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; NO₃⁻, soil nitrate content; NH₄⁺, soil ammonium content; P₂O₅, soil phosphorus content.

| Location | Treatment | pH | OM (g/kg) | Organic C (g/kg) | Total N (100mg/kg) | N-NH ₄ ⁺ (mg/kg) | N-NO ₃ ⁻ (mg/kg) | P-P ₂ O ₅ (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, BiologTM 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 ₄ ⁺) | Available N (NO ₃ ⁻) | Available P (PO ₄ ²⁻) |
|-------------------------------|----------------|-----------|---------|---|---|--|
| 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; NO₃⁻, soil nitrate content; NH₄⁺, soil ammonium content; P₂O₅, 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.