

1 **Stability-associated variation of maturity and plant growth effects of composts**

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33

34 **Abstract**

35

36 *Purpose*

37 The length of composting is positively related with the stability of compost, negatively related to compost
38 phytotoxicity, and has variable effects on plant growth variables, such as nutrient supply and root
39 conditioning. The work aimed in verifying the notion that the evaluation or ranking of finished composts
40 in relation to stability is indicative to their evaluation or ranking in relation to maturity or plant growth
41 effects.

42 *Methods*

43 This study firstly verified whether there is a relationship between the age of compost and its biological
44 properties using common assays of compost characterization and secondly investigated whether Oxygen
45 Uptake Rate (OUR) estimates of three stable composts of different feedstock composition and the same
46 initial C:N ratio could safely indicate the degree of their phytotoxicity and plant growth promotion.

47 *Results*

48 Results indicated that materials at more advanced stages of composting were more stable, less phytotoxic
49 and immobilized nitrogen after incorporation in soil more intensively than immature composts. However,
50 for composts of different feedstock, stability and phytotoxicity did not correlate as the most stable
51 compost was also the most phytotoxic. In contrast, ranking of materials in relation to plant growth effects
52 coincided with stability, but only after dilution with peat.

53 *Conclusions*

54 An adequate assessment of compost effects on plant germination and growth requires the independent
55 characterization not only of its stability but also its phytotoxicity and nutrient supply capacity, as the
56 three biological properties of compost are often not correlated to each other.

57

58 **Keywords: compost stability; compost maturity; compost age; oxygen uptake rate; phytotoxicity;**
59 **plant growth effects**

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61

62 **Introduction**

63

64 Compost market development requires a thorough characterization of compost properties and
65 an association of these properties with its anticipated use. Compost characterization includes a wide
66 range of features. For materials intended to be used in growing media and soil, stability, maturity and
67 nutrient release receive particular attention as they are intimately related to prediction of its impact to
68 plant productivity. These three properties define to a great extent compost quality and potential
69 commercial value [1-3].

70 Stability is one of the most important compost characteristics. It can be defined as the degree of
71 vulnerability of the material to further decomposition. As such, it is expressed by the magnitude of the
72 decomposing microbial activity, which in turn can be determined by oxygen uptake, carbon dioxide
73 production rate or heat released as a result of this activity [4]. A non-stable material can cause poor plant

74 growth and can contribute to consumers' lack of confidence in the compost product. Aerobic
75 decomposition of organic matter can lead to O₂ depletion if unstable compost is applied to soil resulting
76 in both inadequate oxygen supply to plant roots and reduction in nutrient transfer from soil to plant.
77 Besides agronomic implications, stability is particularly important also for commercial reasons. Stable
78 materials can safely be stored and transported without changes in quality and quantity.

79 During composting organic compounds are progressively transformed into more stable organic
80 matter and part of carbon is released as CO₂, so by definition, there is a positive relationship of stability
81 with the length of composting [5].

82 Compost maturity prevailed to refer to the lack of adverse effects of compost on seed
83 germination and plant growth. Phytotoxicity comes from the asphyxiation of roots due to oxygen
84 consumption by the decomposing microorganisms [6], the presence of free ammonia or from the
85 combined effect of several organic intermediates of decomposition such as ethylene oxide and organic
86 acids [7-9].

87 In relation to the progress of the composting process, Tiquia [10] demonstrated the reduction of
88 phytotoxicity with the progress of composting, since microbial production of toxins is high during the
89 initial decomposition stage and it subsides in the following stabilization stage [11]. Consequently,
90 compost maturity is estimated either directly using various phytotoxicity tests or indirectly, by estimating
91 the degree of decomposition of phytotoxic organic substances produced during the composting process
92 [12].

93 Another set of biological characteristics refer to those appearing after application of compost to
94 soil or growing media [13]. Although the incorporation of compost is expected to have positive effects
95 on the physical structure and the microbiological activity of plant supporting systems, effects on nutrient
96 supply have been shown not to be straightforward [14]. Very often compost application increases soil
97 Electrical Conductivity (EC), which is almost always a limiting plant growth factor, and induces nitrogen
98 (N) immobilization. From this point of view composts from woody material such as greenwaste are worse
99 than manures and sewage sludge in providing nutrients to plants [15].

100 The well-documented results that the above biological properties of composts, i.e. stability,
101 maturity and plant growth effects, are related to the progress of composting process and the fact that at
102 least some of their controls are the same (e.g. microbial oxygen consumption for stability and maturity)
103 lead to the intuitive notion that the evaluation or ranking of composts in relation to one property may be
104 indicative of the evaluation or ranking of them in relation to another. However, evidence supporting this
105 hypothesis is not explicit. Oviedo-Ocaña et al. [16] found no correlation between stability and maturity
106 whereas Komilis and Tziouvaras [17] indicated that such correlation existed but it was highly dependent
107 on the kind of seeds used in Germination Index (GI) evaluation. Plant growth effects, which depend on
108 the rate of application, have more rarely correlated with the other biological characteristics. Duong et al.
109 [18], for example, found no correlation between GI and wheat growth effects of compost after compost
110 placement as 2.5 cm thick mulch layer on the soil surface. From the study of a wide range of properties
111 in nine composts of various sources, Paradelo et al. [19] concluded that no single test can be applied to
112 final marketable compost in order to assess maturity and stability and, in particular, that the results of the
113 plant growth tests were not correlated to any other parameter.

114 This study aimed firstly in showing whether compost stability, phytotoxicity and plant growth
115 effect assays can track differences of composting age and describe the relationship between these
116 characteristics in compost of one feedstock, and secondly indicate whether eventual correlation among
117 them holds on composts from different feedstock.

118

119

120 **Materials and Methods**

121

122 The investigation consisted of two experiments. The first intended to verify that the progression
123 of composting is accompanied by an increase in stability, and associated changes in phytotoxicity and N
124 release potential. Nitrogen release (or immobilization) was chosen to depict effects of compost utilization
125 on plant growth. In this experiment material was sampled from composting material at successive
126 occasions and transferred to the lab for analysis. The second experiment intended to show whether three
127 composts originated from different feedstock mixtures of the same C:N ratio showed the same stability
128 estimates after maturation and if eventual differences in these estimates varied in parallel to phytotoxicity
129 and plant growth estimates.

130

131 *Production of composts – Analysis of general characteristics*

132

133 Eight in total turning metal drums of 1.5 m³ were used for composting. Their axes, on which
134 vertical blades had been welded for better blending of materials, were designed so that they could be
135 attached to a moving motor for periodic turnings. Drums were isolated with stone wool. They were
136 opened for temperature measurements using a portable probe, sampling and water addition.

137 Turnings were carried out once a week. Water additions were controlled by regular sampling of
138 materials and gravimetric estimations of moisture content. The targeted water content was not the same
139 for all compost types, ranged between 45 and 70%, and was defined empirically so that the material was
140 dumped.

141 In finished composts pH was measured using EN 13037 [20] and Electrical Conductivity (EC)
142 using EN 13038 [21]. Ash was estimated after organic matter loss on ignition (LOI) at 450°C in a muffle
143 furnace for 12 hours and NO₃⁻ in 2M KCl extracts after passing them through cadmium reduction
144 columns [22].

145

146 *First experiment - Compost from the same feedstock at different composting times*

147

148 Three drums were used to provide the compost (COMP1) for this group of trials. They were
149 filled with municipal tree pruning from lemon, orange, olive, eucalyptus, oleander and acacia trees of
150 unknown proportions. The total mixture which contained small branches and leaves had a C:N ratio of
151 33. Analyses below referred to material composted for 24, 38, 52 and 67 days.

152

153 *Analyses*

154

155 Although stability was measured using the same method at both experiments, tests for
156 phytotoxicity and plant growth effects were selected to be different. In this first experiment, methods
157 were chosen so as to discern the anticipated small differences of the tested materials.

158

159 *OUR tests*

160

161 Oxygen Uptake Rate (OUR) estimates were performed using the OxiTop® measuring system
162 (WTW, Wilhelm, Germany) and method EN 16087-1 [23]. Samples varying in weight but containing
163 always 2 g of organic matter were placed in 1 L vessels and diluted with water containing N- allylthiourea
164 as nitrification inhibitor. Due to aerobic microbial activity the oxygen consumption was measured by the
165 pressure heads that were mounted on the vessels. Organic matter content was measured by LOI. The
166 material from each composting age was measured twice and in some cases three times for OUR. The
167 average of these measurements was used in ANOVA.

168

169 *Phytotoxicity of compost extracts*

170

171 A 1:5 (w/v) compost:distilled water extract was made for each of the previous composts of
172 different maturity by stirring compost-water mixtures for one hour and filtering using a Whatman 2
173 paper. Three ml of extract was placed on three sheets of filter paper in each Petri dish and 25 cress seeds
174 were evenly spread on the paper. Three ml of distilled water was used to moisten the filter papers at the
175 control treatments. The Petri dishes were covered to prevent evaporation of the extract and randomly
176 placed in an incubator for 3 days at $25 \pm 1^\circ\text{C}$ in darkness (adapted from [24]). Each treatment was
177 replicated three times. Numbers of seeds germinated and their root lengths were recorded. The
178 Germination Index (GI) used was the Munoo – Liisa vitality index (MLV) with the concise formula:
179 $\text{MLV (\%)} = (\text{GS} \cdot \text{RL}) * 100 / (\text{GSc} \cdot \text{RLc})$ where,
180 GS and GSc is the average number of Germinated Seeds for sample and control, respectively and RL
181 and RLc is the average Root Length of germinated seeds for sample and control, respectively.

182

183 *Soil incubations and inorganic N determination*

184

185 Mixtures of compost with soil were used to estimate its N mineralization potential in controlled
186 moisture and temperature conditions. Composts were removed from the drums at consecutive dates
187 during the composting process as described previously. Soil used was sampled from a wheat field, air
188 dried and sieved with a 2mm sieve. Visible plant residues and roots were removed by hand. This soil had
189 0.8% organic C content, 0.085% total N, 15% CaCO₃, 39% sand and 35% clay.

190 Incubations of soil + compost mixtures at $25 \pm 2^\circ\text{C}$ were carried out in small plastic 50 ml
191 containers filled with 20 g of soil and 0.1 g of compost material. In order to reduce evaporation water
192 losses, lots of 5-6 containers were enclosed in glass jars with air tight fittings, but with a hole on their
193 lids to allow oxygen replenishment. Water added in containers corresponded to 70% of the WHC of the

194 soil. The amount of mineral N in the soil samples was determined after 10 days from the onset of
195 incubation. Each time, three samples per treatment, were removed from the incubation chamber and soil
196 inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) was extracted with 2M KCl. Samples were shaken for 30 min, sieved
197 through No.2 Whatman filter paper and stored frozen. Inorganic N was determined in extracts by
198 colorimetric methods. Nitrate determination involved first the reduction of nitrate to nitrite through a
199 copper-cadmium column, while the estimation of $\text{NH}_4^+\text{-N}$ was based on the emerald green color formed
200 when ammonia and sodium salicylate react in the presence of sodium hypochlorite.

201

202 *Second experiment – Three composts from different feedstock*

203

204 For the production of these composts, the common material used in all drums was tree pruning
205 of *Ficus microcarpa*, which was mixed either with vetch (drums 1, 2, 3, COMP2), goat manure (drums
206 4, 5, 6, COMP3) or grass clippings (drums 7, 8, COMP4).

207 To attain a predefined C:N ratio of the initial mixture of feedstocks, plant materials and manure
208 were analyzed for their C and N content. Since these results are necessarily taken on a dry weight basis,
209 the water content and bulk density of each of them were also measured, to express element contents per
210 unit of volume of shredded material. The filling of compost drums was then made using the number of
211 20 Lt bin loads of each material needed to achieve the C:N ratio of 30:1.

212

213 *OUR test*

214

215 Compost stability estimates were made using the OxiTop® system that was described before.
216 Material from each composting drum was measured twice. The average of these measurements was used
217 in ANOVA for differences between composts; three replicates for COMP2 and COMP3 and two for
218 COMP4.

219

220 *Square Petri dish test with compost*

221

222 Aiming mainly at the comparison of composts and given that their EC estimates were not very
223 different between them, the square Petri dish test was chosen to be carried out without peat dilution as it
224 is recommended in EN 16086-2 [25] method for compost characterization. Compost materials passed
225 through a 10 mm sieve and moistened to the approximate optimum moisture content according to the fist
226 test. Ten seeds of cress (*Lepidium sativum* L.) were placed at each square Petri dish evenly spaced on a
227 line 15 mm from one edge and pressed gently into the surface of the test material so that there was a good
228 contact with it. A drop of water was also added to each seed using a pipette. As a control, the same
229 procedure was followed for sphagnum peat. The square Petri dishes were placed in an incubator ($25 \pm$
230 1°C in the dark) tilted so that roots growing straight down remain visible [26].

231 The number of germinated seeds was counted and root lengths were measured after 72 h. There
232 were three replications for the material of each of the composting drums. The Germination Index (GI)
233 used was the MLV.

234

235 *Germination/growth test with compost-peat mixtures and compost only in pots*

236

237 The test consisted of directly sowing seeds of Chinese cabbage (*Brassica rapa var. Pekinensis*)
238 in composts filling plastic pot trays (35 cm³ volume) at a rate of 1 seed per pot. Seeds were placed at
239 about 0.5 cm below surface and seed germination was measured after 10 days. The trial was continued
240 to estimate shoot growth after 35 days. A solution with micro and macro nutritional elements was used
241 for watering and fertilizing plants. For each material of a composting drum, 10 pots were filled.

242

243

244 **Results**

245

246 Although the time schedule of measurements during the composting trials was not dense
247 readings showed temperature peaks slightly higher than 60°C in all drums during the first month of
248 composting. Temperatures then dropped until they reached at about 50 days after the initiation of
249 composting the average air temperatures of the season.

250

251 *First experiment – Effect of compost age*

252

253 The maturation of COMP1 was accompanied by an increase in stability and a decrease in
254 phytotoxicity as it was shown by the results of germination trials using compost extracts (Table 1). For
255 the oldest and most stable compost (67-day compost, Table 1) compost extracts appeared to be even
256 phytostimulating at some of the replications.

257 When composts at different stages of maturity were incubated in soil and at optimum conditions
258 of temperature and moisture the extracted mineral N concentrations showed a decreasing trend (Figure
259 1). The incorporation of compost in soil resulted always in soil N immobilization, which was greatest for
260 the oldest material.

261

262 *Second experiment – Composts of different feedstock*

263

264 Table 2 shows some of the chemical characteristics that were measured in the finished composts
265 after 90 days of composting. All composts were alkaline while the highest value of EC (4.8 dS m⁻¹) was
266 found for COMP2 and the lowest (3.7 dS m⁻¹) for COMP4.

267 Stability estimates as they were given by the OxiTop® method varied between the three
268 composts (Table 3). They ranged between 4.5 and 12.8 mmol kg⁻¹OM h⁻¹ and according to them composts
269 ranked in the following order: COMP3 > COMP4 > COMP2. The most stable compost contained manure
270 as one of the feedstocks. Table 3 indicates also statistically significant difference of means.

271 Composts showed great phytotoxic effects either when seeds were placed directly on them in
272 square Petri dishes or when they were placed in plant pot trays (Table 3). COMP3 in particular, showed
273 an almost complete restriction of germination.

274 Initial vegetative development was significantly influenced both by compost type and by mixing
275 with sphagnum peat. In all cases, Chinese cabbage grew more in compost-peat mixtures (1:1, v/v) than
276 in compost only substrates. Among the compost mixtures highest dry biomass yield was shown in
277 COMP3 mixture followed by that at COMP2 and COMP4 (Figure 2) although differences were not
278 statistically significant ($P>0.5$). COMP3 alone completely inhibited seedling growth till the end of the
279 trial (Figure 2).

280

281

282 Discussion

283

284 Stability, phytotoxicity and N mineralization estimates at consecutive times during the course
285 of composting showed that these variables were dynamic. As expected [27], materials that are being
286 composted are becoming more stable and less phytotoxic. Composts at more advanced stages of maturity
287 immobilized N more intensively than immature composts after incorporation in soil presumably due to
288 the elimination of the labile, nitrogen containing substances [28]. Information on N release dynamics of
289 stable and unstable materials in the scientific literature is though inconclusive. Siebert et al. [29], for
290 example, claimed that N dynamics, which is one of the most significant controls of plant growth effects,
291 dependent on the degree of compost stability and that unstable composts can immobilize N. On the
292 contrary, Keeling et al. [2] found that unstable composts release N more than stable ones. Getting insight
293 in these property dynamics may be useful to compost industry where products are probably made by the
294 same initial mixture of feedstock and under the same composting conditions. Depending on market
295 priorities or end uses, composters may seek to stop the composting process at a certain stage with the
296 optimum combination of stability and maturity characteristics. In some cases unstable material has
297 greater positive plant growth effects [30] than mature materials. If this is not compromised by significant
298 increases in phytotoxicity, composters may seek shorter composting periods for acquiring composts with
299 special characteristics.

300 The results of this part of the study showed, however, that although there is a progressive change
301 of the three variables during the course of composting, the overall characterization of the material based
302 on each of them would not be the same. Relying on O_2 consumption rates, COMP1 after 67 days of
303 composting should be characterized as stable whereas N mineralization results, which indicated
304 significant immobilization, would point to a non-stable material [31]. Regarding the methodological
305 approach it has to be underlined that the OUR method EN 16087-1 [23] was proved to be sensitive
306 enough to track composting age effects on stability.

307 Contrary to the first part of the study which showed that stability increase during composting is
308 accompanied by phytotoxicity drop and N release restriction, the second part of the work provided rather
309 confounded results. When composts of different feedstocks were compared between them, stability
310 estimates were not indicative of phytotoxicity and vice versa, despite the fact that some characteristics
311 related to feedstock (*Ficus microcarpa* was always present and initial mixture had the same C:N ratio)
312 and composting process (production was carried out in metallic tumblers) were kept the same.

313 Two of the most essential biological characteristics of compost, stability and phytotoxicity, did
314 not correlate to each other [16, 17, 19]. The most stable compost in this study was shown to be also the
315 most phytotoxic. Microbiologically stable and non-phytotoxic materials are properties related to
316 materials produced at advanced stages of composting but the relationship between the two is such that
317 the one cannot be considered as indicative of the other. It should be taken into account that all composts
318 were characterized as very stable unlike in the first experiment where a number of samples were above
319 15 mmol kg⁻¹OM h⁻¹, which is the critical limit value for use of compost in growing media [32].
320 Comparable results were reported by Blok et al. [33] who found no relation between stability and plant
321 growth when the OUR value was low (< 15 mmol kg⁻¹OM h⁻¹) but a good relation if the range of values
322 were both high and low. Compost characterization, therefore, should contain both properties for a more
323 comprehensive quality description [34].

324 Interestingly, mixing composts with peat not only increased seedling growth [35], but also
325 reduced or even removed their phytotoxic effects in such a way as to modify the compost ranking that
326 was based on GI values (Figure 1). Apparently, either the dilution of chemical substances affecting
327 toxicity or increased microbial degradation of them caused an important reduction of the negative effects
328 of compost on plant growth [36]. Hence, moistening compost for a sufficient period of time and chemical
329 dilution should be considered as necessary conditions to uncover plant growth effects and to allow
330 eventual plant growth promoting agents in it to be manifested. Consequently, full characterization of
331 compost, apart from stability and maturity, requires estimates of plant growth effects after moistening
332 substrate for sufficient time or dilution of compost or other treatment so that the adverse effects of
333 phytotoxicity have been waived. In this study plant growth effects were revealed after one month of
334 seedling growth. Moreover, current results indicated that growth effects were at the same direction as
335 stability estimates would imply, i.e. the more stable composts were also the more plant growth
336 stimulating.

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342

343 **Disclosure statement**

344

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346

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348 **References**

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475 **Figure captions**

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478 **Figure 1:** Net immobilization of nitrogen in μg of mineral-N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) per g of dry soil
479 obtained after 10-day incubation of soil mixed with COMP1 that was sampled at various dates during
480 the composting process. Columns with the same letter are not significantly different (Tukey's post-hoc
481 test, $P>0.05$).

482

483 **Figure 2:** Average production of Chinese cabbage (g/plant) grown in pot trays, using as a substrate either
484 equal volumes of compost and sphagnum peat or only compost. Columns with the same letter are not
485 significantly different (Tukey's post-hoc test, $P>0.05$).

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487

488 **Table 1:** Results of the O₂ consumption rate (OUR in mmol kg⁻¹OM h⁻¹) measured by the OxiTop®
489 device, MLV index using compost extract, pH and EC of COMP1 at different stages of the composting
490 process.

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492

Days of composting	OUR Mean ± SEM	MLV extract Mean ± SEM	pH	EC
24	25,21 ±0,42 a	0,76 ±0,04 a	8,9	3,05
38	21,84 ±0,33 b	0,87 ±0,01 ac	8,6	3,20
52	16,61 ±0,54 c	0,96 ±0,00 bc	8,7	3,20
67	14,27 ±0,03 c	1,18 ±0,07 d	8,4	3,40

493

494 **Table 2:** Some of the chemical characteristics of the composts used at the stability, maturity and plant
495 growth trials.

496

	EC dS m ⁻¹	pH	NO₃⁻ mg kg ⁻¹	Ash %
COMP2	4,8	8,9	1450	24,35
COMP3	4,2	8,8	300	57,80
COMP4	3,7	8,3	925	46,40

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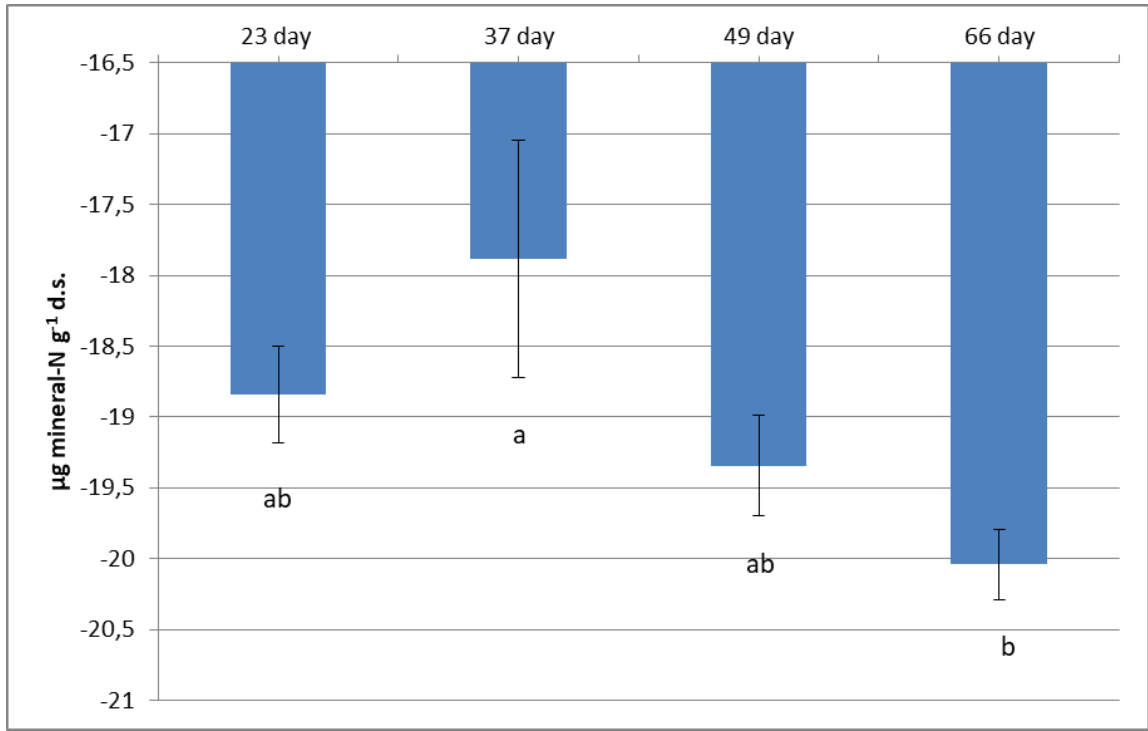
504 **Table 3:** Results for stability and maturity characterization of the three composts as it is manifested by
 505 O₂ consumption (OUR) measured by the OxiTop® device, MLV indices measured using the square Petri
 506 dish test, and % seed germination given by pot tray trials. Numbers indicate mean values ± SEM. When
 507 followed by the same letter are not statistically different (Tukey's post-hoc test, *P*>0.05).

508

	<u>OUR</u> Mean ± SEM	<u>MLV index</u> Mean ± SEM	<u>% seed germination</u>
<u>COMP2</u>	12,78 ±1,70 a	7,45 ±2,94 ab	33,33 ±9,62 ab
<u>COMP3</u>	4,49 ±0,30 b	0,78 ±1,18 a	5,56 ±5,56 a
<u>COMP4</u>	10,48 ±0,52 a	15,88 ±4,50 bc	75,00 ±25,00 bc

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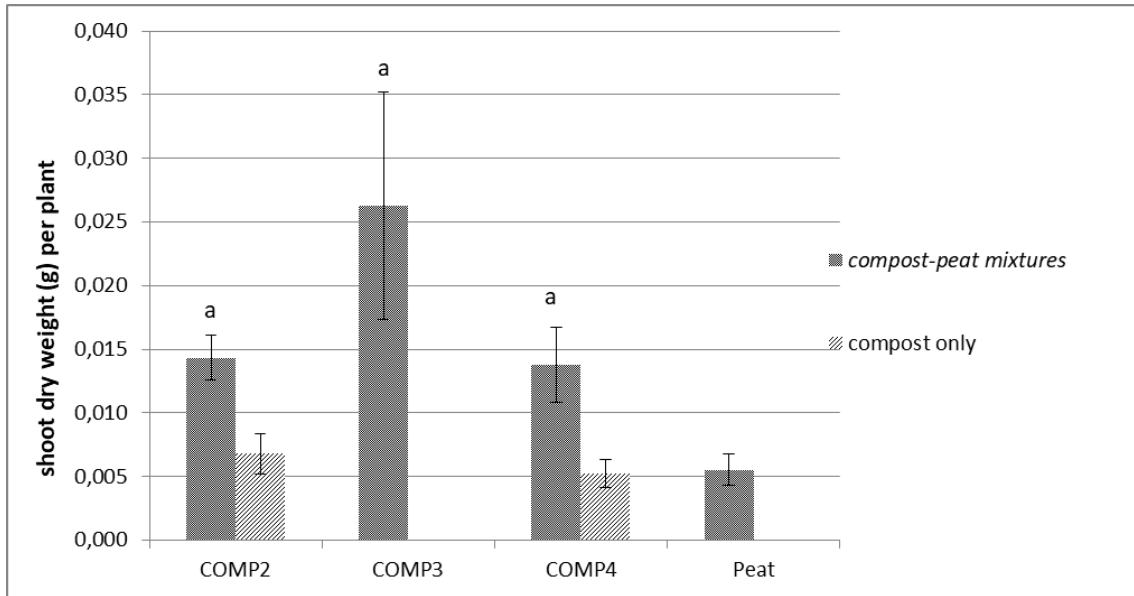


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513 **Figure 1**

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517 **Figure 2**

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