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(Article begins on next page)

1 **Characteristics of compost obtained from winemaking byproducts**

2

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11

12 **Abstract**

13 A model procedure for the sustainable management of plant biomass related to wine production,

14 namely vine branches from agricultural practices in the vineyard and marcs remaining after grapes

15 crushing, was devised. An artificial humification process was set up that could respond to the needs

16 of environmental sustainability and could be a safe way to be reintroduce in the vineyard part of the

17 organic matter previously exported, thus contributing to recover or maintain vineyard soil fertility.

18 Two different strategies for composting were tested, namely a static pile, made by branches and

19 marcs, and a pile that was fed twice a year alternatively with vine branches and grape marcs. The

20 experimentation lasted 710 days, during which environmental parameters, i.e. temperature and

21 rainfalls were monitored. Growth dynamics of the principal functional groups of microorganism were

22 followed. A characterization of the composted material was obtained by measuring several

23 parameters among which, pH, carbon, nitrogen, sulfur and heavy metals content. The characteristics

24 of the produced compost fulfill the requirements prescribed by the Italian legislation regarding the

25 use of compost as soil amendment. Germination tests demonstrated the absence of phytotoxicity and

26 conversely evidenced a stimulating activity towards root development.

27

28 **Keywords:** organic matter, compost, vineyard, grape marcs, pruning residues, microbiota

29

30 **1. Introduction**

31 Wine production in the European Union is rather relevant and in 2015 accounted for about 60% of
32 the world production, including Italy, France and Spain being the main producers [1]. Such high
33 levels of production determine the creation of large amounts of residues, namely vine branches from
34 winter pruning and grape marcs from grape pressing for winemaking.

35 Pruning residues represent a problem for vineyards and at the same time a production cost [2]. Until
36 today the disposal of such wastes follows two main routes: shredding in the field between the rows
37 with possible burial, or branch collecting and burning.

38 Shredding followed by landfilling can be useful in healthy vineyards since in this case shoots do not
39 constitute potential sources of infection or spread of diseases. On the contrary, this practice may
40 represent a phytosanitary problem in case of vineyards affected by diseases such as *Phomopsis* cane
41 and leaf spot (vine escoriosis) favoring the spread of root rot. In many cases shoots are collected and
42 burned close to the fields but currently this solution is banned for its negative environmental returns
43 in terms of air quality, related to emissions of fine particles, and as a precaution to prevent fires.

44 Grape marcs are constituted by the solid parts (skins, seeds and sometimes stalks) that remain after
45 grape pressing. They represent a material rich in phosphorous, potassium and magnesium and
46 potentially bioactive compounds [3] but also present some disadvantages, such as low pH (< 4), high
47 C/N ratio (25-40) and presence of potentially phytotoxic compounds (e.g. ethanol, acetic acid, lactic
48 acid, polyphenols). Grape marcs can be processed in distilleries to produce alcohol or alcoholic
49 beverages, while grape seeds can be used for extraction of oil and hulls can be used as supplement
50 for animal feed [4].

51 Composting is becoming an ecological and economical alternative for reusing plant biomass residues
52 [5] and residues from vitiviniculture, such as pruning residues and grape marcs, can be exploited for
53 production of compost.

54 The microbiota of grape marcs has been studied by classical microbiological techniques [6 - 8] and
55 by metagenomic approaches [9]. The abundant presence of bacteria and yeast provides a strong
56 stimulus to trigger microbial transformations. It has also been reported that yeasts present in marcs
57 possess a wide range of metabolic activities useful for marc transformation [10].

58 To date several agronomic residue types have been tested for producing compost. In particular, grape
59 marcs [11- 15] and branches [16] have been separately composted, also with addition of nitrogen
60 sources, such as manure [17].

61 This work represents the first attempt to combine both kinds of residues coming from vine cultivation
62 and wine production to produce a compost. Microbial dynamics during the entire composting
63 sequence are studied along with physicochemical parameters characterizing the process and the final
64 product. The proposal to produce compost starting from these materials is not particularly new, but
65 the novelty proposed is to try to combine these materials together, also taking into account that they
66 are produced in periods of the year quite apart (branches around February and marcs around
67 September) and also to establish whether it could be possible to install a continuous composting
68 procedure that could be fed twice a year, alternatively with marcs and branches. This composted
69 material could then be periodically removed in part from the pile and used in the field as soil
70 amendment, while the remaining part represents the “inoculum” for the fresh materials added.

71

72 **2. Materials and methods**

73 *2.1. Site description*

74 The study was carried out on a vineyard located in the "D.O.C. Piave" zone (lat. 45° 57' 36" and long.
75 12° 22' 21") at about 83 m above the sea level, a few kilometers from Cordignano (TV) in the Veneto
76 Region, Italy. The site presents climatic characteristics attributable to warm temperate values and

77 belonging to the mesothermal climate. Regarding temperatures, the annual average is 16.1 °C with
78 peaks above 23 °C in summer and below 2 °C in winter. The coldest month is January with average
79 monthly temperature of 4 °C and minimum around 0 °C. The warmest months are July and August
80 with peaks that often exceed 30°C and can reach 37 °C.

81 The average annual rainfall is around 1250 mm, the wettest months are October and November during
82 which rainfalls account for one third of the annual amount. Precipitations are generally abundant
83 during spring (March and April) and scarce in winter. In percentage, the total rainfall is spread 50%
84 in the three autumn months, 25% during the spring, 15% in the months of July-August and the
85 remaining 10% in the winter months. Meteorological data were obtained from the Regional
86 Environmental Protection Agency (Veneto, Italy)

87

88 *2.2. Composting piles setup and sampling*

89 On September 2011, two static piles, hereafter referred to as pile 1 and pile 2, were set up on a field
90 following the criteria indicated by Finstein et al. [18] by mixing 3 t of grape marcs coming from
91 crushing of grapes of Glera vine variety with 3.5 t of mechanically-shredded vine branches (average
92 branch fragments length was from 5 to 10 cm) from the past winter pruning. These materials represent
93 roughly the amount produced from 1 ha of vineyard.

94 In February 2012 the same amount of fresh pruning residues was added to pile 1 only. In September
95 2012, 3 t of fresh grape marcs were added to pile 1 only and in February 2013 about 70% of pile 1
96 was removed and 3.5 t of fresh mechanically-shredded vine branches were added to the pile and
97 mixed with the existing material.

98 For each pile, starting from September 2011 (T_0), samplings were carried out regularly about every
99 two months by pooling three sub-samples taken from different parts of the composting mass, at
100 different depth and exposure.

101 Since the study started in September, when only fresh marc was available, to build the first pile vine
102 branches were obtained from material stored from prunings of the preceding winter. Branches were

103 mechanically ground and mixed with grape marcs. As shown in Fig. 1, pile 1 was periodically fed
104 with marc and branches, while pile 2 was left to stand without any further addition. The evolution
105 was followed during 2 years, from September 2011 to September 2013 by monitoring environmental,
106 chemical and microbiological parameters.

107

108 *2.3. Environmental parameters monitoring*

109 The temperature of the piles was measured by using two probes equipped with a data logger for each
110 experimental pile, embedded into the pile at a depth of about 50 cm.

111 The data loggers were programmed to collect the temperature hourly. Data were elaborated using a
112 MicroLab Lite software (ver. 3.6.5, Fourier Systems, USA).

113 External temperature and rainfall were provided as average daily values by three ARPA
114 meteorological stations (Conegliano, Vittorio Veneto and Gaiarine) located in the site area.

115

116 *2.4 Compost chemical analyses and germination index*

117 Samples of compost from pile 1 and pile 2, collected at the beginning of the composting and during
118 the whole maturation phase, were collected to determine whether compost characteristics were
119 compliant to the requirements defined by the Italian law for compost quality [19]. Sample dry weights
120 were determined from 100 g of wet sample by leaving the samples at 40 °C for 24 h and then at 105°C
121 for 24 h [20].

122 Compost C, N and S content were measured using a CNS automatic analyser (Elementar vario
123 MACRO CNS, Elementar Analysen systeme GmbH, Hanau, Germany). Organic-N was calculated
124 by subtracting NH_4^+ (determined by selective electrode, Sevenmulti Mettler Toledo) from total
125 Kjeldahl N [21]. All analyses were performed in triplicate.

126 Heavy metals were quantified by inductively coupled plasma optical emission spectrometry (ICP-
127 OES) (Ciros Vision EOP, SPECTRO Analytical Instruments GmbH, Kleve, Germany) preceded by

128 acid digestion. Inert residues (plastic, metals, glass and stones) and other additional analyses required
129 by the Italian legislation were performed following the analytical method for compost [22].
130 Germination index (GI, seed germination and root length test) was measured on water extracts by
131 mechanically shaking the fresh sample of mature compost for 1 h (ratio 1:10 sample: distilled water
132 w/v, dry weight basis). Five ml of each extracts were pipetted into a petri dish lined with a Whatman
133 filter paper where 10 cress seeds (*Lepidium sativum* L.) were placed for an incubation period of 48 h
134 at 25° C in the dark. All experiments were performed in triplicate. GI was determined according to
135 Zucconi et al. [23]. If GI is $\geq 60\%$ the compost is defined as “non phytotoxic”. Data were subjected
136 to ANOVA and correlation analysis using Statistica 10.0 package (StatSoft Inc., Tulsa, OK) and
137 Fisher’s Protected Least Significant Difference was calculated for mean comparison.

138

139 2.5. Microbiological analyses

140 The following media were used to enumerate viable microorganisms according to their functional
141 characteristics:

142 TSA (Trypticase Soy Agar) medium (Oxoid) was used for total bacterial counts (TC). After
143 sterilization, 200 $\mu\text{g/ml}$ cycloheximide was added to prevent fungal growth.

144 BC medium (ammonium sulphate 1 g/l, potassium hydrogen phosphate 1 g/l, magnesium sulfate
145 heptahydrate 0.5 g/l sodium chloride 1 mg/l, agar 15 g/l, carboxymethylcellulose 10 g/l) was used for
146 cellulose-degrading bacteria (CB). After sterilization, 200 $\mu\text{g/ml}$ cycloheximide were added to
147 prevent fungal growth.

148 AIA (Actinomycetes Isolation Agar) medium (Oxoid) was used for enumeration of actinobacteria
149 (AC). After sterilization, 200 $\mu\text{g/ml}$ cycloheximide were added to prevent fungal growth.

150 PDA (Potato Dextrose Agar) medium (Oxoid) was used for molds (MO). After sterilization, 100
151 $\mu\text{g/ml}$ chloramphenicol were added to prevent bacterial growth.

152 FC medium (10 g/l of carboxymethylcellulose (Sigma Aldrich), 6.7 g/l of yeast nitrogen base
153 (Oxoid)) was used for enumeration of cellulolytic fungi (CF). After sterilization, 100 µg/ml
154 chloramphenicol were added to prevent bacterial growth.

155 For plate count analyses, 20 g of compost were dissolved into 180 ml of 0.85% NaCl solution and
156 shaken for 2 h at 150 rpm on a rotary shaker at 22°C.

157 Plates were incubated at 30°C for 7 days for mesophilic and at 60°C for 3 day for thermophilic
158 microorganisms. Each sample was analysed in triplicate.

159 Enumeration of viable *Salmonella* and *Escherichia coli* cells were performed according the
160 microbiological methods for compost analysis [24].

161

162 **3. Results and discussion**

163

164 Regarding the weather trend during the study, from September 2011 to September 2013, temperatures
165 remained near the mean values for the region, never exceeded 30°C and went below zero only for
166 few days during winter 2012.

167 Rainfall was as statistically expected for the region, mostly concentrated during springtime and
168 autumn and scarce during winter and summer.

169 The temperature inside the two piles presented remarkable differences throughout the whole period,
170 clearly attributable to the different strategies adopted.

171 In general, every addition of fresh material resulted in a temperature increase inside the piles. In pile
172 2 the temperature remained above 50°C for about 1 month and successively its trend followed the
173 evolution of the external values, remaining from 2 to 20 °C higher.

174 Also pile 1 showed a marked increase at the beginning and a high increase at every addition of fresh
175 material, which was more pronounced following the addition of marc, since this material contains
176 much more readily fermentable sugars than vine branches. In fact following addition of branches the

177 temperature rose up to 50°C while marcs supplementation brought the temperature above 60°C with
178 a peak close to 70°C.

179 This temperature increase is of particular importance regarding the inactivation of possible
180 pathogenic microorganisms and weed seeds. It is reported that a period of at least one week at
181 temperature above 55°C, defined as “thermophilic phase”, is required to reach a good sanitization
182 action, while temperatures between 45°C and 55°C improve the degradation rate [25]. Regarding
183 sanitization, while both piles reached the requested temperature at the beginning of the study, only
184 pile 2 exceeded again 55°C in correspondence of marc addition, due to its high content of simple
185 sugars. Considering the “thermophilic phase”, pile 1 never reached these values even during summer,
186 while pile 2 temperatures, due to fresh material additions, stayed above 45°C for 54% of the time.
187 Hence, both piles guaranteed a good sanitizing action, since pile 1 did not receive new material after
188 its setup and pile 2 underwent a sanitizing step after each addition of new material.

189 The most relevant advantage presented by pile 1 is the longer permanence of thermal conditions
190 favoring the optimal development of composting activities.

191

192 *3.1 Microbiological analyses*

193 The composting process involves the combined action of several microbial species naturally present
194 in the soil and in plant material which transform complex polymeric materials (mainly celluloses and
195 lignins) into compounds of lower molecular weight. Microbial activity is generally high at the
196 beginning of the composting process due to high availability of nutrients (mainly fermentable sugars)
197 and increases when addition of new plant material takes place.

198 Many different techniques are nowadays available to investigate microbial community dynamics
199 during composting, ranging from traditional plate counts and identification of culturable microbiota
200 [26, 27] to novel methods that give information on microbial community composition without
201 cultivation of organisms [28, 29]. However, in spite of the development of new powerful molecular
202 techniques, no single method has proven to be the most reliable for monitoring microbial communities

203 in environmental samples and the traditional techniques are still considered useful in environmental
204 microbiology [30, 31].

205 During the two years of composting we chose to monitor the evolution of five different microbial
206 groups, namely total bacteria, cellulose-degrading bacteria, actinobacteria (actinomycetes), molds
207 and cellulolytic fungi. We decided to focus on functional groups rather than sticking to taxonomic
208 groups that are in general less informative from a functional point of view.

209 Cultivation of each group was performed on specific selective media and growth of each functional
210 group was studied at two different temperatures, 30°C and 60°C, in order to evaluate the thermophilic
211 and mesophilic component of each fraction.

212 The present level of understanding of microbial community dynamics in composting processes is
213 largely based on studies carried out with traditional methods, such as cultivation on a plate, followed
214 by isolation and identification of bacteria, actinobacteria and fungi [32].

215 We performed 11 samplings throughout the study, every two months on average, starting one month
216 after piles were set up to allow the enrichment in the appropriate categories (Fig. 2)

217

218 *3.2 Total counts*

219 Regarding the mesophilic fraction, total counts gave comparable values for pile 1 and pile 2
220 throughout the whole experiment, showing higher values at the beginning of the period (5×10^9 ufc/g)
221 that progressively decreased in both piles down to slightly below 10^9 cfu/g. The only relevant
222 difference was recorded after the last addition of branches, that produced an increase in pile 1,
223 reasonably linked to nutrient supplementation.

224 Thermophilic microbiota had an opposite trend with respect to mesophilics. They started from similar
225 values but had a marked decrease at the third sampling in February, that could probably be ascribed
226 to a shock determined by the dramatic temperature drop inside the mass. After the addition of new
227 material they rapidly resumed their growth and remained at high levels for the rest of the period, with
228 a tendency to increase approximating 10^{10} cfu/g. Pile 2 showed the same initial decrease that was

229 then recovered but population levels did not reach values similar to that of pile 1, particularly towards
230 the end when total population clearly decreased. This seems to evidence that addition of new material,
231 determining an increase in temperature and an overall higher mean temperature, favors the
232 development of a thermophilic population.

233

234 *3.3 Actinobacteria*

235 These Gram positive, filamentous, aerobic or anaerobic bacteria are an important component of the
236 composting microbiota for their action on decomposition and also for their biocontrol potential [33].

237 Their initial number was around 10^9 cfu/g at the beginning in both piles.

238 The mesophilic fraction, after a peak close to 10^{10} cfu/g in both piles at the beginning, maintained a
239 steady level around 10^9 cfu/g but towards the end of the period it started to decrease significantly
240 below 10^8 cfu/g. It is known that composting actinobacteria are mostly thermophilic and perform well
241 between 30°C and 60°C, so this decrease seems to involve a non relevant fraction of this group of
242 microorganisms.

243 Thermophilic actinobacteria showed a similar trend in both piles, although in pile 1 their increase was
244 more rapid, corresponding to the first addition of branches. Both piles showed a marked decrease
245 during the first winter, the only period when the external temperature went below 0°C.

246 After addition of marc, actinobacteria showed a slight decrease with respect to the non-fed pile 2.

247 This could be due to the presence of ethanol produced by yeasts present in marcs, but they also reached
248 the same levels towards the end of the period. The level of thermophilic actinobacteria population
249 was always more than 1 log higher than that of the corresponding mesophilic fraction.

250

251 *3.4 Cellulolytic bacteria*

252 This is a heterogeneous category including gram positive and Gram negative bacteria, aerobic and
253 anaerobic, sharing the capability to enzymatically degrade cellulose.

254 Mesophilic CB had the highest initial value (above 10^9 cfu/g) among all the categories considered
255 and, after an initial peak that brought their presence close to 10^{10} cfu/g they stabilized their number
256 around 10^9 cfu/g throughout the duration of the study in both piles.

257 Thermophilic CB showed values considerably lower, starting from 5×10^8 cfu/g and then slightly
258 increased to values slightly below 10^9 cfu/g at the end. This seems to indicate that thermophilic
259 population takes a considerable time to establish.

260

261 *3.5 Molds*

262 These eukaryotic aerobic microorganisms are among the most diffuse and important agents of
263 degradation of organic substances, particularly plant material, including celluloses and lignins. Being
264 strict aerobes, the amount of this category is in general much less abundant inside the mass.

265 Mesophilic molds were present at 10^7 cfu/g at the beginning, which is about 2 logs lower than bacteria
266 and, after a moderate increase, they tended to slowly decrease and at the end of the period they
267 stabilized around the same population density of the beginning. Pile 2 showed a more stable behavior
268 during summer 2013, while pile 1 evidenced a decreasing trend during the same period. This could
269 be probably ascribed to temperature increase of the mass of pile 2 following the addition of branches.
270 Subsequently they found the same equilibrium of the undisturbed pile 2 around 10^7 cfu/g.

271 Thermophilic molds were the least present fraction at the beginning accounting for around 10^5 cfu/g,
272 but showed in both piles a clear progressive increase (contrary to the correspondent mesophilic
273 counterpart) that determined an increase of more than 2 logs throughout the experimental period.

274

275 *3.6 Cellulolytic fungi*

276 These fungi are capable of degrading cellulose, as confirmed by their growth in a medium having
277 cellulose as the sole energy source. It can be noted that the dynamics of this group, both for the
278 mesophilic and thermophilic fractions, was very similar to that of total molds, thus indicating that the
279 fungal population developing on plant material is composed almost entirely of cellulolytic fungi.

280

281 Overall, from the above data it appears that the two composting strategies do not dramatically modify
282 microbial dynamics, but it can be seen that continuous composting in the long run, appears better
283 from the microbiological point of view, particularly concerning the thermophilic fraction, which
284 established after 2 years at higher values. This procedure appears therefore suitable to select a
285 population that can establish at high levels and is not quantitatively influenced by material addition
286 and mixing.

287

288 *3.7 Analysis of carbon, nitrogen and sulphur content*

289 The results of CNS analysis related to the 11 collected samples (Fig. 3) show the evolution of carbon,
290 nitrogen and of respective ratio and the percentage of sulfur during composting. The first graph shows
291 the classic trend of the process that leads to the increase of nitrogen (% d.m.), the decrease of organic
292 carbon (% d.m.) and then the C/N ratio. This parameter is certainly the most significant because it
293 defines the proper performance of the composting process. If there is shortage of nitrogen, the
294 decomposition of the materials will proceed more slowly, resulting in a slow-down in microorganism
295 action. By contrast with an excess of nitrogenous substances, a release of nitrogen in the form of
296 ammonia occurs. Generally, a C/N ratio equal to 12 is the limit accepted for mature compost [34].
297 The sulfur instead presented a trend almost constant for the whole duration of the process but was
298 maintained at higher values, even if no significant differences with respect to sulfur values were
299 detected for the second pile. This effect can be explained considering the periodic pruning addition
300 (February) that concerned only one of the experimental piles. In fact in viticulture about 38% of plant
301 protection treatments are carried out against fungal diseases using sulfur-based fungicides, that
302 remain on the woody parts of the plant even after a long time and that have been measured during the
303 analysis of compost samples. In the first pile higher final percentages were identified also for nitrogen
304 and carbon, although the second pile presented substantially the same tendency. The differences

305 observed between the two piles related to the content of the three studied elements are consistent with
306 the different management applied to the first pile.

307

308 *3.8 Chemical analyses of compost*

309 Compost characteristics from the two piles are given in Table 1 and are compared with the quality
310 parameters required by the Italian law [19].

311 All parameters respected the requested limits (Table 1), with the exception of the moisture content
312 that was higher than 50% w/w with respected to the required values. This was due to the fact that
313 periodically, during the curing phase, water was added to the piles with the aim of favoring microbial
314 activity. The absence of *Salmonella* and *Escherichia coli*, i.e. typical fecal pathogens presents in
315 slurries, makes it clear that biomass sanitization, i.e. human pathogens reduction, occurred during the
316 composting processes. The presence of inert materials was below the legal limit. This fact suggests
317 that the biomass purity level of these materials is very high. The mean concentration for each heavy
318 metal compared with samples of vine shoot pruning [35] shows values always lower than the Italian
319 legal limit and confirms the high quality of the final compost.

320

321 *3.9 Germination test*

322 As it can appreciated even by eye (Fig.4) the addition of compost suspension induced a better root
323 proliferation corresponding to a germination index above 100% for compost from both piles. If
324 compared to the minimum value required by law, that is equal to 60%, this result indicates not only
325 the total absence of phytotoxicity but instead the presence of a beneficial effects towards the plants.

326

327 **4. Conclusions**

328

329 A novel approach for the reutilization of residues produced by winemaking-related activities was
330 studied in order to obtain a compost that can be reintroduced in the vineyard from where the plant

331 biomass came from. Results suggest that it is possible to set up a continuous composting facility by
332 feeding it twice a year alternatively with marcs and vine branches. The characteristics of the products
333 fulfill the requirements of the Italian law for its use as a fertilizer in the field. Such compost does not
334 show phytotoxic activity, rather it shows some stimulatory action on root elongation in vitro. Further
335 studies will be carried out to confirm the presence of positive traits of the compost in the field, by
336 distributing it in a real vineyard.

337

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342

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346 60A08-0032/11).

347

348 **References**

349

350 [1] OIV, State of the vitiviniculture world market. [http://www.oiv.int/public/medias/4710/oiv-](http://www.oiv.int/public/medias/4710/oiv-noteconjmars2016-en.pdf)
351 [noteconjmars2016-en.pdf](http://www.oiv.int/public/medias/4710/oiv-noteconjmars2016-en.pdf) (2016). Accessed 12 January 2017

352

353 [2] Abbona, E.A., Sarandón, S.J., Marasas, M.E., Astier, M.: Ecological sustainability evaluation of
354 traditional management in different vineyard systems in Berisso, Argentina. *Agric. Ecosyst. Environ.*
355 119, 335–345 (2007).

356

- 357 [3] Sousa, E.C., Uchôa-Thomaz, A.M.A., Carioca, J.O.B., de Moraes, S.M., de Lima, A., Martins,
358 C.G., et al.: Chemical composition and bioactive compounds of grape pomace (*Vitis vinifera L.*),
359 Benitaka variety, grown in the semiarid region of Northeast Brazil. *Food Sci. Technol.* 34, 135–142
360 (2014).
- 361
- 362 [4] Bovo, B., Fontana, F., Giacomini, A., Corich, V.: Effects of yeast inoculation on volatile
363 compound production by grape marcs. *Ann. Microbiol.* 61, 117-124 (2011).
- 364
- 365 [5] Fauci, M.F., Bezdicek, D.F., Caldwell, D., Finch, R.: End product quality and agronomic
366 performance of compost. *Compost Sci. Util.* 7 (2), 17-29 (2013).
- 367
- 368 [6] Bovo, B., Giacomini, A., Corich, V.: Effects of grape marcs acidification treatment on the
369 evolution of indigenous yeast populations during the production of grappa. *J. Appl. Microbiol.* 111,
370 382–388 (2011).
- 371
- 372 [7] Bovo, B., Nardi, T., Fontana, F., Carlot, M., Giacomini, A., Corich, V.: Acidification of grape
373 marc for alcoholic beverage production: Effects on indigenous microflora and aroma profile after
374 distillation. *Int. J. Food Microbiol.* 152, 100–106 (2012).
- 375
- 376 [8] Maragkoudakis, P.A., Nardi, T., Bovo, B., D’Andrea, M., Howell, K.S., Giacomini, A., et al.:
377 Biodiversity, dynamics and ecology of bacterial community during grape marc storage for the
378 production of grappa. *Int. J. Food Microbiol.* 162, 143–151 (2013).
- 379
- 380 [9] Campanaro, S., Treu, L., Vendramin, V., Bovo, B., Giacomini, A., Corich, V.: Metagenomic
381 analysis of the microbial community in fermented grape marc reveals that *Lactobacillus*

382 *fabifermentans* is one of the dominant species: insights into its genome structure. Appl. Microbiol.
383 Biotechnol. 98, 6015–6037 (2014).

384

385 [10] Favaro, L., Corich, V., Giacomini, A., Basaglia, M., Casella, S.: Grape marcs as unexplored
386 source of new yeasts for future biotechnological applications. World J. Microbiol. Biotechnol. 29,
387 1551–1562 (2013).

388

389 [11] Patti, A.F., Issa, G.J., Smernik, R., Wilkinson, K.: Chemical composition of composted grape
390 marc. Water Sci. Technol. 60 (5), 1265-1271 (2009).

391

392 [12] Santos, M., Diáñez, F., del Valle, M.G., Tello, J.C.: Grape marc compost: microbial studies and
393 suppression of soil-borne mycosis in vegetable seedlings. World J. Microbiol. Biotechnol. 24 (8),
394 1493–1505 (2008).

395

396 [13] Carmona, E., Moreno, M.T., Avilés, M., Ordovás, J.: Use of grape marc compost as substrate
397 for vegetable seedlings. Sci. Hortic. 137, 69-74 (2012).

398

399 [14] Manios, T.: The composting potential of different organic solid wastes: experience from the
400 island of Crete. Environ. Int. 29 (8), 1079–1089 (2004).

401

402 [15] Zmora-Nahum, S., Hadar, Y., Chen, Y.: Physico-chemical properties of commercial composts
403 varying in their source materials and country of origin. Soil Biol. Biochem. 39 (6), 1263-1276 (2007).

404

405 [16] Lobo, M.C.: The effect of compost from vine shoots on the growth of Barley. Biol. Wastes 25
406 (4), 281–290 (1988).

407

- 408 [17] Bustamante, M.A., Paredes, C., Marhuenda-Egea, F.C., Perez-Espinosa, A., Bernal, M.P., Moral,
409 R.: Co-composting of distillery wastes with animal manures: carbon and nitrogen transformations in
410 the evaluation of compost stability. *Chemosphere* 72 (4), 551–557 (2008).
- 411
- 412 [18] Finstein, M.S., Miller, F.C., MacGregor, S.T., Psarianos, K.M.: The Rutgers strategy for
413 composting: process design and control, EPA project summary (EPA/600/S2-85/059), US
414 Environmental Protection Agency, Washington, DC (1985).
- 415
- 416 [19] D. Lgs. n.75, 2010. Riordino e revisione della disciplina in materia di fertilizzanti, a norma
417 dell'articolo 13 della legge 7 luglio 2009, n. 88. *Gazzetta Ufficiale* n. 121 del 26 maggio 2010.
- 418
- 419 [20] APHA: Standard methods for the examination of water and wastewater, American Public Health
420 Association, Washington, DC, 18th ed. (1992).
- 421
- 422 [21] Sweeney, R.A.: Generic combustion method for determination of crude protein in feeds:
423 collaborative study. *J. AOAC Int.* 72, 770-774 (1989).
- 424
- 425 [22] ANPA: Metodi di analisi del compost. Manuali e linee guida, 3/2001 (ISBN 88-448-0258-9),
426 (2001).
- 427
- 428 [23] Zucconi, F., Pera, A., Forte, M., De Bertoldi, M.: Evaluating toxicity of immature compost,
429 *BioCycle*. 22 (2), 54–57 (1981).
- 430
- 431 [24] ANPA: Metodi microbiologici di analisi del compost. Manuali e linee guida, 20/2003 (ISBN 88-
432 448-0090-X), (2003).
- 433

- 434 [25] Stentiford, E.I.: Composting control: principles and practice. In: De Bertoldi, M., Sequi, P.,
435 Lemmes, B., Papi, T. (eds.) *The science of composting*, pp. 49–59. Blackie Academic and
436 Professional, Glasgow (1996).
- 437
- 438 [26] Nakasaki, K., Sasaki, M., Shoda, M., Kubota, H.: Change in microbial numbers during
439 thermophilic composting of sewage sludge with reference to CO₂ evolution rate. *Appl. Environ.*
440 *Microbiol.* 49, 37–41 (1985).
- 441
- 442 [27] Gazi, A.V., Kyriacou, A., Kotsou, M., Lasaridi, K.E.: Microbial community dynamics and
443 stability assessment during green waste composting. *Glob. NEST J.* 9 (1), 35–41 (2007).
- 444
- 445 [28] Fracchia, L., Dohrmann, A.B., Martinotti, M.G., Tebbe, C.C.: Bacterial diversity in a finished
446 compost and vermicompost: differences revealed by cultivation-independent analyses of PCR-
447 amplified 16S rRNA genes. *Appl. Microbiol. Biotechnol.* 71 (6), 942–952 (2006).
- 448
- 449 [29] Tiquia, S.M.: Microbial community dynamics in manure composts based on 16S and 18S rDNA
450 T-RFLP profiles. *Environ. Technol.* 26 (10), 1101-1114 (2005).
- 451
- 452 [30] Roberts, M.S., Klamer, M., Frazier, C., Garland, J.L.: Community profiling of fungi and bacteria
453 in an in-vessel composter for the NASA advanced life support program. In: Michel, F.C., Rynk, R.F.,
454 Hoitink, H.A.J. (eds.) *Proceedings of the 2002 International Symposium ‘Composting and Compost*
455 *Utilization’*, pp. 139–155. Emmaus: JG Press, Columbus, OH (2002).
- 456
- 457 [31] Ryckeboer, J., Mergaert, J., Coosemans, J., Deprins, K., Swings, J.: Microbiological aspects of
458 biowaste during composting in a monitored compost bin. *J. Appl. Microbiol.* 94, 127-137 (2003).
- 459

- 460 [32] Alfreider, A., Peters, S., Tebbe, C.C., Rangger, A., Insam, H.: Microbial community dynamics
461 during composting of organic matter as determined by 16S Ribosomal DNA Analysis. *Compost Sci.*
462 *Util.* 10 (4), 303-312 (2002).
- 463
- 464 [33] Cuesta, G., García-de-la-Fuente, R., Abad, M., Fornes, F.: Isolation and identification of
465 actinomycetes from a compost-amended soil with potential as biocontrol agents. *J. Environ. Manage.*
466 95, S280–S284 (2012).
- 467
- 468 [34] Bernal, M.P., Paredes, C., Sánchez-Monedero, M.A., Cegarra, J.: Maturity and stability
469 parameters of composts prepared with a wide range of organic wastes. *Bioresour. Technol.* 63 (1),
470 91-99 (1998).
- 471
- 472 [35] Bustamante, M.A., Restrepo, A.P., Albuquerque, J.A., Pérez-Murcia, M.D., Paredes, C., Moral,
473 R., et al.: Recycling of anaerobic digestates by composting: effect of the bulking agent used. *J. Clean.*
474 *Prod.* 47, 61-69 (2013).
- 475
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Parameter	legal limits	Compost pile 1	Compost pile 2
Humidity	<500 g kg ⁻¹ FM	592.3 ± 23.1	581.7 ± 33.2
pH	6.0 - 8.5	8.24 ± 0.04	8.04 ± 0.07
TOC	>200 g kg ⁻¹ DM	287.2 ± 4.1	299.5 ± 6.9
Humic acid + Fulvic acid	>70 g kg ⁻¹ DM	135.1 ± 2.3	121.3 ± 3.7
TN	g kg ⁻¹ DM	20.5	19.7
Org-N	>80% TN	88.94 ± 0.7	90.31 ± 0.9
C/N	<25	13.01	12.53
S	g kg ⁻¹ DM	3.28	1.88
Cd	<1.5 mg kg ⁻¹ DM	0.04 ± 0.01	0.03 ± 0.01
Cr ^{VI}	<0.5 mg kg ⁻¹ DM	<0.4	<0.4
Hg	<1.5 mg kg ⁻¹ DM	0.04 ± 0.01	0.02 ± 0.01
Ni	<100 mg kg ⁻¹ DM	1.07 ± 0.13	0.98 ± 0.09
Pb	<140 mg kg ⁻¹ DM	0.87 ± 0.11	0.81 ± 0.21
Cu	<230 mg kg ⁻¹ DM	26.55 ± 0.33	23.63 ± 0.23
Zn	<500 mg kg ⁻¹ DM	13.42 ± 0.81	12.92 ± 0.72
<i>Salmonella</i>	MPN absent	absent	absent
<i>Escherichia coli</i>	<1000 UFC/g	absent	absent
GI (<i>Lepidium sativum</i> L.)	>60%	108.92	102.66
Plastic, glass, metals (ø>2 mm)	<5 g kg ⁻¹ DM	0.3	0.1
Stones (ø >5 mm)	<50 g kg ⁻¹ DM	1.8	0.7

478 FM: fresh matter; DM: dry matter; TOC: total organic carbon; Org-N: organic nitrogen; TN: total nitrogen;
479 GI: germination index.

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482 Table 1. Characteristics of compost from pile 1 and 2 at the end of the humification process compared with

483 legal limits according to the Italian legislation [19] determined as indicated in ANPA [22].

484

485 **Figure legends**

486

487 Fig. 1. Trend of temperature and rainfall during composting. Black arrows: vine branches addition;
488 white arrows: grape marcs addition; dashed arrows: sampling times. The dashed region indicates the
489 “thermophilic phase” according to Stentiford [25].

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491 Fig. 2. Microbial populations dynamics during composting. MO: molds; CF: cellulolytic fungi; TC:
492 total bacteria count; CB: cellulolytic bacteria; AC: actinobacteria.

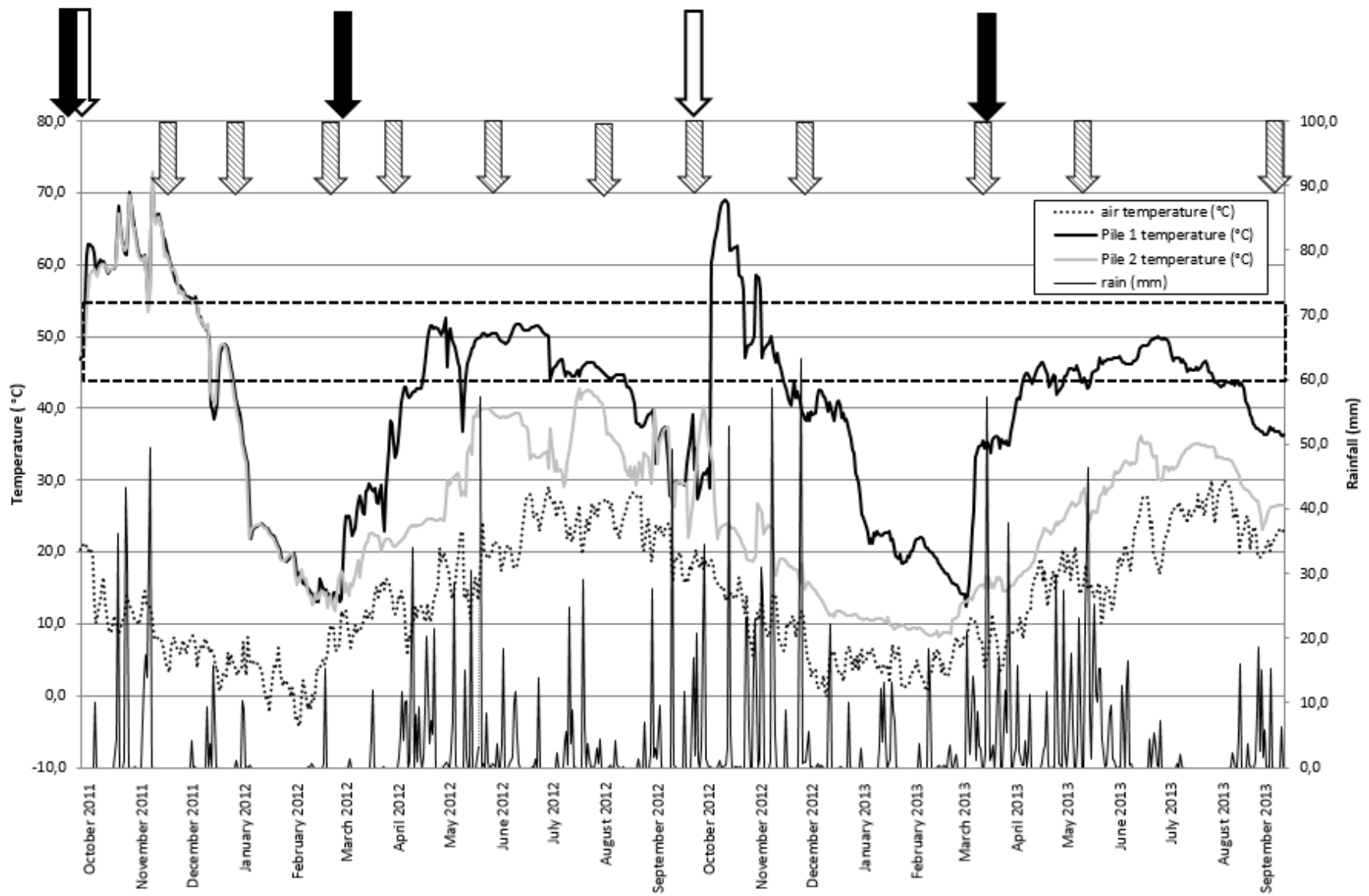
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494 Fig. 3. Percentages of carbon, nitrogen, sulfur, and C/N ratio in the two experimental piles during the
495 humification process. Black: pile1; grey, pile 2.

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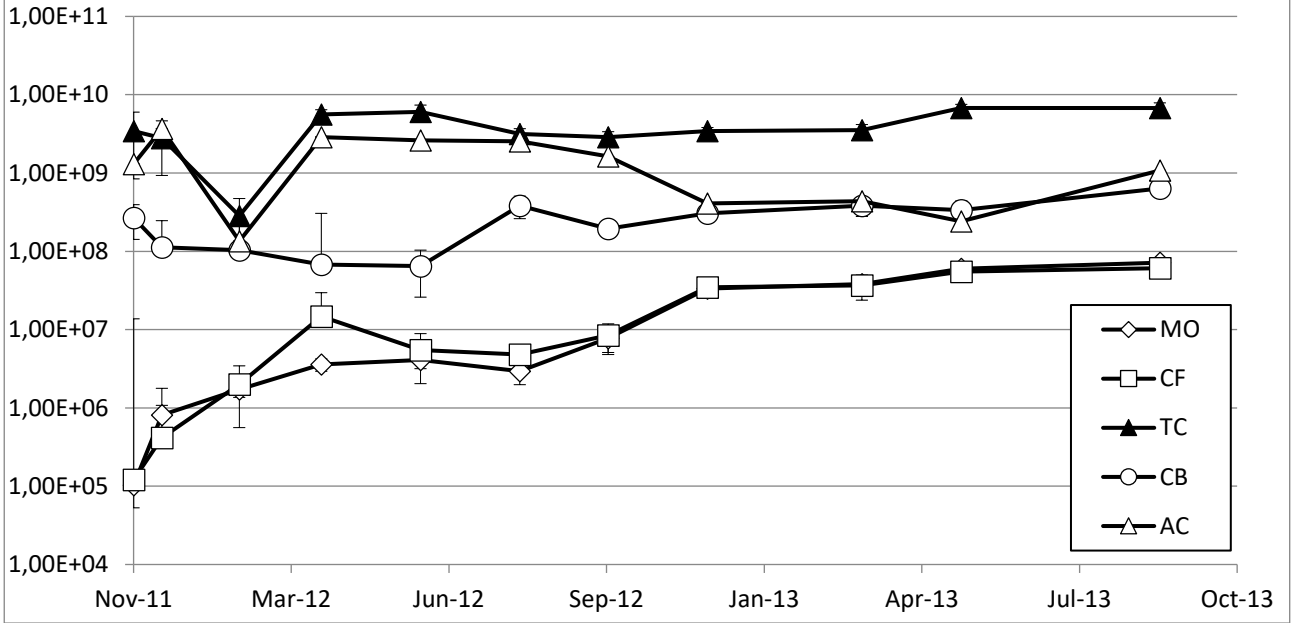
497 Fig. 4. Germination test. Seeds in the upper part received compost-shaken suspension, while those in
498 the lower part received 100% water.

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501 **Fig. 1.**

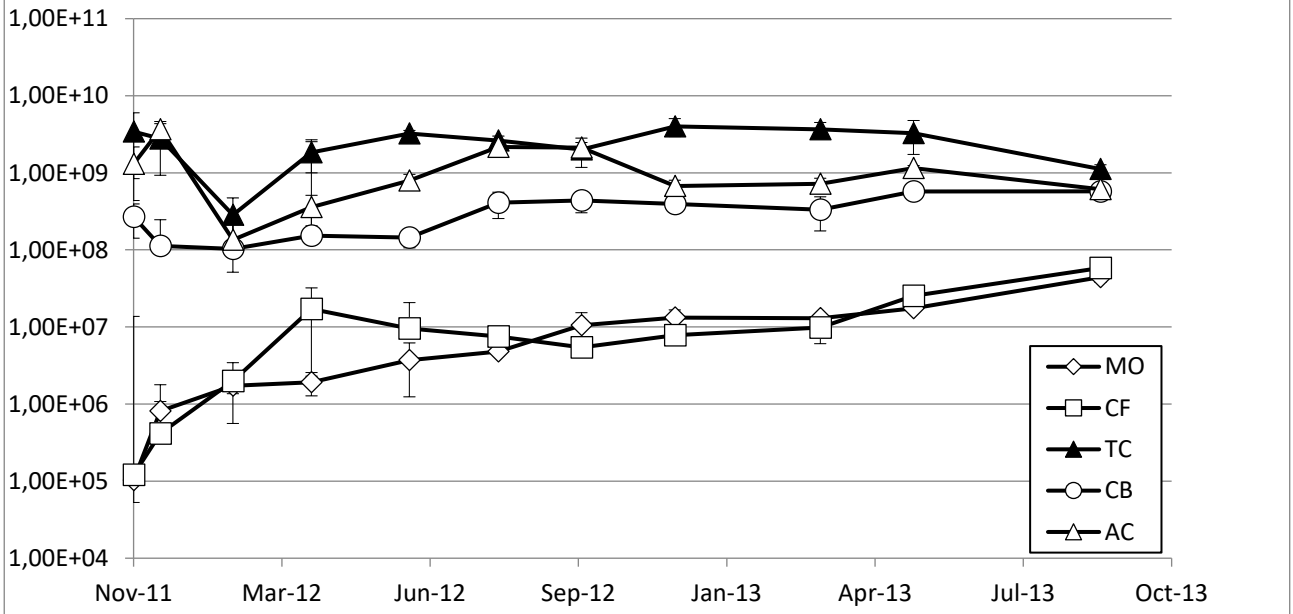
Thermophilic - Pile 1



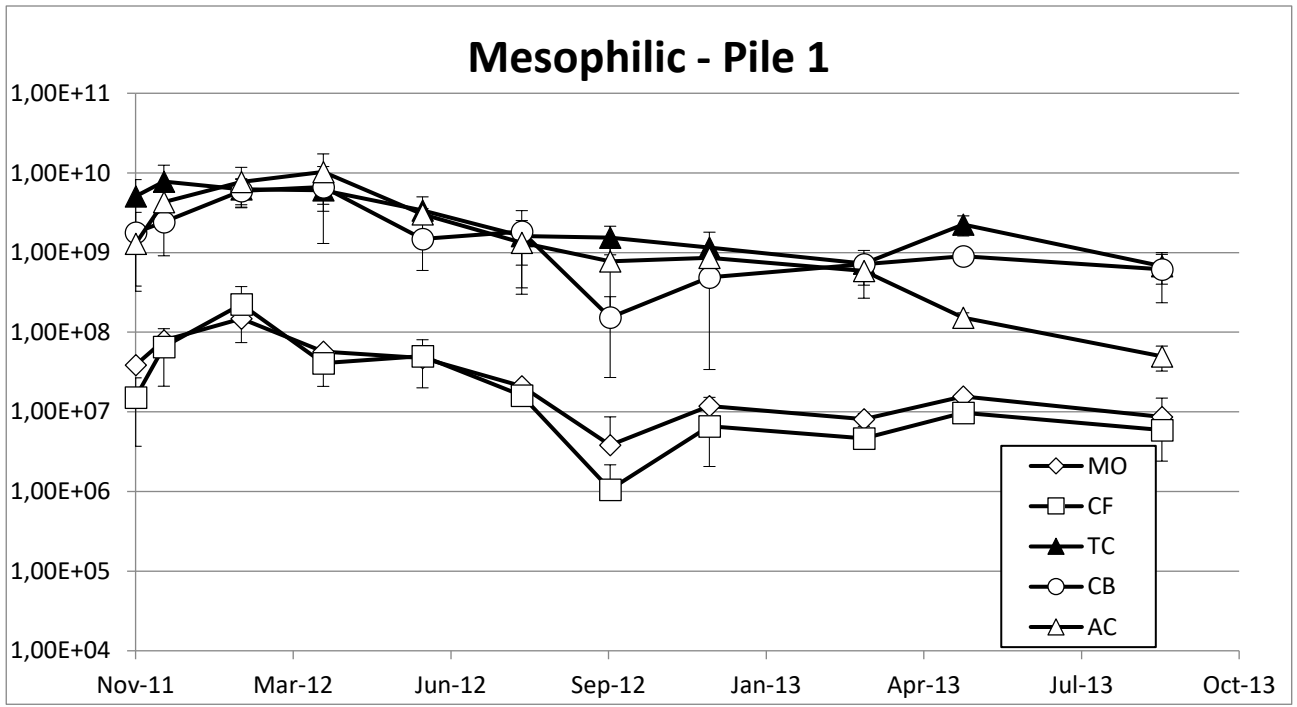
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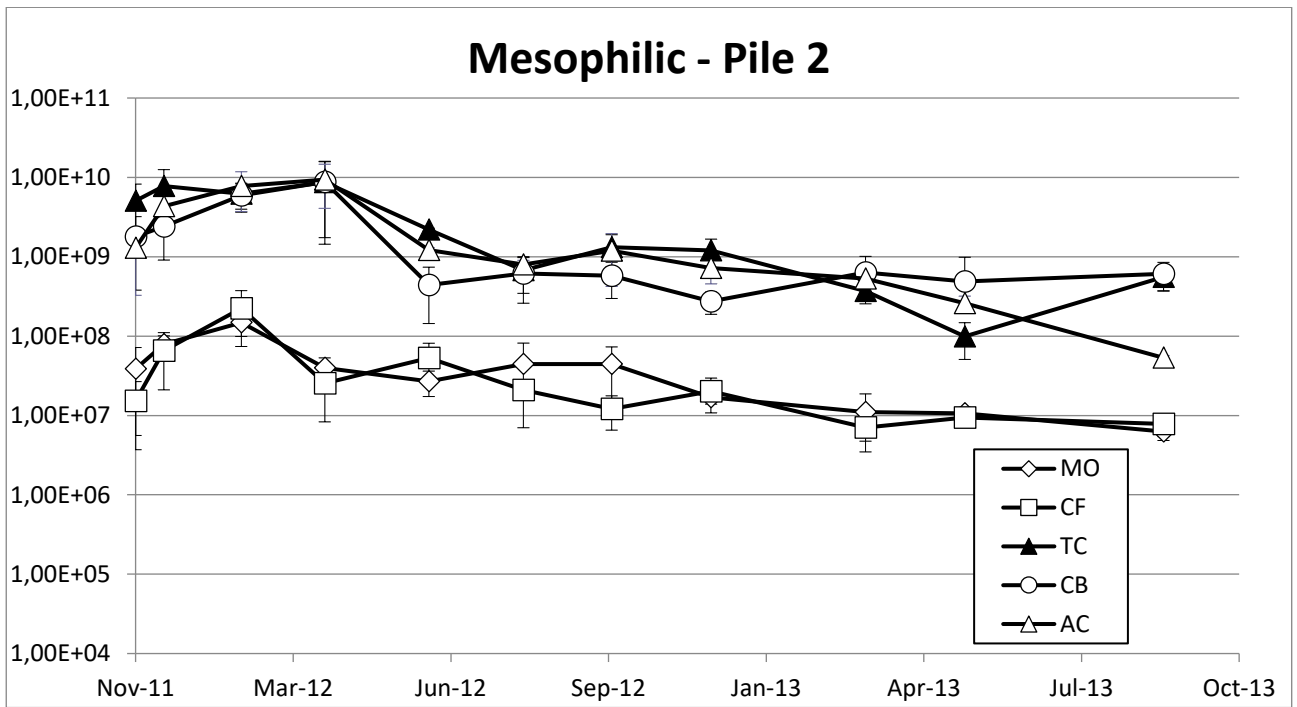
Thermophilic - Pile 2



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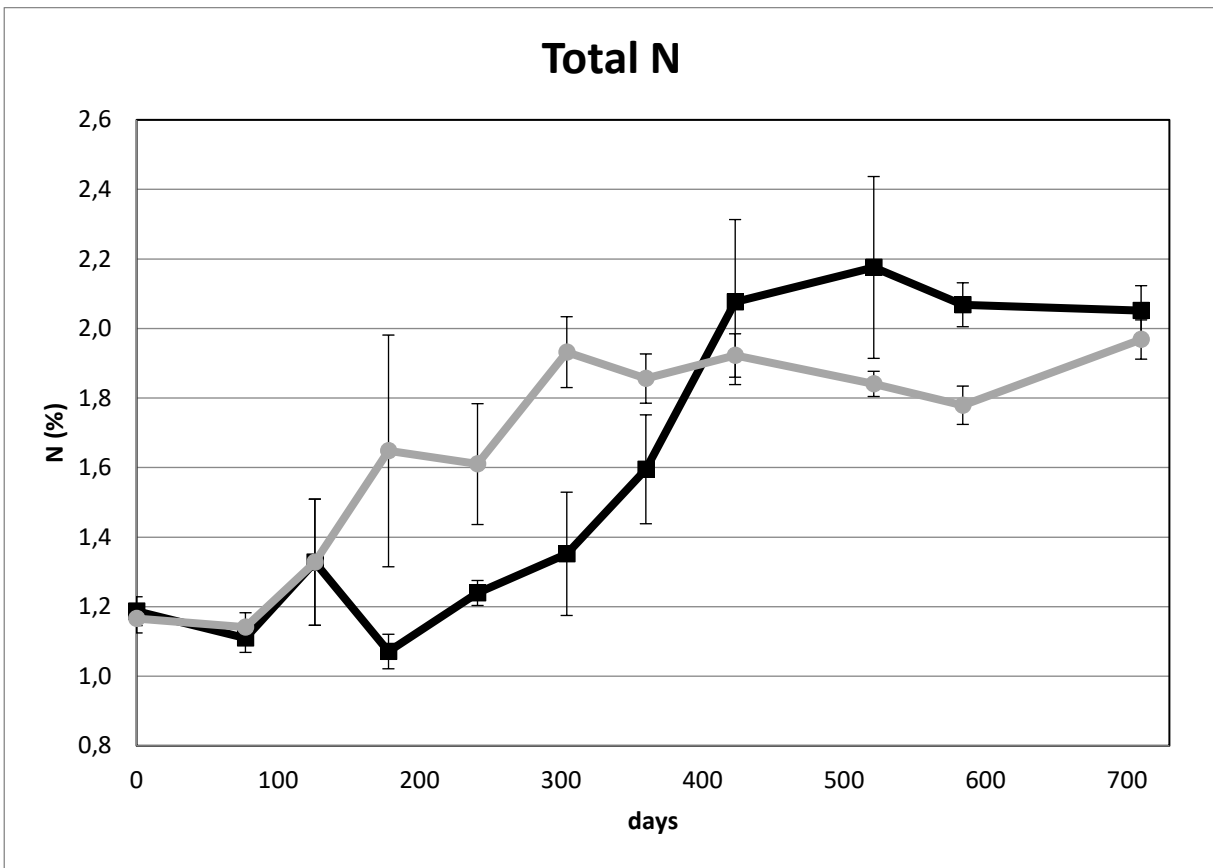
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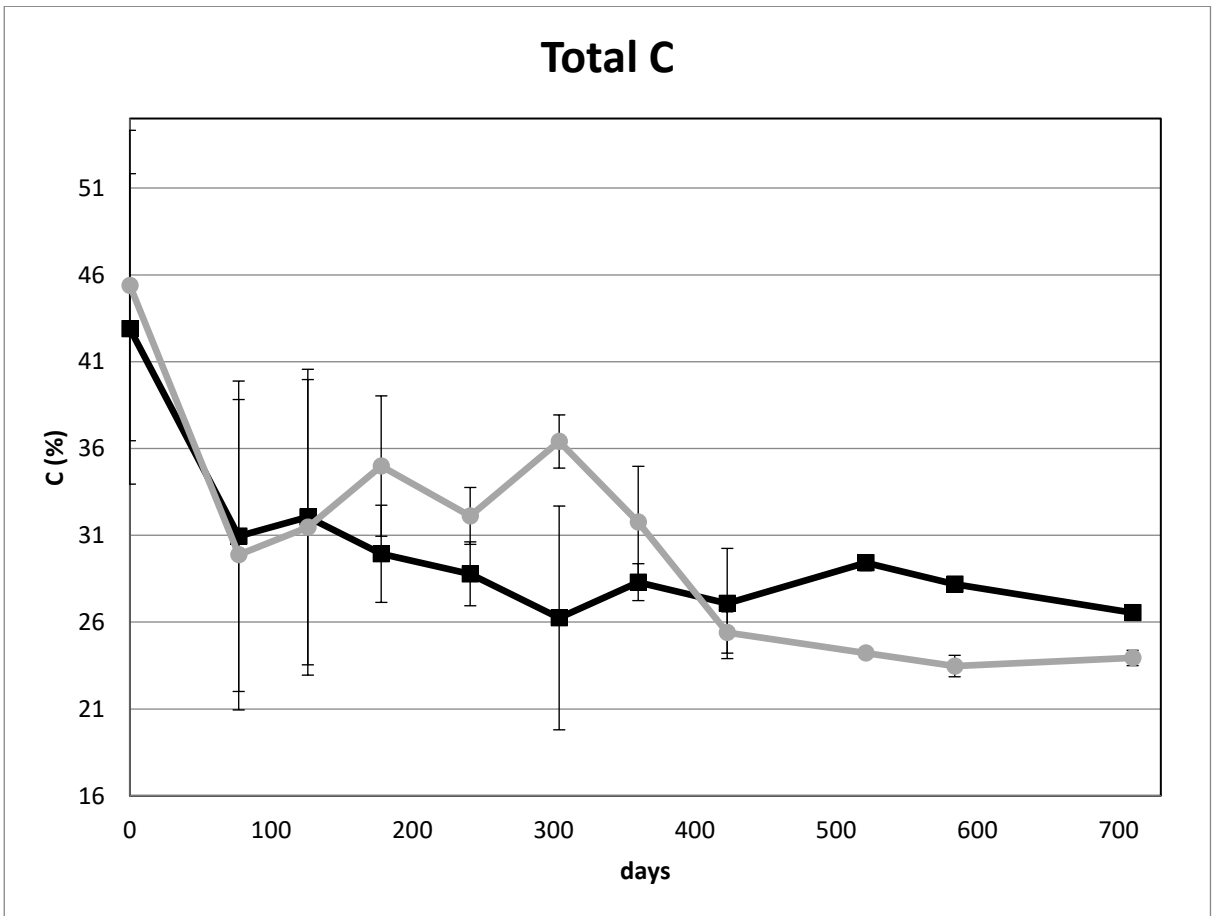
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Fig. 2.

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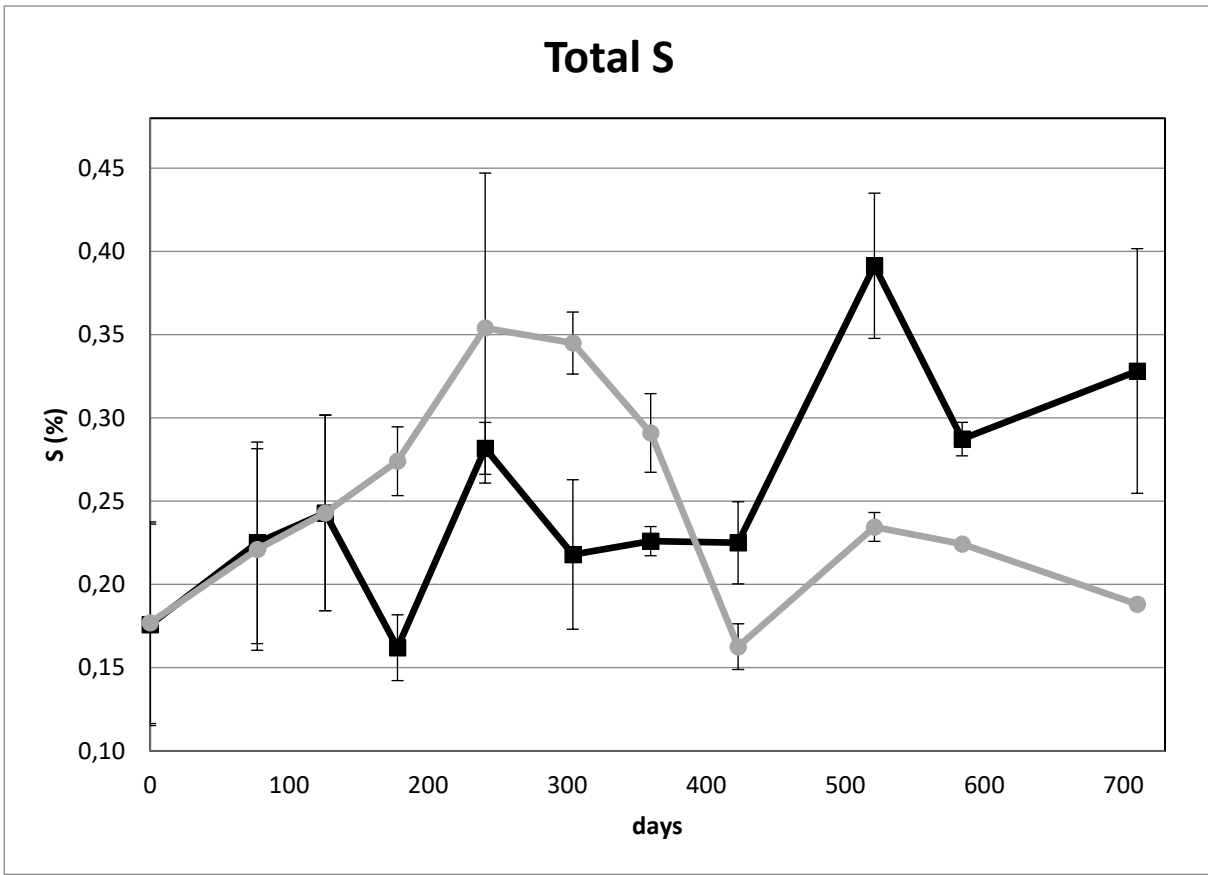


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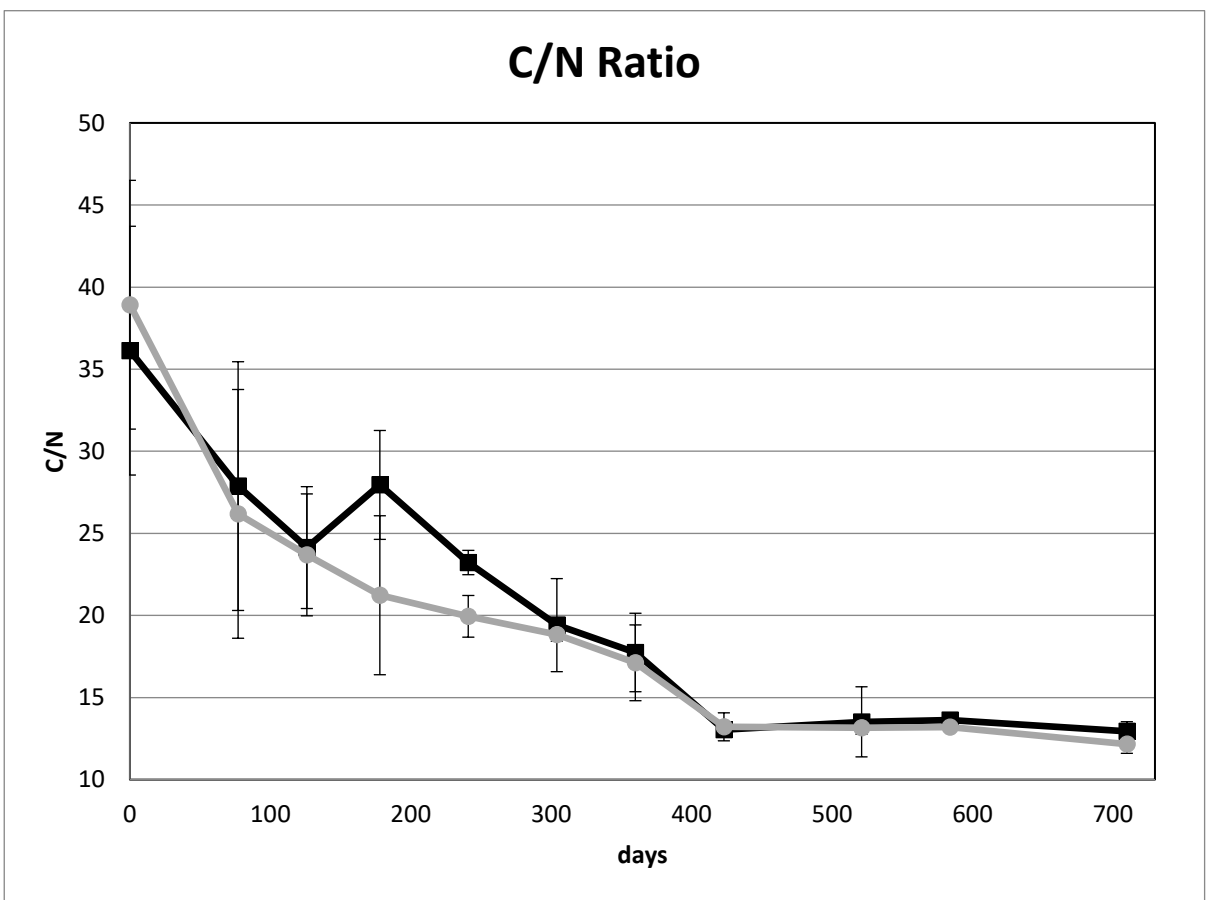


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Fig. 3.

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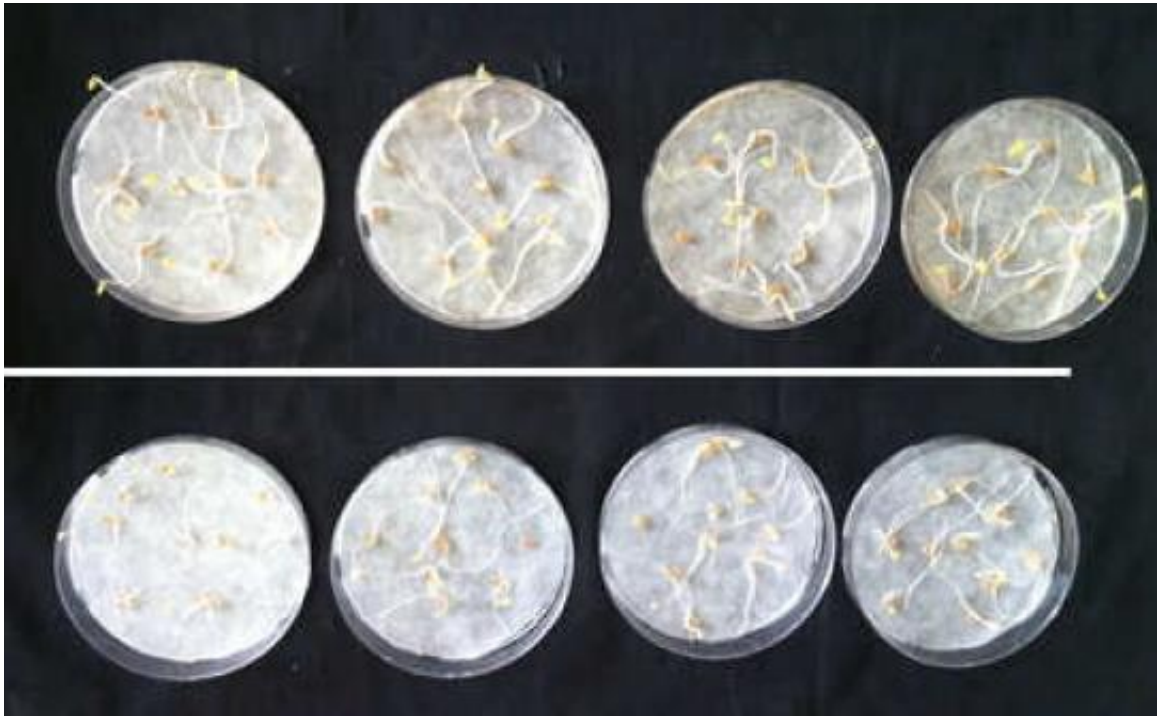


Fig. 4