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**1Comparison of soil biosolarization with mesophilic and thermophilic solid
2digestates on soil microbial quantity and diversity**

3

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28Abstract

29Soil biosolarization (SBS) is a pest control technique that combines passive solar
30heating and fermentation of amended organic matter. The extreme soil conditions
31generated during SBS could decrease microbial biomass and restructure the soil
32microbiome, which could impact soil quality. Digestates from anaerobic digesters may
33harbor microbial communities tolerant of the oxygen, moisture, and temperature
34stresses encountered during SBS as these conditions may also occur in digesters.
35Digestate microbial communities may contribute to soil fermentation during SBS and
36affect organic matter turnover in soils treated with SBS. The objective of this study was
37to assess the effect of SBS on soil microbial diversity and quantity when solid digestates
38from thermophilic (TD) and mesophilic (MD) anaerobic digesters were used as soil
39amendments. In the soils amended with TD, communities showed the greatest
40divergence from the initial soil state whereas MD amendment resulted in a microbiome
41more similar to the non-amended soil. The microbial biomass of the biosolarized soils
42was significantly greater than the non-amended, solar-heated soil. The microbial
43biomass in the biosolarized soils was dominated by K-strategic or “native” species.
44Solar heating of the non-amended soil mainly affected “native” species, leading to
45conditions where other opportunistic species become more dominant. Further studies
46are needed to elucidate whether the persistent microbes in the soil are benign or
47pathogenic and to understand their roles in pest inactivation and nutrient cycling during
48and following SBS.

49Keywords

50Soil Biosolarization; digestates; microbial diversity; microbial activity; microbial
51community

52

531. Introduction

54 Soil fumigation is an important agronomic practice in the production of many
55 high-value vegetable and fruit crops. Traditional soil fumigants used to eliminate
56 pathogens and weed seeds in agricultural soils, such as methyl bromide, are harmful for
57 the environment and humans. Alternative soil fumigants such as chloropicrin or 1,3-
58 dichloropropene present less risk to the ozone layer (Ajwa et al., 2013), but still present
59 health concerns for humans, making these fumigants undesirable and especially
60 dangerous for urban farms (Sanchez-Moreno et al., 2009). Moreover, these fumigants
61 do not discriminate between undesirable pests and beneficial microorganisms (Momma,
62 2015). It is therefore necessary to find alternative, sustainable ways of controlling
63 soilborne pests.

64 Soil biosolarization (SBS) can be a sustainable soil pest control technique as it
65 avoids the use of synthetic pesticides. SBS has successfully inactivated fungal,
66 nematode, insect, and weed pests (Bonanomi et al., 2008; Yao et al., 2016). SBS is a
67 combination of soil solarization, where moist soil is covered with a transparent tarp to
68 increase the soil temperature via passive solar heating (Katan et al., 1976), and
69 anaerobic soil disinfestation (ASD) where soil is amended with organic matter prior to
70 tarping to promote anaerobic microbial activity (Lamers et al., 2010). The addition of
71 organic matter can enhance pest inactivation through several mechanisms. First, the
72 additional microbiota and nutrient source associated with the amendment can enhance
73 soil heating through biological heating. For example, amending soil with compost and
74 organic matter yielded temperature increases between 2 and 5°C greater than non-
75 amended soil when both were solar heated (Achmon et al., 2015; Simmons et al., 2013;
76 Simmons et al., 2016). Secondly, anaerobic microbial fermentation of organic matter
77 can result in the production of organic compounds such as volatile fatty acids (VFAs)

78that accumulate due to the physical barrier of the plastic tarp. VFAs may accumulate to
79levels that result in a decrease in soil pH and increase in toxicity to soil biota and weed
80seeds (Achmon et al., 2017; Gamliel and Stapleton, 1997; Huang et al., 2015a; Katase et
81al., 2009). Thirdly, microbiota may directly affect pest organisms through competition,
82such as between beneficial fungi and pathogenic fungi, or infection, such as microbial
83degradation of the seed coat of weed seeds (Huang et al., 2015b; Rokhbakhsh-Zamin et
84al., 2011). The combined thermal, biochemical and ecological action of the organic
85amendment in SBS can significantly increase the efficiency of the process,
86counteracting the need for high solar radiation and long treatment durations of up to 6
87weeks, which can infringe on the most productive time of the growing season for
88farmers.

89 The soil microbiome plays an essential role in maintaining soil fertility. Soil
90biosolarization will likely affect soil microbial biomass and diversity although these
91impacts not yet been quantified. It has been reported that solar heating of the soil (i.e.,
92solarization) is beneficial to soil microflora because it stimulates fluorescent
93*Pseudomonas* spp. (Gamliel and Katan, 1991). Soil solarization has also been shown to
94have favorable effects on soil microbiota as evidenced by increased amino acid
95synthesis (Chen et al., 2000). Significant changes in the microbial diversity within the
96soil profile have also been observed after soil solarization or soil biosolarization
97(Simmons et al., 2014; Simmons et al., 2016). Specifically, a significant decrease of the
98phylum Firmicutes was observed in solarized soils, particularly at greater depths.
99Additionally, a significant increase of bacteria from the phylum Proteobacteria was
100observed with increasing soil depth in biosolarized soils. These shifts were attributed to
101temperature gradients established during solarization and changes in the composition of
102the soil aqueous and gaseous phases.

103 Soil temperatures above 50 °C are considered to be lethal to most soil-borne
104 pathogens and most mesophilic microbes (Stapleton, 1996) and temperatures around
105 60°C have been shown to significantly decrease microbial metabolism and survival in
106 general (Palese et al., 2004). For instance, it has been observed that soil solarization
107 decreased microbial activity as well as the activities of phosphatase and β -glucosidase
108 enzymes in different solarized plots compared to non-solarized controls (Scopa et al.,
109 2009).

110 Elucidating the impact of SBS on soil biological activity and microbial
111 community structure is important for understanding post-treatment implications for
112 agriculture. The effects of biotic and abiotic stress on soil organisms can be assessed by
113 measuring changes in biological activity, microbial biomass, soil respiration and
114 enzyme activities (Scopa and Dumontet, 2007). In addition, active microbial soil
115 biomass and diversity can be used to understand microbial decomposition of soil
116 organic matter (SOM), a critical element of the soil phytonutrient cycle (Stenstrom et
117 al., 1998).

118 The objective of this study was to assess the composition and activity of
119 microbial communities in biosolarized soils amended with two different solid digestates
120 from anaerobic digestion of mixed organic wastes. Like compost, which has been
121 shown to be an effective inoculum for biosolarization (Simmons et al., 2013), digestates
122 contain robust, anaerobic, organic matter-degrading microbial communities that may
123 also tolerate biosolarization. Studies have shown that SBS can increase the proportion
124 of facultative and obligate anaerobic microorganisms in the soil (Yao et al., 2016). As a
125 result, digestate amendment could influence soil microbial community restructuring
126 during biosolarization and help prime the soil with active bacteria following treatment.
127 This could be important for occupying soil niches that pathogens may otherwise

128recolonize. Furthermore, enriching the soil with biomass-degrading bacteria from
129digestate could benefit nutrient cycling in the soil. In this study, soil microbial biomass
130following biosolarization was estimated using substrate-induced respiration (SIR) with
131measurement of the respiration response kinetics (Anderson and Domsch, 1978;
132Panikov and Sizova, 1996; Stenstrom et al., 1998). These data can provide information
133on the physiological state of the microbial biomass by estimating the ratio of growing
134(r-strategic) versus non-growing (K-strategic) (Chen et al., 2012b; Stenstrom et al.,
1351998). The taxonomic diversity of microbial communities was also analyzed via next
136generation 16S rRNA gene sequencing. These results will help gauge the valorization
137potential of digestates in agricultural to improve soil microbial activity and diversity as
138well as provide guidelines for application of digestate in biosolarization.

1392. Materials and Methods

1402.1 Soil and digestate description

141

142 Dry topsoil (Hanford sandy loam) was collected from the 0–15 cm depth range
143at UC Kearney Agricultural Research and Extension Center (KARE) in Parlier, CA
144(36.6_N; 119.5_W; elevation 97 m a.s.l.), sieved through a 2 mm screen and stored at
145room temperature (S). The contents of organic matter, sand, silt and clay were 0.015 g
146g⁻¹, 0.41 g g⁻¹, 0.37 g g⁻¹ and 0.22 g g⁻¹, respectively.

147 Two solid digestates from two anaerobic digesters with different operational
148conditions and of feedstocks were used in the experiment. A thermophilic digestate (TD)
149was acquired from an anaerobic digester located on the University of California, Davis
150campus in Davis, CA. The UC Davis digester processes mixed organic waste (food,
151agriculture, and green wastes). The digester utilizes sequential thermophilic hydrolysis
152and methanogenesis (55°C) with low solids loading (5-10% total solids in the

153methanogenesis phase). The solid digestate was periodically separated from the liquid
154phase of the methanogenic sludge and dewatered by pressing. The Yolo County Landfill
155(Woodland, CA) provided a mesophilic digestate (MD) from anaerobic digestion of
156food, manure and green wastes. Digestion occurred under high solids loading (40-60%
157of moisture content) and mesophilic conditions (35°C). Both digestates were air-dried,
158ground and sieved (<2mm) prior to mixing with the sampled soil. The total N of the
159soil, the TD and the MD amendments was 0.04, 1.48 and 1.03%, respectively. The total
160C of the soil, the TD and the MD amendments was 0.38, 47.10 and 41.53%,
161respectively.

1622.2 Soil mesocosm preparation

163

164 Soil mesocosms served as experimental units in field studies as described in
165previous studies (Achmon et al., 2017; Simmons et al., 2013). Soil mixtures for
166mesocosms were prepared by amending dry soil with dry thermophilic (STD) or
167mesophilic digestate (SMD) to achieve 1.5% loading (dry weight basis). Soil without
168amendment was used as a control (S). Soil mixtures were wetted to their respective field
169capacities and allowed to incubate overnight at 4°C so that moisture could equilibrate
170between the various soil components. Equilibrated soil mixtures were packed into 3.8 L
171black plastic grow bags (neHydro, Southampton, MA). The bags contained drainage
172holes to facilitate moisture and gas exchange with the surrounding soil. Compact
173temperature sensors and data loggers (Thermochron iButtons model 1922L, Embedded
174Data Systems, Lawrenceburg, KY) were embedded in the center of each microcosm at
17515 cm depth. The diameter and height of the filled mesocosms were 17.8 cm and 22.5
176cm, respectively.

1772.3 Field experiment

178 The field site was also located at KARE and it was prepared as previously
179described (Achmon et al., 2017; Simmons et al., 2013). Each field plot measured 1.8 x
1808.5 m and contained one mesocosm from each treatment and the arrangement of
181mesocosms was randomized. Mesocosms were buried in field plots. Plots were sprinkler
182irrigated to bring the soil moisture to field capacity (~11% wet basis) at the depths
183sampled in this study. The plots were then covered with clear plastic tarp ('Huskey Film
184Sheeting'; Poly-America, Inc., Grand Prairie, TX) to initiate biosolarization. Care was
185taken to minimize the amount of air trapped beneath the tarp. Five replicate plots were
186prepared. An identical set of mesocosms without temperature loggers were prepared and
187incubated in parallel at room temperature (RT, 22-27°C). They were loosely covered
188with plastic tarp to avoid water loss. After 8 days of solarization, the mesocosms were
189extracted from the field and divided into three sections representing different soil depths
190(H=0-7.5 cm, M=7.5-15 cm and L=15-22.5 cm depth). The contents of RT mesocosms
191were thoroughly mixed at the end of the incubation period, as no depth effect was
192expected due to the absence of solar heating or burial in field soil. All soil samples were
193stored at -20°C for further analysis.

1942.4 DNA isolation, sequencing and data processing and analysis

195 Genomic DNA was purified from the soil microbial communities at the
196beginning of the experiment and after solar heating or incubation at RT. DNA
197extraction, purification, amplification and sequencing were performed as described
198elsewhere (Fernandez-Bayo et al., 2016; Simmons et al., 2014). Raw sequencing reads
199were processed using the Quantitative Insights into Microbial Ecology (QIIME) toolkit
200(Caporaso et al., 2010). To filter the reads, the default values used were: $r = 3$; $p = 0.75$
201total read length; $q = 3$; $n = 0$; $c = 0.005\%$ (Bokulich et al., 2013). Filtered reads were

202 clustered into operational taxonomic units (OTU) with at least 97% sequence similarity
203 (Caporaso et al., 2010). Ecological analyses were performed using RStudio (version
204 0.98.1103) (Racine, 2012) with the vegan (Dixon, 2003) and entropart (version 1.2.1,
205 <http://CRAN.R-project.org/package=entropart>) packages. Prior to analysis, singletons
206 were removed from operating taxonomic unit (OTU) read count data to reduce noise.
207 Shannon diversity (H') indices, relative abundance, Bray-Curtis dissimilarity and non-
208 metric multidimensional scaling (NMDS) analyses were calculated for each community
209 as described elsewhere (Simmons et al., 2014).

210 2.6 Microbial biomass analysis

211 The soil microbial biomass at different depths in biosolarized and control soils
212 were estimated via measurement of respiration kinetics following substrate addition to
213 the soil. This analysis allowed estimation of the lag phase, the total microbial biomass,
214 exponential growth rate and the substrate-induced response of the microbial
215 communities. For this analysis, 250 ml aerated bioreactors (previously described by
216 Achmon et al., 2015 and Yu et al., 2015) containing 100 g (dry weight equivalent) of
217 field soil samples were incubated under controlled aerated conditions at room
218 temperature (24°C). The samples were incubated for for 3 days at which point the
219 respiration rate was observed to be constant. Soil samples were then spiked with 1 ml of
220 a 100 mg mL⁻¹ sterile glucose solution (the final soil concentration was 1 mg g⁻¹ soil).
221 Following addition of the substrate, CO₂ evolution rate (CER) was measured as
222 described elsewhere (Achmon et al., 2015; Yu et al., 2015). CER values were
223 normalized by the dry weight of sample in the reactor and calculated based on the C
224 content in the CO₂ (mg C-CO₂ g soil⁻¹ h⁻¹).

225 The CER data after glucose amendment were fitted to the following kinetic
226 respiration model (Eq. 1) to describe the microbial biomass in the soil (Panikov and

227Sizova, 1996; Stenstrom et al., 1998; Wutzler et al., 2012).

$$228 \quad CER_{(t)} = K + r \cdot e^{(\mu \cdot t)} \quad \text{Eq 1}$$

229This model assumes that mineralization of an easily degradable substrate in the soil,
230such as glucose, is performed by two main groups of microorganisms; those which grow
231exponentially as a result of the substrate addition ($r \cdot e^{(\mu t)}$) and those which increase their
232respiration to a high constant rate without increasing much in number (K). Thus, K is
233the constant respiration rate performed by non-growing microorganisms ($\text{mg C-CO}_2 \text{ g}$
234 $\text{soil}^{-1} \text{ h}^{-1}$), r is the initial respiration rate of growing microorganisms ($\text{mg C-CO}_2 \text{ g soil}^{-1}$
235 h^{-1}) and μ is the maximum specific growth rate. The SIR-rate is defined as the
236respiration rate obtained immediately after the addition of the substrate to the samples.
237Thus

$$238 \quad SIR = r + K \quad \text{Eq. 2}$$

239which is obtained by solving Eq. 1 when $t = 0$. The beginning of the exponential phase
240is often dominated by physiological adaptation of cells to new environmental
241conditions, called the lag-phase. During this phase often only a small increase in
242microbial biomass is observed (Wutzler et al., 2012). This lag phase (T_{lag}) can be
243estimated by the equation (Chen et al., 2012)

$$244 \quad T_{\text{lag}} = \frac{\ln \left(\frac{K}{r} \right)}{\mu} \quad \text{Eq. 3}$$

245The total microbial biomass (TMB) was calculated using Eq. (4).

$$246 \quad TMB = \frac{r}{r_0 \cdot Q} \quad \text{Eq 4}$$

247The parameter r_0 is the physiological state index of the microbial biomass before
248substrate addition,

$$249 \quad r_0 = \frac{K \cdot (1 - \lambda)}{r + K \cdot (1 - \lambda)} \quad \text{Eq 5}$$

where λ is a basic stoichiometric constant, which has an accepted value of 0.9 (Panikov and Sizova, 1996). Q, the total specific respiration activity, was calculated as follows:

$$Q = \mu / \lambda * Y_{CO_2} \quad \text{Eq 6}$$

where Y_{CO_2} is the microbial biomass yield per unit of glucose-C, which was assumed to be constant, with a mean value of 0.6 (Chen et al., 2012).

It can be challenging to determine a discrete SIR rate immediately after the addition of the substrate due to disturbances to the soil and measuring equipment during sample preparation and handling (Stenstrom et al., 1998). Thus, values of the parameters r and K were obtained by fitting observed CER measurements to Eq. 1 by a non-linear least square regression method (“nls” function in R software). The CER values used for modeling included data from immediately after the glucose addition to the last time point before the maximal respiration rate value was observed. Data following the maximal CER value were not included to ensure the microbial community was in the growth phase. Finally, goodness of fit was assessed using the coefficient of determination (R^2) according to (Wutzler et al., 2012),

$$R^2 = 1 - (SS_{err} / SS_{tot}) \quad \text{Eq 7}$$

Where SS_{err} is the residual sum of squares of the non-linear regression (difference between the predicted respiration rate and the observed respiration rate) and SS_{tot} is the total sum of squares (difference between the mean respiration rate for all time points and the observed respiration rate).

2.7 Statistical analysis

ANOVA and Tukey’s Honest Significant Difference (HSD) post hoc test were used to compare means at a significance level of 0.05. Statistical analyses were performed using JMP-IN software (version Pro 12, SAS, Cary, NC).

2753. Results

2763.1 Temperature evolution during soil biosolarization

277 The treatments showed similar diurnal trends in soil temperature during solar
278heating. The mean, minimum and maximum temperature recorded for every treatment at
27912.7 cm depth during the 8 days of the experiment were 35, 41, and 29°C, respectively
280(Table 1). No significant differences between the treatments were observed.

2813.2 Microbial diversity in biosolarized soils

282 The Shannon diversity index (H' , Figure 1) of the non-amended soil was always
283significantly higher than amended samples ($P<0.001$). The microbial diversity of the top
284layer of the non-amended soil slightly but significantly decreased after solarization
285compared to the initial soil ($P=0.012$). While amendment with either MD or TD
286resulted in depressed diversity values immediately following amendment, the diversity
287values for both amendment treatments increased after incubation at RT or solar heating
288in the field. The diversity of the STD communities was significantly greater than the
289SMD communities ($P<0.001$). Only at the deepest solarized layer was the diversity
290index of the SMD and STD samples not significantly different.

2913.3 Dissimilarity between microbial communities in biosolarized and control soils

292 The NMDS analysis of community dissimilarity showed three different clusters
293corresponding to the non-amended soil, STD, and SMD treatments (Figure 2). The
294communities in all treatments diverged significantly from the initial non-amended soil
295community (T0) in agreement with the Bray-Curtis dissimilarity results (Figure 1SI).
296For the control soil, both solar heating and RT incubation led to deviation from the
297initial community state. By comparison, the amended soils exhibited greater shifts from
298their respective initial community states after solar heating or RT incubation. Initially,

299 digestate amendment resulted in markedly different soil community compositions
300 relative to non-amended soil. For both digestate amendments, community structure
301 became more similar to that of the non-amended soil following solar heating or RT
302 incubation. However, distinct differences in community structure relative to non-
303 amended soil remained at the end of the experiment for both SMD and STD treatments.
304 At the end of the incubation period, SMD samples from RT incubation and the medial
305 and lower depths of the solar heated soil were the most similar to the non-amended soil
306 community structure. In general, all STD samples had greater dissimilarity from the
307 non-amended soil compared to SMD samples.

308

309 **3.4 Phylum composition of soil microbial communities**

310 Five different phyla represented at least 84.5% of the relative abundance for each
311 the samples (Table 2). In the original non-amended soil, the phylum dominating the
312 microbial community was Proteobacteria (41.05%). Incubation of the non-amended
313 samples at RT significantly decreased the Actinobacteria and Firmicutes and increased
314 Bacteroidetes ($P < 0.05$). Solarization also significantly decreased the abundance of
315 Actinobacteria at all depths, but to a lower extent than in samples incubated at RT. The
316 addition of the MD to the soil raised the abundance of Firmicutes to more than 70%.
317 Solarization and incubation at RT significantly decreased ($P < 0.05$) the relative
318 abundance of this phylum at all soil depths examined. In contrast, the relative
319 abundance of Proteobacteria and Bacteroidetes increased after the experiment in all the
320 SMD samples. The Actinobacteria also decreased significantly after incubation at RT
321 and for the lower layers of the solarized samples ($P < 0.05$). Finally, the levels of
322 Acidobacteria after solarization significantly decreased in the top layer and increased in
323 the low layer of solarized soil ($P < 0.05$). The STD samples were also dominated by

324Proteobacteria. The incubation at RT significantly increased this phylum, Acidobacteria
325and Bacteroidetes whereas the abundance of Firmicutes decreased ($P<0.05$).
326Solarization significantly decreased the relative abundance of Proteobacteria and
327increased Actinobacteria at the lowest layer ($p<0.05$).

328

3293.5 OTU contributions to community dissimilarity

330

331 Table 3 shows the five most abundant OTUs for each sample. The most
332abundant OTU in the non-amended soil, both before incubation or when incubated at
333RT, was affiliated with *Kaistobacter*. At the top layer of the solarized, non-amended soil,
334the most abundant genus was affiliated with *Thermomonas*. The most abundant genus in
335the lower solarized layers was affiliated with *Flavisolibacter*. For all the SMD samples,
336the most abundant genus represented was *Bacillus*, although it significantly decreased
337($P<0.01$) from 46.93% to less than 12% in all the samples after the experiment. After
338incubation at RT or solarization, *Thermomonas* and *Flavisolibacter* were the other most
339abundant genera found in the SMD samples. Finally, for the STD samples, the most
340abundant OTU in the non-incubated soil was the *Acinetobacter* (21.60%). This genus
341significantly decreased ($P<0.01$) after incubation at RT and solarization. At the upper
342and middle solarized layers, the most abundant OTU was *Balneimonas* and for the
343lowest solarized layer the most abundant OTU corresponded to *Pseudomonas*.

344 Table 4 shows the five OTUs that contributed most to the overall Bray-Curtis
345dissimilarity for various pairwise comparisons of treatments. The data show that the
346differentiating OTUs between the non-amended control soil and the SMD soil are
347largely microorganisms common to both communities that differ in relative abundance.
348In contrast, the primary differentiating OTUs between the non-amended soil and the
349STD soil contain a greater number of microorganisms that are exclusive to the

thermophilic digestate. The SMD soil also lacked some of the most abundant OTUs of the STD soil. Comparing non-amended and SMB soils after incubation, *Bacillus* and *Flavisobacter* genera were among the prominent differentiating bacteria across multiple soil depths in solar heated samples as well as for RT incubated samples. In the RT treatment and in the lower soil depth of the solar heated samples, where more moderate temperatures are expected, bacteria from genus *Lysobacter* and order RB41 were also major differentiators between the non-amended and SMB communities. In the uppermost soil layer, where the greatest soil temperatures occurred, the *Thermobacillus* genus, which was unique to the SMB mixture, was the second greatest contributor to dissimilarity between the SMB and non-amended soil communities. A variety of OTUs contributed to the dissimilarity between the non-amended and STD soil communities across the RT and solar heated treatments. *Balneimonas* and *Brevibacillus* genera were the greatest contributors to dissimilarity between STD and non-amended soil communities in the uppermost layer of the solar heated soil. These OTUs also distinguished the SMD community from that in the non-amended soil at the same depth. Although both conditions represented more moderate soil temperatures, different OTUs were largely responsible for differences between the communities in the STD soils from RT incubation and the solarized lower soil depths and the communities from the similarly treated non-amended soils. For instance, the *Pseudomonas* genus contributed most to differences between the STD and non-amended soils in the lower depth of the solarized soil, but was not among the top 5 contributors for the RT treatment. Instead, the *Acinetobacter* genus was the dominant contributor to dissimilarity at RT. When compared against one another, the biosolarized SMD and STD communities differed primarily due to the abundance of *Bacillus* bacteria in the SMD soils. Similarly, the greater levels of *Thermomonas* in the SMD soils was also a major contributor to

375dissimilarity across all samples. STD-exclusive OTUs such as *Pseudomonas*, *Serpens*,
376*Acinetobacter*, and *Cellvibrio* were substantial differentiators between the SMD and
377STD communities as well.

3783.6 Soil microbial biomass

379 The residual microbial biomass and activity after biosolarization was assessed by
380analyzing the following growth kinetic parameters, which were fitted from CER data
381(Figure S2): lag phase, total microbial biomass (TMB), substrate-induced response
382(SIR) and exponential growth rate (μ , Figure 3). The untreated soil and the solarized,
383non-amended soil had a lag phase less than 10 h. This lag phase significantly ($p<0.001$)
384increased to more than 30h for both biosolarized soils (Figure 3). Within the same
385treatment, only the SMD samples presented a significantly higher lag phase in the upper
386layer (41.52 ± 7.20 h) than the lower layer (28.83 ± 4.00 , $P=0.002$, and 31.12 ± 0.81 h,
387 $P=0.007$, for the middle and lower layers, respectively). The TMB did not present
388significant differences between the original untreated soil and the solarized, non-
389amended soil (S). Both the SMD and STD biosolarized soils contained significantly
390greater TMB than the original untreated soil and non-amended, solarized soil ($P<0.001$).
391However, the biosolarized STD soil showed significantly ($P<0.001$) higher TMB than
392the SMD. The TMB did not show significant differences with depth for any of the
393treatments. The SIR did not show significant differences between the original untreated
394soil and the SMD solarized samples. However, the SMD samples were significantly
395higher than the non-amended, solarized soil but significantly lower than the STD
396samples ($P<0.001$). Within each treatment, the only depth effect detected was for the
397STD soil, where the uppermost layer presented significantly lower SIR than the lower
398depths ($P<0.001$). Finally, the exponential growth rate was greatest in the original
399untreated soil. Solarization of the non-amended soil and biosolarization with either

400 digestate amendment decreased the exponential growth rate significantly ($P < 0.001$) with
401 the value being the lowest for both biosolarized soils. Differing depth effects were
402 observed across the treatments. The uppermost layer of the non-amended, solarized soil
403 presented a significantly higher exponential growth rate than the lowermost layer
404 ($P < 0.001$), whereas for both amended solarized soils, the uppermost layer showed
405 significantly lower microbial growth rates than the lowermost layer ($P < 0.001$).

406 4. Discussion

407 The lower microbial diversity of the amended soils was likely related to the
408 lower diversity values of the digestate communities. Previous work with the
409 thermophilic sludge used in this study revealed an H' value of 2.1 for the sludge
410 community (Fernandez-Bayo et al., 2016). As digestate amendment to the soil adds
411 OTUs, which promotes increased diversity through elevated species richness, the only
412 way the digestate could have a net negative effect on diversity is by decreasing the
413 evenness of the OTU relative abundance distribution in the microbial communities. In
414 the non-amended soil, none of the OTUs showed a relative abundance higher than 5%,
415 whereas for the MD and STD soil *Bacillus* and *Acinetobacter* represented 46.93% and
416 21.60% of the initial relative abundance, respectively (Table 3). After the experiment,
417 the abundance of these microorganisms dropped drastically which explains the recovery
418 of the microbial diversity (Figure 2).

419 Despite the convergence in diversity index values following biosolarization, the
420 NMDS analysis showed that microbial communities from the SMD and STD treatments
421 maintained notable phylogenetic differences that were influenced by the application of
422 soil heating and soil depth. Nevertheless, microbial communities from SMD showed
423 more similarity to the non-amended soil. Specifically, a *Bacillus* OTU (Firmicutes) was
424 naturally abundant in the non-amended soil. The SMD soil presented a particularly large

relative abundance of this OTU (Table 3). Despite the sharp drop in the relative abundance after treatment, the SMD soil remained enriched with *Bacillus*. This enrichment may be of interest, some *Bacillus* sp. have shown to be antibiotic-producers and may act as plant pest antagonists against pathogenic *Fusarium* fungi (Urano et al., 1997), a pest that is often treated with solarization (Basallote-Ureba et al., 2016). Moreover, *Bacillus* spp. play an important role in nitrogen fixation (Owarnah et al., 2014), and can effectively degrade biopolymers such as proteins, starch, and pectin, thus playing a significant role in the biological cycles of carbon and nitrogen (Mandic-Mulec and Prosser, 2011). *Thermobacillus* was one of the most abundant genera in the top layer of the solarized SMD soil. One OTU corresponding to *Thermobacillus composti*, which is considered to be a thermophilic aerobe, has been isolated in compost bioreactors (Touzel and Prensier, 2015; Watanabe et al., 2007).

On the other hand, many of the most abundant OTUs present in the STD soil were nonexistent or of very low abundance in the original non-amended soil (Table 3). Specifically, enterobacteriaceae family, *Cellvibrio*, *Acinetobacter*, *Pseudomonas*, *Serpens* and *Gracilibacter* were enriched in the STD samples compared to non-amended or SMD samples. Siderophore-producing *Acinetobacter* strains have shown in vitro inhibition of *Fusarium oxysporum*, a fungal phytopathogen, under iron-limited conditions (Rokhbakhsh-Zamin et al., 2011). Certain *Cellvibrio*, *Pseudomonas*, and *Bacillus* spp. are potential biofertilizers through nitrogen fixation and nutrient solubility in soils (Owarnah et al., 2014; Suarez et al., 2014). Studies on the effect of solarization on tomato plants measured population densities of fluorescent pseudomonads up to 130-fold higher in the rhizosphere of plants in solarized soils. Organisms recovered from solarized soil included *Pseudomonas putida*, *P. fluorescens*, and *P. alcaligenes* (Gamliel and Katan, 1991). The OTU changes may provide information about oxygen levels in

the soil under various biosolarization conditions. The OTU *Serpens* was identified as *S. flexibilis*, reclassified as *Pseudomonas flexibilis* (Shin et al., 2015). This species, which is known to grow in aerobic conditions (Bergey and Holt, 1994; Hespell, 2006), persisted in the top layer of the STD solarized soil. On the other hand, a *Gracilibacter* sp. that belongs to the anaerobic class clostridia (Kim et al., 2010; Lee et al., 2006) was enriched in the lower solarized layer.

Finally, *Balneimonas* was enriched in the upper layer (H and M) of both solarized SMD and STD samples. *Balneimonas* has been reclassified as *Microvirga*, an organism known to include at least three species known to form root-nodules and have a significant role in nitrogen fixation (Ardley et al., 2012).

The biosolarized samples showed significantly higher microbial biomass than the solarized and non-solarized samples. Moreover, it is not surprising that the STD soil presented the highest TMB values as the microbial community associated with this amendment was originally thermophilic. Studies where solarization was applied for 30d showed a significant negative impact on the microbial biomass due to solarization of a non-amended soil and the same soil amended with compost (Scopa and Dumontet, 2007). In agreement with our study, the solarized compost-amended samples showed higher microbial biomass than the solarized, non-amended soil. This suggests that soil biosolarization is better able to preserve soil microbial activity compared to traditional solarization due to the addition of organic matter and microbial biomass from the amendment.

TMB did not show significant differences with soil depth within each treatment. However, the significantly higher lag-phase in the SMD soil in the uppermost layer, the lower SIR of the STD soil in the uppermost layer (Figure 3) and the positive correlation of μ with depth ($P < 0.001$) are indicators of a negative effect of solarization on the

475microorganisms at the top layer. Another indicator of the negative effect on the
476microbial communities is the slight but significantly lower microbial diversity of the top
477layer of the non-amended soil. These negative impacts were attributed to the higher
478temperatures in the upper soil layer. Although the mean maximum temperature value
479recorded at 12.5 cm during the field experiment was 41°C (Table 1), during the final
480days of solarization, maximum temperatures at this depth reached up to 45°C (data not
481shown). Due to the temperature gradient expected in soil during solarization, it can be
482assumed that the upper layer reached temperatures closer to 50°C, which would cause
483more rapid mortality to most soil-borne plant pathogens and mesophilic microbes
484dominating the majority of soils worldwide (Stapleton, 1996).

485 Finally, it has been suggested that soil microorganisms exhibiting active growth
486(r-strategic) are zymogeneous organisms, meaning they increase to large numbers when
487nutrients are applied and then they decrease quickly when the nutrients are depleted,
488becoming dormant or decreasing in number until conditions are suitable for active
489growth again (Stenstrom et al., 1998). In contrast, the autochthonous microorganisms
490are always numerous in soil and their numbers do not fluctuate much, carrying out
491activities that require no nutrients or energy other than those normally present in soil,
492for instance lignin-decomposing fungi (Gerson and Chet, 1981). These active
493autochthonous organisms are more related to K-strategic organisms (Stenstrom et al.,
4941998). The r/K ratio of the control soil and the amended soils was always very low
495(<0.06 Table S1). These results may indicate that the majority of the greater microbial
496biomass in the amended soil is attributed not to non-native opportunistic
497microorganisms, but rather to the soil endemic species that flourished due to the
498addition of nutrients, carbon, and colonizable substrate from the amendment. This
499notion is supported by the NMDS analysis, which showed that biosolarized soil

500microbial communities became more similar to those in the non-amended soil following
501treatment. On the other hand, the r/K values of the solarized non-amended soils ranged
502between 0.21 and 0.41 (Table S1), which indicates that solarization can affect the
503autochthonous organisms when no amendment is applied. Since, soil biogeochemical
504processes are primarily driven by physiologically active soil microbes (Blagodatskaya
505and Kuzyakov, 2013), the decrease of the r/K ration in the solarized non-amended soils
506can be assumed as a negative effect.

507

5085. Conclusions

509 This study showed that the microbial biomass increased in soil that was
510biosolarized using anaerobic digestates. Additionally, biosolarization produced
511significant shifts in microbial community composition that were related to the origin of
512the amendment. The overall trend of the soil microbial community was to shift towards
513the composition of the original soil community during biosolarization, which was more
514evident in the mesophilic digestate-amended soil. Thermophilic digestate introduced
515new OTUs to the soil, some of which are known as potential inhibitors of pests in
516biosolarized soils. Both digestate amendments also promoted the growth of beneficial
517microorganisms during biosolarization such as *Balneimonas* (or *Microvirga*) spp.
518Solarization of non-amended soil affected active non-growing species, leading to an
519enrichment of opportunistic species. This is of great relevance as active growing species
520play a significant role in soil biogeochemical processes. For genera that are susceptible
521to biosolarization, foreknowledge of the presence of beneficial or detrimental species in
522the soil is needed to fully gauge the risks and benefits of biosolarization.

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689

690Figure 1. Shannon diversity indices (H') of microbial communities from the non-
691amended, mesophilic digestate- (SMD) and thermophilic digestate- (STD) amended
692soils prior to solarization (T0), after incubation at room temperature (RT) and following
693solarization at different depths (H=0-7.5cm, M=7.5-15cm, L=15-22cm). Error bars
694represent one standard deviation ($n = 5$).

695Figure 2. Non-metric multidimensional scaling (NMDS) of microbial communities from
696the non-amended and mesophilic- (SMD) and thermophilic- (STD) digestate-amended
697soil prior to solarization (T0), after incubation at room temperature (RT) and following
698solarization at different depths (H=0-7.5cm, M=7.5-15cm, L=15-22cm).

699Figure 3. Microbial activity responses of the original non-amended soil and the
700solarized non-amended and biosolarized soils, estimated from CER values, at different
701depths: Lag Phase (a), Total Microbial Biomass (TMB) (b), Substrate-Induced Response
702(SIR) (c) and exponential growth rate (μ) (d). Different capital letters designate
703significant differences ($P < 0.05$) between amendment treatments (Control soil $n=5$;
704solarized soil (S), mesophilic (SMD) and thermophilic (STD) digestate-amended soils
705 $n=15$). Lowercase letters designate significant differences among samples of the same
706treatment and different depths ($n=5$).

707Table 1. Average and standard deviation of the mean, maximum and minimum
708temperatures recorded during solarization at 12.5 cm for every non amended soil (S),
709and amended with mesophilic (SMD) and thermophilic (STD) digestates ($n = 5$, except
710for STD where $n=4$).

	$T_{\text{mean}} (^{\circ}\text{C})$	$T_{\text{max}} (^{\circ}\text{C})$	$T_{\text{min}} (^{\circ}\text{C})$
S	34.97 $\pm$ 0.18	40.89 $\pm$ 0.62	29.28 $\pm$ 0.23
SMD	34.95 $\pm$ 0.09	40.56 $\pm$ 0.44	29.18 $\pm$ 0.22
STD	34.78 $\pm$ 0.06	40.23 $\pm$ 0.28	29.29 $\pm$ 0.16

711

712

713Table 2. Mean relative abundance (n=5) of the 5 dominant bacterial phyla in
 714communities from untreated soil (S) and Thermophilic (STD) and mesophilic (SMD)
 715amended soils prior to (T0) and following solarization at different soil depths (H=0-
 7167.5cm, M=7.5-15cm, L=15-22cm) or incubation at room temperature (RT).

Phylum	Proteobacteria	Actinobacteria	Bacteroidetes	Acidobacteria	Firmicutes
S-T0	41.05ab ^{†‡}	14.83a	11.92bc	11.57ab	10.87ab
S-RT	46.62a	4.77c	16.62a	15.37a	2.74c
S-H	41.15ab	9.80b	8.84c	9.93b	18.26a
S-M	38.73b	10.66b	13.79ab	14.60a	7.81bc
S-L	37.79b	9.49b	15.17ab	15.41a	6.82bc
SMD-T0	8.41c	8.50a	1.89d	5.24b	71.65a
SMD-RT	43.39ab	5.20b	23.85a	5.91b	14.90c
SMD-H	41.86b	9.93a	12.52c	2.38c	28.85b
SMD-M	45.98a	5.13b	19.49b	5.43b	17.59c
SMD-L	45.76a	4.00b	17.77b	7.57a	17.36c
STD-T0	53.96b	5.74bc	10.62b	1.52c	26.47ab
STD-RT	61.78a	4.07c	19.50a	4.40a	5.94d
STD-H	54.74ab	7.49ab	11.31b	3.93ab	17.48bc
STD-M	58.91ab	8.26ab	11.48b	5.44a	11.33cd
STD-L	39.13c	9.97a	13.93b	2.65bc	31.80a

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718

719[†]Different letters indicate significant differences in relative abundance for each phylum
 720for samples within the same treatment (P<0.05, n=5); [‡]Shading reflects general higher
 721relative abundance

722Table 3. Mean relative abundance (n=5) of the most dominant operational taxonomic
 723units (OTUs) in communities from untreated soil (control) and amended soil prior to
 724and following solarization at different soil depths or incubation at room temperature
 725(RT). Most dominant were defined by collating the five most abundant OTUs per simple
 726from all treatments (blue cells). Red color indicates that the OTU was not observed.

727 Samples with 0.00 non colored in red have relative abundance <0.01. Bold values indicate the highest value for that sample.

OTU [†]	Soil					SMD					STD				
	T0	RT	H	M	L	T0	RT	H	M	L	T0	RT	H	M	L
<i>Kaistobacter</i> [‡]	4.6	5.6	2.7	3.1	2.7	0.38	2.8	1.46	2.09	1.57	0.42	1.54	0.93	0.63	0.38
<i>RB41(o)</i>	3.5	4.0	2.1	3.5	3.7	1.52	0.8	0.28	0.60	0.93	0.47	0.58	0.48	0.53	0.30
<i>Bacillus</i>	3.1	0.8	3.1	2.0	1.7	46.9	9.9	10.17	11.5	11.43	5.31	1.00	3.01	2.52	3.61
<i>Bacillus</i>	2.4	0.5	1.7	1.7	1.5	0.78	0.2	0.33	0.30	0.31	0.23	0.19	1.16	0.87	1.10
<i>Oxalobacteraceae(f)</i>	1.9	0.5	0.1	0.1	0.2	0.11	0.1	0.03	0.01	0.03	0.05	0.62	0.04	0.19	0.02
<i>Thermomonas</i>	1.8	3.2	5.3	3.0	2.2	0.11	4.7	6.04	4.82	5.09	0.11	1.41	2.37	1.15	0.21
<i>Flavisolibacter</i>	1.6	3.4	4.1	4.6	4.8	0.12	7.2	7.64	10.3	7.89	0.08	4.25	6.14	4.05	4.22
<i>Balneimonas</i>	1.4	0.3	1.0	1.0	1.8	0.18	1.8	4.58	2.60	1.58	0.28	0.96	6.89	5.28	1.06
<i>Bacillales(o)</i>	1.0	0.1	0.9	0.5	0.4	5.67	0.8	0.76	0.71	0.75	2.88	0.48	1.32	1.16	0.37
<i>Lysobacter</i>	0.9	2.7	1.6	1.6	1.4	0.14	6.7	1.66	3.52	4.38	0.06	2.21	1.18	0.89	0.18
<i>Aquicola</i>	0.9	1.4	3.4	1.8	2.2	0.10	2.3	3.48	2.34	2.94	0.11	1.27	2.52	1.61	0.53
<i>Flavisolibacter</i>	0.6	0.3	0.2	0.3	0.3	0.08	0.6	1.64	1.10	0.70	0.04	2.21	1.01	1.33	2.91
<i>Bacillus</i>	0.4	0.1	0.4	0.3	0.3	1.83	0.5	0.49	0.77	0.83	0.61	0.18	0.49	0.33	1.14
<i>Bacillus</i>	0.4	0.0	0.4	0.2	0.2	2.10	0.3	0.32	0.29	0.31	1.56	0.21	0.56	0.46	0.22
<i>Methylibium</i>	0.2	0.5	0.2	0.4	0.4	0.05	0.2	0.30	0.41	0.51	0.08	1.08	0.81	4.49	0.98
<i>Bacillus</i>	0.1	0.0	0.2	0.1	0.1	2.57	0.9	0.15	0.48	0.65	3.17	0.10	0.42	0.38	2.44
<i>Sorangium</i>	0.0	1.3	0.1	0.2	0.1	0.03	0.2	0.13	1.77	1.84	0.01	2.38	0.10	2.65	0.25
<i>Acinetobacter</i>	0.0	0.0	0.0	0.0	0.0	0.01	0.0	0.01	0.00	0.01	21.6	6.58	3.04	1.39	1.86
<i>Acinetobacter</i>	0.0	0.0	0.0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	2.93	0.74	0.12	0.13	0.32
<i>Pseudomonas</i>	0.0	0.0	0.0	0.0	0.0	0.07	0.0	0.00	0.01	0.00	7.06	1.26	1.26	0.83	5.08
<i>Enterobacteriaceae(f)</i>	0.0	0.0	0.0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	0.72	2.95	1.17	0.97	0.42
<i>Pseudomonas</i>	0.0	0.0	0.0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	0.56	2.47	0.31	1.21	1.47
<i>Cellvibrio</i>	0.0	0.0	0.0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	0.07	4.45	0.24	0.32	0.27
<i>Serpens</i>	0.0	0.0	0.0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	0.07	0.05	3.29	0.53	0.10
<i>Thermobacillus</i>	0.0	0.0	0.0	0.0	0.0	0.01	0.0	4.36	0.08	0.00	0.00	0.00	0.53	0.00	0.00
<i>Gracilibacter</i>	0.0	0.0	0.0	0.0	0.0	0.00	0.0	0.00	0.01	0.15	0.00	0.22	0.03	0.17	3.24
<i>Brevibacillus</i>	0.0	0.0	4.0	0.0	0.0	0.00	0.0	1.09	0.00	0.00	0.00	0.00	0.92	0.01	0.02

729 [†]Taxonomy is given at the best taxonomic resolution (genus; o, order; f, family); [‡]Color label

730 matches with same OTU in table 4

731 Table 4. Five predominant operational taxonomic units (OTUs) contribute the most to Bray–Curtis dissimilarity (SIMPER analysis)
732 among the non-amended, the TD and the SMD samples, at the top and bottom solarized layers and the samples incubated at room
733 temperature. Same color indicates same OTU

	Relative Abundance (%)		% to BC†	Taxonomy‡	Relative Abundance (%)		% to BC	Taxonomy	Relative Abundance (%)		% to BC	Taxonomy
	Soil	S			S	ST			ST	SMD		
Top solarized layer (0-7.5 cm)	3.14	10	6.96	Bacillus	1.	6.8	5.83	Balneimonas	3.0	10.17	7.67	Bacillus
	0.00	4.	11.28	Thermobacillus	4.	0.9	9.35	Brevibacillus	0.5	4.36	11.77	Thermobacillus
	4.13	7.	14.75	Flavobacter	0.	3.2	12.65	Serpens	2.3	6.44	15.71	Thermomonas
	1.09	4.	18.	Balneimonas	0.	3.0	15.	Acinetobacter	3.2	0.	19.24	Serpens

			21				70			0		
	□ 4.08	□ 1.	□ 21.39	□ <i>Brevibacillus</i>	□ 5.	□ 2.3	□ 8.69	□ <i>Thermomonas</i>	□ 3.0	□ 0.01	□ 22.48	□ <i>Acinetobacter</i>
□	□											
□ Lower solarized layer (15-22.5 cm)	□ 1.72	□ 11	□ 10.79	□ <i>Bacillus</i>	□ 0.	□ 5.0	□ 3.46	□ <i>Pseudomonas</i>	□ 3.6	□ 1.43	□ 5.90	□ <i>Bacillus</i>
	□ 4.89	□ 7.	□ 14.57	□ <i>Flavisolibacter</i>	□ 3.	□ 0.3	□ 5.80	□ <i>RB41(o)</i>	□ 5.0	□ 0.00	□ 9.49	□ <i>Pseudomonas</i>
	□ 1.48	□ 4.	□ 17.79	□ <i>Lysobacter</i>	□ 0.	□ 3.2	□ 8.00	□ <i>Gracilibacter</i>	□ 0.2	□ 5.09	□ 12.93	□ <i>Thermomonas</i>
	□ 2.23	□ 5.	□ 20.97	□ <i>Thermomonas</i>	□ 0.	□ 2.7	□ 9.86	□ <i>Jonesia</i>	□ 0.1	□ 4.38	□ 15.89	□ <i>Lysobacter</i>
	□ 3.74	□ 0.	□ 24.09	□ <i>RB41(o)</i>	□ 0.	□ 2.9	□ 11.62	□ <i>Flavisolibacter</i>	□ 4.2	□ 7.89	□ 18.49	□ <i>Flavisolibacter</i>
□	□											
□ Room	□ 0.86	□ 9.	□ 10.	□ <i>Bacillus</i>	□ 0.	□ 6.5	□ 5.8	□ <i>Acinetobacter</i>	□ 1.0	□ 9.9	□ 8.46	□ <i>Bacillus</i>

Temperature			18				7			5		
	□ 2.78	□ 6.	□ 14.66	□ <i>Lysobacter</i>	□ 0.	□ 4.4	□ 9.83	□ <i>Cellvibrio</i>	□ 6.5	□ 0.00	□ 14.67	□ <i>Acinetobacter</i>
	□ 3.43	□ 7.	□ 18.93	□ <i>Flavolibacter</i>	□ 5.	□ 1.5	□ 13.52	□ <i>Kaistobacter</i>	□ 2.2	□ 6.77	□ 18.98	□ <i>Lysobacter</i>
	□ 4.05	□ 0.	□ 22.57	□ <i>RB41(o)</i>	□ 4.	□ 0.5	□ 16.62	□ <i>RB41(o)</i>	□ 4.4	□ 0.00	□ 23.19	□ <i>Cellvibrio</i>
	□ 5.67	□ 2.	□ 25.73	□ <i>Kaistobacter</i>	□ 0.	□ 2.9	□ 19.25	□ <i>Enterobacteriaceae(f)</i>	□ 1.4	□ 4.72	□ 26.32	□ <i>Thermomonas</i>

734 □ †Cumulative % Contribution to Bray Curtis dissimilarity; #Taxonomy is given at the best taxonomic resolution (genus; o, order; f, family)

Figure 1

Figure 2

739 □

740 □ Figure 3

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