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

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ABSTRACT

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

1. Introduction

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

Soil biosolarization (SBS) can be a sustainable soil pest control technique as it avoids the use of synthetic pesticides. SBS has successfully inactivated fungal, nematode, insect, and weed pests (Bonanomi et al., 2008; Yao et al., 2016). SBS is a combination of soil solarization, where moist soil is covered with a transparent tarp to increase the soil temperature via passive solar heating (Katan et al., 1976), and anaerobic soil disinfestation (ASD) where soil is amended with organic matter prior to tarping to promote anaerobic microbial activity (Lamers et al., 2010). The addition of organic matter can enhance pest inactivation through several mechanisms. First, the additional microbiota and nutrient source associated with the amendment can enhance soil heating through biological heating. For example, amending soil with

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compost and organic matter yielded temperature increases between 2 and 5 °C greater than non-amended soil when both were solar heated (Achmon et al., 2015; Simmons et al., 2013, 2016). Secondly, anaerobic microbial fermentation of organic matter can result in the production of organic compounds such as volatile fatty acids (VFAs) that accumulate due to the physical barrier of the plastic tarp. VFAs may accumulate to levels that result in a decrease in soil pH and increase in toxicity to soil biota and weed seeds (Achmon et al., 2017; Gamliel and Stapleton, 1997; Huang et al., 2015a; Katase et al., 2009). Thirdly, microbiota may directly affect pest organisms through competition, such as between beneficial fungi and pathogenic fungi, or infection, such as microbial degradation of the seed coat of weed seeds (Huang et al., 2015b; Rokhbakhsh-Zamin et al., 2011). The combined thermal, biochemical and ecological action of the organic amendment in SBS can significantly increase the efficiency of the process, counteracting the need for high solar radiation and long treatment durations of up to 6 weeks, which can infringe on the most productive time of the growing season for farmers.

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

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

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

The objective of this study was to assess the composition and activity of microbial communities in biosolarized soils amended with two different solid digestates from anaerobic digestion of mixed organic wastes. Like compost, which has been shown to be an effective inoculum for biosolarization (Simmons et al., 2013), digestates contain robust, anaerobic, organic matter-degrading microbial communities that may also tolerate biosolarization. Studies have shown that SBS can increase the proportion of facultative and obligate anaerobic microorganisms in the soil (Yao et al., 2016). As a result, digestate amendment could influence soil microbial community restructuring during biosolarization and help prime the soil with active bacteria following treatment. This could be important for occupying soil niches that pathogens may otherwise recolonize. Furthermore, enriching the soil with biomass-degrading bacteria from digestate could benefit nutrient cycling in the soil. In this study, soil microbial biomass following biosolarization was estimated using substrate-induced respiration (SIR) with measurement of the respiration response kinetics (Anderson and Domsch, 1978; Panikov and Sizova, 1996; Stenstrom et al., 1998). These data can provide information on the physiological state of the microbial biomass by estimating the ratio of growing (r-strategic) versus non-growing (K-strategic) (Chen et al., 2012b). The taxonomic diversity of microbial communities was also analyzed via next generation 16S rRNA gene sequencing. These results will help gauge the valorization potential of digestates in agricultural to improve soil microbial activity and diversity as well as provide guidelines for application of digestate in biosolarization.

2. Materials and methods

2.1. Soil and digestate description

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

Two solid digestates from two anaerobic digesters with different operational conditions and ofeedstocks were used in the experiment. A thermophilic digestate (TD) was acquired from an anaerobic digester located on the University of California, Davis campus in Davis, CA. The UC Davis digester processes mixed organic waste (food, agriculture, and green wastes). The digester utilizes sequential thermophilic hydrolysis and methanogenesis (55 °C) with low solids loading (5–10% total solids in the methanogenesis phase). The solid digestate was periodically separated from the liquid phase of the methanogenic sludge and dewatered by pressing. The Yolo County Landfill (Woodland, CA) provided a mesophilic digestate (MD) from anaerobic digestion of food, manure and green wastes. Digestion occurred under high solids loading (40–60% of moisture content) and mesophilic conditions (35 °C). Both digestates were air-dried, ground and sieved (< 2 mm) prior to mixing with the sampled soil. The total N of the soil, the TD and the MD amendments was 0.04, 1.48 and 1.03%, respectively. The total C of the soil, the TD and the MD amendments was 0.38, 47.10 and 41.53%, respectively.

2.2. Soil mesocosm preparation

Soil mesocosms served as experimental units in field studies as described in previous studies (Achmon et al., 2017; Simmons et al., 2013). Soil mixtures for mesocosms were prepared by amending dry soil with dry thermophilic (STD) or mesophilic digestate (SMD) to achieve 1.5% loading (dry weight basis). Soil without amendment was used as a control (S). Soil mixtures were wetted to their respective field capacities and allowed to incubate overnight at 4 °C so that moisture could equilibrate between the various soil components. Equilibrated soil mixtures were packed into 3.8 l black plastic grow bags (neHydro, Southampton, MA). The bags contained drainage holes to facilitate moisture and gas exchange with the surrounding soil. Compact temperature sensors and data loggers (Thermochron iButtons model 1922L, Embedded Data Systems, Lawrenceburg, KY) were embedded in the center of each microcosm at 15 cm depth. The diameter and height of the filled mesocosms were 17.8 cm and 22.5 cm, respectively.

2.3. Field experiment

The field site was also located at KARE and it was prepared as previously described (Achmon et al., 2017; Simmons et al., 2013). Each field plot measured 1.8 × 8.5 m and contained one mesocosm from each treatment and the arrangement of mesocosms was randomized.

Mesocosms were buried in field plots. Plots were sprinkler irrigated to bring the soil moisture to field capacity (~11% wet basis) at the depths sampled in this study. The plots were then covered with clear plastic tarp ('Huskey Film Sheeting'; Poly-America, Inc., Grand Prairie, TX) to initiate biosolarization. Care was taken to minimize the amount of air trapped beneath the tarp. Five replicate plots were prepared. An identical set of mesocosms without temperature loggers were prepared and incubated in parallel at room temperature (RT, 22–27 °C). They were loosely covered with plastic tarp to avoid water loss. After 8 days of solarization, the mesocosms were extracted from the field and divided into three sections representing different soil depths (H = 0–7.5 cm, M = 7.5–15 cm and L = 15–22.5 cm depth). The contents of RT mesocosms were thoroughly mixed at the end of the incubation period, as no depth effect was expected due to the absence of solar heating or burial in field soil. All soil samples were stored at −20 °C for further analysis.

2.4. DNA isolation, sequencing and data processing and analysis

Genomic DNA was purified from the soil microbial communities at the beginning of the experiment and after solar heating or incubation at RT. DNA extraction, purification, amplification and sequencing were performed as described elsewhere (Fernandez-Bayo et al., 2016; Simmons et al., 2014). Raw sequencing reads were processed using the Quantitative Insights into Microbial Ecology (QIIME) toolkit (Caporaso et al., 2010). To filter the reads, the default values used were: r = 3; p = 0.75 total read length; q = 3; n = 0; c = 0.005% (Bokulich et al., 2013). Filtered reads were clustered into operational taxonomic units (OTU) with at least 97% sequence similarity (Caporaso et al., 2010). Ecological analyses were performed using RStudio (version 0.98.1103) (Racine, 2012) with the vegan (Dixon, 2003) and entropart (version 1.2.1, http://CRAN.R-project.org/package=entropart) packages. Prior to analysis, singletons were removed from operating taxonomic unit (OTU) read count data to reduce noise. Shannon diversity (H') indices, relative abundance, Bray-Curtis dissimilarity and non-metric multidimensional scaling (NMDS) analyses were calculated for each community as described elsewhere (Simmons et al., 2014).

2.5. Microbial biomass analysis

The soil microbial biomass at different depths in biosolarized and control soils were estimated via measurement of respiration kinetics following substrate addition to the soil. This analysis allowed estimation of the lag phase, the total microbial biomass, exponential growth rate and the substrate-induced response of the microbial communities. For this analysis, 250 ml aerated bioreactors (previously described by Achmon et al., 2016 and Yu et al., 2015) containing 100 g (dry weight equivalent) of field soil samples were incubated under controlled aerated conditions at room temperature (24 °C). The samples were incubated for 3 days at which point the respiration rate was observed to be constant. Soil samples were then spiked with 1 ml of a 100 mg mL^{-1} sterile glucose solution (the final soil concentration was 1 mg g^{-1} soil). Following addition of the substrate, CO_2 evolution rate (CER) was measured as described elsewhere (Achmon et al., 2016; Yu et al., 2015). CER values were normalized by the dry weight of sample in the reactor and calculated based on the C content in the CO_2 (mg C-CO_2 g $soil^{-1}$ h^{-1}).

The CER data after glucose amendment were fitted to the following kinetic respiration model (Eq. (1)) to describe the microbial biomass in the soil (Panikov and Sizova, 1996; Stenstrom et al., 1998; Wutzler et al., 2012).

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

This model assumes that mineralization of an easily degradable substrate in the soil, such as glucose, is performed by two main groups of microorganisms; those which grow exponentially as a result of the substrate addition ($r \cdot e^{(\mu t)}$) and those which increase their respiration to a high constant rate without increasing much in number (K). Thus, K is the constant respiration rate performed by non-growing microorganisms (mg C-CO_2 g $soil^{-1}$ h^{-1}), r is the initial respiration rate of growing microorganisms (mg C-CO_2 g $soil^{-1}$ h^{-1}) and μ is the maximum specific growth rate (h^{-1}). The SIR-rate is defined as the respiration rate obtained immediately after the addition of the substrate to the samples. Thus

$$SIR = r + K \quad (2)$$

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

$$T_{lag} = \frac{\ln\left(K/r\right)}{\mu} \quad (3)$$

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

$$TMB = \frac{r}{r_0 \cdot Q} \quad (4)$$

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

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

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:

$$Q = \mu / \boldsymbol{\lambda} \times Y_{CO_2} \quad (6)$$

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),

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

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).

2.6. 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).

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

	T_{mean} (°C)	T_{max} (°C)	T_{min} (°C)
S	34.97 ± 0.18	40.89 ± 0.62	29.28 ± 0.23
SMD	34.95 ± 0.09	40.56 ± 0.44	29.18 ± 0.22
STD	34.78 ± 0.06	40.23 ± 0.28	29.29 ± 0.16

3. Results

3.1. Temperature evolution during soil biosolarization

The treatments showed similar diurnal trends in soil temperature during solar heating. The mean, minimum and maximum temperature recorded for every treatment at 12.7 cm depth during the 8 days of the experiment were 35, 41, and 29 °C, respectively (Table 1). No significant differences between the treatments were observed.

3.2. Microbial diversity in biosolarized soils

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

3.3. Dissimilarity between microbial communities in biosolarized and control soils

The NMDS analysis of community dissimilarity showed three different clusters corresponding to the non-amended soil, STD, and SMD treatments (Fig. 2). The communities in all treatments diverged significantly from the initial non-amended soil community (T0) in agreement with the Bray-Curtis dissimilarity results (Fig. S1 in SI). For the control soil, both solar heating and RT incubation led to deviation from the initial community state. By comparison, the amended soils exhibited greater shifts from their respective initial community states after solar heating or RT incubation. Initially, digestate amendment resulted in markedly different soil community compositions relative to non-amended soil. For both digestate amendments, community structure became more similar to that of the non-amended soil following solar heating or RT incubation. However, distinct differences in community structure relative to non-amended soil remained at the end of the experiment for both SMD and STD treatments. At the end of the incubation period, SMD samples from RT incubation and the medial and lower depths of the solar heated soil were the most similar to the non-amended soil community structure. In general, all STD samples had greater dissimilarity from the non-amended soil compared to SMD samples.

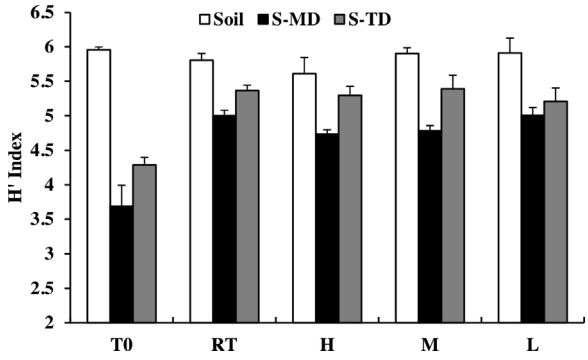

Fig. 1. Shannon diversity indices (H') of microbial communities from the non-amended, mesophilic digestate- (SMD) and thermophilic digestate- (STD) amended soils prior to solarization (T0), after incubation at room temperature (RT) and following solarization at different depths (H = 0–7.5 cm, M = 7.5–15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

3.3.1. Phylum composition of soil microbial communities

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

3.3.2. OTU contributions to community dissimilarity

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

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

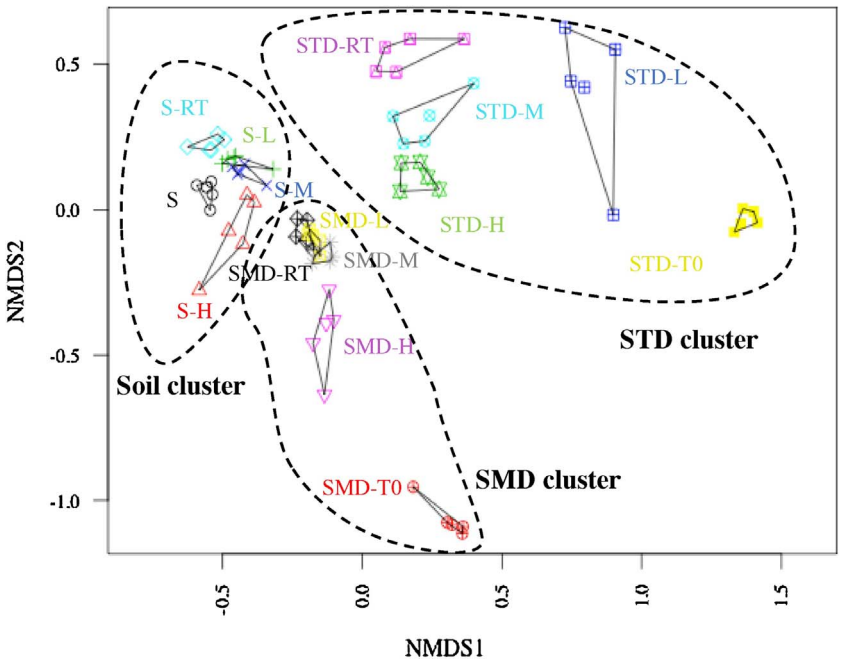

Fig. 2. Non-metric multidimensional scaling (NMDS) of microbial communities from the non-amended and mesophilic- (SMD) and thermophilic- (STD) digestate-amended soil prior to solarization (T0), after incubation at room temperature (RT) and following solarization at different depths (H = 0–7.5 cm, M =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

Table 2
Mean relative abundance (n = 5) of the 5 dominant bacterial phyla in communities from untreated soil (S) and Thermophilic (STD) and mesophilic (SMD) amended soils prior to (T0) and following solarization at different soil depths (H = 0–7.5 cm, M = 7.5–15 cm, L = 15–22 cm) 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

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

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 SMD communities. In the uppermost soil layer, where the greatest soil temperatures occurred, the *Thermobacillus* genus, which was unique to the SMD mixture, was the second greatest contributor to dissimilarity between the SMD 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 dissimilarity across all samples. STD-exclusive OTUs such as *Pseudomonas*, *Serpens*, *Acinetobacter*, and *Cellvibrio* were substantial differentiators between the SMD and STD communities as well.

Table 3
Mean relative abundance (n = 5) of the most dominant operational taxonomic units (OTUs) in communities from untreated soil (control) and amended soil prior to and following solarization at different soil depths or incubation at room temperature (RT). Most dominant were defined by collating the five most abundant OTUs per simple from all treatments (blue cells). Red color indicates that the OTU was not observed. Samples with 0.00 non colored in red have relative abundance < 0.01. Bold values indicate the highest value for that sample. (For interpretation of the references to colour in this table, the reader is referred to the web version of this article.)

OTU†	Soil					SMD					STD				
	T0	RT	H	M	L	T0	RT	H	M	L	T0	RT	H	M	L
Kaistobacter‡	**4.68**	**5.67**	2.73	3.15	2.72	0.38	2.86	1.46	2.09	1.57	0.42	1.54	0.93	0.63	0.38
RB41(o)	3.52	4.05	2.13	3.53	3.74	1.52	0.80	0.28	0.60	0.93	0.47	0.58	0.48	0.53	0.30
Bacillus	3.19	0.86	3.14	2.05	1.72	**46.93**	**9.95**	**10.17**	**11.58**	**11.43**	5.31	1.00	3.01	2.52	3.61
Bacillus	2.42	0.55	1.72	1.77	1.58	0.78	0.24	0.33	0.30	0.31	0.23	0.19	1.16	0.87	1.10
Oxalobacteraceae(f)	1.98	0.52	0.10	0.19	0.24	0.11	0.15	0.03	0.01	0.03	0.05	0.62	0.04	0.19	0.02
Thermomonas	1.87	3.28	**5.35**	3.02	2.23	0.11	4.72	6.04	4.82	5.09	0.11	1.41	2.37	1.15	0.21
Flavisolibacter	1.65	3.43	4.13	**4.63**	**4.89**	0.12	7.25	7.64	10.31	7.89	0.08	4.25	6.14	4.05	4.22
Balneimonas	1.40	0.34	1.09	1.08	1.88	0.18	1.88	4.58	2.60	1.58	0.28	0.96	**6.89**	**5.28**	1.06
Bacillales(o)	1.03	0.19	0.94	0.52	0.45	5.67	0.80	0.76	0.71	0.75	2.88	0.48	1.32	1.16	0.37
Lysobacter	0.96	2.78	1.63	1.60	1.48	0.14	6.77	1.66	3.52	4.38	0.06	2.21	1.18	0.89	0.18
Aquincola	0.94	1.45	3.47	1.87	2.24	0.10	2.37	3.48	2.34	2.94	0.11	1.27	2.52	1.61	0.53
Flavisolibacter	0.69	0.34	0.27	0.39	0.31	0.08	0.65	1.64	1.10	0.70	0.04	2.21	1.01	1.33	2.91
Bacillus	0.45	0.10	0.40	0.34	0.32	1.83	0.54	0.49	0.77	0.83	0.61	0.18	0.49	0.33	1.14
Bacillus	0.41	0.09	0.43	0.23	0.21	2.10	0.30	0.32	0.29	0.31	1.56	0.21	0.56	0.46	0.22
Methylibium	0.29	0.50	0.20	0.44	0.47	0.05	0.28	0.30	0.41	0.51	0.08	1.08	0.81	4.49	0.98
Bacillus	0.12	0.05	0.23	0.17	0.17	2.57	0.92	0.15	0.48	0.65	3.17	0.10	0.42	0.38	2.44
Sorangium	0.07	1.33	0.17	0.24	0.10	0.03	0.21	0.13	1.77	1.84	0.01	2.38	0.10	2.65	0.25
Acinetobacter	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.01	**21.60**	**6.58**	3.04	1.39	1.86
Acinetobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.93	0.74	0.12	0.13	0.32
Pseudomonas	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.01	0.00	7.06	1.26	1.26	0.83	**5.08**
Enterobacteriaceae(f)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.72	2.95	1.17	0.97	0.42
Pseudomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	2.47	0.31	1.21	1.47
Cellvibrio	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	4.45	0.24	0.32	0.27
Serpens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.05	3.29	0.53	0.10
Thermobacillus	0.00	0.00	0.00	0.00	0.00	0.01	0.00	4.36	0.08	0.00	0.00	0.00	0.53	0.00	0.00
Gracilibacter	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.15	0.00	0.22	0.03	0.17	3.24
Brevibacillus	0.00	0.00	4.08	0.00	0.00	0.00	0.00	1.09	0.00	0.00	0.00	0.00	0.92	0.01	0.02

†Taxonomy is given at the best taxonomic resolution (genus; o, order; f, family); ‡Color label matches with same OTU in Table 4.

3.4. Soil microbial biomass

The residual microbial biomass and activity after biosolarization was assessed by analyzing the following growth kinetic parameters, which were fitted from CER data (Fig. S2): lag phase, total microbial biomass (TMB), substrate-induced response (SIR) and exponential growth rate (μ, Fig. 3). The untreated soil and the solarized, non-amended soil had a lag phase less than 10 h. This lag phase significantly

Table 4
Five predominant operational taxonomic units (OTUs) contribute the most to Bray–Curtis dissimilarity (SIMPER analysis) among the non-amended, the TD and the SMD samples, at the top and bottom solarized layers and the samples incubated at room temperature. Same color indicates same OTU. (For interpretation of the references to colour in this table, the reader is referred to the web version of this article.)

	Relative Abundance (%)		% to BC†	Taxonomy‡	Relative Abundance (%)		% to BC	Taxonomy	Relative Abundance (%)		% to BC	Taxonomy
	Soil	SMD			Soil	STD			STD	SMD		
Top solarized layer (0-7.5 cm)	3.14	10.17	6.96	*Bacillus*	1.09	6.89	5.83	*Balneimonas*	3.01	10.17	7.67	*Bacillus*
	0.00	4.36	11.28	*Thermobacillus*	4.08	0.92	9.35	*Brevibacillus*	0.53	4.36	11.77	*Thermobacillus*
	4.13	7.64	14.75	*Flavisolibacter*	0.00	3.29	12.65	*Serpens*	2.37	6.04	15.71	*Thermomonas*
	1.09	4.58	18.21	*Balneimonas*	0.00	3.04	15.70	*Acinetobacter*	3.29	0.00	19.24	*Serpens*
	4.08	1.09	21.39	*Brevibacillus*	5.35	2.37	18.69	*Thermomonas*	3.04	0.01	22.48	*Acinetobacter*
Lower solarized layer (15-22.5 cm)	1.72	11.43	10.79	*Bacillus*	0.00	5.08	3.46	*Pseudomonas*	3.61	11.43	5.90	*Bacillus*
	4.89	7.89	14.57	*Flavisolibacter*	3.74	0.30	5.80	*RB41(o)*	5.08	0.00	9.49	*Pseudomonas*
	1.48	4.38	17.79	*Lysobacter*	0.01	3.24	8.00	*Gracilibacter*	0.21	5.09	12.93	*Thermomonas*
	2.23	5.09	20.97	*Thermomonas*	0.00	2.73	9.86	*Jonesia*	0.18	4.38	15.89	*Lysobacter*
	3.74	0.93	24.09	*RB41(o)*	0.31	2.91	11.62	*Flavisolibacter*	4.22	7.89	18.49	*Flavisolibacter*
Room Temperature	0.86	9.95	10.18	*Bacillus*	0.00	6.58	5.87	*Acinetobacter*	1.00	9.95	8.46	*Bacillus*
	2.78	6.77	14.66	*Lysobacter*	0.00	4.45	9.83	*Cellvibrio*	6.58	0.00	14.67	*Acinetobacter*
	3.43	7.25	18.93	*Flavisolibacter*	5.67	1.54	13.52	*Kaistobacter*	2.21	6.77	18.98	*Lysobacter*
	4.05	0.80	22.57	*RB41(o)*	4.05	0.58	16.62	*RB41(o)*	4.45	0.00	23.19	*Cellvibrio*
	5.67	2.86	25.73	*Kaistobacter*	0.00	2.95	19.25	*Enterobacteriaceae(f)*	1.41	4.72	26.32	*Thermomonas*

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

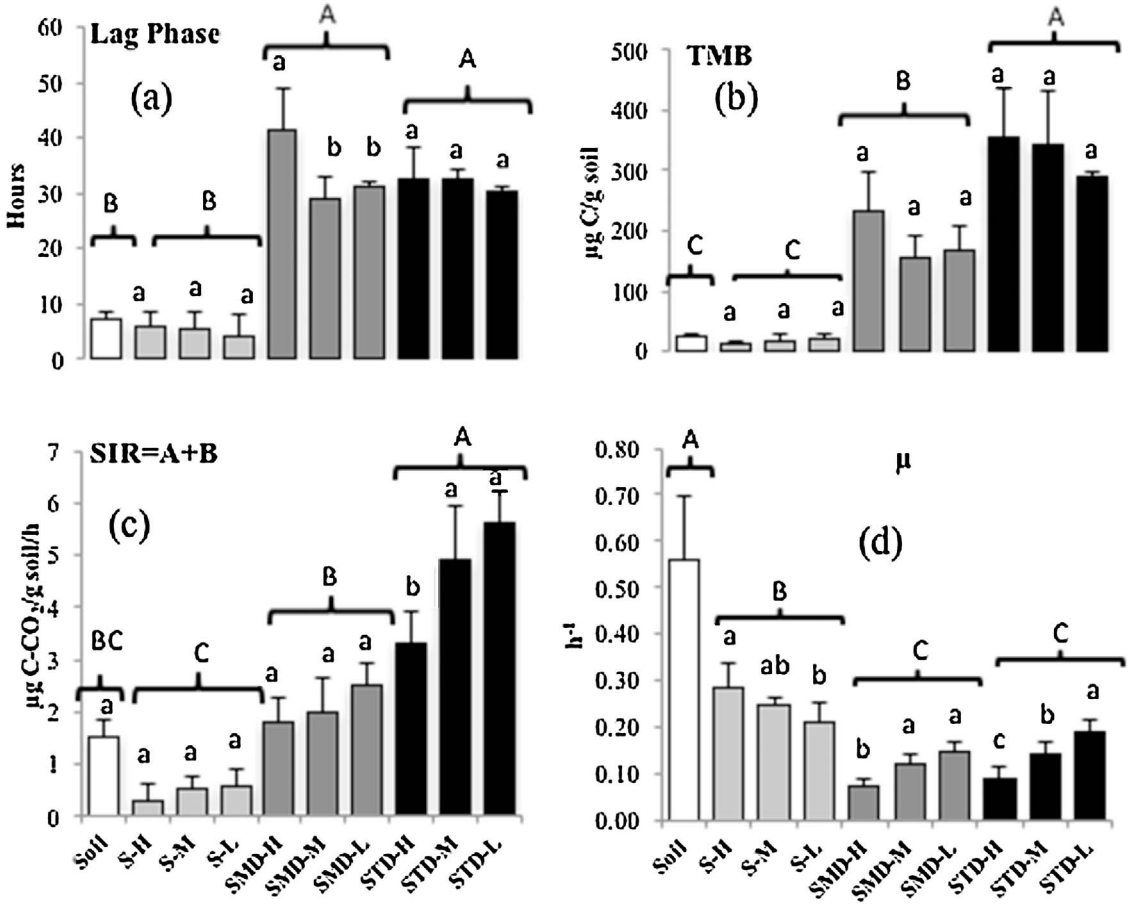

Fig. 3. Microbial activity responses of the original non-amended soil and the solarized non-amended and biosolarized soils, estimated from CER values, at different depths: Lag Phase (a), Total Microbial Biomass (TMB) (b), Substrate-Induced Response (SIR) (c) and exponential growth rate (µ) (d). Different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

($p < 0.001$) increased to more than 30 h for both biosolarized soils (Fig. 3). Within the same treatment, only the SMD samples presented a significantly higher lag phase in the upper layer (41.52 ± 7.20 h) than the lower layer (28.83 ± 4.00, $P = 0.002$, and 31.12 ± 0.81 h, $P = 0.007$, for the middle and lower layers, respectively). The TMB did not present significant differences between the original untreated soil and the solarized, non-amended soil (S). Both the SMD and STD biosolarized soils contained significantly greater TMB than the original untreated soil and non-amended, solarized soil ($P < 0.001$). However, the biosolarized STD soil showed significantly ($P < 0.001$) higher TMB than the SMD. The TMB did not show significant differences with depth for any of the treatments. The SIR did not show significant differences between the original untreated soil and the SMD solarized samples. However, the SMD samples were significantly higher than the non-amended, solarized soil but significantly lower than the STD samples ($P < 0.001$). Within each treatment, the only depth effect detected was for the STD soil, where the uppermost layer presented significantly lower SIR than the lower depths ($P < 0.001$). Finally, the exponential growth rate was greatest in the original untreated soil. Solarization of the non-amended soil and biosolarization with either digestate amendment decreased the exponential growth rate significantly ($P < 0.001$) with the value being the lowest for both biosolarized soils. Differing depth effects were observed across the treatments. The uppermost layer of the non-amended, solarized soil presented a significantly higher exponential growth rate than the lowermost layer ($P < 0.001$), whereas for both amended solarized soils, the uppermost layer showed significantly lower microbial growth rates than the lowermost layer ($P < 0.001$).

4. Discussion

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

Despite the convergence in diversity index values following biosolarization, the NMDS analysis showed that microbial communities from the SMD and STD treatments maintained notable phylogenetic differences that were influenced by the application of soil heating and soil depth. Nevertheless, microbial communities from SMD showed more similarity to the non-amended soil. Specifically, a *Bacillus* OTU (Firmicutes) was 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 as 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 layers (H and M) of both solarized SMD and STD samples. *Balneimonas* has been reclassified as *Microvirga*, a genus known to include at least three species that 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 (Fig. 3) and the positive correlation of μ with depth ($P < 0.001$) are indicators of a negative effect of solarization on the microorganisms at the top layer. Another indicator of the negative effect on the microbial communities is the slight but significantly lower microbial diversity of the top layer of the non-amended soil. These negative impacts were attributed to the higher temperatures in the upper soil layer. Although the mean maximum temperature value recorded at 12.5 cm during the field experiment was 41 °C (Table 1), during the final days of solarization, maximum temperatures at this depth reached up to 45 °C (data not shown). Due to the temperature gradient expected in soil during solarization, it can be assumed that the upper layer reached temperatures closer to 50 °C, which would cause more rapid mortality to most soil-borne plant pathogens and mesophilic microbes dominating the majority of soils worldwide (Stapleton, 1996).

Finally, it has been suggested that soil microorganisms exhibiting active growth (r-strategic) are zymogeneous organisms, meaning they increase to large numbers when nutrients are applied and then they decrease quickly when the nutrients are depleted, becoming dormant or decreasing in number until conditions are suitable for active growth again (Stenstrom et al., 1998). In contrast, the autochthonous microorganisms are always numerous in soil and their numbers do not fluctuate much, carrying out activities that require no nutrients or energy other than those normally present in soil, for instance lignin-decomposing fungi (Gerson and Chet, 1981). These active autochthonous organisms are more related to K-strategic organisms (Stenstrom et al., 1998). The r/K ratio of the control soil and the amended soils was always very low (< 0.06 Table S1). These results may indicate that the majority of the greater microbial biomass in the amended soil is attributed not to non-native opportunistic microorganisms, but rather to the soil endemic species that flourished due to the addition of nutrients, carbon, and colonizable substrate from the amendment. This notion is supported by the NMDS analysis, which showed that biosolarized soil microbial communities became more similar to those in the non-amended soil following treatment. On the other hand, the r/K values of the solarized non-amended soils ranged between 0.21 and 0.41 (Table S1), which indicates that solarization can affect the autochthonous organisms when no amendment is applied. Since, soil biogeochemical processes are primarily driven by physiologically active soil microbes (Blagodatskaya and Kuzyakov, 2013), the decrease of the r/K ration in the solarized non-amended soils can be assumed as a negative effect.

5. Conclusions

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

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2017.06.016.

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