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Effect of soil preparation techniques on the biochemical properties and microbial communities of a citrus orchard after replanting and conversion into organic management

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Abstract

Adequate levels of organic matter and biological activity in soil under organic management are key for crop nutrition and protection against soil-borne pathogens. Before replanting, soil must therefore be prepared accordingly. In this study, the effects of commonly used techniques (vegetal cover, organic amendment, solarization and organic amendment+ solarization) on the main biochemical properties and microbial communities of a replanted organic citrus soil were investigated during a full season. The results clearly showed negative solarization effects that degraded the soil organic

matrix by the moisture and heat combination, which affected all enzyme activities, save chitinase. These effects were particularly severe and long-lasting for ammonium oxidation-related activities and communities. Solarization was also the main factor to affect soil microbial communities, although differences between treatments disappeared in 6 months, save *Bacillus*-like bacteria. Given that nutrient cycling is essential for fertilisation in organic cropping, caution must be taken when considering the use of solarization to reduce pathogens from soil.

1. Introduction

Adequate levels of soil biological activity and diversity are paramount in organic farming. Not only does crop nutrition largely rely on the biological decomposition of organic materials, but also soil suppressiveness to diseases, which originates from several biological mechanisms, and is arguably the most important defence against soil-borne pathogens. As conventional soils usually have lower levels of biological activity than those in soils under organic management (Albiach et al., 1999; Ge et al., 2013; van Diepeningen et al., 2006), conversion period may be problematic since soil has conventional characteristics, but the agrosystem can be managed only with organic techniques. As replanting is particularly delicate given the susceptibility of plantlets to pathogens, soil disinfection treatments must usually be applied beforehand.

Despite being authorised in organic farming, some of these disinfection methods are known to damage soil biological activity; e.g., heat-based techniques such as steam disinfection (Roux-Michollet et al., 2008) and solarization (Bonanomi et al., 2008; Pane et al., 2012; Scopa et al., 2008) have been shown to bring about detrimental changes in soil microbial biomass and structure. The methodologies that rely totally or partially on fresh organic matter applications could, therefore, be preferable to lessen the impact of

1 disinfection on soil properties. There are no doubts about the intimate relationship
2 between soil organic matter and biological activity. Many studies with bioindicators
3 have shown that applying organic materials directly or indirectly enhances soil
4 biological activity (Diacono and Montemurro, 2010). The result of this enhanced
5 activity is increased soil suppressiveness to diseases as this capacity is based partially
6 on competition for resources. Nevertheless, the main disinfection mechanism involved
7 in these methodologies is the release of volatile substances during decomposition (Klein
8 et al., 2011). The term biofumigation was coined in the early 1990s to identify methods
9 to suppress soil-borne pests by releasing volatile isothiocyanates from the decomposing
10 residues of some *Brassica* species (Angus et al., 1994). Nowadays however, it is
11 routinely used for all practices that involve using fresh organic matter, including those
12 based on releasing ammonia from N-rich residues as a disinfecting agent (Arnault et al.,
13 2013; Martínez et al., 2012). Solarization and biofumigation are often combined for the
14 twofold purpose of enhancing disinfection and reducing damage to soil organic and
15 biological components (Núñez-Zofío et al., 2012; Núñez-Zofío et al., 2013). During
16 solarization, the degradation rates of organic waste residues are increased by higher
17 temperature and soil moisture, and the disinfection action of volatile substances is
18 strengthened by confinement under the plastic layer that soil is covered with. Moreover,
19 organic matter and the microbial community within residues compensate the reductions
20 in both parameters that solarization may cause in soil (Gelsomino et al., 2006; Pane et
21 al., 2012; Núñez-Zofío et al., 2013).

22 Despite all this information, there are many factors that strongly influence the success of
23 these practices, such as edaphoclimatic variables, which affect biological activity in soil,
24 the availability and characteristics of local organic materials, or legislative limits, which
25 must be respected in different areas, such as the maximum amounts of nitrogen that can

be applied to organic farming. More information that links local conditions and management practices to changes in relevant soil properties is, therefore, necessary to ensure the most beneficial results without harming agrosystems.

Cultural practices are known to strongly influence soil quality and disease suppressiveness (Janvier et al., 2007). Several factors have been proposed as soil quality components, and methods are variable to measure some of them. Among these numerous factors, it is important to be able to select the most interesting ones as indicators because they are fundamental to choose the most adequate soil management. Although the impact of agronomic practices on soil quality has been customarily evaluated by determining soil physicochemical variables, soil biological properties related to the quantity, structure, diversity and activity of soil microbial communities are increasingly being used for their sensitivity, rapid response and ecological relevance (Núñez-Zofío et al., 2012). In organic farming, soil biological activity indices, especially those based on enzymatic activities in connection with nutrient cycles, are particularly interesting as they reflect soil functioning (Canet et al., 2000). In this work, the effects on different bioindicators, soil microbial communities and soil biochemical properties of several commonly used practices to prepare soil before replanting in Mediterranean organic orchards were investigated during a full cropping season.

2. Materials and Methods

This study was carried out in a field trial set in an 1.2-hectare orchard on an old farm located at Gandía (Valencia, east Spain), wherein citrus has been cultivated for more than a century and many *Phytophthora* attacks have been recorded in recent decades of intensive management. Before replanting and converting the orchard into organic

management, different techniques to control soil-borne pathogens, based on the disinfection and/or enhancement of soil microbial activity, were tested:

- untreated control (C)
- solarization (S): carried out from the start of July to mid-September
- organic amendment (OA): ovine manure applied in June and incorporated at the rate of 16 t ha⁻¹ (fresh weight). Ovine manure characteristics: pH (1.25 aqueous solution) 8.41, EC (1:5, 25°C) 12.7 dSm⁻¹, total organic C 25.3%, total N 2.43%, C/N ratio 10.4, P₂O₅ 1.34%, K₂O 2.96%, MgO 1.51%, moisture 50.1%
- organic amendment + solarization (OA+S): carried out from the start of July to mid-September with the same ovine manure used in the OA
- biodisinfestant vegetal cover (VC): mixture of *Sinapis alba* (at a seed rate of 16.5 kg ha⁻¹) and *Brassica carinata*, *B. rapa* and *B. juncea* (at a seed rate of 25 kg ha⁻¹ each). Seeding at the beginning of March and incorporated in June. Seed rates were much higher than usual because the experiment schedule imposed very late seeding and a short growing period before the cover had to be cut and ploughed into soil.

For solarization, recently-irrigated soil was covered with 300-gauge transparent polyethylene film and was immediately sealed. The soil temperatures reached during the process were monitored by iButton electronic probes inserted at 15 cm (average sampling depth) immediately before laying plastic covers. The obtained data certified successful increases in temperature up to 53°C in all the solarized subplots. All the treatments were evaluated in triplicate in the experimental subplots. At the beginning of October, when treatments finished, eight 1-year-old orange trees (*Citrus sinensis* var.

1 *Salustiana*), grafted on Citrange Carrizo rootstocks (*C. sinensis* x *Poncirus trifoliata*),
2 were planted at 6x4 m in each subplot. The water and nutritional demands of the
3 plantation were homogeneously met for all the trees by means of a fertirrigation system.
4 All the fertilisers and application rates were certified for use in organic production
5 (Commission Implementing Regulation (EU) No. 354/2014). Three soil samplings were
6 performed during the trial: after treatments and before planting (start sampling), 6
7 months later (mid-season sampling) and 1 year later (end-season sampling). For all the
8 samplings, 16 subsamples were taken at a depth of 0-30 cm in each subplot from both
9 sides of trees. Upon the start sampling, subsamples were taken after considering the
10 places that trees would occupy after plantation, which was supported by high-precision
11 GPS. After collections, all the subsamples were carefully mixed into a composite
12 sample per subplot, which was then prepared for the biochemical and microbiological
13 analyses.

14 The studied soil biochemistry parameters were: microbial biomass carbon (Vance et al.,
15 1987), alkaline phosphomonoesterase activity (Tabatabai and Bremner, 1969),
16 phosphodiesterase activity (Browman and Tabatabai, 1978), urease activity (Kandeler
17 and Gerber, 1988), arylsulphatase activity (Tabatabai and Bremner, 1970), β -D-
18 glucosidase activity (Tabatabai, 1982), N-acetyl- β -D-glucosaminidase (chitinase)
19 activity (Parham and Deng, 2000), dehydrogenase activity (Casida et al., 1964), and the
20 ammonium oxidation potential (Elsgaard et al., 2001). Soil organic matter content was
21 determined according to standard methods (MAPA, 1986). The soil microflora
22 components were quantified by a Pasteur approach based on employing appropriate
23 growth media for each one; they were: bacteria (yeast peptone glucose agar with
24 cycloheximide, 100 mg l⁻¹, 25°C, 2 days), fungi (malt agar with antibiotics, citric acid
25 250 mg l⁻¹, chlortetracycline 50 mg l⁻¹ and streptomycin 100 mg l⁻¹, 25°C, 2 days),

actinomycetes (Vargas Gil et al., 2009), *Bacillus*-like bacteria (Yi et al., 2012), fluorescent pseudomonads (Simon and Ridge, 1974), and nitrogen cycle communities: denitrifying bacteria, ammonia-oxidizing bacteria and nitrite-oxidizing bacteria (Roux-Michollet et al., 2008). At least 3-fold laboratory replicates were performed.

To investigate whether significant differences between treatments existed, a statistical analysis of the results was performed by ANOVA (F-test and LSD Multiple Range Test at $p < 0.05$). The overall impact of treatments was evaluated with a principal component analysis (PCA) of the standardised data. Subsequently, an ANOVA (F-test and LSD Multiple Range Test at $p < 0.05$) was performed with the two principal components that explained the highest percentage of variance (PC1 and PC2). All these statistical calculations were carried out using the Statgraphics 5.1 (Manugistics Inc.) software package.

3. Results

Figure 1 displays the effect of treatments on the organic matter and microbial biomass contents, together with dehydrogenase activity. In the three samplings, soil organic matter after the S treatment was significantly lower than in the control C. The two treatments which included solarization (S and OA+S), very significantly decreased microbial biomass and dehydrogenase activity. Even though a clear trend towards recovery at the end of the full year study was noted, both decreases were most remarkable until the mid-season sampling, although the effect was significant only at $p < 0.06$ for microbial biomass.

Figures 2 and 3 display the effects of treatments on the different enzymatic activities under study. Except for chitinase, which was largely unaffected, treatments S and OA+S severely reduced activity, and this effect lasted the full season in many cases. Urease

activity decreased three fold, and the activity values were similar for all treatments only at the end of the experiment. The most severe changes were found in the ammonium-oxidation potential, which nearly disappeared after the S and OA+S treatments and remained below half the control level after 1 year. In the activities related to the phosphorus cycle, alkaline phosphomonoesterase and phosphodiesterase, the negative effect of solarization was once again intense, and was stronger and longer-lasting in the former. Similarly, carbohydrate degradation was depressed by solarization, precisely as the reduction in β -D-glucosidase activity in the S and S+OA treatments indicated (see Fig. 3). After the remarkable initial reductions, activity levels came close and were statistically undistinguishable from the mid-season sampling. The sulphur cycle was also disrupted in the S and OA+S treatments since arylsulphatase activity was depressed throughout the year.

Figure 4 displays the effects of treatments on total bacteria, fungi and the actinomycetes soil communities. Bacterial density reduced in the S and S+OA treatments to half the control value, although the effect was not long-lasting as density recovered half a year later. In fungi and the actinomycetes community, differences between treatments were not statistically significant. The number of fluorescent pseudomonads was variable throughout the experiment (see Figure 5). The counts in the C, OA and VC treatments were notably higher at the beginning of the study than during the rest of the year. The treatments S and OA+S led to a 10-fold reduction, although communities recovered and treatments were statistically indistinguishable 6 months later. Similarly, solarization was the main factor behind the changes noted in the *Bacillus* community, which were 100-fold higher after the S and S+OA treatments (Figure 5). Even though their sizes reduced in the mid and end-season samplings, differences with the other treatments remained highly significant. Figure 6 displays the effect of treatments on three microbial groups

that are closely related with the N cycle. Statistically significant differences were only found in the denitrifying bacteria and in the ammonia-oxidizing bacteria. As seen in S and OA+S, solarization increased the denitrifying bacteria and depressed the ammonia oxidizing bacteria at the start sampling, but treatments were statistically indistinguishable half a year later.

A PCA analysis was performed to study the variability of the total data (Figure 7). The soil biochemical properties, except for chitinase, and soil microbial parameters total bacteria, fluorescent pseudomonads and ammonia-oxidizing bacteria, intercorrelated upon the start sampling, as can be seen in the biplot performed with the two principal components that explained 73.3% of the variance in the data. When the effect of treatments on these two components was analysed, solarization was the main cause of variation, as shown by the ANOVA performed with PC1 and PC2 (Table 1). The initial changes persisted throughout the experiment, but to a lesser extent. Upon the mid-season sampling, the soil biochemical properties still correlated (biplot with PC1+PC2 explaining 63.7% of variance), and the S and OA+S treatments continued to produce significant differences in PC1. Six months later, the intercorrelation was less evident and the PC2 value significantly lowered as a result of the effect of the OA+S treatment.

4. Discussion

According to the trial results, treatments which included solarization, seemed to have a deleterious effect on the soil organic component, which affected both the biochemical and microbial parameters. Indeed some bioindicators remained severely depressed even 1 year after applying treatments. The untreated control showed organic matter, microbial biomass content and dehydrogenase activity, within the usual ranges expected for Mediterranean citrus soils, but they came much closer to those in conventional

orchards, which is expected at the beginning of the conversion into organic management (Albiach et al., 1999). When the S treatment was applied, soil organic matter significantly reduced, which is in line with what other authors have pointed out (Bonanomi et al., 2008; Scopa et al., 2008). Nevertheless in our study, an increase in microbial activity was not likely to be the main cause because both treatments which included solarization (S and OA+S), significantly reduced microbial biomass and dehydrogenase activity. A more likely explanation would, therefore, be that high temperature and soil moisture during solarization favour the extraction of stabilised organic matter, which eventually degrades even at lower biological activity rates. The reductions in microbial biomass and dehydrogenase activity caused by solarization were very similar in size and extent terms, which suggests that general soil activity simply lowered due to the smaller microbial community. The vegetal covers brought about a temporary stimulation in dehydrogenase activity, probably due to the action of the plant root exudates still present in soil.

S and OA+S treatments depressed enzymatic activities, except for chitinase. Although some recovered throughout the experiment, others remained inhibited 1 year after treatments. Soil enzymes can be found in cell debris, and may be extracellularly free in the soil solution and associated in various ways with humic molecules (Nannipieri et al., 2002). So solarization can affect them because the high temperature and moisture combination may favour the extraction of the most exposed or less-stable humic compounds that some enzymes are stabilised into, and the degradation and denaturalisation of the free and extracted enzymes also accelerate. Special attention should be paid to the reduction in the activities related to nitrogen cycling: urease and the ammonium-oxidation potential. The urease activity levels significantly lowered after the S and OA+S treatments, and their values were not similar to those of the control

1 until after 1 year. These results agree well with those reported by Ros et al. (2008), who
2 observed recovered activity after 8 months from biofumigation. However, they disagree
3 with those reported by Núñez-Zofio et al., 2013, who observed no decrease in urease
4 activity after biofumigation, but an increase 1 year later. The ammonium-oxidation
5 potential was hardly detected after the S and OA+S treatments, and was still severely
6 depressed by the end the experiment. From the fertilisation point of view, the decreases
7 noted in these two activities are particularly troubling as both are intimately related to
8 the production of nitrates (Subbarao et al., 2006), the preferential N source for plants.
9 Apparently, these results disagree with the large amount of nitrate that usually appears
10 immediately after solarization (Stapleton and DeVay, 1986; Gelsomino et al., 2006),
11 which was also observed in this work (data not shown). Nevertheless, the involved
12 biological systems were negatively affected, but not inactivated. Hence this early nitrate
13 release is due to the degradation of freshly-extracted soil organic matter, and also
14 derives from ovine manure in the case of OA+S.

15 The chitinase results are much more difficult to explain inasmuch as it was the only
16 activity not affected by solarization. As for the rest of enzymes, high temperature and
17 soil moisture would have negatively affected it, but other factors could stimulate the
18 activity that counteracted that effect. As mentioned in a review by Beier and Bertilsson
19 (2013) about the principal steps and mechanisms involved in chitin degradation, chitin
20 availability seems to promote chitin degradation rates. The large quantity of dead
21 invertebrates and fungi generated during solarization could, therefore, have stimulated
22 chitinase activity. In the same way, solarization boosted the *Bacillus* community and, as
23 they are chitin degradation mediators, their growth could have increased chitinase
24 activity.

1 The effects of treatments on the microbial soil communities were studied at different
2 integration levels: a global level by measuring the total microbial densities of bacteria,
3 fungi and actinomycetes; at a more specific level by analysing shifts in plant growth-
4 promoting microorganisms, these being fluorescent pseudomonads and *Bacillus*, and
5 key nitrogen-cycle communities: ammonia-oxidizing bacteria, nitrite-oxidizing bacteria
6 and denitrifying bacteria. Treatments brought about evident changes in several
7 parameters, although the statistical significance of the results was negatively affected by
8 the usual wide variability of the microbiological results. There is very little doubt that
9 the remaining from the eliminated old trees contributed to such variability. It is well-
10 known that presence of decomposing plant residues in soil produces considerable
11 microbiological heterogeneity, with residue itself being the zone where the most major
12 changes in microbial community composition take place over time (Gaillard et al.,
13 2003; Nicolardot et al., 2007).

14 Despite this variability in the data, solarization seems, once again, to be the main factor
15 for changes, as shown by the PCA and the individual analysis of each community. It is
16 well-known that solarization affects a wide range of soil microorganisms, but somewhat
17 contrasting results have been reported as to the fate of soil microflora in response to soil
18 solarization: both positive and negative effects on total bacterial and fungal
19 communities have been reported (Culman et al., 2006; Bonanomi et al., 2008; Scopa et
20 al., 2008). Our results show that solarization clearly and negatively affected total
21 microflora, just as the reduction in microbial biomass content indicated, but the effect
22 was different for bacteria, fungi and actinomycetes. This should not be considered
23 surprising giving the different tolerance of these microbial groups to heat.

1 Solarization affected the two analysed plant growth promoting communities in the
2 opposite way: reducing fluorescent pseudomonads and increasing the *Bacillus*
3 community. With the C, OA and VC treatments, the counts of fluorescent
4 pseudomonads were larger at the start sampling than at the end of the study. This is not
5 surprising since pseudomonads are very sensitive bacteria to environmental conditions,
6 whose survival is depressed by soil dryness and high temperatures (Agaras et al., 2014).
7 The community was depressed by the S and OA+S treatments, but the effect was not
8 long-lasting. Fluorescent pseudomonads have been frequently associated with the
9 stronger plant growth response observed in solarized soils. Although their populations
10 usually drop after soil solarization, they rapidly re-establish and colonise the
11 rhizosphere and roots of plants, and grow to a greater extent than in untreated soil
12 (Gamliel and Katan, 1991). In bulk soil, Patrício et al., 2006 did not note any significant
13 effects 1 month after solarization. However, Bonanomi et al., 2008 reported different
14 results depending on soil: counts increased in sandy soil, but remained unaffected in
15 clay soil. The exact opposite effect was found in *Bacillus* community, where the S and
16 S+OA treatments boosted the number of bacteria until the end of the study. Under
17 stress, *Bacillus* is able to generate heat-resistant spores. This is a clear competitive
18 advantage for regrowth after solarization and the most likely explanation for our
19 obtained findings.

20 Denitrifying bacteria and ammonia-oxidizing bacteria are the two microbial groups of
21 the three related with the N cycle that showed statistically significant differences after
22 treatments were applied. At the beginning of the study, denitrifying bacteria were
23 increased by S and OA+S, but at the mid-season sampling resilience were observed.
24 Because denitrifying bacteria are chemoorganotrophs than can use organic carbon for
25 their growth under aerobic conditions (Tiedje et al., 1989), one explanation is that

1 solarization could release soluble organic compounds allowing higher community size.
2 On the other hand, the ammonia-oxidizing bacteria were reduced by S and OA+S. These
3 results agree with the high susceptibility of ammonia-oxidizing bacteria to heat, as
4 reported in different studies on steam soil disinfestations (Tanaka et al., 2003; Roux-
5 Michollet et al., 2008; Yamamoto et al., 2008). The initial reduction in ammonia-
6 oxidizing bacteria is in accordance with the drops recorded in the ammonium oxidation
7 potential. Half a year later however, these bacterial members recovered, but the
8 potential microbial activity did not. This discrepancy can be explained as other
9 microorganisms with different heat sensitivity and resilience capacities, such as
10 ammonio-oxidizing archaea, some fungi and heterotrophic bacteria, are also able to
11 nitrify (Wertz et al., 2012).

12 This study reveals that soil disinfection by thermal methods, such as solarization, is a
13 risk in organically-managed orchards given damaged soil organic activity. Even though
14 no treatment rendered positive results against *Phytophthora* infections in the
15 experimental citrus orchard (Pérez-Piqueres et al., 2016), those which included
16 solarization (S and S+OA), resulted in severe damage to the organic matrix of soil,
17 especially in its biochemically-active fractions, that lasted longer than a full season in
18 some cases. N metabolism-related activities and communities were those were the most
19 intensely affected. This is very important for organic cropping not only because N is the
20 main limiting factor for agricultural productivity, but also because its annual input is
21 limited to 170 kg/ha, completely from organic sources. For many crops, this limit is
22 already most restricting, and any negative change to organic matter degradation
23 dynamics may act as an additional limit to productivity. Besides, given the very severe
24 and long-lasting decrease induced to the ammonium-oxidation process, NH_4^+ may be

1 favoured as the available N source, even though NO_3^- is naturally preferred by plants
2 (Britto and Kronzucker, 2013).

3 The experiment results also show how the choice of indicators to evaluate changes in
4 the soil biological status in different management alternatives is critical, as other works
5 have also suggested (Burns and Dick, 2002; Garcia and Hernández, 2000). Microbial
6 parameters were extremely variable and volatile compared with the biochemical
7 parameters, with the latter being both sensitive and long-lasting and, therefore, more
8 adapted to long-term studies. In addition, in the soil that underwent organic cropping,
9 where nearly all fertilisation depended on both biological nutrient fixation and the
10 further decomposition of produced or added organic matter, profound knowledge of
11 nutrient cycles is undoubtedly crucial to choose adequate soil management alternatives.
12 The determination of biochemical parameters, particularly the levels of different
13 enzymatic activities, is therefore a very useful tool to diagnose both the actual and
14 potential fertility of soil that undergoes organic cropping.

15 As a practical consequence of this study, solarization, and by extension thermal
16 methods, could be discouraged in orchards that are subjected to organic cropping given
17 their likely effects on the all-too necessary soil biological activity. In tree crops it is
18 relegated to preplanting and damage may be limited, but in other cases such as vegetal
19 production, it may be used many times and can have negative effects that are eventually
20 misdiagnosed as 'soil exhaustion'. When strong soil disinfection is genuinely needed,
21 solarization should be relegated to the very first organic conversion stages, and be
22 combined with additions of active organic matter or, more advisably, with a rotation of
23 green covers, which enhances the disinfectant effect, increases via fresh organic matter

incorporation, secretion of radicular exudates and microbiological activity, and preserves biodiversity in soil.

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Table 1. Effect of the treatments on the principal components extracted from the data from the three sampling dates.

	Start		Mid-season		End	
	Comp 1	Comp 2	Comp 1	Comp 2	Comp 1	Comp 2
C	2.76b	0.363a	2.79b	0.045a	1.64a	0.931b
S	-3.90a	0.032a	-2.88a	-0.523a	-2.57a	-1.14ab
OA	1.87b	-0.625a	2.31b	-0.070a	1.11a	1.27b
S+OA	-3.78a	0.253a	-3.67a	0.883a	-1.31a	-2.57a
VC	3.05b	-0.024a	1.46b	-0.336a	1.12a	1.50b
<i>F-test (p)</i>	<i>0.001</i>	<i>NS</i>	<i>0.001</i>	<i>NS</i>	<i>NS</i>	<i>0.05</i>

† C: control, S: solarization, OA: organic amendment, VC: vegetal cover.

†† Data standardized by subtracting their means and dividing by their standard deviation. Values followed by the same letter are not significantly different according to LSD Multiple Range Test at the 0.05 level of probability.

Figure 1. Content of organic matter (OM), microbial biomass (MB), and dehydrogenase activity (dHase) in soils after treatments: control (C), solarization (S), organic amendment (OA), organic amendment + solarization (OA+S), vegetal covers (VC). Values in the bars with the same letter are not significantly different according to LSD Multiple Range Test at the 0.05 level of probability.

Figure 2. Urease activity (Uase), ammonium oxidation potential (AO), phosphomonoesterase activity (Pase) and phosphodiesterase activity (Pdiase) in soils after treatments: control (C), solarization (S), organic amendment (OA), organic amendment + solarization (OA+S), vegetal covers (VC). Values in the bars with the same letter are not significantly different according to LSD Multiple Range Test at the 0.05 level of probability.

Figure 3. β -D-glucosidase activity (β -Gase), chitinase activity (Chase) and arylsulphatase activity (Asase) in soils after treatments: control (C), solarization (S), organic amendment (OA), organic amendment + solarization (OA+S), vegetal covers (VC). Values in the bars with the same letter are not significantly different according to LSD Multiple Range Test at the 0.05 level of probability.

Figure 4. Bacterial, fungal and actinomycetes communities in soils after treatments: control (C), solarization (S), organic amendment (OA), organic amendment + solarization (OA+S), vegetal covers (VC). Values in the bars with the same letter are not significantly different according to LSD Multiple Range Test at the 0.05 level of probability.

Figure 5. Fluorescent *Pseudomonas* and *Bacillus*-like bacteria in soils after treatments: control (C), solarization (S), organic amendment (OA), organic amendment + solarization (OA+S), vegetal covers (VC). Values in the bars with the same letter are not significantly different according to LSD Multiple Range Test at the 0.05 level of probability.

Figure 6. Denitrifying, ammonia-oxidizing (AO) and nitrite-oxidizing (NO) bacteria in soils after treatments: control (C), solarization (S), organic amendment (OA), organic amendment + solarization (OA+S), vegetal covers (VC). Values in the bars with the same letter are not significantly different according to LSD Multiple Range Test at the 0.05 level of probability. Absence of letters indicates no significant effect.

Figure 7. PCA of the standardised data at start (PC1 61.0%, PC2 12.3%), mid-season (PC1 47.4%, PC2 16.3%) and end-season (PC1 42.6 %, PC2 25.1 %) samplings. A: OM, B: MB, C: dHase, D: Uase, E: AO potential, F: Pase, G: Pdiase, H: β -Gase, I: Chase, J: Asase, K: Bacteria, L: Fungi, M: Actinomycetes, N: Fluorescent *Pseudomonas*, O: *Bacillus*-like bact., P: Denitrifying bact., Q: AO bact., R: NO bact.

Start

Mid-season

End-season