AIMS Geosciences

AIMS Geosciences, 7(3): 415–439.
DOI: 10.3934/geosci.2021024
Received: 03 June 2021
Accepted: 19 August 2021
Published: 30 August 2021

http://www.aimspress.com/journal/geosciences

Research article

Organic versus inorganic fertilizers: Response of soil properties and crop yield

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Abstract: The decrease in soil productivity and quality caused by the continuous and abusive use of mineral fertilizers makes necessary to adopt more sustainable agricultural soil management strategies that help to maintain soil edaphic fertility. In light of these considerations, we have evaluated the effect of organic vs. inorganic fertilization on soil microbial communities, soil quality, and crop yield in a melon crop. The following treatments were tested: i) aerobic sewage sludge from a conventional wastewater treatment plant (WWTP) using aerobic bacteria (SS); ii) aerobic sewage sludge from a WWTP using a bacteria-microalgae consortium (B); iii) N-P-K mineral fertilizer (M); iv) a treatment in which 50% of the N was contributed by SS and 50% by mineral fertilizer (M + SS); v) a treatment in which 50% of the N was contributed by B and 50% by mineral fertilizer (M + B); and vi) a no-fertilized control soil. Melon yield and fruit quality were determined in addition to several soil physical, chemical, biochemical and microbiological parameters. Organic fertilizers (SS and B) increased the percentage of soil water-stable aggregates (52 and 60% respectively) as well as the content of organic C (18 and 31%), water soluble C (21 and 41%), N (15 and 41%) and available P content (41 and 82%) compared to inorganic fertilization. They also stimulated bacterial and fungal abundance to a greater extent than mineral fertilizers (189 and 242% vs 85%, and 57 and 122% vs 29%, respectively), as well as soil respiration, and dehydrogenase, β-glucosidase, phosphatase, urease, and glycine aminopectidase activities. The analysis of principal components with parameters linked to soil quality clearly showed that organic fertilizers cause a greater improvement in soil

characteristics and microbial community than mineral fertilizers. Results demonstrate that organic and combined fertilization could be used as substitutes for nitrogen mineral fertilizers in melon crop, since these treatments led to similar melon production and quality while improving soil characteristics and microbial population size and activity.

Keywords: crop yield; enzymatic activity; organic and mineral fertilization; Shannon diversity index; sewage sludge; soil respiration; soil microbial diversity, water-stable aggregates

1. Introduction

The need to increase food production in order to feed an increasingly large population has promoted the use of synthetic mineral fertilizers capable of supplying crops with the amounts of nutrients they need for development. However, the long-term and abusive use of chemical fertilizers leads to soil degradation and to a reduction in soil microbial biomass and activity and microbial diversity, and can cause groundwater contamination problems due to excessive nutrient leaching [1,2]. Zhou et al. [3], reported that long-term inorganic fertilization led to a significant reduction in bacteria diversity and abundance, this reduction being greater as fertilizer concentration increased. It is therefore necessary to adopt more sustainable agricultural soil management strategies that make current food production possible without mortgaging future production—strategies that improve soil characteristics for maintaining soil edaphic fertility [2,4]. Although mineral fertilizers provide the plant directly with the nutrients it needs to develop, these fertilizers are not capable of exerting beneficial effects on soil characteristics as organic amendments do. Narsheh and Mousa [5], in a study comparing the effects of organic, inorganic, and combined fertilization on soil properties and cucumber plant yield, indicated that the addition of compost increased soil available water, soil porosity and organic C content compared to inorganic fertilization. Hernandez et al. [6], indicated that after two successive lettuce crops using manure and sewage sludge composts and inorganic fertilization, composts-treated soils showed improved physical characteristics as well as higher C, N and P content, higher microbial biomass, soil respiration and enzyme activity than the soils receiving conventional inorganic fertilization.

Therefore, the use of organic fertilizers as a total or partial alternative to mineral fertilizers can be a good option, provided that they are properly managed. This strategy also contributes to the solution of another issue—that of increasing organic waste production and the resulting accumulation of such waste, which can be worrying.

A great part of the nutrients provided by organic amendments are found in the form of organic compounds that are not available to plants, and a mineralization process is necessary for them to become available [7]. This must be taken into account when amendments are used as an alternative to mineral fertilization, so that yields are not diminished.

There are many studies aimed to evaluate the application of different organic residues in different crops as an alternative to mineral fertilizers [6,8–10]. However, the results obtained are sometimes contradictory, possibly due to the different nature of the amendments applied, the

application rate, and/or the climatic conditions, which influence the rate of mineralization of the organic matter provided [11].

The use of organic amendments leads to improved soil aggregation, porosity and water infiltration capacity, an increase in soil organic matter and nutrient content, and to the stimulation of microbial populations [6,7,12,13]. Therefore, organic fertilization can be an economically and environmentally viable alternative to mineral fertilizers.

Cai et al. [7], in a long-term fertilization experiment using organic and inorganic fertilizers indicated that organic and combined fertilization resulted in higher yield and soil available N and available P content than inorganic treatment alone. Rezácová et al. [12], in a field experiment observed that the application of compost and digestates improved soil aggregation increasing soil fertility. Xin et al. [13], in a 23-year field experiment also found that compost application increased soil water-stable aggregates. Hernandez et al. [14], indicated that 5 year after the application of composted urban waste to a degraded soil, amended soils showed higher water holding capacity, water-stable aggregates, nutrients and total and water-soluble C content than the control soil as well as greater bacteria and fungi abundance and hydrolase enzyme activity.

Most of the existing studies have focused on the effect of organic amendments on crop yield and on soil physical and chemical properties, whereas information on the effects of organic fertilizers on the activity and diversity of microbial communities in semi-arid environments is still scarce.

Microorganisms play a key role in the decomposition of organic matter and nutrient cycles and are involved in stabilizing soil structure. Plant residue degradation is mainly driven by the relative abundance (e.g. fungal-bacterial ratio) and activity of soil microorganisms and by soil-plant microorganism interactions [15]. Fungi are more efficient than bacteria in breaking down low-quality and resistant residues [16] and are therefore associated with unfertile soils and drought. In turn, bacteria prosper in environments in which inputs of labile forms of C dominate [17].

Soil microorganisms release hydrolytic extracellular enzymes capable of breaking down complex compounds into simpler structures available to themselves and other organisms and plants, being actively and directly involved in geochemical nutrient cycles, such as those of C, N, P and S, thereby establishing the ideal conditions for crop development. Hernandez et al. [9], in a barley and wheat crop experiment using inorganic, organic (sewage sludge compost and manure compost) and combined fertilization, observed that organically-treated soils showed after harvest greater global microbial activity (soil respiration and dehydrogenase activity) and greater β-glucosidase and alkaline phosphatase activity than the inorganically-treated soil.

Among the methods for analyzing the soil microbial diversity include the phospholipid fatty acid profile (PLFA), which gives us information on the structural microbial diversity, and the community level physiological profile (CLPP), an analysis of the bacterial response to different C sources (bacterial functional diversity). Although the results of the latter technique are not necessarily related to the functional potential of bacteria present in a given soil [18], and the technique is prone to biases inherent in the method used to measure diversity in growing conditions [19], it can be used effectively for a global view of functional diversity [20] and is recognized as a useful tool for comparing soil bacterial communities [18,21]. The measurement of soil microbial respiration and enzyme activities can also be used as a reliable indicator of soil quality and functionality, given the important role these activities play in nutrient cycling.

The agricultural application of sewage sludge is regulated by the Directive 86/278/EEC of the European Union (EU) [22], and there are numerous works on the beneficial effect of sewage sludge application on soil characteristics. However, there are no studies on the potential agronomic value of sludge generated by the use of bacteria-microalgae consortium in wastewater treatment systems. Rehman et al. [23], in a study on the effect of several sewage sludges (SS) and their biochar on soil properties and P uptake in wheat with and without P fertilizer, indicated that SS application increased grain yield and P concentration in plant. Koutroubas et al. [24], in an experiment of two successive wheat crops to investigate the influence of sewage sludge application on wheat yield and N accumulation, use and translocation, observed similar grain yield and N accumulation with the application of sludge that with the application of inorganic fertilization and concluded that sludges could be used as a fertilizer in wheat cultivation.

In light of these considerations, we carried out a study on melon cultivation using mineral and organic fertilization in order to evaluate the effects on soil microbial community, soil characteristics, and melon yield of either, mineral fertilization or alternative organic fertilizers: sewage sludge from an aerobic conventional wastewater treatment plant (WWTP) and sewage sludge from a bacteria-microalgae WWTP, used alone or combined with mineral fertilizers.

Hypothesis. Our starting hypothesis was that sewage sludges, obtained by conventional aerobic bacteria digestion or by bacteria-microalgae digestion, can improve soil physical, chemical and microbiological fertility as compared to conventional mineral fertilizers while producing similar crop yields.

2. Materials and methods

2.1. Experimental design

The melon crop was grown at a farm sited in the municipality of Mazarrón (Murcia) in SE Spain. The treatments were distributed in 6 ridges with drip irrigation lines; there were three treatments per ridge, with the corresponding separations between treatments. The separation between ridges was 2.5 m. The treatments tested in this experiment were as follows: i) aerobic sewage sludge (SS) from a conventional WWTP; ii) aerobic sewage sludge from a WWTP using a bacteria-microalgae consortium (B); iii) N-P-K mineral fertilizer (M); iv) a treatment in which 50% of the N was contributed by SS and 50% by mineral fertilizer (M + SS); v) a treatment in which 50% of the N was contributed by B and 50% by mineral fertilizer (M + B); and (vi) no fertilization (control).

The characteristics of the sludges used as organic fertilizers in the crop are shown in Table 1. Heavy metal concentrations in both sludges were under the limits established by EU legislation for the use of sewage sludge in agriculture [22].

The total amount of N contributed by the different treatments was 100 kg N/ha. Based on a previous mineralization assay (data not shown), the amount of the organic materials to be added was calculated considering a mineralization rate of 40% of the organic N contained in these materials. All treatments were established in triplicate and randomly distributed. For the application of the treatments, a trench was opened for the application of the fertilizer product in each of drip irrigation lines.

Two days after the treatment application, the melon plants were transplanted. The chosen variety was “Piel de Sapo”, and the plants were placed 40 cm apart. Irrigation was provided by an

integrated dropper with a flow rate of 3 liters/hour. The initial irrigation after planting lasted 30 minutes. Two days after transplanting, the plants were treated against thrips with Merusol. Since the mineral fertilization (M) provided more K to the soil than the rest of the treatments, the necessary amount of K was added to the other treatments, using a K_2SO_4 solution, to equalize all the treatments.

The melon crop was maintained for 110 days. After the melons were harvested, the soils were sampled and analyzed for physical-chemical, chemical, biological, biochemical and microbiological parameters. Some fruit quality parameters (average fruit size, acidity, firmness, ºBrix, and nitrate, sodium and potassium content) were measured on selected fruits.

Soil samples were stored at 4 ºC for chemical and biochemical analysis, and at −20 ºC for microbiological analysis.

Table 1. Characteristics of the conventional aerobic sewage sludge (SS) and the sewage sludge from an aerobic bacteria-microalgae waste-water treatment plant (B) used as organic fertilizers (d. wt.).

	SS	B
Moisture, %	76.68	97.81
Organic matter, %	60.25	67.03
pH	7.31	6.08
Electrical conductivity, dS m^{-1}	2.70	3.69
C, %	26.85	36.9
N, %	4.10	6.43
P, %	0.75	2.63
K, %	0.13	0.39
Ca, %	2.77	3.29
B, mg kg^{-1}	17.17	24.19
As, mg kg^{-1}	8.9	5.5
Cd, mg kg^{-1}	<0.01	0.55
Ni, mg kg^{-1}	20.8	24.5
Cu, mg kg^{-1}	448.7	400.0
Pb, mg kg^{-1}	62.2	30.7
Cr, mg kg^{-1}	43.9	52.3
Zn, mg kg^{-1}	1146	1020
E. coli, UFC/g	$1.5\ 10^3$	$1.4\ 10^3$
Salmonella in 25 g	Absence	absence

2.2. Soil analysis

Water-stable aggregates (WSA) were determined by the method of Lax et al. [25]. Four grams of 4 mm sieved soil placed on a 0.250 mm sieve were subjected to 150 ml of 270 Jm^2 artificial rain. The remaining soil on the sieve was dried at 105 ºC and weighed (P1). The soil was then soaked for 2 h in distilled water and then forced to pass through the same 0.250 mm sieve to break up the remaining aggregates. The residue remaining on the sieve was dried and weighed (P2). WSA were calculated as: % WSA = (P1 − P2) × 100/(4 − P2).

Soil water-holding capacity (WHC) was determined as the water retained in a saturated paste of the soil after submitting it to 33 kPa of pressure.

Organic C (Corg) and N were determined on a LECO TruSpec C/N/S automatic elemental analyzer (St. Joseph MI USA). Nutrient and heavy metal content in vegetal and soil were analyzed after microwave digestion in 65% HNO_3 using inductively coupled plasma-optical emission spectrometry (ICP-OES, model ICAP 6500 DUO THERMO, Thermo Scientific Wilmington, DE, USA). Water soluble C (WSC) and N (WSN) were measured in a water 1:5 (w:v) extract on a C-N analyzer for liquid samples (Multi N/C 3100, Analytic Jena, Germany). This water extract was also used to measure pH and electrical conductivity using a Crison GLP 21 pH-meter and a Crison CM 2200 conductimeter (Crison Hach Lange, Alella, Spain), respectively. Available P and K were determined by 1:10 (w:v) extraction with 0.1 M pH 8.5 $NaHCO_3$ and 1 M pH 7 ammonium acetate, respectively.

Soil respiration was determined by measuring in an infrared gas analyzer (Toray PG-100, Toray Engineering Co., Ltd., Japan) the CO_2 evolved from 15 g of moistened soil during a 27 day incubation period [26]. Basal respiration indicates the amount of CO_2 evolved per day during the incubation period and it is expressed as mg CO_2-C/kg soil day. Dehydrogenase activity was measured using p-iodonitrotetrazolium chloride (INT) as substrate according to Garcia et al. [27]. For β-glucosidase and phosphomonoesterase activities, modified universal buffer (MUB; pH 6) and 0.025 M p-nitrophenyl-β-D-glucopyranoside or MUB (pH 11) and 0.025 M pnitrophenyl-phosphate, were used, respectively [28]). Urease activity, was measured using urea (0.48%) as substrate and borate buffer (pH 10) [29]. Glycine-aminopeptidase activity was determined by the addition to 0.5 g of soil of 2 ml of 50 mN tris-HCl buffer at pH 7 and 2 ml of 50 mM glycine p-nitroaniline substrate, and colorimetric measurement of the p-nitroaniline formed after incubating the suspension for 2 hours at 40 ºC [30].

Bacterial functional diversity was evaluated using Biolog-Ecoplates (Biolog Inc., Hayward, CA, USA) [31]. The colorimetric indicator used by this system is a tetrazolium salt, which is colorless in its oxidized form but pinkish in its reduced form. For each sample, 1 g of dry soil was shaken with 10 ml distilled water (MilliQ quality) for 1 hour. After centrifuging and determining the optical density of each sample by spectrophotometer at a wavelength of 590 nm, the necessary dilutions of each sample were performed until similar optical density readings for the extracts were obtained, in order to obtain extracts with a similar microbial biomass; 100 µl of extract was added to each well of the Biolog EcoPlate, which was incubated at 28 ºC in darkness, and well color evolution was monitored periodically by optical reading at 595 nm.

The average well color development (AWCD) was calculated as: AWCD = Σ TD/n, where TD (transformed absorbance) is the absorbance value of the ith substrate following incubation (ƛ = 595 nm)—the absorbance value of its own first reading, and n is the number of carbon sources on the EcoPlate (n = 31).

In this study, data obtained after 90 h of sample incubation were used to characterize the bacterial metabolic diversity. The 31 C sources were grouped into the following groups: carboxylic acids, polymeric compounds, carbohydrates, polyphenols, amino acids and amines [32].

Richness and Shanon (H') indices were used to evaluate functional diversity. The richness index is the number of wells in a plate with values of transformed absorbance higher than 0.25 [33]. The Shannon index (H′) is calculated as H' = −Σpi* ln pi, where pi is the transformed absorbance of the ith substrate divided by the sum of the transformed absorbance of all the substrates.

The microbial community structure was assessed by phospholipids fatty acid analysis. Phospholipids were extracted from 6 g of soil using chloroform-methanol extraction [34], and were separated using a silica-bonded phase column (Sep-Pak Vacc 3; Silica Cartrides) (Sep-Pak Vacc 3; Silica Cartrides) and transformed by alkaline methanolysis into fatty acid methyl esters (FAMEs) [35,36]. FAMEs were quantified with a gas chromatograph (Trace GC Ultra Thermo Scientific) fitted with a 30 m capillary column (Thermo TR-FAME 30m × 0.25 mm ID × 0.25 μm film) and using helium as carrier gas. Metyl nonadecanoic acid (21:0) in isooctane was used as internal standard. Based on the Literature, PLFAs were assigned to different taxonomic groups (Table 2)

The species diversity was estimated by the Shannon index (H') = $-\sum$pi* ln pi, where pi is the PLFA abundance for a particular specie divided by the total PLFA concentration. This index considers the number of species that exist in the sample and the relative number of individuals there are for each species. Its value in the soil usually ranges from 2.3 to 4, and values less than 2 are considered ecosystems with a relatively low diversity of species, while those greater than 3 reflect high diversity levels.

Table 2. PLFAs assignation to taxonomic groups.

Taxonomic group	PLFAs	Reference
Bacteria	i15:0, a15:0, 15:0, i16:0, i17:0, cy17:0, cy19:0, 16:1–7c, 16:1–7t, 18:1–	[35]
	9c, 18:1–9t, 10 Me 16:0, and 10 Me 18:0	[70]
Fungi	18:2w6	[71]
Gram positive bacteria	i15:0, a15:0, i16:0, i17:0, 10 Me 16:0, and 10 Me 18:0	[35,70]
Gram negative bacteria	cy17:0, cy19:0, 16:1–7c, 16: –17t, 18:1–9 c, 18:1–9 t	[35,70]
Actinobacteria ($Gram^+$)	10 Me 16:0 and 10 Me 18:0	[71]

2.3. Fruit analysis

On selected fruits some quality parameters were measured. For firmness determination a TA.XT Plus Texture Analyzer (Stable Micro Systems Godalming, UK) was used. º Brix were measured with an Atago N1 alpha refractometer (0–32 º) (Atago Bellevue, WA, USA), and the titratable acidity was measured in a Metromhm 848 Titrino plus titroprocessor (Metrohm Herisau, Switzerland).

2.4. Statistical analysis

One-way analysis of variance (ANOVA) was used to test the significance of differences between treatments. The normality (Shapiro-Wilk test) and homoscedasticity (Levene's test) of data were previously tested, and non-normal variables were transformed prior to ANOVA. Post-hoc analyses were based on Tukey's honestly significant difference (HSD) test ($p \leq 0.05$). A principal component analysis (PCA) with 28 different soil parameters was performed to discriminate between the effects of organic and mineral fertilization on soil quality and soil microbial communities. SPSS v.19.0 (SPSS, Inc.) software was used for data analysis.

3. Results

3.1. Soil characteristics after melon cultivation

All the treatments tended to slightly decrease soil pH after melon cultivation, although differences with the control were only statistically significant ($P \leq 0.05$) for the B-treated soil. Electrical conductivity increased slightly with the addition of organic materials, although EC values were not high in any case, and differences between treatments were not statistically significant (Table 3).

No significant increases in the water holding capacity of organically-treated soils were observed with respect to the control. Nevertheless, soil aggregation was favored by the addition of both sludges (SS and B) whereas in the soil with mineral fertilization only (M), the percentage of water stable aggregates was similar to that of the control (Table 3).

The soils treated with SS or B, either alone or combined with mineral fertilizers, showed higher levels of organic C, N and available P than both the control and the M-treated soil (Table 4). The higher N content corresponds, in part, to the organic N provided with organic fertilizers that was not mineralized during the cultivation period, along with some mineralized N that was not absorbed by the plant. The B-treated soil showed the highest N content after cultivation, and C, N and available P contents in this soil were higher than in the SS-treated soil (Table 4).

The water-soluble C fraction (WSC) integrates the most easily degradable organic matter compounds. It is a very dynamic fraction that is in a continuous process of formation and degradation. As can be seen in Table 4, the soils treated with the organic materials showed higher concentrations of this fraction than the treatment with mineral fertilization only or the control. The B-treated soil showed the highest WSC content.

The fraction of water-soluble N (WSN), which contains those forms of N available to plant, was higher in M- and B-treated soils than in the rest of treatments (Table 4). The nitrate content, a component of the WSN fraction, was also higher in the M- and B-treated soils matching the WSN data (Table 4). No noteworthy differences were observed between treated and control soils in terms of total P and K content or available K (Table 4), or for the other macro and micronutrients analyzed (data not shown).

Unsurprisingly, since the organic materials used had low heavy metal content, the control and the fertilized soils (data not shown) showed similar heavy metal content, indicating that the use of SS and B sludge does not pose any risk of heavy metal contamination.

Table 3. Soil physical and physical-chemical characteristics after melon crop (d. wt.).

Treatment	pH	Electrical conductivity, dS/m	Water holding capacity, %	Water stable aggregates, %
Control	9.02 ± 0.20b	0.651 ± 0.16a	51.88 ± 0.76a	13.16 ± 5.70a
Mineral fertilizer	8.95 ± 0.11b	0.726 ± 0.14a	50.35 ± 1.07a	16.27 ± 3.91a
SS	8.74 ± 0.15b	0.975 ± 0.36a	52.76 ± 0.23a	26.24 ± 2.73b
B	8.33 ± 0.21a	0.886 ± 0.61a	51.78 ± 1.07a	24.77 ± 2.50b
Mineral+SS	8.83 ± 0.05b	0.968 ± 0.21a	52.36 ± 0.01a	21.41 ± 0.94ab
Mineral + B	8.74 ± 0.07b	0.917 ± 0.14a	52.99 ± 1.42a	19.70 ± 2.70ab

Note: SS:conventional aerobic sewage sludge; B: sewage sludge from an aerobic bacteria-microalgae waste-water treatment plant. For each parameter, data followed by the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).

Table 4. Soil nutrient content after melon crop (d. wt.).

	Control	Mineral fertilizer	SS	B	Mineral + SS	Mineral + B
Organic C, %	0.63 ± 0.06a	0.62 ± 0.12a	0.73 ± 0.07b	0.81 ± 0.01b	0.67 ± 0.09ab	0.81 ± 0.18b
Water soluble C, mg kg^{-1}	225.7 ± 3.06a	202.5 ± 6.11a	245.9 ± 6.16ab	285.2 ± 3.47b	238.5 ± 4.16a	242.2 ± 3.80ab
Nitrogen, %	0.058 ± 0.00a	0.068 ± 0.01a	0.078 ± 0.01ab	0.096 ± 0.01b	0.085 ± 0.00b	0.074 ± 0.01ab
Water soluble N, mg kg^{-1}	21.2 ± 0.84a	101.58 ± 13.52bc	46.1 ± 4.75ab	128.1 ± 10.56c	42.8 ± 2.45ab	64.5 ± 3.55ab
Nitrates, mg kg^{-1}	47.8 ± 1.41a	447.0 ± 61.09b	112.0 ± 14.91a	566.8 ± 129.02c	86.3 ± 9.21a	230.7 ± 30.32ab
Ammonium-N, mg kg^{-1}	2.4 ± 0.33a	3.7 ± 0.61b	4.2 ± 0.43b	4.3 ± 0.41b	4.2 ± 6.42b	4.3 ± 0.31b
P, %	0.083 ± 0.01a	0.087 ± 0.09a	0.086 ± 0.06a	0.093 ± 0.02a	0.100 ± 0.09a	0.102 ± 0.05a
Available P, mg kg^{-1}	12.52 ± 0.75a	12.93 ± 1.59a	18.18 ± 3.30b	23.48 ± 5.01b	13.48 ± 1.28a	16.81 ± 1.20a
K, %	0.38 ± 0.12a	0.46 ± 0.09a	0.45 ± 0.06a	0.41 ± 0.02a	0.43 ± 0.09a	0.38 ± 0.05a
Available K, mg kg^{-1}	118.61 ± 10.66[a]	104.54 ± 2.41a	105.73 ± 10.14a	104.44 ± 8.93a	111.50 ± 6.69a	117.29 ± 11.28a

Note: SS: conventional aerobic sewage sludge; B: sewage sludge from an aerobic bacteria-microalgae waste-water treatment plant. For each parameter, data followed by the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).

3.2. Soil biochemical and microbiological characteristics

3.2.1. Microbial respiration and enzyme activities

Both, microbial respiration and dehydrogenase activity (DA) are good indices of the global microbial metabolic activity. After melon cultivation, the soils with organic fertilization, either alone or combined with mineral fertilization, showed higher values of basal respiration and dehydrogenase activity than the control soil or the soil with mineral fertilization only (Figure 1). The B-treated soil

showed the highest basal respiration and dehydrogenase activity values, while the soil with mineral fertilization only (M) showed values of these parameters similar to those of control.

The enzyme β-glucosidase catalyzes the hydrolysis of glycosydic terminal groups up to glucose. Its activity therefore ensures an energy source for microorganisms. β-glucosidase activity was greater in the B- and SS-treated soils than in the control, whereas in the M-treated soil the activity of this enzyme was similar to that of the control (Figure 2).

Phosphatases catalyze the hydrolysis of organic P compounds to inorganic P compounds, making this element available to plants and microorganisms; these enzymes thus play a key role in soil fertility. Alcaline phosphomonoesterase activity was higher in the soil with organic or combined fertilization than in the control and M-treated soil (Figure 2). The soils treated with B and M+B showed the highest activity values for this enzyme.

Urease and glycine aminopeptidase are enzymes involved in the N cycle. Urease catalyzes the hydrolysis of urea-to-ammonium, and high activity levels of this enzyme can result in N loss by ammonium volatilization. Although all treatments showed higher activity levels of this enzyme than the control soil, increases only were statistically significant ($p \leq 0.05$) for the B treatment (Figure 2). Glycine aminopeptidase acts as a catalyst in the hydrolysis of peptide bonds, intervening in protein degradation. M- and B-treated soils showed glycine-aminopeptidase activity levels similar to those of the control, while the rest of the treated soils showed greater glycine-aminopeptidase activity than the control (Figure 2).

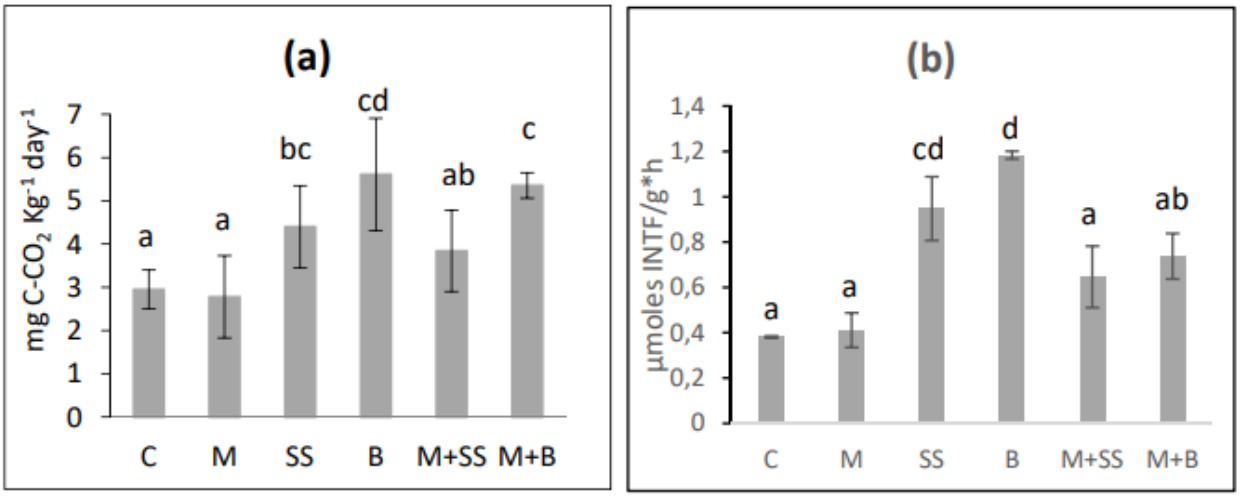

Figure 1. Basal respiration (a) and dehydrogenase activity (b) in the soils after melon crop. C: control; M: mineral fertilization; SS: conventional sewage sludge; B: sewage sludge from aerobic bacteria-microalgae waste-water treatment plant; M + SS: 50% N from mineral fertlizer and 50% N from SS; M + B: 50% N from mineral fertlizer and 50% N from B. For each parameter bars with the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).

3.2.2. Bacterial functional diversity

The average well color development (AWCD) within the Biolog Ecoplate plates is the measure of the average response of the metabolic community [31] and indicates the ability of bacteria to consume the substrate present in the well. The higher the AWCD, the greater the capacity of the bacterial population to consume the carbonated substrate.

As shown in Figure 3, the AWCD values after 196 h of incubation were higher for organically-treated soils than for the control. The SS-treated soil showed the highest AWCD values, whereas the M-treated soils showed lower AWCD values than the control, suggesting a decrease in functional activity.

Figure 4 represents the use of the different carbon sources by the control and the fertilized soils. As can be seen, there are significant differences in the consumption of the different substrates between the control soil and the treated soils, as well as between the different treatments tested, highlighting the susceptibility of microbial communities to soil treatments. We thus, observed a greater consumption of carbohydrates (Figure 4a), carboxylic acids (Figure 4b), polymers (Figure 4c), and amino acids (Figure 4e) in the SS-treated soil than in the control and the other treatments, which showed a lower or similar consumption of these substrates with respect to the control (with the exception of M+B, which was more efficient than the control in using polymers).

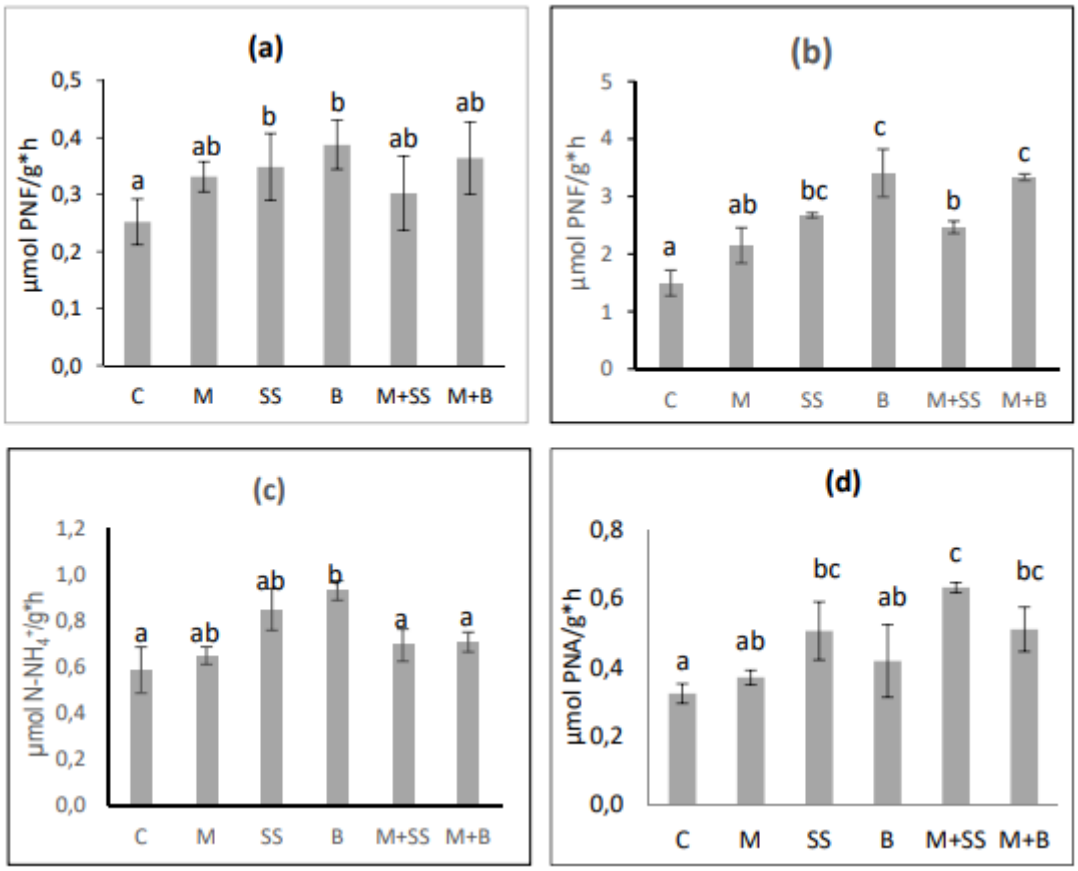

Figure 2. β-glucosidase (a), Phosphatase (b), urease (c) and glycine aminopeptidase (c) activities in the soils after melon crop. For abbreviations and significant differences between tratments see Figure 1.

Table 5. Physiological diversity (Shanon index, H') and richness of the bacterial communities.

	Control	Mineral fertilizer	SS	B	Mineral + SS	Mineral + B
Shanon index	3.26 ± 0.00cd	2.97 ± 0.08a	3.36 ± 0.03cd	3.21 ± 0.02bc	3.14 ± 0.03b	3.25 ± 0.05bc
Richness	27.33 ± 1.15bc	20.00 ± 1.73a	30.33 ± 0.58c	21.33 ± 1.53b	25.00 ± 1.00b	28.33 ± 1.15bc

Note: SS:conventional aerobic sewage sludge; B: sewage sludge from an aerobic bacteria-microalgae waste-water treatment plant. For each parameter, data followed by the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).

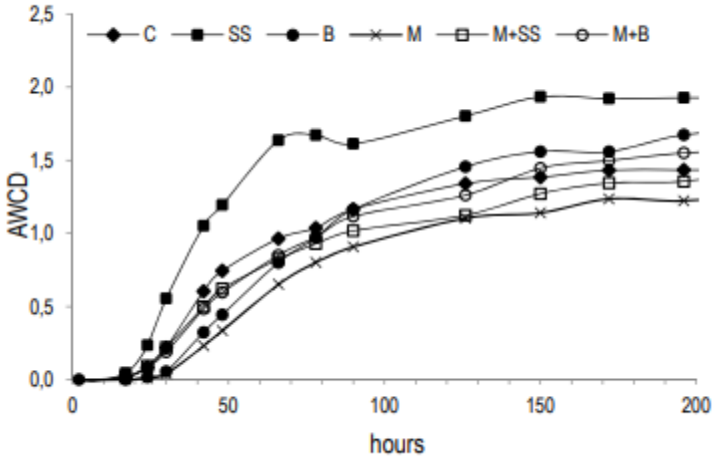

Figure 3. Average Well Color Development (AWCD) with time in the soils of the different treatments after melon crop. For abbreviations see Figure 1.

Soil populations in the B-treated soil behaved similar to control in the consumption of the different substrates, although they did show significantly lower consumption levels of amines and higher consumption levels of amino acids.

In the M + B-treated soil, the consumption of polymers (Figure 4c) and amines (Figure 4f) was significantly greater than that of the control and the M-treated soil, whereas the consumption of phenolic compounds (Figure 4d) and carbohydrates was significantly lower. Similarly, the consumption of carbohydrates was significantly lower in the M + SS-treated soil. Mineral fertilization (M) led to the highest consumption of phenolic compounds but to lower amine consumption.

The inorganically treated soil showed significantly ($P \leq 0.05$) lower physiological diversity, measured as both richness and according to the Shannon index, than the organically treated soils and the control, (Table 5).

3.2.3. Microbial structural diversity

The SS- and B-treated soils showed greater total bacteria, Gram+ bacteria, Gram− bacteria (only B treated soil) and fungal abundance than the control and the M treated soil, total PLFAs being significantly greater in the B-treated soil than in the SS treated soils (Figure 5). Moreover, the B-treated soil showed the greatest increases in Gram− and Gram+ bacterial and fungal abundance with respect to the control, followed by SS, M, and soils with combined fertilization, in this order.

Actinobacteria (Gram+ bacteria) play a key role in the breakdown of compounds such as cellulose and chitin; these bacteria renew nutrient stores in the soil and are fundamental in the formation of humus; SS, B and M treated soils increased the abundance of this type of bacteria with respect to control (Figure 5).

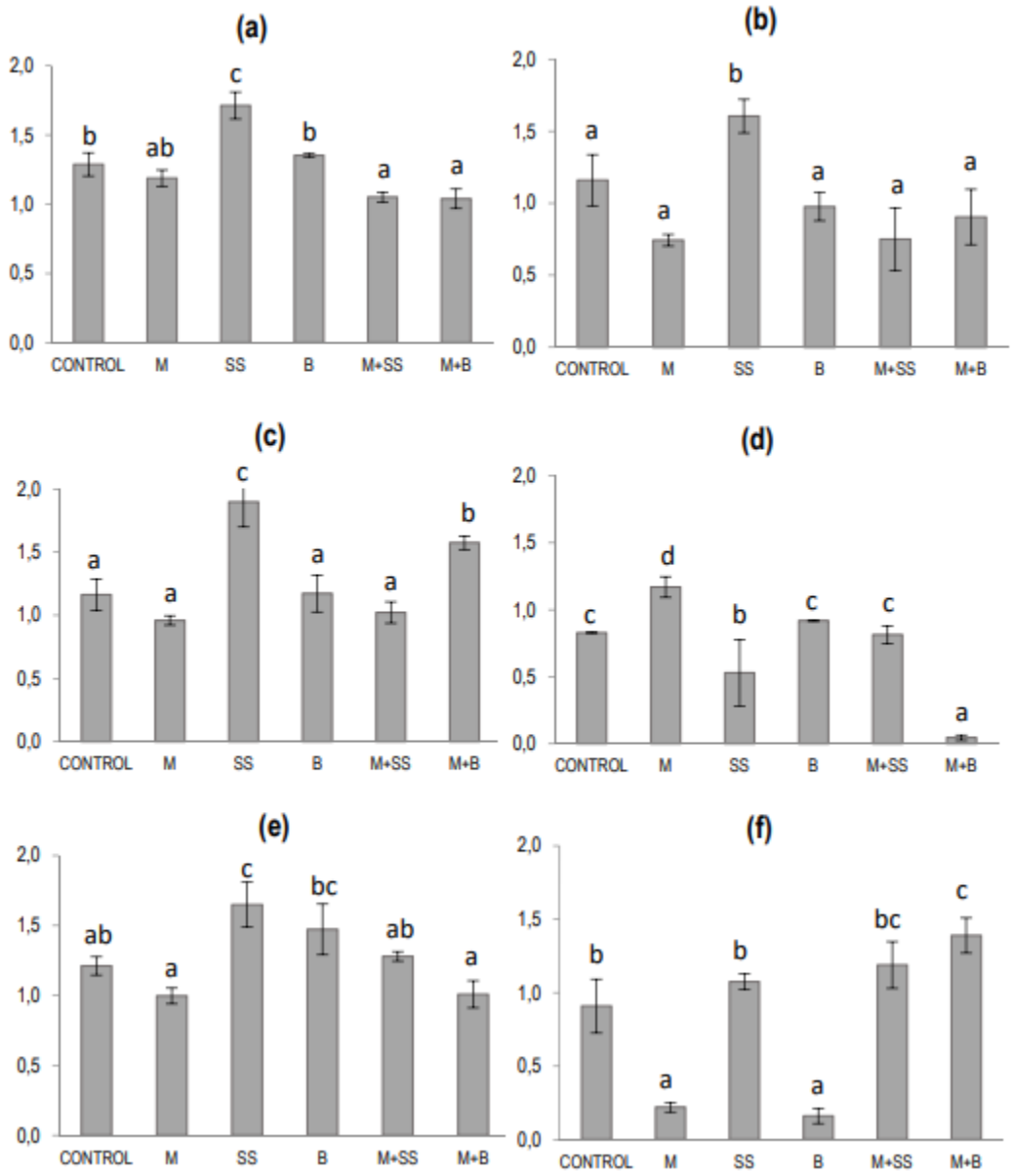

Figure 4. Changes in carbohydrates (a), carboxylic acids (b), polymers (c), phenolic compounds (d), amino acids (e), and amines (f) utilization by bacteria in the soils of the different treatments. For abbreviations and significant differences between tratments see Figure 1.

The SS- and B-treated soils showed greater abundance of saturated and mono-unsaturated acids than the soils with mineral or combined fertilization (Table 6). The fungi/bacteria, $Gram^{+}/Gram^{-}$ and mono-unsaturated/saturated acid ratios were greater in all the treated soils than in the control, with little differences among treatments (Table 6).

The soils with organic or combined fertilization (SS, B, M + SS and M + B) showed significantly ($p \leq 0.05$) greater Shannon index values than the control soil, whereas microbial structural diversity in the inorganically-treated soil (M) was similar to that of the control (Table 6).

Table 6. Saturated (Sat) and monounsaturated (Mono) fatty acid abundance (µmol/g dry soil), Mono/Sat and Fugi/bacteria ratios, and Shanon and Richness indices in the soils after melon crop. In bracket standard deviation.

	Control	Mineral fertlizer	SS	B	Mineral + SS	Mineral + B
Saturated acids	45.51 ± 1.69a	111.99 ± 0.64b	124.38 ± 3.33b	151.58 ± 7.86c	47.46 ± 7.92a	61.25 ± 5.53a
Mono-unsaturated acids	1.73 ± 0.47a	26.70 ± 4.45c	31.60 ± 2.39c	43.24 ± 3.24d	12.77 ± 1.69b	12.91 ± 2.56b
Gram+/Gram-	1.42 ± 0.08a	2.80 ± 0.49ab	3.86 ± 0.39b	3.11 ± 0.30b	2.92 ± 0.44b	4.41 ± 0.39b
Fungi/Bacteria	0.08 ± 0.00a	0.16 ± 0.02c	0.14 ± 0.03b	0.16 ± 0.00c	0.12 ± 0.01b	0.13 ± 0.02bc
Mono/Sat	0.03 ± 0.01a	0.24 ± 0.02b	0.25 ± 0.03b	0.29 ± 0.02b	0.27 ± 0.02b	0.21 ± 0.02b
Specific richness	3.95 ± 0.25a	4.21 ± 0.10a	4.29 ± 0.00a	4.12 ± 0.04a	5.30 ± 0.20c	4.78 ± 0.11b
Shanon index	2.40 ± 0.06a	2.47 ± 0.03ab	2.52 ± 0.04bc	2.53 ± 0.02bc	2.61 ± 0.03c	2.54 ± 0.02bc

Note: SS:conventional aerobic sewage sludge; B: sewage sludge from an aerobic bacteria-microalgae waste-water treatment plant. For each parameter, data followed by the same letter are not significantly different according to the Tukey test (P≤0.05)

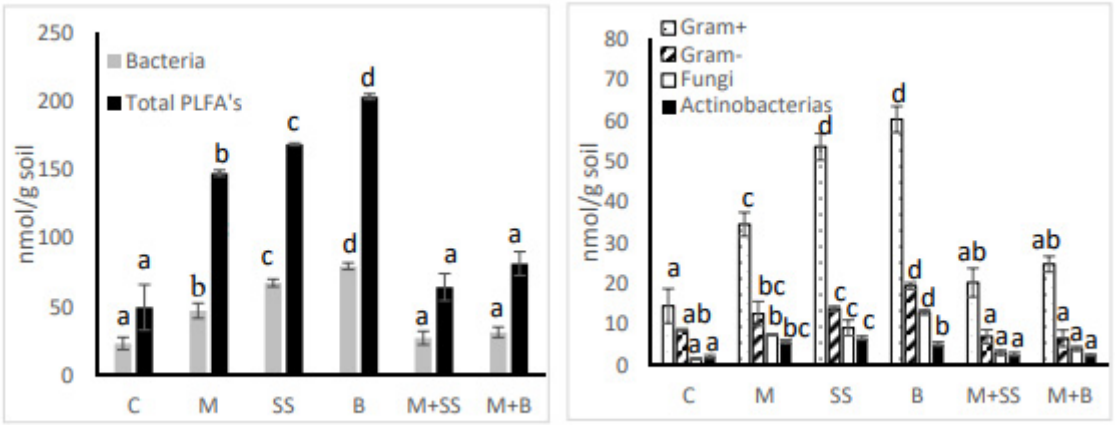

Figure 5. Concentration of total PLFAs and total bacteria, Gram$^+$, Gram$^-$, fungi and actinobacteria in the soils after melon crop. For abbreviations and significant differences between tratments see Figure 1.

3.3. Principal component analysis (PCA)

To differentiate between treatments, a PCA was carried out with most of the different parameters determined in the soil after melon cultivation (28 parameters). PCA provided two factors explaining 66.2% of the variance observed in the results: PC1 explained 50.79% of the variance and PC2 explained 15.39% (Figure 6). As shown in Figure 6, PC1 allows for discrimination between the different treatments, so that four groups can be significantly differentiated: one group covering the

control and M+SS-treated soils, a second group including M, M + SS and M+B, a third group with the SS-treated soils, and a fourth group with the B-treated soils. In turn, PC2 discriminates between the soils with mineral fertilization only and those subjected to the other treatments.

PC1 was positively correlated with most soil chemical (Corg, WSC, N, WSN, and P), biochemical (basal respiration, DHA, PHA, GA, and UA) and microbiological (total PLFAs, Bacteria, Fungi, Gram+, Gram-, saturated and monounsaturated acids, and consumption of phenolic compounds) parameters analyzed. The SS and B treatments caused higher scores than the control, M, M + SS and M + B treatments along PC1, and the score was higher for B than for SS. Along PC2, positively correlated with the consumption of C substrates (carboxylic acids, carbohydrates, polymers, and amino acids) and actinobacteria, profile scores for the M, SS and B treatments, in this order, were higher than those for the control and combined fertilization.

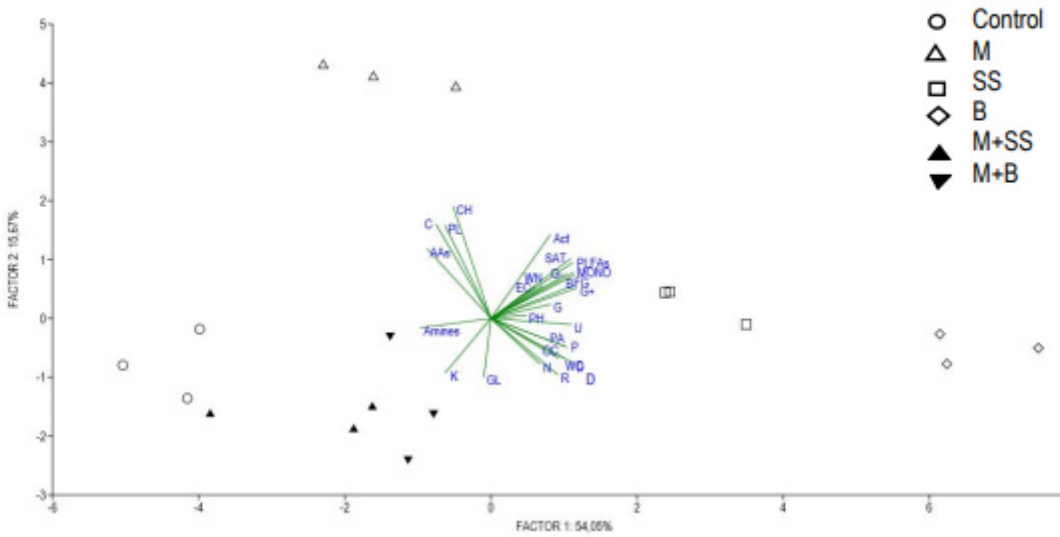

Figure 6. Principal component analysis of the main soil characteristics after melon crop. AAs:aminoacids, PL: polymers, C: carboxylic acids, CH: carbohydrates, PH: phenolic compounds, Act: actinobacteria, SAT: saturated fatty acids, MONO: monounsaturated fatty acids, B: total bacterias, FG: fungi, PLFAs: total PLFAs, G^+: Gram+ bacteria, G^-: $Gram^-$ bacteria, OC: organic carbon, WC: Water soluble C, WN: water soluble N, EC: electrical conductivity, P: soil phosphorus content, K: soil potassium content, R: microbial basal respiration, D: dehydrogenase activity, PA: phosphatase activity, G: β-glucosidase activity, U: urease activity, GL: glycine-aminopeptidase activity, N: total nitrogen.

3.4. Melon yield and fruit characteristics

As shown in Figure 7, the soil with organic fertilization, both when applied alone (SS and B treatments) and when applied in combination with mineral fertilization (MSS and MB), showed higher yields than the treatment with mineral fertilization only (M), although due to the dispersion of data values, the differences were not statistically significant.

Organic and mineral fertilization led to a similar fruit size and produced fruits with similar characteristics, showing similar pulp firmness, sweetness (º Brix), and acidity (Table 7). Likewise, no

differences worthy of mention were found in terms of potassium or nitrate concentrations in the fruits of the different treatments.

Table 7. Parameters of fruit quality.

Treatments	Average fruit weight	Sweetness ºBrix	Acidity (g citric ac. kg^{-1}	Firmness	NO_3^- mg kg^{-1}	K^+ mg kg^{-1}
Control	1.86 ± 0.48a	12.63 ± 0.61a	2.00 ± 0.18a	2.65 ± 0.18a	176 ± 1.92a	1511 ± 50.92bcd
Mineral fertilizer	2.38 ± 0.38a	12.74 ± 0.55a	2.04 ± 0.34a	2.55 ± 0.14a	183 ± 0.00a	1489 ± 19.25bc
SS	2.28 ± 0.42a	12.51 ± 0.50a	2.00 ± 0.27a	2.69 ± 0.26a	174 ± 1.92a	1467 ± 0.00b
B	2.37 ± 0.22a	12.36 ± 0.42a	2.09 ± 0.45a	2.65 ± 0.18a	207 ± 0.00a	1367 ± 0.00a
Mineral+SS	2.49 ± 0.17a	12.54 ± 0.10a	1.89 ± 0.21a	2.83 ± 0.33a	184 ± 1.92a	1544 ± 19.25cd
Mineral+B	2.47 ± 0.27a	12.67 ± 0.49a	1.88 ± 0.38a	2.73 ± 0.12a	158 ± 3.85a	1567 ± 0.00d

Note: SS:conventional aerobic sewage sludge; B: sewage sludge from an aerobic bacteria-microalgae waste-water treatment plant. For each parameter, data followed by the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).

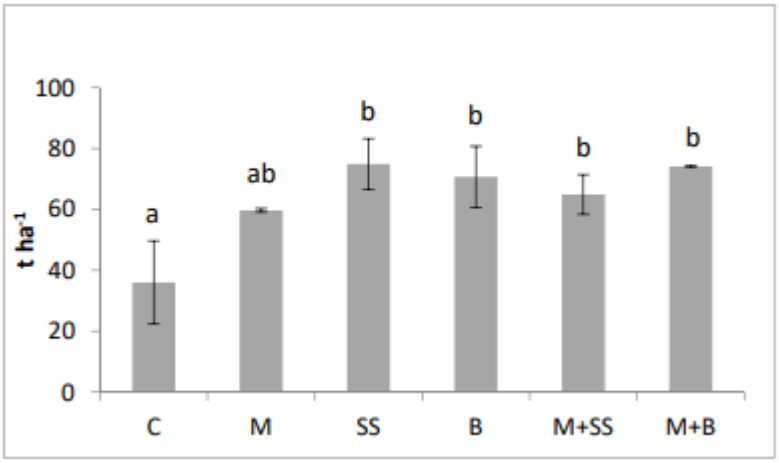

Figure 7. Melon yield with the different treatments. For abbreviations and significant differences between tratments see Figure 1.

4. Discussion

4.1. Effects on soil characteristics

Although it is known that organic amendments tend to favor the retention of water in the soil [6,13,14], in this experiment, no significant increases were observed in the soil water holding capacity (WHC) of the organically-treated soils. This can be attributed to the fact that the organic materials were added to the soils only once, and new organic material contributions are probably necessary in subsequent years for the soil WHC and soil nutrient reserve to be increased [7,37]. Even so, and unlike mineral fertilization, soil aggregation was favored by the addition of both sludges. This can be explained by the higher soil Corg, N and WSC content observed in the organically-

treated soils in comparison to the inorganically-treated soil, as well as by the greater microbial population size and activity of the organically-treated soils, since microorganisms improve the formation and stabilization of soil aggregates through the excretion of compounds that bind clays and organic materials [38,39,40]. Rezácová et al. [12], in a field experiment within Central Europe indicated that the improvement in soil aggregation caused for different organic fertilizers was associate with WSC, C, N and S content and the abundance of eubacterias and total glomalins. The beneficial effect of organic fertilizers on soil aggregate stability has been reported by several authors [14,41]. Mangalassery et al. [42], in a cashew growth experiment, also observed a higher percentage of water stable aggregates with the addition of organic fertilizers than with the addition of mineral fertilization.

It has been reported that the repetitive addition of organic fertilizers to the soil increases the soil nutrient reserve due to both, the activation of nutrient cycles through the synthesis of hydrolytic enzymes by soil microorganisms and through the nutrients organic amendment itself provides to the soil [43]. Our results also show that the addition of organic fertilization increased the content of Corg, N, available P, WSC and WSN in comparison with inorganic fertilization, and considerably stimulates the development and activity of soil microbial populations, as suggested by the higher levels of respiration and dehydrogenase activity and some hydrolase activities in the soils with organic or combined fertilisation (SS, B, M + SS and M + B) compared to the inorganically-treated soil (M) and the control. This effect was influenced by the characteristics of the organic material: we observed behavioral differences between B and SS, and, in general, B fertilization was more effective than SS in increasing microbial population growth and activity. This enhancement of soil enzyme activities, resulting from the organic matter provided with the organic fertilization, gives rise to a marked mineralization and activation of nutrient cycling, regulating soil nutrient availability for plant and microbial growth and improving soil fertility [11]. Zhao et al. [44], in a two-year experiment to study the impact of organic amendments on soil properties, winter wheat yields, and root growth, observed that organic amendments increased soil organic matter, N, water stable aggregates, and macroporosity compared to the soil treated with only mineral fertilizers. Toor et al. [45] also observed increases in Corg, N, and available P contents in a long-term experiment with long-lasting applications of organic wastes.

4.2. Effects on microbial functional and structural diversity

The AWCD indicated a higher rate of bacteria substrate utilization and a greater functional diversity of the organically-treated soils relative to the inorganically-treated soil, with the SS treatment presenting the highest AWCD values. This is probably because the added organic matter provides readily decomposable carbon sources to microorganisms. As indicated by several authors, C is the main factor driving microbial growth, and the improvement in C content, derived from the addition of the organic fertilizers, leads to changes in the use of carbon substrates by microorganisms [46,47]. This, in turn, increases microbial diversity and activity [48]. According to Hoogmoed et al. [49], changes in soil physical and chemical properties affect the microorganism environment and consequently the species activity and distribution.

Although higher global and specific microbial activity and total PLFAs were detected in the B-treated soil than in the control and the rest of the treatments, few differences were observed between the B treatment and the control in terms of substrate utilization. The SS treatment, on the other hand,

showed significantly greater substrate utilization than the control. This could be because only the metabolic activity of the bacteria is recorded by the Biolog technique, since the B-treated soil showed the greatest fungal population abundance.

The low consumption of phenolic compounds detected in the M + B treatment is striking, and could be attributable to antagonistic interactions between the microbiota [50].

Chakraborty et al. [51], observed by Biolog analysis, an increase in the use of carbohydrates in amended soils, while polymers, carboxylic acids, phenolic compounds, amino acids and amines presented similar or lower values than control. Authors like Rashedul et al. [52], found that carbohydrates, carboxylic acids, amines and amino acids were used more than polymers in soils that had been amended with organic compost. Therefore, it is evident that the application of soil amendments affects the use of different carbon sources by soil microorganisms, although, generally speaking, the behavior patterns have not been entirely clarified, indicating the complexity of soil microbial communities.

In our study, PLFA analysis indicated that soil fertilization resulted in a shift in the microbial communities due to the incorporation of C and N sources, which strongly encourages microbial growth and activity [53]. This effect was greater with organic fertilization than with inorganic fertilization. Thus, compared to inorganic fertilizers, organic fertilizers produced a greater increase in bacterial and fungal abundance along with a greater microbial community size. It should be noted that the B-treated soil presented, in general, the highest values for the different PLFAs, and that the organically and inorganically-treated soils differed significantly in most cases.

In agreement with Bray et al. [54], the addition of organic fertilizers alone (SS and B) increased both Gram− and Gram+ abundance and fungal community abundance to a greater extent than mineral fertilization. Some authors [55,56], have indicated that Gram+ bacteria have preference for using older carbon, whereas Gram- bacteria prefer to use fresh material from the plants. This could explain the fact that the differences between the organically-treated soils and the inorganically-treated soils are greater for Gram+ than for Gram− bacteria. The increased Gram+ bacteria abundance in soils, suggest a change from chemilithotrophic microbial communities (many of them Gram−) towards a more heterotrophic community, due to increased carbon levels [57].

Monounsaturated fatty acids are important indicators mainly associated with Gram− bacteria, and they increase with increases in available organic substrates [58]. This agrees with our study results, in which, organically-treated soils, particularly those treated with B, showed the greatest monounsaturated fatty acid abundance.

According to Bossio et al. [59], the monounsaturated to saturated fatty acid ratio (Mono/Sat) can be a reflection of C availability in the soil. This agrees with the fact that the B-treated soil which presented the highest value of WSC also presented the highest value of this ratio, as well as with the higher WSC values recorded in all fertilized soils in comparison with the control.

Organic fertilizers supply both recalcitrant and labile organic carbon. The latter acts as an energy source for microorganisms, enhancing the decomposition of the added and native organic matter. Added recalcitrant carbon, on the other hand, despite being difficult to degrade, can also improve the growth of some fungi and bacteria [60,61]. Generally, fungi decompose more complex and stable organic matter, while most bacteria favor the decomposition of labile biopolymers [62,63]. Our results suggest that fertilizer application, particularly organic fertilizer, has a greater incidence on fungal than on bacterial communities; this, in turn, suggests that soil fungi are more responsible

for the decomposition of added organic matter than bacteria [64]. Banerjee et al. [65], also found a greater response of fungal communities to fertilization, especially to organic fertilization. Broeckling et al. [66], indicated that the majority of fungi are heterotrophs and depend on exogenous organic C to grow, which would explain the greater fungal abundance found in organically-treated soils.

Contrary to inorganic fertilization, organic and combined fertilization significantly ($p \leq 0.05$) increased structural microbial diversity (Shannon index) with respect to the control. Other authors have also reported a higher microbial diversity using organic amendments than with inorganic fertilization [11]. This supports our hypothesis that the use of organic fertilizers instead of mineral N fertilizer is beneficial for the resilience of microbial diversity and soil productivity [67].

Regarding the fungal/bacterial ratio, all the treatments increased this ratio with respect to the control, indicating that the fungal population was stimulated to a greater extent in the different treatments than in the control, and that this stimulation was proportionally greater than that of the bacterial population. This relationship was similar in all of the treated soils, which showed similar increases in bacterial or fungal PLFAs. Accordingly, Montiel-Rozas et al. [68], also observed an increase in the fungal/bacterial ratio with the addition of the organic amendments to a degraded soil in a rehabilitation experiment using leonardite and biosolid compost.

4.3. Principal component analysis (PCA)

The PCA carried out on the different parameters analyzed in the soil after melon harvest confirmed the greater positive effect of organic fertilization on soil quality as compared to mineral fertilizers. The organically-treated soils showed a greater contribution of most of the parameters analyzed, with high scores in both the parameters related to microbial abundance and activity and the nutritional and organic C parameters. PCA analysis clearly discriminated between sludge-treated soils and inorganically-treated soils. This analysis also highlighted the greater beneficial effect of B sludge application on soil quality with respect to conventional sewage sludge.

4.4. Effects on melon yield and fruit quality

Crop yields are typically limited by the availability of nutrients for the plant, particularly N. The similar, or even higher, yields obtained in the organically-treated soils (SS, B, M + SS, and M + B) in comparison with the inorganically treated soil are probably due to the positive effect of the amendment on soil properties, encouraging root exploration and nutrient absorption by the plant in addition to microbial population growth and activity. Our results also suggest that the N amount provided by the organic matter contained in SS and B was enough to meet the plant's nutritional requirements. Lopedota et al. [69]. reported similar findings for melon crops. Cai et al. [8], in a 25-year fertilization experiment on wheat and maize growth under mineral, organic and combined fertilization, concluded that organic fertilization led to increased crop yields in relation to mineral fertilization, by improving soil fertility. In our study, no differences in melon yield between SS and B were observed.

The different indexes of melon quality, measuring sweetness, firmness, acidity and º Brix, were quite similar in the organically and inorganically treated soils, indicating that the organoleptic quality of the fruits obtained with organic or combined fertilization was similar to that obtained with mineral fertilization only.

All this emphasizes that both SS and B can be used at suitable doses, either alone or in combination with mineral fertilization, as alternative fertilizers in melon cultivation, yielding fruits of similar quality to those obtained with mineral fertilization while improving soil characteristics, particularly soil microbiological properties.

5. Conclusions

Organic fertilizers can improve soil biological, chemical and physical characteristics compared to mineral fertilizers.

Both, sludge from a conventional aerobic WWTP (SS) and from a bacteria-microalgae WWTP (B) provide beneficial effects to both, plant and soil. These organic materials improve soil aggregation and increase soil nutrient content. Likewise, these organic materials stimulate bacterial and fungal growth as well as the overall and specific activity of soil microorganisms, B sludge being, in general, more effective than conventional sewage sludge in improving soil quality.

The melon yields obtained with SS and B, used alone or in combination with mineral fertilization, were similar and even somewhat superior to those obtained with traditional mineral fertilization for this crop, yielding fruits of similar quality to those obtained with conventional mineral fertilization while improving soil characteristics (increased organic matter, nutrients and microbial community size and activity).

It can therefore be concluded that these organic amendments can be used as substitutes for nitrogen mineral fertilizers, with the environmental and energy-saving benefits that this entails.

Despite the short term duration of the experiment (only one crop) and the fact that a sole addition of organic amendment was carried out, we were able to observe positive effects of the organic fertilization on soil characteristics, and after cultivation, the soil remained enriched in certain nutrients and microbial populations. The edaphic fertility thus increased in these soils, and more noticeable improvements in crop yield and soil quality could potentially be observed in subsequent cultivations.

Acknowledgements

This work was supported by the Spanish Ministry of Science, Innovation and Universities within the project: MICROALBAC, Ref. RTC-2015-3245-5. The authors also thank to the "Fundación Séneca" of the autonomic Government of the Region of Murcia, for its financial support as Excellence Research Group of the Region of Murcia, Spain. The authors also thank the Castellon City Council (Spain) for their technical support.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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