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Use of biostimulants on soil restoration: Effects on soil biochemical properties and microbial community

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ABSTRACT

Four biostimulants (BS): WCDSs, wheat condensed distiller solubles; PA-HE, hydrolyzed poultry feathers; CGHE, carob germ enzymatic extract; and RB, rice bran extract were applied annually at 4.7 t organic matter (OM) ha^{-1} for a 3-year period to a Xerollic Calciorthid soil to evaluate their efficiency in soil restoration. Their effects on the plant cover, soil enzymatic activities and the structure of the soil microbial community by analysing phospholipid fatty acids (PLFAs) were determined. Application of BS that contain higher amounts of protein and higher percentage of peptides under 3 kDa had a greater effect on the soil biological properties, possibly due to the low molecular weight protein content can be easily assimilated by soil microorganisms. Following 3 years of successive soil amendment, the dehydrogenase activity was 4.6, 9.6, and 17.6% higher in PA-HE-amended soils than in the RB, CGHE and WCDS-amended soils, respectively. The urease activity was 5.3, 14.5, and 28.8% higher in PA-HE-amended soils than in the RB, CGHE and WCDS-amended soils, respectively. The phosphatase activity was 8, 15.3, and 20.2% higher in PA-HE-amended soils than in the RB, CGHE and WCDS-amended soils, respectively. The arylsulfatase activity was 16, 21.1, and 27.2% higher in PA-HE-amended soils than in the RB, CGHE and WCDS-amended soils, respectively. Total soil phospholipid fatty acid (PLFA) concentration was significantly ($p < 0.05$) higher in BS-amended soil than control soil. Principal component analysis discriminated between the BS-amended soils, mainly based on content of lower molecular weight peptides. Thus, PA-HE and RB were grouped and differentiated from CGHE and WCDS, respectively. After 3 years of treatment, vegetal cover was 11.4, 17.7, 24.1, and 85.8% higher in PA-HE-amended soils than in the RB, CGHE, WCDS treatments and control soil. These results suggested that under semiarid climatic conditions the application of BS with higher amounts of protein (>50%) and a higher percentage of peptides under 0.3 kDa (>60%) notably increased the soil enzymatic activities, induced changes in microbial community because the protein with lower molecular weight can be more easily absorbed by soil microorganisms, and also favoured the establishment of vegetation, which will protect the soil against erosion and will contribute to its restoration.

1. Introduction

Increasingly, organic soil amendments are being examined for their potential use in soil restoration and for preventing soil erosion. Recent years have seen an increase in the application to semiarid soils for soil restoration purposes of organic wastes with a high organic matter content, usually composted, such as urban waste (Ros et al., 2003), plant materials derived from the municipal landscape (Walker, 2003), cotton gin compost (Tejada et al., 2006a), beet vinasse composted with a crushed cotton gin compost (Tejada et al., 2006b), vermicomposts (Tejada et al., 2010), etc.

In the recent years, there has been increasing use of hydrolysates organic biofertilizers or biostimulants (BS) obtained from different organic materials by hydrolysis reactions. These BS, generally comprising peptides, amino acids, polysaccharides, humic acids, phytohormones, etc., are directly absorbed by soil microorganisms and plants which spend a smaller amount of energy in the absorption process (García-Martínez et al., 2010a,b). This has a positive effect not only on growth but also on the quality and production of the fruit or grain harvested (Parrado et al., 2008). The aim of these products is not to provide nutrition, but rather to encourage and stimulate plant metabolism, stress reduction, etc. (Parrado et al., 1991, 2006, 2007, 2008). Therefore, the development of new BS has become the focus of interest in research.

Normally, soils degraded by erosion processes are characterized by low organic matter content and therefore, low microbial

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activity, which ultimately hinders the establishment of plant cover (Tejada et al., 2009, 2010). To recover these degraded soils, the most effective way is to improve quality by adding organic materials. Therefore, this suggests that land application of BS to the soil could improve the soil's microbial population quickly and thus support the development of plant cover.

Biologically and biochemically mediated processes in soils are of the utmost importance to ecosystem function. Soil microbes are the driving force behind many soil processes including the transformation of organic matter (Miltner et al., 2004), nutrient release (Wichern et al., 2007) and degradation of xenobiotics (Zabaloy et al., 2008). Many studies have shown that biological parameters have been used to assess soil quality and health as affected by agricultural practices (Gianfreda et al., 2005; Truu et al., 2008; García-Ruiz et al., 2009). In this respect, soil enzymes can be used as potential soil quality indicators for sustainable management because they are sensitive to ecological stress and land management practices (Tejada, 2009). Enzymes may react to changes in soil management more quickly than other variables and therefore may be useful as early indicators of biological changes (Zabaloy et al., 2008).

On the hand, the number of physiological groups of bacteria has also to be useful when measuring structural changes in soil due to several anthropogenic factors (Ratcliff et al., 2006; Zabaloy et al., 2008; Zhang et al., 2010). Therefore, the comparison of the soil enzymatic activities and biodiversity could be of help when evaluating the impacts of BS on soils.

Currently there are no studies that examine the incidence of BS in the restoration of degraded soils. The aim of this paper was to study the influence of different BS on soil biological activity, soil microbial community and soil restoration in a semiarid Mediterranean ecosystem.

2. Materials and methods

2.1. Site description

The study was conducted from October 2003 to October 2006 near Córdoba (Guadalquivir Valley, Andalusia, Spain). The climate of the study area is semiarid with an average annual precipitation of 400 mm for the three experimental years, concentrated in the spring and autumn months. The mean annual temperature of the three experimental years was 17.3 °C and the mean potential evapotranspiration was 700 mm/year^{-1}. Thus, the long-term water deficit, calculated by the Thorntwaite method, is 436 mm. July and August are the driest months.

The area is a fragile environment, strongly marked by erosion. Harsh physical conditions and inadequate soil use by man have resulted in a dissected landscape where furrows, rills, and gullies scour both the hill slopes and the weak deposits which fill the low-lying regions.

The study was conducted on a Xerollic Calciorthid soil (Soil Survey Staff, 1987) with an 8% slope. The general properties of the soil (0–25 cm) are shown in Table 1. Soil pH was determined in distilled water with a glass electrode (soil:H_2O ratio 1:2.5), as was soil electrical conductivity (soil:H_2O ratio 1:5). Soil texture was determined by Robinson's pipette method (SSEW, 1982) and dominant clay types were determined by X-ray diffraction. Total $CaCO_3$ was measured by quantifying the CO_2 produced by adding HCl to the soil (MAPA, 1986). Organic carbon in the soil was determined by oxidizing the organic matter in the soil samples with $K_2Cr_2O_7$ in sulphuric acid (96%) for 30 min, and measuring the concentration of Cr^{3+} formed (Yeomans and Bremner, 1988). Total N in soil was determined by the Kjeldahl method (MAPA, 1986). Soil bulk density was determined using the core method, weighing and drying the soil at 105 °C for 48 h before determining bulk density as the ratio between soil dry weight and the ring volume, according to the official methods of the Spanish Ministry of Agriculture (MAPA, 1986).

Table 1
Initial soil characteristics. Data are the means of four samples.

pH	7.6 ± 0.1
Electrical conductivity (dS m^{-1})	0.22 ± 0.07
Clay (g kg^{-1})	316 ± 14
Silt (g kg^{-1})	256 ± 11
Sand (g kg^{-1})	428 ± 13
Texture	Clay loam
Dominant clay types	Illite, illite-montmorillonite (interstratified)
Bulk density (Mg m^{-3})	1.45 ± 0.04
$CaCO_3$ (g kg^{-1})	341 ± 12
Total N (g kg^{-1})	0.3 ± 0.03
Total C (g kg^{-1})	1.1 ± 0.08

Mean ± st. error.

2.2. Properties of biostimulants

The BS were produced using an enzymatic attack process by endoproteases (subtilisin) as the hydrolytic agent on different raw organic materials (Romero et al., 2007). The process was carried out in a bioreactor with controlled temperature (60 °C) and pH (pH 8) using the pH-stat method, which controls the pH through continuous alkali uptake during the hydrolytic process. The proteins from the raw material were efficiently hydrolyzed. The enzymatic hydrolysis process is detailed in García-Martínez et al. (2010a,b).

The BS obtained were: (1) wheat condensed distiller soluble (WCDS) enzymatic hydrolysate, where the raw material is a by-product of ethanol fermentation provided by Abengoa-Bioenergy (Bioethanol Galicia, Teixero, Spain) (García-Martínez et al., 2010a,b), (2) hydrolyzed poultry feathers (PA-HE), where the raw material is poultry feathers, (3) carob germ enzymatic extract (CGHE), where raw material is carob germ by-product from the food industry (Parrado et al., 2008), and (4) rice bran (RB) extract, where the raw material is rice bran, a major product in the rice industry (Parrado et al., 2003).

The general properties of BS are shown in Table 2. Organic matter was determined by dry combustion. Total soluble carbohydrates were determined after extraction with a mixture of ethanol/water (2/3) for 2 h. After centrifugation at 4000 × *g*, the supernatant was filtered through no. 1 Whatman paper, and total soluble sugars were estimated colorimetrically by the phenol–sulphuric acid method, using a standard glucose curve (Dubois et al., 1956). The protein content was determined by multiplying the total nitrogen by a conversion factor of 6.25 (Uncu and Cekmecelioglu, 2010). Fat was determined gravimetrically after extraction with hexane for 12 h in a Soxhlet extractor. After nitric and perchloric acid digestion, P was determined by the Guitian and Carballas (1976) method, and K, Ca, Mg, Fe, Cu, Mn and Zn were measured by ICP-S.

Molecular-mass distribution of protein in the samples was determined by size-exclusion chromatography using an ÄKTA-purifier (GE Healthcare), according to the procedure described by Bautista et al. (1996), using a Superdex Peptide^{TM} 10/300GL column (optimum separation range 0.1–7 kDa). Samples were centrifuged at 13,300 × *g* for 15 min at 4 °C to remove insolubles, and the supernatant was passed through a 0.2 μm filter and loaded into a 0.1 ml loop connected to an Äkta purifier system. The column was equilibrated, and eluted with 0.25 M Tris–HCl buffer (pH 7.00) in isocratic mode, at a flow-rate of 0.5 ml/min, and proteins/peptides were detected at 280 and 215 nm with a GE Healthcare UV900 module coupled to the column elution. A standard protein mixture (cytochrome C, 12,500 Da; aprotinin, 6512 Da; vitamin B_{12},

Table 2
Biostimulant chemical characteristics. Data are the means of four samples.

	WCDS	CGHE	PA-HE	RB
Density ($g\,ml^{-1}$)	1.12 ± 0.07	1.17 ± 0.05	1.20 ± 0.08	1.19 ± 0.06
Organic matter ($g\,kg^{-1}$)	234a† ± 25	492b ± 20	541b ± 43	530b ± 25
Total soluble carbohydrates ($g\,kg^{-1}$)	49.8c† ± 5.2	38.0c ± 1.6	6.5a ± 1.9	19.0b ± 1.1
Protein ($g\,kg^{-1}$)	40.2a† ± 3.8	46.5a ± 1.2	83.0b ± 8.4	50.0a ± 2.3
Fat ($g\,kg^{-1}$)	4.2b† ± 0.4	6.5b ± 0.2	2.0a ± 0.6	4.0b ± 0.9
N-Kjeldahl ($g\,kg^{-1}$)	6.4a† ± 1.8	7.4a ± 1.2	13.3b ± 8.4	8.0a ± 2.3
P ($g\,kg^{-1}$)	3.5c† ± 0.2	7.7d ± 0.6	0.50a ± 0.08	1.60b ± 0.24
K ($g\,kg^{-1}$)	6.8b† ± 0.6	22.8c ± 1.2	0.86a ± 0.021	Nd
Na ($g\,kg^{-1}$)	0.82a† ± 0.08	0.9a ± 0.1	0.86a ± 0.24	Nd
Ca ($g\,kg^{-1}$)	≤0.5a†	3.1b ± 0.2	0.78a ± 0.09	0.59a ± 0.10
Fe ($g\,kg^{-1}$)	≤0.2b†	≤0.1b	≤0.05a	≤0.02a
Cu ($g\,kg^{-1}$)	Nd	≤0.05	Nd	Nd
Mg ($g\,kg^{-1}$)	1.2b† ± 0.21	3.5c ± 0.2	≤0.32a	0.79b ± 0.07
Mn ($g\,kg^{-1}$)	≤0.05	Nd	Nd	Nd
Zn ($g\,kg^{-1}$)	≤0.05a†	≤0.05a	1.4b ± 0.14	Nd

Nd: not determined.
† Files (mean ± standard errors) followed by the same letter(s) are not significantly different according to the Tukey test ($p < 0.05$).

1255 Da; cytidine, 246 Da; glycine 75 Da) was used to cover the range of 100–7000 Da.

2.3. Experimental layout

The experimental layout was a randomised, complete-block design with five treatments and three replicates per treatment. The plot size was 7 m × 4 m, and the treatments were the following: (1) C, control soil (no organic amendment); (2) WCDS, amended with 50 l of WCDS (4.7 t organic matter (OM) ha^{-1}); (3) CGHE, amended with 22.8 l of CGHE (4.7 t OM ha^{-1}); (4) PA-HE, amended with 20.2 l of PA-HES (4.7 t OM ha^{-1}); and (5) RB, amended with 20.8 l of RB (4.7 t OM ha^{-1}). The BS were diluted with 500 l ha^{-1} of water and were applied to the soil surface on 16 October 2003, 20 October 2004, and 23 October 2005, respectively, and incorporated to a depth of 25 cm by chisel ploughing and disking the day after application. The chemical compositions of the BS remained unchanged throughout the 3-year experimental period. The BS were kept refrigerated at 0 °C for years and thawed and applied each year.

2.4. Soil sampling and analytical determinations

Soil samples (0–25 cm) were collected from each plot with a gauge auger (30-mm diameter) on 14 October 2003, 19 October 2004, 21 October 2005, and 20 October 2006, respectively. Four subsamples were collected from each plot and ground to pass through a 2-mm sieve before being stored in sealed polyethylene bags at 4 °C until analysis.

Five soil enzymes' activity levels were measured. Dehydrogenase activity was measured by reducing 2-*p*-iodo-3-nitrophenyl 5-phenyl tetrazolium chloride to iodonitrophenyl formazan (García et al., 1993). Urease activity was determined by the buffered method of Kandeler and Gerber (1988), using urea as substrate. Phosphatase activity was measured using *p*-nitrophenyl phosphate as substrate (Tabatabai and Bremner, 1969). Arylsulfatase activity was determined using *p*-nitrophenylsulfate as substrate (Tabatabai and Bremner, 1970).

At the beginning and the end of the experimental period and for each treatment, phospholipids were extracted from 4 g of soil using a chloroform–methanol extraction based on Bligh and Dyer (1959), fractionated and quantified using the procedure described by Frostegard et al. (1993a) and Bardgett et al. (1996). Twenty-six separated fatty acid methyl esters were identified using gas chromatography and a flame ionization detector. Phospholipids were transformed into fatty acid methyl esters (FAMEs) by alkaline methanolysis, which were quantified by gas chromatography (GC/FID, AutoSystem XL Gas Chromatograph, Varian Saturno 2000) fitted with a 50-m capillary column and helium as carrier gas. The injector temperature was 260 °C, the flame ionization detector temperature was 280 °C, and the initial temperature was 70 °C (for 2 min); it was increased to 160 °C at 30 °C min^{-1} and then to 280 °C at 3 °C min^{-1}.

PLFAs were defined by standard nomenclature: the total number of carbon atoms, followed by a colon, and the number of double bonds. The position of the first double bond is indicated by "ω" followed by the number of carbon atoms counted from the aliphatic end. The suffixes "c" for cis and "t" for trans refer to geometric isomers. The prefixes "a" and "i" refer to anteiso- and iso-branched fatty acids. The prefix "10Me" indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule. The prefix "cy" denotes cyclopropane fatty acids. To estimate the various proportions of the main taxa in samples by PLFAs, biomarkers as i15:0, a15:0, i16:0, 16:1ω7c, 17:0, i17:0, cy17:0, 18:1ω9c, and cy19:0 were used to represent bacterial biomass (bacPLFA) (Frostegard et al., 1993b; Bardgett et al., 1996), and 18:2ω6 (fungPLFA) was taken to indicate fungal biomass (Federle et al., 1986). The $Gram^+$ specific fatty acids i15:0, a15:0, i16:0, and i17:0 and the $Gram^-$ specific fatty acids cy17:0, 18:1ω9c, and cy19:0 were taken as a measure of the ratio between $Gram^+$ and $Gram^-$ bacterial biomass ($Gram^+$:$Gram^-$). All results are given in nmol g^{-1}.

Plant cover, or the percentage of soil covered by the octagonal projection of the aerial part of each plant, was determined by the linear intercept method (Canfield, 1941).

2.5. Statistical analysis

Data were submitted to two-way ANOVA with treatment and sampling time as factors followed by Tukey significant difference as a post hoc test using the Statgraphics Plus 2.1. For the ANOVA, triplicate data were used for each treatment and every experimental season.

The concentrations of individual PLFAs were used as input values in Principal Components Analysis (PCA) to determine if the PLFAs signatures of microbial communities varied after application of BS. PCA was conducted using the same software package previously described. The factor loading scores for the individual PLFAs were used to assess the relative importance of each individual PLFA in the calculation of the principal component axes.

Table 3
Molecular weight (Daltons) distribution of biostimulants. Data are the means of four samples.

Molecular weight (Daltons)	WCDS (%)	CGHE (%)	PA-HE (%)	RB (%)
>10,000	12.2c† ± 1.3	6.1b ± 1.7	2.8a ± 0.5	3.3a ± 0.9
10,000–5000	5.7b† ± 0.4	4.8b ± 0.9	1.6a ± 0.3	2.8a ± 1.1
5000–1000	14.2b† ± 2.6	9.1a ± 1.1	8.3a ± 1.4	10.4a ± 1.9
1000–300	27.1a† ± 3.1	20.8a ± 2.3	20.4a ± 3.6	19.7a ± 2.6
<300	40.8a† ± 6.9	59.1b ± 7.6	66.9c ± 8.8	63.8c ± 7.1

† Files (mean ± standard errors) followed by the same letter(s) are not significantly different according to the Tukey test ($p < 0.05$).

3. Results

3.1. Characterization of soil biostimulants

The final enzymatic vegetable extracts are brown syrup, completely soluble in water. Table 2 shows the main chemical characteristics, proteins and carbohydrates are the main components, being the protein content higher than in the original raw material due to the use of proteases that solubilize and hydrolyze the original proteins, increasing the protein concentration in the vegetable extracts.

We chose different BS in order to know the influence of chemical composition on biochemical soil features. The chemical composition of different BS is related to the raw material used in the enzymatic process. Thus CGHE is a BS with a high content of fat; PA-HE, is a BS composed mainly of proteins (83 g kg^{-1}), with a nitrogen content almost twice that of the rest of BS.

The WCDS and CGHE are carbohydrate-rich BS, but the main difference between them is that the protein/carbohydrate ratio is higher in CGHE (2.5) than in WCDS (0.8).

Molecular weight distribution of the protein content indicated that more than 80% of all BS are formed by low molecular weight peptides (<1 kDa), being PA-HE the BS with higher percentage of peptides under 0.3 kDa (Table 3). Thus the enzymatic process of protein hydrolysis makes the amino acids and peptides available for an easy absorption by microorganisms.

3.2. Soil analysis

The statistical analysis indicates that the addition of the four amendments has the same effects on the tested enzymes (Fig. 1). The activity of the dehydrogenase enzyme was significantly stimulated during the experimental period in the biostimulant-amended soils. In this respect, and after 3 years of treatment, soil dehydrogenase activity significantly increased 95%, 95.9%, 97.4% and 97.6%, respectively, in soils amended with WCDS, CGHE, RB and PA-HE, respectively when compared to the control soil.

With respect to the hydrolase enzymes, urease activity was also strongly stimulated by the addition of biostimulants to the soil (Fig. 1). After 3 years of treatment, and compared to the control soil, this stimulation was higher in soil amended with PA-HE (97%), followed by RB (96.9%), CGHE (95.5%) and WCDS (94.8), respectively. At the end of the experiment and between organic treatments, statistical analysis only shows significant differences ($p < 0.05$) between PA-HE and RB treatments with WCDS treatment. Also, the evolution of phosphatase and arylsulfatase activities during the experimental period was similar to that of the urease activity.

Regarding the phospholipid fatty acid profile, Table 4 shows the bacterial $Gram^+$ and $Gram^-$ and fungal PLFA concentration in control and BS-amended soils after 3 years of treatment. There was a significant ($p < 0.05$) increase in the total amount of each fatty acid in BS-amended soil with respect to the control. Also, significant differences between soils amended with different BS were observed, finding the highest total amount of each fatty acid in PA-HE amended soil, followed by RB, CGHE and WCDS, respectively.

The $Gram^+$:$Gram^-$ ratio was significantly ($p < 0.05$) higher in all BS-amended soils than in the control, although there were no significant differences in the bacPLFA/fungPLFA ratio in the different treatments due to the increase in PLFA in both.

PCA was used to examine treatment-related impacts on the microbial community structure using PLFA (Fig. 2). The *x* and *y* axis accounted for 98.56% and 1.02% of the total variation, respectively, and PCA showed clear discrimination between soils under different fertilizer treatments. In this respect, there is a clear distinction between control and Bs-amended treatments. With respect to the organic treatments, there was also a clear differentiation between the various BS applied to soil. This differentiation is mainly based on the content of lower molecular weight peptides. Thus, PA-HE and RB were grouped and differentiated from CGHE and WCDS.

3.3. Plant cover

One year after the application of the BS to soil, the spontaneous vegetation growth increased in the treated soils with respect to the control. The most abundant species were *Borago officinalis* L., *Diplotaxis muralis* L., *Moricandia arvensis* L., *Paronychia argentea* Lam. and *Silene colorata* Poiret. Fig. 3 shows the evolution of plant cover percentage after the application of BS during the experimental period. After 3 years, the plant cover percentage increased with respect to the control soil in the following order: plot treated with PA-HE (87.3% plant cover) > plot treated with RB (85.7% plant cover) > plot treated CGHE (83.1% plant cover) > plot treated with WCDS (80.6% plant cover). At the end of the experiment and between the organic treatments, the statistical analysis shows only significant differences ($p < 0.05$) between PA-HE and RB treatments with WCDS treatment.

4. Discussion

Our results suggest that the chemical composition of biostimulants applied to the soil influenced the soil enzymatic activities stimulation differently. These results are in agreement with those obtained by Bastida et al. (2008) and Tejada et al. (2006a,b, 2008, 2009, 2010) when they applied different sources of organic matter such as manure, cotton compost, plant residues, sewage sludge, vermicomposts, etc., indicating that land application of organic materials with higher fulvic acid content in its chemical composition results in a higher stimulation of soil enzymatic activities.

However, and comparing our results with those obtained by the authors mentioned above, the stimulation of enzymatic activities in BS-amended soils was higher than when other sources of organic matter applied to soil. In this respect, the stimulation of enzymatic activities by applying BS to the soil is 3–15% higher than the stimulation obtained by Tejada et al. (2010) by applying 6 t C ha^{-1} of two vermicomposts obtained from cow dung and green forage. Also, the stimulation of enzymatic activities found by Bastida et al. (2008), when applied to the soil 12 kg ha^{-1} of sewage sludge and sewage sludge compost as bulking agent + straw during his first trial year was 35–40% lower than the stimulation obtained by applying the BS. This may be caused by the application of BS to soil with high contents of lower molecular weight proteins is of great environmental interest, as these proteins can be directly assimilated by soil microorganisms. This leads to a higher stimulation of the soil microbial community (Parrado et al., 2008). These authors also suggested that the fat content of these biostimulants also plays an essential role in nutrient and peptides absorption by soil microorganisms, indicating that a lower fat content favours nutrient and peptides

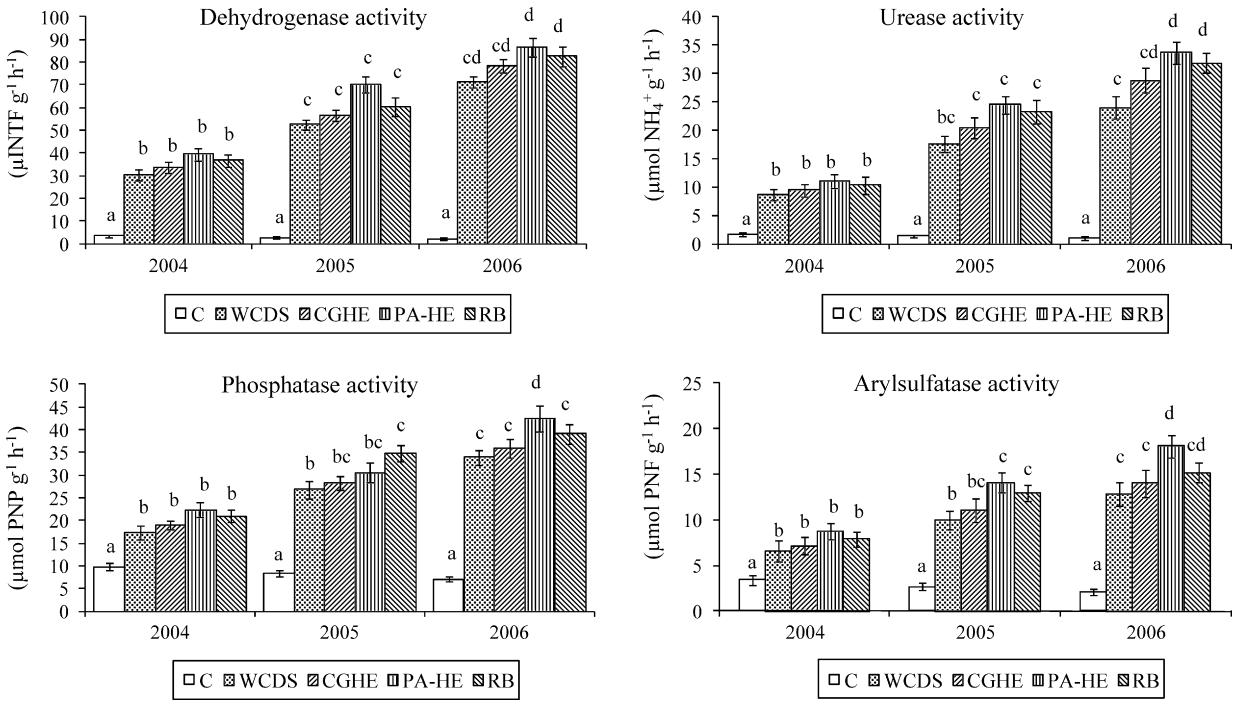

Fig. 1. Dehydrogenase, urease, phosphatase and arylsulfatase activities in soils to which biostimulants were applied Columns (mean ± SE) followed by the same letter(s) are not significantly different according to the Tukey test ($p < 0.05$) INTF: 2-*p*-iodo-3-nitrophenyl; PNP: *p*-nitrophenol; PNF: *p*-nitrophenyl.

absorption. Possibly for this reason soil enzymatic activities were higher in soil amended with PA-HE compared with other BS.

The organic amendments stimulated both bacterial and fungal proliferation, as observed by Bossio et al. (1998), Marschner et al. (2003) and Bastida et al. (2008). This was mainly due to the nutrient inputs for microbial development derived from the organic amendments. Also, the application of BS caused a change in the soil microbial community. These results are in agreement with those of Saison et al. (2006) who found changes in the microbial community structure of an organic amended soil, using multivariable analysis with PLFAs. Also, Marschner et al. (2003) showed that the bacterial community structure was altered by low, but regular, inputs of organic matter to soil. Using PCR-DGGE, Ros et al. (2006) demonstrated a change in the diversity of the bacterial soil community after compost amendment compared to control, yet no differences between different types of compost were found.

However, the PCA showed that the bacteria and fungi growth was very different depending on the BS applied to the soil. The PCA results indicated a higher microbial proliferation in soils amended with BS with higher percentage of peptides under 0.3 kDa (PA-HE and RB treatments), followed by CGHE and WCDS treatments. These results suggest that the chemical composition of organic matter strongly influences the soil microbial community's evolution. Furthermore, these results confirm that BS with the highest content of lower molecular weight protein can be more easily absorbed by soil microorganisms, thus promoting their growth.

In the present study, bacterial and fungal PLFA biomass both increased over time in the restoration sites. Some authors have observed an increase in bacterial biomass after the addition of organic matter to soil, whereas fungal biomass decreases – probably due to the existence of strong competition for the substrates easily assimilated in the soil (Bittman et al., 2005; Mille-Lindblom et al., 2006). Bacteria are thought to be more competitive regarding easily available carbon sources, such as simple carbohydrates, especially under nitrogen-rich conditions, but this type of substrates is also preferentially used by fast-growing opportunistic fungi, like

Table 4
Bacterial Gram$^+$ and Gram$^-$, fungal PLFA concentration, Gram$^+$:Gram$^-$ and bacteria:fungi ratios in control and BS-amended soils at the end of the experimental period.

PLFA (ng g^{-1})	Control	WCDS	CGHE	PA-HE	RB
Bacterial PLFA					
Gram$^+$					
i15:0	4.93a† ± 0.21	6.06b ± 0.33	11.32c ± 1.02	12.93d ± 0.96	12.25cd ± 0.79
a15:0	2.07a† ± 0.11	6.15b ± 0.24	7.85c ± 0.88	8.88c ± 0.37	8.36c ± 0.85
i16:0	2.31a† ± 0.17	7.02b ± 0.15	8.25c ± 0.93	9.93d ± 0.55	9.11cd ± 0.41
i17:0	2.96a† ± 0.21	6.49b ± 0.22	6.63b ± 0.29	7.19c ± 0.48	7.02b ± 0.39
Total	12.27a† ± 0.70	25.72b ± 0.94	34.05c ± 3.12	38.93d ± 2.36	36.74cd ± 2.44
Gram$^-$					
cy17:0	0.86a† ± 0.09	1.06b ± 0.08	1.29b ± 0.11	1.63b ± 0.09	1.56b ± 0.13
18:1ω9c	3.04a† ± 0.40	5.02b ± 0.32	5.31b ± 0.23	5.82b ± 0.61	5.76b ± 0.44
cy19:0	4.05a† ± 0.31	6.13b ± 0.29	6.38b ± 0.55	6.66b ± 0.42	6.56b ± 0.35
Total	7.95a† ± 0.80	12.21b ± 0.69	12.98b ± 0.89	14.11c ± 1.12	13.88bc ± 0.92
Gram$^+$:Gram$^-$ ratio	1.54a† ± 0.37	2.11b ± 0.41	2.62bc ± 0.43	2.76c ± 0.39	2.65c ± 0.40
Fungal PLFA					
18:2ω6	5.23a† ± 0.61	11.24b ± 1.12	12.38b ± 1.58	15.17c ± 1.32	14.66c ± 1.19
Bacteria:fungi ratio	3.87a† ± 0.44	3.37a ± 0.31	3.49a ± 0.35	3.50a ± 0.48	3.45a ± 0.52

† Files (mean ± standard errors) followed by the same letter(s) are not significantly different according to the Tukey test ($p < 0.05$).

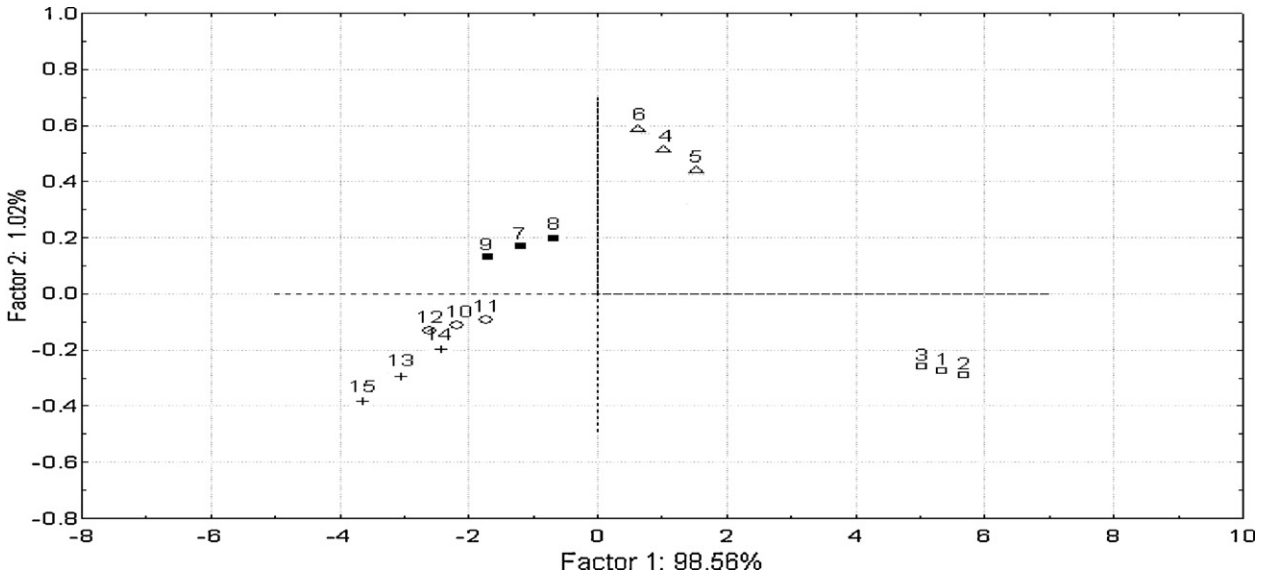

Fig. 2. Principle components analysis of PLFAs from control (1, 2 and 3 samples), WCDS treatment (4, 5 and 6 samples), CGHE treatment (7, 8 and 9 samples), RB treatment (10, 11 and 12 samples), and PA-HE treatment (13, 14 and 15 samples). Values on axes 1 and 2 represent the percent of total variance explained by the axes.

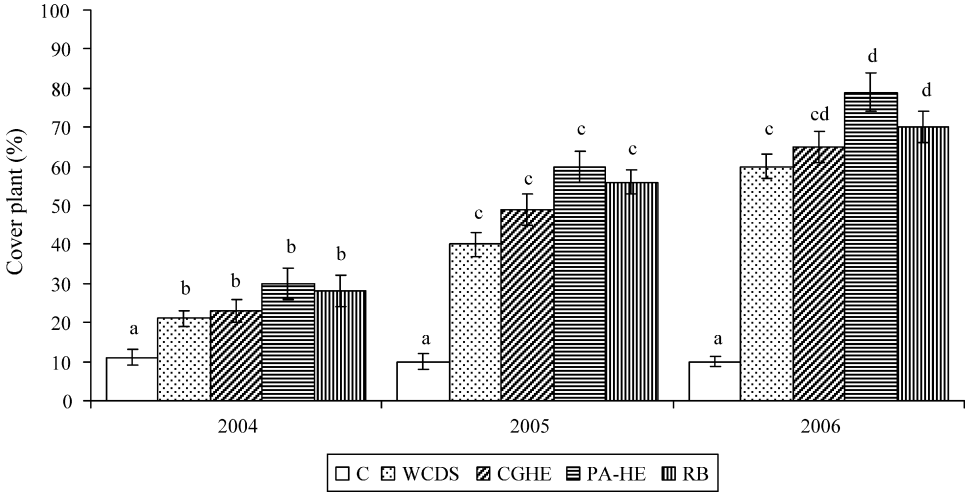

Fig. 3. Plant cover on soils to which biostimulants were applied Columns (mean ± SE) followed by the same letter(s) are not significantly different according to the Tukey test ($p < 0.05$).

so-called sugar fungi and yeast (Meidute et al., 2008). However, in our study the bacterial and fungal biomass increased after the addition of the BS to the soil. These results are in agreement with Meidute et al. (2008), who found an increase in bacterial and fungal biomass in soils amended with a available source of C. Possibly, the high content of easily available C substrates for microorganisms decreased competition between bacteria and fungi and therefore bacterial and fungal biomass was higher in the plots amended with PA-HE, followed by RB, CGHE and WCDS, respectively.

Similar to soil enzymatic activities and soil bacteria and fungi biomass, plant cover in the third experimental season was higher than those of the second and first experimental seasons, respectively, due to the residual effect of the organic matter of each BS applied to the soil.

Since soil enzymatic activities are responsible for important cycles such as C, N, P and S, plant cover increased significantly in the plots amended with BS. Again, the plant cover density was higher in the plots amended with PA-HE, followed by RB, CGHE and WCDS, respectively, probably due to the highest availability to plants of nutrients and peptides of lower molecular weight. These results are very important, principally in the arid zones where the risk of desertification and loss of soil is a great problem (Tejada et al., 2009).

5. Conclusions

The application of biostimulants had a positive effect on soil biological properties, and also favoured the establishment of vegetation which will protect the soil against erosion and contribute to its restoration. Therefore the addition of this type of organic waste may be considered a good strategy for recovering semiarid areas. Our results indicated that soil enzymatic activities and bacteria and fungal biomass were highest in soil amended with biostimulants with a higher percentage of peptides under 0.3 kDa (>60%), because the protein with lower molecular weight can be more easily absorbed by soil microorganisms, and thus promote their growth. Since plant cover is directly related to the soil's biological properties, the study of soil enzymatic activities and soil PLFA profiles might be a good indicator of the plant cover evolution.

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