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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},