Available online at www.sciencedirect.com
SCIENCE DIRECT®
Applied Soil Ecology 30 (2005) 3–10
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Use of microbiological indicators for evaluating success in soil restoration after revegetation of a mining area under subtropical conditions

I. Izquierdo [a], F. Caravaca [b,*], M.M. Alguacil [b], G. Hernández [a], A. Roldán [b]

[a] *Instituto de Ecología y Sistemática-CITMA, Carretera de Varona Km 3 1/2, Capdevila Rancho Boyeros, Ciudad de La Habana, Cuba*
[b] *CSIC-Centro de Edafología y Biología Aplicada del Segura, Department of Soil and Water Conservation, P.O. Box 164, Campus de Espinardo, 30100 Murcia, Spain*

Received 30 November 2003; received in revised form 15 July 2004; accepted 8 February 2005

Abstract

Physical (aggregate stability and bulk density) and microbiological (enzyme activities and labile carbon fractions) properties were studied in soils from a degraded mining site and two areas revegetated with *Casuarina equisetifolia* L. ex J.R. & G. Forst. or *Anacardium occidentale* L. under subtropical conditions. An adjacent soil under natural vegetation was used as a standard of local, high quality soil. Six years after planting, revegetation with *C. equisetifolia* or *A. occidentale* had resulted in higher soil total organic C, water soluble C and carbohydrates, total N content and aggregate stability compared with the bare soil. Soil bulk density decreased sharply after planting of both the tree species, approaching that of soil under native vegetation. Protease-BAA and β-glucosidase activities were higher in the soil revegetated with *C. equisetifolia* than in that revegetated with *A. occidentale*, while the remaining activities reached similar values in both the revegetated soils, these being higher than those of the bare soil. It may be concluded that revegetation with *C. equisetifolia* or *A. occidentale* rapidly improved soil physical and microbiological properties of a mining area under subtropical conditions. Soil enzyme activities and labile carbon fractions were very sensitive indicators of the improvement in soil quality resulting from the revegetation. Over the duration of this experiment, revegetated soils were still far from reaching the quality levels of the soil under natural vegetation.

Keywords: *Casuarina equisetifolia*; *Anacardium occidentale*; Enzyme activities; Aggregate stability; Bulk density

1. Introduction

Soil is an important natural resource that needs to be preserved and if possible, its quality and productive capacity must be improved. The ecological equilibrium of soil can be perturbed easily by human intervention, resulting in a consistent decrease in its

* Corresponding author. Tel.: +34 968 396337; fax: +34 968 396213.
E-mail address: fcb@cebas.csic.es (F. Caravaca).

0929-1393/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2005.02.004

natural quality. Mining activity, for example, may be considered a major threat to soil physical and biological quality (Gil-Sotres et al., 1992). Intensive mining activity promotes the destruction of the plant cover, deficiency of organic matter, great relief depression and the degradation of soil structure which, in turn, leads to an increase in the erosion risk.

Revegetation constitutes the most widely accepted and useful way to reduce erosion and protect soils against degradation (Morgan, 1986). Plantings of fast growing native and exotic trees, such as *Anacardium occidentale* L., in revegetation programmes for areas disturbed by mining activity have been encouraged by the agroforestry policy of the Cuban government. Mine restoration efforts have also focussed on nitrogen-fixing trees, such as *Casuarina equisetifolia* L. ex J.R. & G. Forst., because they facilitate the development of vegetation by raising the soil nutrient content through litter fall and nitrogen fixation (Parrotta, 1999). It is one of the most extensively introduced tree species outside its natural range due to its invasive nature and rapid growth rate, especially in the Caribbean (Pinyopusarerk and Williams, 2000).

Recently, there has been widespread agreement on the importance of measuring the soil biochemical and labile organic matter fractions related to microbial activity and soil structure, in order to evaluate soil quality (Nannipieri et al., 1990; Ghani et al., 2003). Microbial populations and activities are fundamental for maintaining soil quality by mediating the processes of organic matter turnover and nutrient cycling (Jeffries et al., 2003). Enzymes are potential indicators of the extent to which soil disturbance by a given activity may affect the immediate environment (Pascual et al., 2000). Soil enzyme activities also have been used early as sensitive indicators for reflecting the degree of quality reached by a soil in the rehabilitation process (Caravaca et al., 2003). Due to the complex interactions and dynamic nature of young mine soils, numerous researchers have emphasised the need for monitoring biological properties with time, in order to achieve a successful land reclamation. However, there are relatively few studies regarding the use of such properties as indicators of the improvement in mine soils quality (Trasar-Cepeda et al., 2003), and the information is even more scarce under tropical climates.

The objective of this study was to assess and compare the influence of the revegetation of a degraded mining site with *C. equisetifolia* or *A. occidentale* under subtropical conditions on soil properties considered to be indicators of soil quality such as physical (aggregate stability and bulk density) and microbiological (enzyme activities and labile carbon fractions) properties. The recovery of the quality of the revegetated soils was evaluated by comparison with an adjacent soil under native vegetation.

2. Materials and methods

This research was conducted at a serpentine-mined area located at Moa, in Holguín, Northeastern Cuba. In this area exist great nickel–chrome–cobalt deposits, which have been opencast exploited since 1963. The climate is subtropical with an average annual temperature of 25 °C and an average rainfall of 2000 mm. Undisturbed soils in the area are classified as Oxisol (Soil Survey Staff, 1999). The soils of this area are ultramafic, moderately acidic with low cation exchange capacity (<6 cmol kg^{-1}) and consequently low productivity. The topography of the area is mainly flat. The climax vegetation of this area, which has almost disappeared due to mining activity, is currently represented by *Pinus cubensis* Griseb. as the dominant species and some scattered trees like *Guapira rufescens, Byrsonima biflora, Ilex macfdayenii* and *Coccoloba shaferi.* After the area was mined, topsoil stored before mining and stockpiled until mining operations were completed, was spread directly onto the stone mine floor and then ripped with a bulldozer to a depth of 1.5 m.

2.1. Experimental design and layout

Experimental plantations were established in November 1997 in an area of 1500 m^2 following a full randomised design with five replication plots of 10 $m^2 \times 10$ m^2. Each planting occupied an area of 500 m^2, and the remaining area non-planted (500 m^2), it was used as control. *C. equisetifolia* or *A. occidentale* seedlings were planted in individual holes. Initial tree spacing within plantations was 1 $m^2 \times 1$ m^2. At least 25 seedlings per replication plot were planted.

2.2. Soil sampling

Four and 6 years after planting, five soil samples of each plant species were collected. Each sample consisted of five bulked subsamples (200 cm^3 soil cores), randomly collected at 0–20 cm depth in the rhizospheres of five individual plants. As a standard of local high quality soil, the soil under natural vegetation adjacent to the cultivated area was also collected. Field-moist soil samples were divided into two subsamples. One soil subsample was sieved at 2 mm and stored at 2 °C for biochemical analysis and another soil subsample was allowed to dry at room temperature for physical–chemical, physical and chemical analysis.

2.3. Soil physical–chemical, chemical and biochemical analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous extract. Total nitrogen was determined by the Kjeldahl method, and the total organic C according to Yeomans and Bremner (1988).

In soil aqueous extracts, water soluble carbon was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of the absorbance at 590 nm (Sims and Haby, 1971). Water soluble carbohydrate was determined by the method of Brink et al. (1960).

Dehydrogenase activity was determined according to García et al. (1997). For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 ml of 0.4% 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride (INT) in distilled water for 20 h at 22 °C in the dark. The iodo-nitrotetrazolium formazan (INTF) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtering through Whatman no. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-α-benzoyl-L-argininamide (BAA) hydrolyzing protease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used as the substrates, respectively. Two millilitres of buffer and 0.5 ml of substrate were added to 0.5 g of the sample, which was incubated at 30 °C (for urease) or 39 °C (for protease) for 90 min. Both the activities were determined as the NH_4^+ released in the hydrolysis reaction (Nannipieri et al., 1980).

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two millilitres of 0.5 M sodium acetate buffer at pH 5.5 using acetic acid (Naseby and Lynch, 1997) and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 2 °C for 15 min. Then, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added and the mixture was centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm (Tabatabai and Bremner, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as a substrate. This assay is based on the release and detection of PNP. Two millilitres of 0.1 M maleate buffer, pH 6.5, and 0.5 ml of substrate was added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethano (THAM) according to Tabatabai (1982). The amount of PNP was determined by spectrophotometry at 398 nm (Tabatabai and Bremner, 1969).

2.4. Physical analysis

The percentage of stable aggregates was determined by the method described by Lax et al. (1994). A 4 g aliquot of sieved (0.2–4 mm) soil was placed on a small 0.250 mm sieve and wetted by spray. After 15 min, the soil was subjected to an artificial rainfall of 150 ml with energy of 270 J m^{-2}. The remaining soil on the sieve was placed in a previously weighed capsule (T), dried at 105 °C and weighed (P1). Then, the soil was soaked in distilled water and after 2 h, passed through the same 0.250 mm sieve with the assistance of a small stick to break the remaining aggregates. The residue remaining on the sieve, which was made up of plant debris and sand particles, was dried at 105 °C and weighed (P2). The percentage of stable aggregates with regard to the total aggregates was calculated by (P1–P2) × 100/(4–P2+T).

2.5. Statistical analysis

The data were tested for normality and subjected to analysis of variance, and comparisons among means were made using the least significant difference (LSD) test, calculated at $p < 0.05$. Statistical procedures

were carried out with the software package SPSS for Windows.

3. Results and discussion

3.1. Plant cover

Six years after planting, the plant cover in the *C. equisetifolia* area was 45%, while in the *A. occidentale* area only 30%. Litter depths were significantly greater in plots with *C. equisetifolia* (3–4 cm) than in those with *A. occidentale* (1–2 cm). Increased litter and plant cover have been shown to reduce soil erosion and improve soil water retention (Cerdá, 1997). However, deep litter found underneath Casuarina could impede the establishment of colonists such as herbs and grasses from the surrounding areas (Cherrier, 1990).

3.2. Soil physical–chemical and chemical properties

The pH and electrical conductivity values of soil revegetated with *C. equisetifolia* or *A. occidentale* were significantly lower than those of the bare soil (Table 1). For both the plant species, soil pH did not vary in the growth period from 4 to 6 years, remaining above that of the native soil. However, electrical conductivity of the revegetated soil, for both the plant species, clearly decreased to reach the value of the soil under native vegetation.

The main input of organic matter to soil is plant litter, decaying aerial parts and roots and rhizomes which remain in the soil. Other sources are polysaccharides, excreted by the roots or resulting from the microbial and animal activities. Revegetation with *C. equisetifolia* or *A. occidentale* resulted in a higher soil total organic C content compared with that of the bare soil (Table 1), particularly in the soil revegetated with *C. equisetifolia* after 6 years (about 22.2-fold higher than the bare soil). The revegetated soils had higher total N contents than the bare soil, although there were no significant differences between plant species during all of the growth period (Table 1).

Recent soil organic matter studies have focussed on identifying the bio-reactive or labile fractions of soil organic matter pools, that is, the plant and microbial materials that are being transformed actively or mobilised through the biological processes that contribute to soil fertility and quality. Several authors have considered that these fractions, mainly water soluble C and carbohydrates, could indicate the soil's potential microbial activity, which is sensitive to land use and management (Ghani et al., 2003; Roldán et al., 2003). As for total organic C, revegetation with either plant species increased water soluble C and carbohydrates (Table 1), pointing to a greater degree of biological activity. In fact, we have observed positive correlations between the soluble C fractions and enzyme activities ($p < 0.05$). The highest increases from years 4 to 6 were recorded in the soil revegetated with *C. equisetifolia*. Six years after planting *C. equisetifolia* and *A. occidentale*, total organic matter and water soluble C fractions were still considerably less than in nearby soil under native vegetation.

Table 1
Physical–chemical properties, organic matter and carbon fractions of the soil under native vegetation, bare soil and of the soil revegetated with *Casuarina equisetifolia* and *Anacardium occidentale*, 4 and 6 years after planting ($n = 5$)

	pH (H_2O)	EC ($\mu S\ cm^{-1}$)	TOC ($g\ kg^{-1}$)	WSC ($\mu g\ g^{-1}$)	WSCH ($\mu g\ g^{-1}$)	Total N ($g\ kg^{-1}$)
Native vegetation	5.69 a[a]	194 ab	50.3 e	589 e	171 e	3.29 c
Bare soil	6.44 c	296 d	0.5 a	18 a	6 a	0.06 a
C. equisetifolia						
4 Years after planting	6.06 a	252 c	1.9 b	127 b	32 b	0.21 b
6 Years after planting	6.07 a	187 a	11.1 d	244 d	86 d	0.22 b
A. occidentale						
4 Years after planting	6.21 b	252 c	1.2 ab	152 c	44 c	0.20 b
6 Years after planting	6.22 b	205 b	6.9 c	246 d	52 c	0.18 b

EC: Electrical conductivity; TOC: total organic carbon; WSC: water soluble carbon; WSCH: water soluble carbohydrates.
[a] Values in columns sharing the same letter do not differ significantly ($p < 0.05$) as determined by the LSD test.

Table 2
Biochemical properties of the soil under native vegetation, bare soil and of the soil revegetated with *Casuarina equisetifolia* and *Anacardium occidentale*, 4 and 6 years after planting ($n = 5$)

	Dhase (μg INTF g^{-1} soil)	Urease (μg NH_3 g^{-1} h^{-1})	Protease (μg NH_3 g^{-1} h^{-1})	Phosphatase (μg PNP g^{-1} h^{-1})	β-glucosidase (μg PNP g^{-1} h^{-1})
Native vegetation	12.90 e[a]	5.44 d	88.70 e	248.8 d	130.6 e
Bare soil	0.49 a	0.07 a	0.14 a	29.2 a	0.6 a
C. equisetifolia					
4 Years after planting	3.62 c	0.76 b	0.51 b	225.2 c	2.2 c
6 Years after planting	4.26 d	1.36 c	1.27 d	318.3 e	9.7 d
A. occidentale					
4 Years after planting	2.22 b	0.60 b	0.60 b	192.4 b	1.1 b
6 Years after planting	4.60 d	1.19 c	0.85 c	314.1 e	2.8 c

Dhase: dehydrogenase.
[a] Values in columns sharing the same letter do not differ significantly ($p < 0.05$) as determined by the LSD test.

3.3. Soil biochemical properties

Soil microorganisms and soil microbial processes are very sensitive to changes in the quantity and quality of soil organic matter (Kandeler et al., 1999). In particular, a higher rhizodeposition of soluble C fractions is expected to stimulate microbial activity in the rhizosphere. A direct measurement of the microbial population is the dehydrogenase activity, which was significantly higher in the revegetated areas than in the bare soil (Table 2). Dehydrogenase is an oxidoreductase, which is only present in viable cells. This enzyme has been considered as a sensitive indicator of soil quality in degraded soils (García et al., 1997) and it has been proposed as a valid biomarker to indicate the changes in total microbial activity due to changes in soil management under different agronomic practices and climates (Ceccanti et al., 1994). Six years after planting, the reactivation of microbial populations did not depend on the tree species selected for revegetation.

Measurement of soil hydrolases provides an early indication of changes in soil fertility since they are related to the mineralisation of such important nutrient elements as N, P and C (Ceccanti et al., 1994). Four years after planting, urease, protease-BAA, acid phosphatase and β-glucosidase activities were significantly higher in the revegetated soils than in the bare soil (Table 2). With the exception of protease-BAA activity, the highest increase in enzyme activities was provoked by revegetation with *C. equisetifolia*. These differences with respect to the bare soil had generally increased 6 years after planting both the plant species. All the activities are correlated significantly with each other ($p < 0.001$ in most cases). Thus, there is clearly a strong interrelationship among enzymatic processes involved in the carbon, nitrogen and phosphorus cycles. At the end of the growth period, protease-BAA and β-glucosidase activities were higher in the soil revegetated with *C. equisetifolia* than in that revegetated with *A. occidentale*, while the remaining activities reached similar values in both the revegetated soils. The fact that the protease-BAA and β-glucosidase activities, related with the N and C cycles, respectively, were higher in the *C. equisetifolia* area could indicate a higher accumulation of organic C and N in this area. Most enzyme activities were still significantly lower in the revegetated soils than in the native soil. In contrast, it is worth noting that both the revegetated soils had phosphatase activity values even higher than those of the soil under native vegetation. Increased acid phosphate activity in the rhizosphere of *C. equisetifolia* and *A. occidentale* trees may be due to the direct fungal secretion or an induced secretion by the plant roots, as pointed out by Haussling and Marschner (1989).

3.4. Soil physical properties

The revegetation of degraded ecosystems must be carried out with plants selected on the basis of their

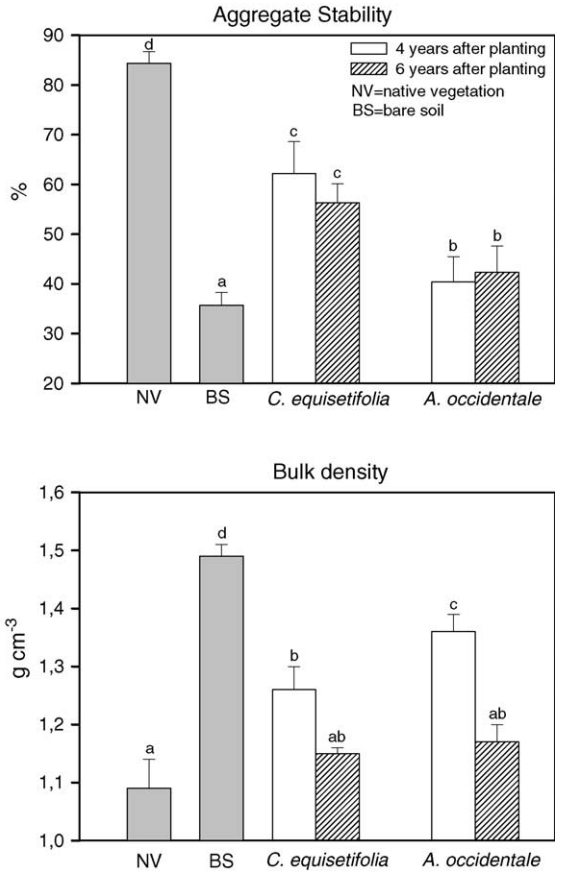

Fig. 1. Aggregate stability and bulk density of the soil under native vegetation, bare soil and of the soil revegetated with *C. equisetifolia* and *A. occidentale*, 4 and 6 years after planting ($n = 5$).

ability to survive and regenerate in the local environment, and on their ability to stabilise the soil structure (Caravaca et al., 2002). Revegetation with *C. equisetifolia* and *A. occidentale* increased soil aggregate stability by about 66 and 16%, respectively, relative to the bare soil, although this difference did not vary with time for either species (Fig. 1). The mechanisms involved in aggregate stabilisation are based on the enmeshment of soil particles by hyphae and roots, and the exudation of polysaccharides (Roldán et al., 1994; Bearden and Petersen, 2000). The water soluble C fraction is also regarded as one of the key labile components of organic matter responsible for soil aggregation (Puget et al., 1999). The increased levels of stable aggregates resulting from revegetation experiments can be attributed also to the greater stimulation of the rhizosphere microbial population and, particularly, to the proliferation of fungal hyphae (Roldán et al., 1994; Jeffries and Barea, 2000). According to Roldán et al. (1994), the binding effect of roots and hyphae is long-lived, while that of polysaccharides is transient because they are decomposed rapidly by microbes. In our study, the improvements in soil aggregate stability depended on the tree species selected, *C. equisetifolia* being more effective. *C. equisetifolia* develops soils with high aggregate stability, probably due to its high root density and productivity (Parrotta, 1999). The symbiosis between arbuscular mycorrhizal fungi and plants has been shown to increase the stability of soil macroaggregates (>250 μm) (Bearden and Petersen, 2000). *C. equisetifolia* and *A. occidentale* form symbioses with arbuscular mycorrhizal fungi (Osundina, 1998), which also suggests the involvement of such fungi in soil aggregate stabilisation. On the other hand, the fact that the highest microbial activity was in the revegetated areas might be due to the high levels of stable aggregates which protect the organic fraction (on which enzymes are immobilised) from microbial degradation (Nannipieri, 1994).

The incorporation of organic material from vegetation reduces the soil bulk density and hence increases total porosity which, in turn, has a positive effect on plant growth. Soil bulk density decreased sharply from 1.49 to 1.26 and 1.36 g cm^{-3}, 4 years after planting of *C. equisetifolia* and *A. occidentale*, respectively (Fig. 1). It has been suggested that improvement in the physical properties of the soil, particularly bulk density, may affect its biological and biochemical activities, including enzymatic activities (Giusquiani et al., 1995). Thus, the soil bulk density was correlated negatively with the water soluble carbon and carbohydrate contents and with all the enzyme activities. At the end of the growth period, the bulk density of soil revegetated with either plant species approached that of soil under native vegetation. However, the revegetated soils did not reach the percentages of stable aggregates of the soil under native vegetation.

It may be concluded that the revegetation with *C. equisetifolia* or *A. occidentale* rapidly improved the soil physical and biochemical properties of a mining area under subtropical conditions. In particular, the revegetation with *C. equisetifolia* can be more

effective for improving soil structural stability. Soil enzyme activities and labile carbon fractions were very sensitive indicators of the improvement in soil quality resulting from the revegetation. Over the duration of this experiment, revegetated soils were still far from reaching the quality levels of the soil under natural vegetation.

References

Bearden, B.N., Petersen, L., 2000. Influence of arbuscular mycorrhizal fungi on soil structure and aggregate stability of vertisols. Plant Soil 218, 173–183.

Brink, R.H., Dubach, P., Lynch, D.L., 1960. Measurements of carbohydrates in soil hydrolyzates with anthrone. Soil Sci. 89, 157–166.

Caravaca, F., Alguacil, M.M., Figueroa, D., Barea, J.M., Roldán, A., 2003. Re-establishment of *Retama sphaerocarpa* as a target species for reclamation of soil physical and biological properties in a semiarid Mediterranean land. Forest Ecol. Manag. 182, 49–58.

Caravaca, F., Hernández, M.T., García, C., Roldán, A., 2002. Improvement of rhizosphere aggregates stability of afforested semi-arid plant species subjected to mycorrhizal inoculation and compost addition. Geoderma 108, 133–144.

Ceccanti, B., Pezzarossa, B., Gallardo-Lancho, F.J., Masciandaro, G., 1994. Bio-tests as markers of soil utilization and fertility. Geomicrobiol. J. 11, 309–316.

Cerdá, A., 1997. The effect of patchy distribution of *Stipa tenacissima* L. on runoff and erosion. J. Arid Environ. 36, 37–51.

Cherrier, J-F., 1990. Reverdissement des terrains miniers en Nouvelle Calédonie. Bois et Forêts des Tropiques 225, 5–23.

García, C., Hernández, M.T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun. Soil Sci. Plant Anal. 28, 123–134.

Ghani, A., Dexter, M., Perrott, K.W., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biol. Biochem. 35, 1231–1243.

Gil-Sotres, F., Trasar-Cepeda, M.C., Ciardi, C., Ceccanti, B., Leirós, M.C., 1992. Biochemical characterization of biological activity in very young mine soils. Biol. Fertil. Soils 13, 25–30.

Giusquiani, P.L., Pagliai, M., Gigliotti, G., Businelli, D., Benetti, A., 1995. Urban waste compost: effects on physical, chemical, and biochemical soil properties. J. Environ. Qual. 24, 175–182.

Haussling, M., Marschner, H., 1989. Organic and inorganic soil phosphates and acid phosphatase activity in the rhizosphere of 80-year-old Norway spruce [*Picea abies* (L.) Karst.] trees. Biol. Fertil. Soils 8, 128–133.

Jeffries, P., Barea, J.M., 2000. Arbuscular mycorrhiza–a key component of sustainable plant-soil ecosystems. In: Hock, B. (Ed.), The Mycota IX. Fungal Associations Springer, Berlin, pp. 95–113.

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K., Barea, J.M., 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol. Fertil. Soils 37, 1–16.

Kandeler, E., Tscherko, D., Spiegel, H., 1999. Long-term monitoring of microbial biomass. Biol. Fertil. Soils 28, 343–351.

Lax, A., Díaz, E., Castillo, V., Albaladejo, J., 1994. Reclamation of physical and chemical properties of a salinized soil by organic amendment. Arid Soil Res. Rehab. 8, 9–17.

Morgan, R.P.C., 1986. Soil Erosion and Conservation. Longman Scientific & Technical, London.

Nannipieri, P., 1994. The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., Grace, P.R. (Eds.), Soil Biota: Management in Sustainable Farming Systems. CSIRO, Australia, pp. 238–244.

Nannipieri, P., Ceccanti, B., Cervelli, S., Matarese, E., 1980. Extraction of phosphatase, urease, protease, organic carbon and nitrogen from soil. Soil Sci. Soc. Am. J. 44, 111–116.

Nannipieri, P., Grego, S., Ceccanti, B., 1990. Ecological significance of the biological activity in soils. In: Bollag, J.M., Stotzky, G. (Eds.), Soil Biochemistry, vol. 6. Marcel Dekker, New York, pp. 293–355.

Naseby, D.C., Lynch, J.M., 1997. Rhizosphere soil enzymes as indicators of perturbation caused by a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. Soil Biol. Biochem. 29, 1353–1362.

Osundina, M.A., 1998. Nodulation and growth of mycorrhizal *Casuarina equisetifolia* J.R. and G. First in response to flooding. Biol. Fertil. Soils 26, 95–99.

Parrotta, J.A., 1999. Productivity, nutrient cycling, and succession in single- and mixed-species plantations of *Casuarina equisetifolia*, *Eucalyptus robusta*, and *Leucaena leucocephala* in Puerto Rico. Forest Ecol. Manag. 124, 45–77.

Pascual, J.A., García, C., Hernández, T., Moreno, J.L., Ros, M., 2000. Soil microbial activity as a biomarker of degradation and remediation processes. Soil Biol. Biochem. 32, 1877–1883.

Pinyopusarerk, K., Williams, E.R., 2000. Range-wide provenance variation in growth and morphological characteristics of *Casuarina equisetifolia* grown in Northern Australia. Forest Ecol. Manag. 134, 219–232.

Puget, P., Angers, D.A., Chenu, C., 1999. Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. Soil Biol. Biochem. 31, 55–63.

Roldán, A., Caravaca, F., Hernández, M.T., García, C., Sánchez-Brito, C., Velásquez, M., Tiscareño, M., 2003. Effect of reduced tillage, rates of straw and legume cropping on selected soil quality characteristics under maize in Patzcuaro watershed (MEXICO). Soil Till. Res. 72, 65–73.

Roldán, A., García-Orenes, F., Lax, A., 1994. An incubation experiment to determine factors involving aggregation changes in an arid soil receiving urban refuse. Soil Biol. Biochem. 26, 1699–1707.

Sims, J.R., Haby, V.A., 1971. Simplified colorimetric determination of soil organic matter. Soil Sci. 112, 137–141.

SSS Soil Survey Staff, 1999. Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys. USDA

Natural Resources Conservation Service. Agric. Hdbk. 436. US Government Printing Office, Washington, DC.

Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, E.M., Keeney, D.R., (Eds.), Methods of Soil Analysis Part 2, second ed. Agron. Monogr., vol. 9. ASA and SSSA, Madison, pp. 501–538.

Tabatabai, M.A., Bremner, J.M., 1969. Use of *p*-nitrophenol phosphate in assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307.

Trasar-Cepeda, C., Leirós de la Peña, M.C., García-Fernández, F., Gil-Sotres, F., 2003. Soil biochemical properties as indicators of soil quality. In: Lobo, M.C., Ibáñez, J.J. (Eds.), Preserving Soil Quality and Soil Biodiversity. IMIA-Consejería de Economía e Innovación Tecnológica, Madrid, pp. 119–140.

Yeomans, J.C., Bremner, J.M., 1988. A rapid and precise method for routine determination of organic carbon in soil. Commun. Soil Sci. Plant Anal. 19, 1467–1476.