Micro-Scale analytical approach in the study of a soil ecosystem affected by different levels of erosion

C. Macci[(1)], S. Doni[(1)], G. Masciandaro[(1)], Hernandez[(2)], C. Garcia[(2)], B. Ceccanti[(1)]

(1) Institute for the Ecosystem Study -National Research Council (CNR). Via Moruzzi, 1. 56124 Pisa (Italy). E-mail: cristina.macci@ise.cnr.it
(2)CEBAS-CSIC, Campus Universitario de Espinardo, Murcia (Spain). E-mail: cgarizq@cebas.csic.es

ABSTRACT

In this paper the evolution of the humic-enzyme complexes, characterized by analytical isoelectric focussing technique, have been investigated for characterizing three different soil degradation forms caused by the plant cover loss: I) a natural soil with a wide shrub-grass cover (Forest); II) a partially degraded soil with a scanty grass cover (Shrub); and III) a bare soil originated by landslides (Bare).
Chemical (total organic carbon, total nitrogen, C/N ratio and water soluble carbon) and biochemical (dehydrogenase, biomass carbon, basal respiration and β-glucosidase) parameters only discriminate between the most degraded bare soil from the natural and partially degraded soils. A clear discrimination between the three soil ecosystems was given by the microbial cell constituent (ATP), humic carbon, extracellular β-glucosidase activity and humic carbon-bound β-glucosidase, which distributed the soils in a decrease order of degradation: natural (Forest), partially degraded (Shrub) and Bare. The results confirmed the previous findings that the biochemical properties of humic substances determined at microscale level, help in achieving a completeness of information capable of discriminating even little differences in soil ecosystem quality and functioning.
The study was carried out in the framework of the European project “Indicators and threshold for desertification, soil quality and remediation” (INDEX- STREP n. 505450, 2004-06).

Keywords: Soil degradation, biochemical parameters, Iseoelectric focussing, humic carbon, humic carbon β-glucosidase activity.

INTRODUCTION

Soil degradation is one of the most important environmental problem worldwide recognized. The most evident effect of soil degradation is a progressive loss of stable plant cover, usually caused by biological and physical alterations in consequence of improper soil management or climate adversities. The importance of vegetal cover in the soil physical protection from degradation and sediment transport has been assessed (Morgan, 2005). In fact, the presence of vegetation reduces water-caused erosion by intercepting rainfall, increasing water infiltration, intercepting runoff at surface level, and mechanically stabilizing soil by means of the roots (Bochet et al., 2000). Moreover, the vegetation contributes also to significant enhancement of organic matter in soil thus increasing soil water holding capacity and soil biological fertility (Garcia et al., 1994).
A degradation process generally begins with the microbiological and enzymatic alterations which may further be accelerate in semiarid conditions. Some extracellular enzymes, as β-glucosidase, a key enzyme in the turnover of organic carbon in soil, could be stabilized and retain their activity through interactions with humic substances (humic-enzyme complexes), which protect them against proteolysis and other denaturing agents (Lähdesmäki and

Piispanen, 1992).
The active proportion of stabilized soil enzymes represents a reservoir of potential enzyme activity that may be important under unfavourable conditions for soil microorganisms.
In this paper the evolution of the humic-β-glucosidase complexes have been investigated for characterizing three different soil degradation forms caused by the plant cover loss.

MATERIALS AND METHODS

Sampling sites

The sites selected for the study are located in Basilicata region in the South of Italy. The climate in this area is predominantly Mediterranean with dry hot summer and cold winter, an average annual temperature of 16.6 °C and an average rainfall of 46.2 mm. In this area, three different soil degradation forms caused by the plant cover loss has been sampled: I) a natural soil with a wide shrub-grass cover (forest); II) a partially degraded soil with a scanty grass cover (shrub); and III) a bare soil originated by landslides (Bare). Three samples were taken from each sites: each sample consisted of eight subsamples taken from the top 15 cm of soil.
The subsamples were mixed, homogenised, sieved (2 mm) and stored at room temperature until laboratory analysis.

Methods

Total C and N contents were determined by dry combustion with a RC-412 multiphase carbon and a FP-528 protein/nitrogen determinator respectively (LECO corporation). Water-soluble carbon (WSC) and pyrophosphate (0.1M, pH 7.1) extractable carbon >10.000 Da (PEC) were extracted using the methods reported by Garcia et al. (1990) and Ceccanti et al. (2008), respectively. The C content of PEC and WSC were determined by dichromate oxidation (Yeomans and Bremner, 1988). ATP was extracted from soil using the Webster et al. (1984) procedure and measured as recommended in Ciardi and Nannipieri (1990).
Microbial biomass *C* was determined by the fumigation–extraction method (Vance et al., 1987) using the Shimadzu TOC5050A. Soil respiration was measured following Bastida et al. (2006) method. Dehydrogenase activity was determined by the method of Masciandaro et al. (2000). The methods used to assay β-glucosidase is described by Garcia et al. (1993).
The PEC characterization by isoelectric focussing (IEF) technique and the β-glucosidase activity on humic bands after IEF were performed following Ceccanti et al. (2008) method.

RESULTS AND DISCUSSION

Total organic carbon, total nitrogen and water soluble carbon (WSC), as aspected, resulted higher in Forest site but they were not significantly different from that measured in Shrub site (table 1). The scanty grass cover of Shrub site seems to be sufficient to preserve soil organic matter content. In addition, the higher content of WSC observed in these sites with respect to Bare site, mainly due to the inputs from the root exudates and the amount of fresh residues released by plant into soil (Garcia et al., 1997), was probably the reason of the higher total β-glucosidase activity, an enzyme related to the carbon cycle (table1).
Also the dehydrogenase activity, microbial biomass *C*, basal respiration and ATP, resulted higher in plant cover sites (table 1), indicating the better condition for soil microrganisms to growth and carried out their activity. However, these biochemical parameters, with the exception of ATP, were able to only discriminate between the most degraded Bare soil from the natural (Forest) and partially degraded (Shrub) soils. Sodium pyrophosphate extract, that was generally considered a good selective extractant of humic compounds (PEC) and humus-associated enzymes (Ceccanti et al., 2008), permitted a better discrimination of the different soil ecosystems. In fact, the PEC content and the extracellular β-glucosidase activity (EG) clearly discriminated between Forest, Shrub and Bare soils (table 2). The higher PEC

and EG found in Forest soil indicated a better humic carbon and biochemical energy preservation.

Table 1. Chemical and biochemical parameters of soil samples

Site	TOC	TN	C/N	WSC	TG	DH-ase	BC	BR	ATP
Forest	31,4a	2,72a	11,5a	97,1a	761a	3,77a	766a	15,0a	1299a
Shrub	29,9a	2,57a	11,6a	79,2a	866a	4,42a	682a	14,6a	540b
Bare	5,2b	0,61b	8,52b	20,4b	155b	1,28b	125b	0,0b	67c

Different letters indicate statistically different values (*P<0.05*). TOC, Total Organic Carbon (mgC g^{-1}); TN, Total Nitrogen (mgC g^{-1}); WSC, Water Soluble Carbon (µgC g^{-1}); TG, Total β-glucosidase activity (µgPNP g^{-1} h^{-1}); DH-ase, Dehydrogenase (µgINTF g^{-1} h^{-1}); BC, Biomass Carbon (mgC kg^{-1}); BR, Basal Respiration (mgC-CO_2 kg^{-1} d^{-1}); ATP (ng g^{-1}).

The information obtained from IEF profiles defined the level of stability of humic substances isolated from each soil ecosystem on the basis of its isoelectric focussing pattern and they could be useful to the better understanding of the soil resilience and pressure-response mechanism to a given stress. The more intense IEF peaks were focussed in the pH range 4.5-4.2 (AHC) and they resulted higher in Forest soil (figure 1, table 2).

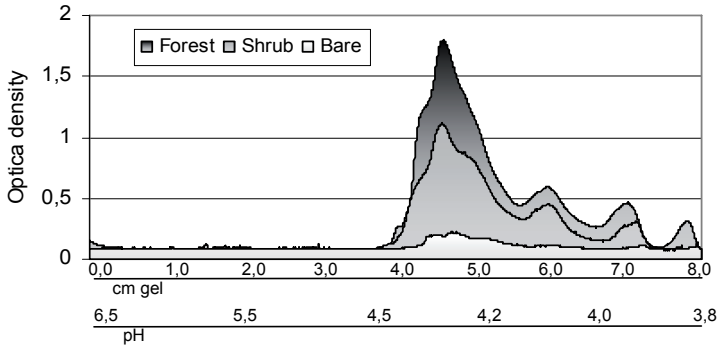

Figure1. IEF profiles of humic matter extracted from Forest, Shrub and Bare soils.

Table 2. β-Glucosidase activity in soil pyrophosphate extract (fraction>10^4Da) and enzymatically active humic carbon in the pH range 4.5-4.2 after isoelectric focussing.

Site	PEC>10^4	EG	AHC	HEG
Forest	2,86a	23,8a	1329a	2,53a
Shrub	1,52b	16,7b	634b	1,96b
Bare	0,44c	3,2c	292c	1,06c

Different letters indicate statistically different values (*P<0.05*). PEC>10^4, Pyrophosphate Extractable Carbon fraction >10^4Da (mgC g^{-1}); EG, Extracellular β-glucosidase activity (mgPNP kg^{-1} h^{-1}); AHC, active humic carbon calculated from the IEF peak areas focused in the pH range 4.5-4.2 (mgC kg^{-1}); HEG, humic-bound β-glucosidase activity pH 4.5-4.2 (mgPNP kg^{-1} h^{-1}).

Furthermore the β-glucosidase activity determined on humic band after IEF in the pH range 4.5-4.2 (HEG) resulted higher in the site with the greater plant cover (table 2), suggesting a higher metabolic efficiency in the Forest site. Finally, therefore the biochemical properties of humic substances resulted able to distributed the soils in a decrease order of degradation: natural (Forest), partially degraded (Shrub) and bare soils (Bare).

CONCLUSIONS

Chemical and biochemical parameters only discriminate between the most degraded bare soil from the natural and partially degraded soils. A clear discrimination between the three soil ecosystems was given by the microbial cell constituent (ATP), humic carbon, extracellular β-glucosidase activity and humic carbon-bound β-glucosidase enzyme, which distributed the soils in a decrease order of degradation: natural (forest), partially degraded (shrub) and bare. The results confirmed the previous findings that the biochemical properties of humic substances, determined at microscale level, usually help in improving the information capable to discriminate even little differences in soil ecosystem quality and functioning.

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