Use of molecular separative techniques (UF and IEF) to study humic-enzyme complexes in different agricultural soil managements

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ABSTRACT

In this paper, membrane ultrafiltration (UF) and isoelectric focusing (IEF) techniques were used to fractionate and purify a humic-bound β-glucosidase complex in two soil types, both under organic (biological) and mineral (conventional) management. This complex could be more effective and consistent indicator of management induced changes to soil quality than conventional parameters. The study showed that the β-glucosidase-humic complex focussed in the pH range 4.5-4.2 and resulted higher in the organic than in mineral fertilization systems. This result clearly indicated a better preservation of stable humic-carbon and biochemical energy in soils treated with the organic fertiliser. In conclusion, UF and IEF techniques may be successfully adopted for the study, at molecular scale, of the impact of different management practices (organic and mineral management) on soil quality. 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).

Keyswords: Isoelectric focussing, humic carbon, β-glucosidase-humic complex, soil management.

INTRODUCTION

The long-term effects of continuous and indiscriminate use of inorganic fertilisers have seriously damaged soil structure, caused biological degradation, loss of soil organic matter (SOM) and environmental pollution (Roldan et al., 2003). This has encouraged the development and promotion of ecological management systems such as organic fertilization, crop rotation, mulching and shallow ploughing. These biological management can enhance soil moisture retention, improve soil structure, and increase SOM and nutrient levels (Lal et al., 1997). Better understanding of the effects of land uses on soil properties is of importance for developing rational soil management. In this context, it is essential to identify key soil parameters which can be used as indicators of soil quality and fertility. For instance, differences between conventional and biological fertilisers have generally been assessed through the study of organic matter evolution and biochemical properties of soil (Bending et al., 2004). In particular, the activity of extracellular β-glucosidase enzymes, which are involved in the dynamics of soil carbon cycling and energy transfer, can be modified by management practices (Martens et al., 1992). These enzymes can be associated with humic substances resulting protected against thermal denaturation, proteolysis, dehydration or decomposition. The humic-enzyme complexes, being part of a persistent enzyme pool independent of the existing microorganisms, do not react readily to environmental changes, like the soil microbial biomass or other biological parameters related to microbial activity, and therefore represent a more stable indicator of biological processes occurring in soils (Miller and Dick, 1995).

Biomolecular techniques to deepen investigation on the evolution, structure and functionality of stable humic fractions and humic-β-glucosidase complexes are available and based on the extraction, separation and characterization of soil organic matter (Ceccanti et al., 2008).
The aim of this paper is to use the combination of ultrafiltration (UF) and isoelectric focussing (IEF) techniques as methodology to underline, trough the quantification of humic bound β-glucosidase activity and humic substances preservation and/or incorporation, the impact of organic and mineral management practices on soil quality.

Materials and methods

Sites description

Soil samples were collected from two different climatic area: 1) Tuscany (Centre of Italy) in a mild Mediterranean climate and 2) Basilicata (South of Italy) in a dry-semiarid climate. Two types of soil treatment for each site have been chosen: biological agriculture and conventional agriculture. In the biological agriculture soil (biological procedure for five years), green manure (1,5 t ha^{-1} y^{-1}) was applied to the fields, while in the conventional agriculture soil, the fields received mineral fertilizer (0,2 t ha^{-1} y^{-1}): ammonium nitrate for Tuscany site and ammonium phosphate and nitrate (mineral complex with 25 %N and 15 %P) for Basilicata site and regular pesticide application. All soils were cropped with *Triticum durum* and the soil samples were collected from the top soil (0-15 cm) at the beginning of the second year of cultivation. Each experimental plot was divided longitudinally into tree areas (tree subplots per treatment). Tree soil samples for each treatment (one per subplot) were collected. Each sample consisted of nine bulked sub-samples (150 cm^3 cores) randomly collected.

Methods

Sodium pyrophosphate (0.1M, pH 7.1) was used to extract humic-enzyme compounds (Masciandaro and Ceccanti, 1999); the extract was filtered using a 0.22 μm membrane and passed through an ultrafiltration AMICON PM10 cut-off membrane to obtain the fraction>10^4Da. The C content of pyrophosphate extract fraction>10^4DA (PEC>10^4Da) was determined by dichromate oxidation (Yeomans and Bremner, 1988). Total organic C and total N contents were determined by dry combustion with a RC-412 multiphase carbon and a FP-528 protein/nitrogen determinator, respectively (LECO corporation). The method used to test extracellular β-glucosidase activity is described by Garcia et al. (1993). The pyrophosphate extracted fraction>10^4Da was further characterised by isoelectric focussing (IEF) technique (Ceccanti et al., 2008). The β-glucosidase activity after IEF was assayed on a slice of gel containing the humic band. Each gel slice was pre-washed and conditioned for 1 h with 2 ml 0.1M phosphate buffer, pH 6.4, (Ceccanti et al., 1989) before incubation for 17h at 37°C with 4-nitro-phenyl-β-D-glucanopyranoside substrate (PNG).

RESULTS AND DISCUSSION

Soil management practices can influence the quality and quantity of soil organic matter (Roldan et al., 2003; Lal et al., 1997). Both Centre and South of Italy sites under organic fertilization showed higher values of total organic carbon (TOC) and humic carbon (PEC fraction>10^4 Da) with respect to the mineral treated sites (table 1). The management effects on soil organic matter could also influence soil biological activity. The higher extracellular β-glucosidase activity (EG) in organically amended soils could be due to the increase of humus amount, which protect the extracellular enzymes, and to the addition of organic C substrates, which could induce the synthesis and release of such enzyme by microorganisms. β-Glucosidase activity represents, in fact, the soil potential to hydrolyze organic carbon compounds and produce glucose, which is an important energy source for soil microbial populations. The specific enzyme activity (SEG), calculated as the ratio between EG and PEC>10^4Da (table 1), resulted unable to discriminate the effect of organic and mineral management on soil quality, but confirmed the relationship between carbon content and biochemical soil properties.

Table 1. Chemical and biochemical parameters of the Centre and South of Italy agricultural sites both under organic (OM) and mineral (MM) management. For each treatment different letters indicate statistically different values (ANOVA-LSD, p<0.05).

Site	TOC	PEC>10^4	TN	EG	SEG
Centre of Italy-OM	1,82±0,05a	949±131a	0,164±0,008a	8,08±0,94a	7,46±1,03a
Centre of Italy-MM	1,31±0,18b	691±52b	0,081±0,018b	6,00±0,91b	8,69±0,40a
South of Italy-OM	1,63±0,10a	982±68a	0,066±0,018a	6,41±0,13a	6,53±0,38a
South of Italy-MM	1,32±0,03b	831±49b	0,069±0,005a	5,00±0,50b	6,02±0,37a

TOC, Total Organic Carbon in %, PEC>10^4, Pyrophosphate Extractable Carbon fraction>10^4Da in mgC kg^{-1}; TN, Total Nitrogen in %; EG, Extracellular β-glucosidase activity in mgPNP kg^{-1} h^{-1}; SEG: EG/PEC>10^4Da in mgPNP gC^{-1} h^{-1}.

The information obtained from IEF profiles defined the level of stability of humic substances isolated from each soil agro-ecosystem on the basis of its isoelectric focussing pattern (Ceccanti et al., 2008) (figure 1).

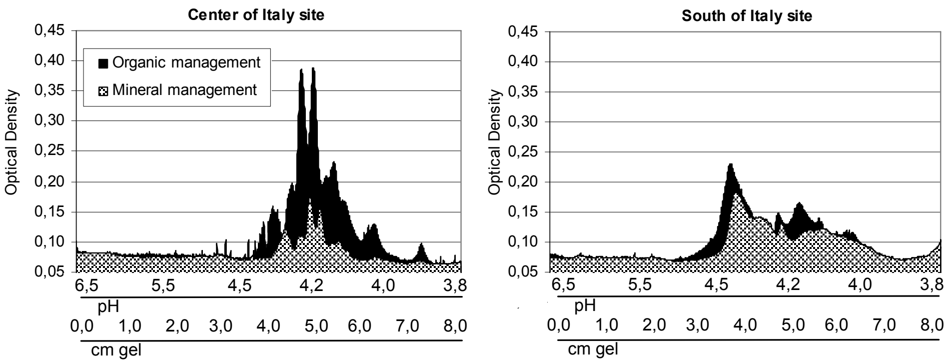

Figure 1. IEF profiles of humic matter extracted from Centre and South of Italy agricultural sites both under organic and mineral farming systems.

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. For each treatment different letters indicate statistically different values (ANOVA-LSD, p<0.05).

site	AHC	HEG	SHEG
Centre of Italy-OM	339±25a	10,5±0,97a	30,9±1,6a
Centre of Italy-MM	299±2b	8,14±0,11b	27,2±1,4b
South of Italy-OM	312±2a	7,01±0,12a	22,5±0,4a
South of Italy-MM	252±10b	1,54±0,05b	6,12±0,1b

OM, Organic Management; MM, Mineral Management. AHC, active humic carbon calculated from the IEF peak areas focused in the pH range 4.5-4.2 in mgC kg^{-1}; HEG, humic-bound β-glucosidase activity pH 4.5-4.2 in mgPNP kg^{-1} h^{-1}; SHEG: HEG/AHC, in mgPNP gC^{-1} h^{-1}.

After IEF, the peaks in the pH range 4.5-4.2, which can be considered a quantitative index of the humification level in soil (Ceccanti et al., 2008), resulted higher in both, Centre and South Italian sites under organic with respect to mineral farming system, confirming a better preservation and/or incorporation of humic carbon (AHC) (figure 1, table 2). This was particularly evident in the Centre of Italy site. Also both absolute (HEG) and specific (SHEG) humic bound β-glucosidase activity in the pH range 4.5-4.2, resulted higher in the organic with respect to the mineral treatment (table 2), suggesting that the higher stable organic matter content gives protective capacity to extracellular enzymes which are independent from soil microbial biomass.

CONCLUSIONS

The purification of a humic-enzyme complex, trough the combination of UF and IEF techniques, resulted able to underline the impact of organic and mineral management practices on soil quality. The results indicate that the humic-enzyme complex could be more effective and consistent indicator of management induced changes to soil quality than conventional chemical and biological parameters. The higher humic carbon and humic bound β-glucosidase activity in the organic management with respect to mineral fertilization systems clearly indicated a better carbon sequestration and biochemical energy preservation in soils treated with the organic fertiliser.

REFERENCES

- Bending GD, Turner MK, Rayns F, Marx MC,Wood M., 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. *Soil Biology and Biochemistry* 36, 1785–92.
- Ceccanti, B., Bonmati-Pont, M., Nannipieri, P., 1989. Microdetermination of protease activity in humic bands of different sizes after analytical isoelectic focusing. *Biology and Fertility of Soils* 7, 202-206.
- Ceccanti, B., Doni, S., Macci, C., Cercignani, G., Masciandaro, G., 2008. Characterization of stable humic–enzyme complexes of different soil ecosystems through analytical isoelectric focussing technique (IEF). *Soil Biology and Biochemistry* 40, 2174-2177.
- Garcia, C., Hernandez, T., Costa, F., Ceccanti, B., Masciandaro, G., Ciardi, C., 1993. A study of biochemical parameters of composted and fresh municipal wastes. *Bioresource Technology* 44, 17-23.
- Lal, R., Kimble, J., Follett, R., 1997. Land use and soil C pools in terrestrial ecosystems. p. 1-10. *In* R. Lal et al. (ed.) *Management of carbon sequestration in soil*. CRC Press, Boca Raton, FL..
- Martens, D., Johanson, J., Frankenberger, J., 1992. Production and persistence of soil enzymes with repeated addition of organic residues. *Soil Science* 153, 53–61.
- Masciandaro, G., Ceccanti, B., 1999. Assessing soil quality in different agro-ecosystems through biochemical and chemical structural properties of humic substances. *Soil Tillage Research* 51, 129-137.
- Miller M., Dick R.P., 1995. Thermal stability and activities of soil enzymes as influenced by crop rotations. *Soil Biology and Biochemistry* 27, 1161-1166.
- Roldan, A., Caravaca, F., Hernandez, M.T., Garcıa, C., Sanchez- Brito, C., Velasquez, M., Tiscareno, M., 2003. No-tillage, crop residue additions, and legume cover cropping effects on soil quality characteristics under maize in Patzcuaro watershed (Mexico). *Soil Tillage Research* 72, 65–73.
- Yeomans, J.C., Bremner, J.M., 1988. A rapid and precise method for routine determination of organic carbon in soil. *Soil Science and Plant Analysis* 19, 1467–1476.