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1 Free and immobilized acid phosphatase in DOM extracted from two forest soils: preliminary results

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Most of the information available on the stability of enzymes in soils is derived from work on urease, acid phosphatase and arylsulfatase. The first evidence that soil enzymes are more stable than those added to soils was obtained by Conrad (1940). It is known that organic matter and mineral particles protect enzymes against microbial degradation. Now it is generally accepted that enzymes are immobilized within a network of organo-mineral complexes.

The aim of this work was to assess kinetic parameters of acid phosphatase in two different dissolved fractions: (i) free and (ii) immobilized enzyme. The soil samples were taken from two forest sites in Central Italy, one with deciduous and the other with coniferous plants, both soils were classified as *Dystrochrept (Soil Taxonomy)*. Horizon A (5-20 cm depth) was sampled in four cores from each site (10 m^2).

The total activity of acid phosphatase was determined following the method reported by Garzillo et al. (1996). DOM was extracted with 4 mM $CaCl_2$ solution; suspended particles and microbial cells were concentrated on 0.2 μm polycarbonate filters and immersed in 1.4 ml of DOM. The kinetics of acid phosphatase associated to particles and microbial cells (dissolved immobilized fraction), was studied using p-nitrophenylphosphate (pNPP) as substrate in a range of concentrations from 0.066 mM to 0.820 mM. Potassium acetate was added as buffer solution pH 5. Following

incubation for 16 h at 37˚C on a shaker at 100 rpm, the concentration of p-nitrophenol formed in the reaction was determined spectrophotometrically (wavelength 400 nm). The remained phosphatase enzyme (dissolved free fraction: <0.2 μm) was concentrated from 250 ml to 5 ml of filtered DOM using a membrane Vivacel 5000 MWCO size and the activity was tested using the same incubation conditions. To study the phosphatase kinetics for the dissolved free fraction was used pNPP ranging from 0.032 mM to 0.820 mM. The results showed a significant difference in terms of enzyme-substrate affinity between two soils and dissolved fractions. As expected, the dissolved free phosphatase fraction had lower Km and Vmax values than the dissolved immobilized fraction, meaning a higher enzyme-substrate affinity and lower enzyme "quantity" in the free fraction.