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Comparison of β-glucosidase kinetics using colorimetric and fluorimetric assays under organic and conventional soil managements

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Soil enzymes responsiveness to environmental changes and management practices makes them a potential indicator of soil biological quality. Specifically, organic management has been reported to enhance enzyme activities particularly those involved in C and N cycles. Different methodologies for a wide range of soil enzymes have been developed; in particular colorimetric and fluorimetric methods. In organic soils, however, soluble organic compounds can interfere and the quantification of the end product pNP (p-nitrophenol, commonly used in colorimetric methods) may be biased by pNP or substrate adsorption. Therefore, the use of conjugates of the fluorescent compound 4-methylumbelliferone (MUB), whose adsorption is negligible, has gained increasing interest. Several studies compared both methods efficacy in detecting changes in soils under different management /practices or organic matter content; however, to our knowledge, few of these used β-glucosidase which is reported to perform best as indicator of soil quality. In a four-years experiment a conventional system (traditional agricultural practices) was compared with an organic one (green manure and organic fertilizers). Both systems have a three-years crop rotation: pea – durum wheat – tomato. Soil sampling was performed during winter 2006 under durum wheat. Aims of the study were: 1) to compare the kinetic parameters of β-glucosidase (V_{max}, K_m and K_a) under organic and conventional managements using the colorimetric and the fluorimetric approach, 2) to highlight which method performed best in detecting changes under the two systems. The importance of the specificity constant K_a (k_{cat}/K_m) is that it relates the reaction rate to the concentration of free, rather than total, enzyme; furthermore K_a determines the specificity for competing substrates. The results showed

that enzyme affinity for each substrate was comparable (similar K_m values), while the V_{max} was significantly higher under organic management suggesting an increase of β-glucosidase content in these soils. Within each treatment, the K_a values showed that β-glucosidase degraded MUF-G (4-methylumbelliferyl β-D-glucopyranoside) ca. 180 times more easily than pNG (p-nitrophenyl-β-D-glucopyranoside).