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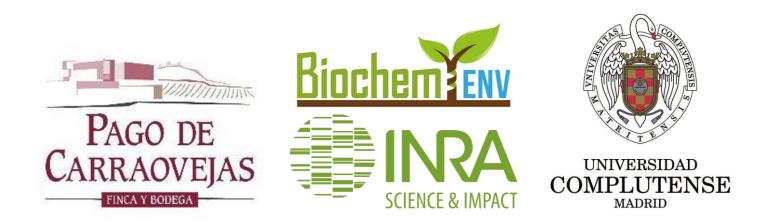
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ENZYMATIC ANALYSIS NORMALIZATION IN VINE SOILS: DETERMINATION OF QUALITY AND BIOLOGICAL PRODUCTIVITY

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INTRODUCTION

The agricultural practices and amendments apply to contribute nutrients to the soil increasing the performance and the quality of the vintage. The application of an amendment (synthetic or of biological origin) or a phytosanitary can cause a local change that modify the edaphic microbiota, disturb the soil natural balance. This local perturbation, beneficial for the vineyard, can cause a future global change in the microbial composition of the vineyard soil and destabilize completely the soil-plant system. This situation can produce biodiversity loss and can be an infection opportunity for vine pathogens (Yesca, Petri disease). By this reason, is important to soil monitoring of the through enzymatic robust methods.

The main causes of soil enzymes presence are microorganisms like worms can excrete enzymes in the soil. Soil enzymatic analysis allows the prevention of possible lacks or excess nutritional state. Also enzymatic assays can determinate the origin of the amendments: in the last years are being used active slimes of SDRW (Station Debugger of Residual Waters) and compost polluted with VOCs (Volatile Organic Compounds) or others harmful compounds. So this interferes in soil enzymatic activity and, therefore, the biological productivity.

AIM

Nowadays there is an increase to care vineyards soils. For this reason it is precise to use technics to determine soil functional state on the most representative vine physiological states. Therefore, the aims of this work are:

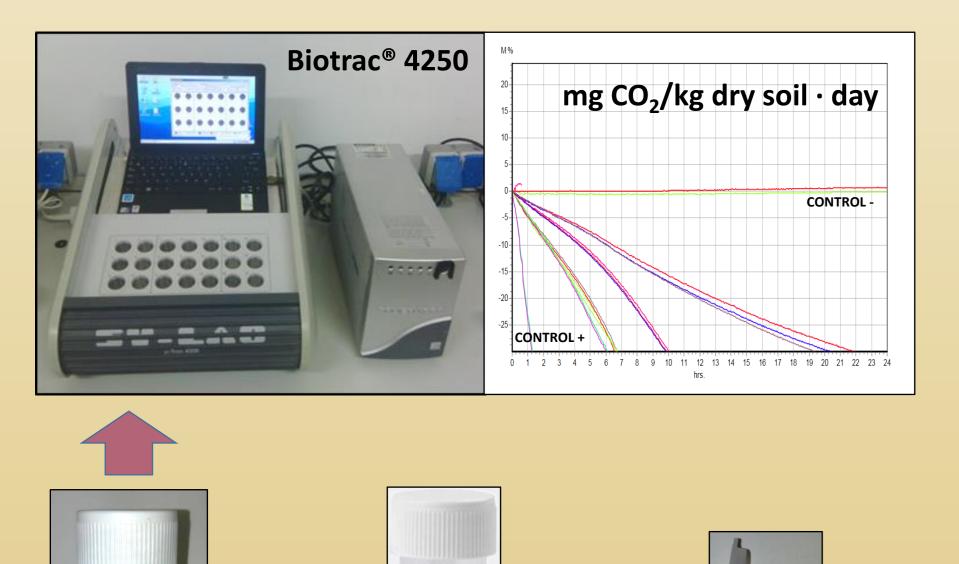
- Develop a method to measure enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates (ISO CD20130) to use in vine soils like standard of enzymatic analysis.

- Monitoring soils biological activity using Substrate-Induced Respiration (SIR).

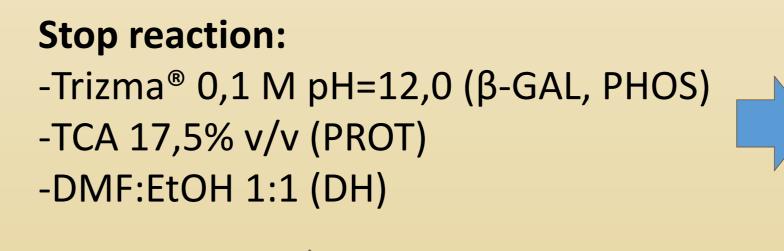
SUBSTRATE-INDUCED RESPIRATION

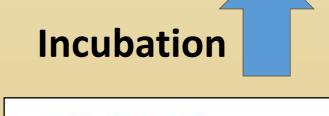
ENZYMATIC ANALYSIS

Optical Density (OD):

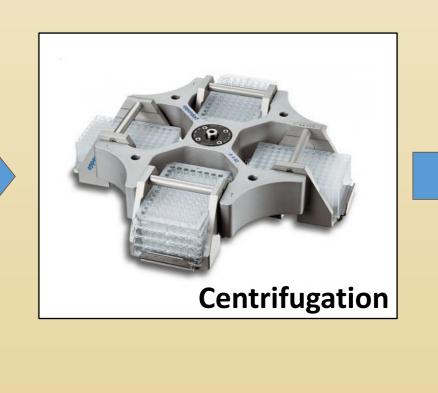


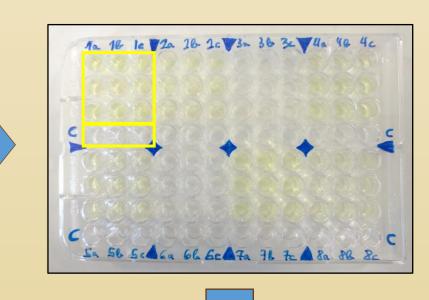










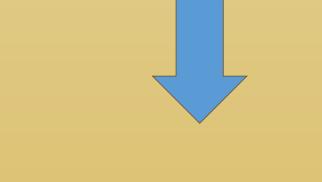


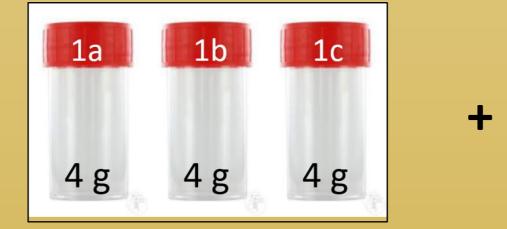


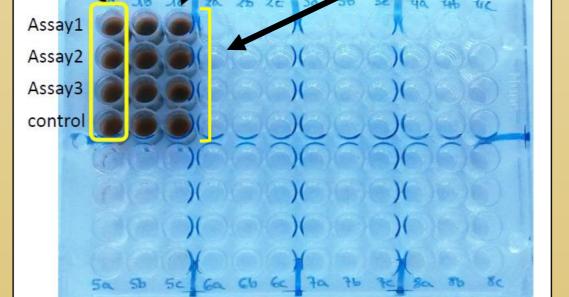


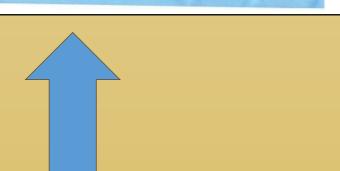
| Enzymes analyzed | Abbrevs. | E.C. Number | Soil cycle |
|--|----------|-----------------------------|---------------------------|
| β-Galactosidase | β-GAL | E.C. 3.2.1.22 | Carbon |
| Phosphatase | PHOS | E.C. 3.1.3.(1,2) | Phosphorus |
| Protease [◊] | PROT | E.C. 3.4.21.92 | Nitrogen |
| Deshidrogenase* | DH | E.C. 1.5.1.(30,37,38,39,45) | Microbial redox system |
| Substrate-Induced Respiration ⁺ | SIR | | Carbon |



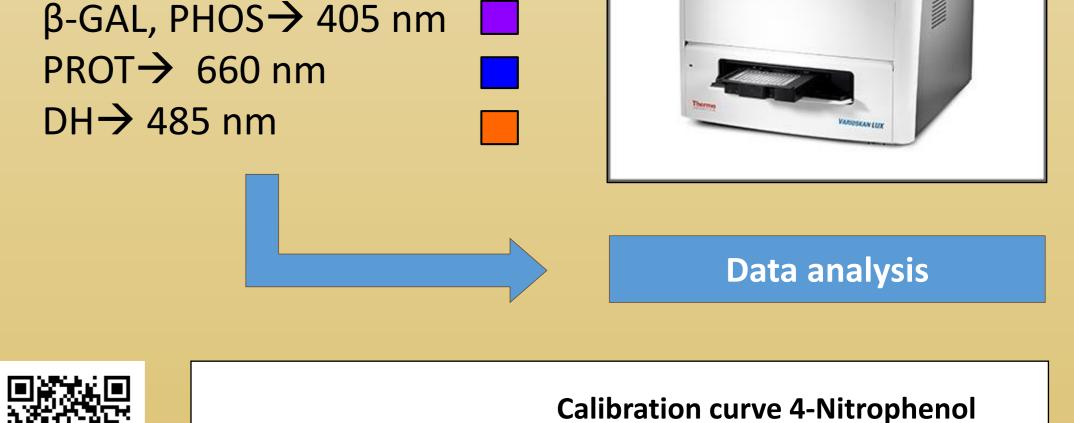


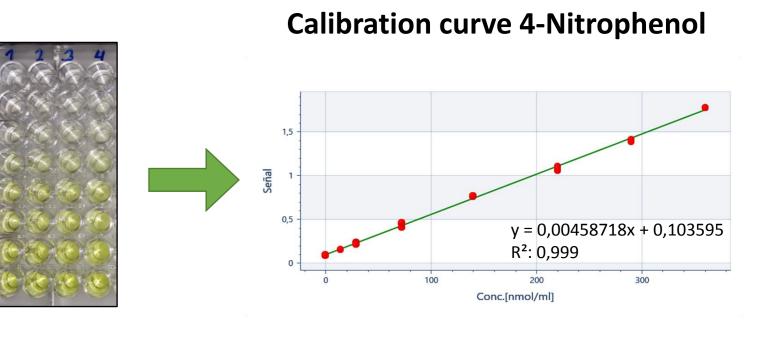




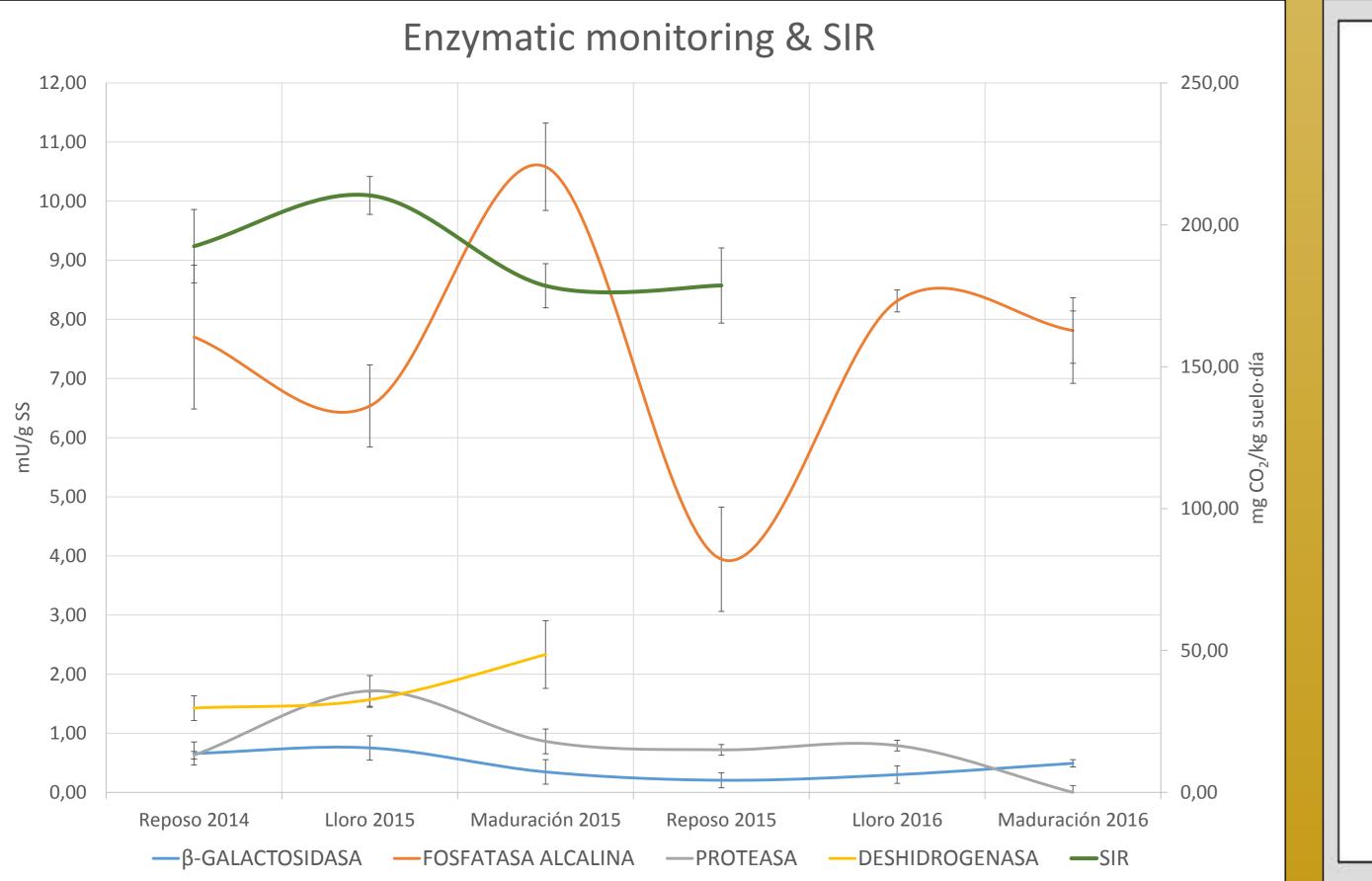


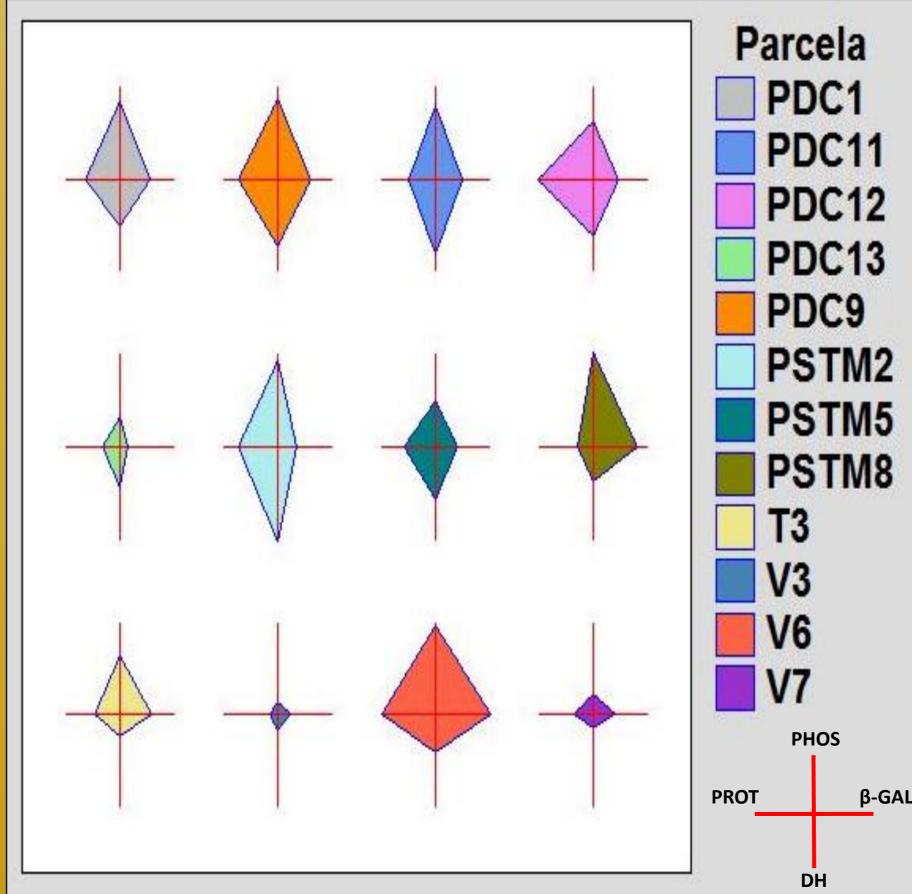
Distilled water (β-GAL, PHOS) Trizma[®] base 0,1M pH=8,1 (PROT) Trizma[®] base 1,0M pH=7,5 (DH)





RESULTS





CONCLUSIONS

1- This method of enzymatic activities meassurement in vine soils is fast, economic, repetitive and precise. It is allow to estimate fertility indexes.

2- Substrate-Induced Respiration is an excellent method to indirectly determine the soil biological activity, therefore it is a fertility meassure.

3- The use of method described in ISO Substrate-Induced and CD20130 meassurement are been Respiration OIV with the proposed to aim to implement a standardised method to allow compare different international level of fertility, quality and productivity parameters in vine soils.

The graphic shows the average of enzymatic tests performed in 12 vine plots in Ribera del Duero, during the three physiological stages of the vine. Desviation bars indicate inter-plots variability in rest, cry and harvest physiological state.

Soil enzymatic glyphs (β-Galactosidase, phosphatase, protease, deshidrogenase) in different plots analyzed during a period of two years.

This ISO procedure is a working draft or committee draft and is copyright-protected by ISO. We are under confidentiality agreement and we do not show the details of the procedure.

According to Ladd & Butler Method's (1972), modified by Bonmatí et al. (1991). * According to Trevors Method's et al. (1982) modified by García et al. (1993). + ISO 17155 Soil quality – Determination of abundance and activity of soil microflora using respiration curves.