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SENSITIVE MONITORING OF SOIL FERTILITY IN THE VALPOLICELLA VALLEY BY THE "FERTIMETRO" TOOL AND A HIGH-THROUGHPUT ENZYME METHOD

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INTRODUCTION

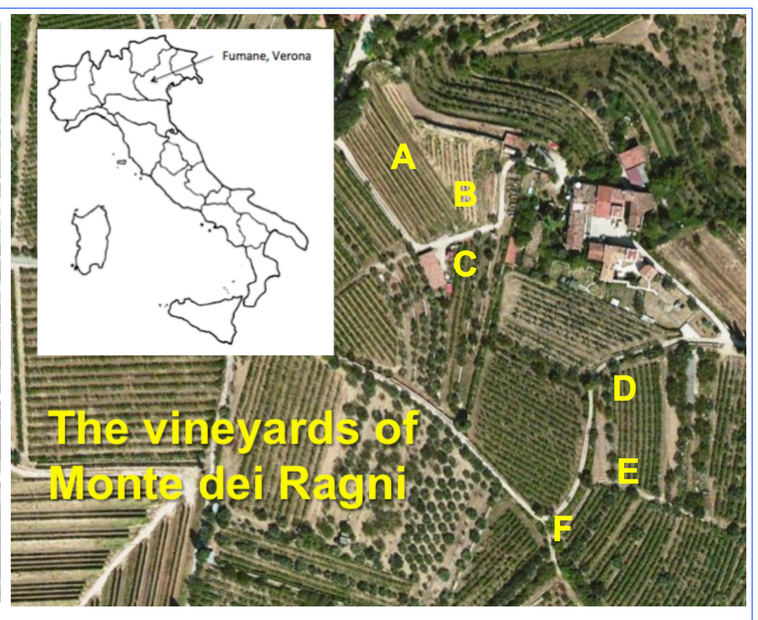
The "Fertimetro" (Patent Cooperation Treaty PCT/IB2012/001157 - Squartini, Concheri, Tiozzo) is a tool based on the degradation of cotton and silk threads (both of which either untreated or pre-treated with N or P solutions) kept in contact with the topsoil horizon. The cellulolytic (cotton) and proteolytic (silk) activities are common to most of the telluric microflora and usually correlate well with its activity and efficiency. Furthermore, the degradation of the threads treated with N or P, compared to that of the untreated controls provides an estimate of their availability and therefore may help to dose the fertilizers. The aim of this study was to evaluate soil fertility of biodynamic vs conventional vineyards in the well-renowned area of Valpolicella Region (Verona, Italy) by the "Fertimetro" tool and several enzyme activities involved in the biogeochemical cycles of C, N, S, P. Arylsulfatase, beta-glucosidase, chitinase, acid- and alkaline-phosphomonoesterase, pyrophosphate-phosphodiesterase, leucine-aminopeptidase and acetate-esterase were determined by a microplate, desorption-based enzyme assay. Soil microbial biomass was measured as double-strand DNA content in crude (unpurified) extracts. The detailed description of the multi-faceted results obtained with the "Fertimetro" method and enzyme analysis plus dsDNA quantitation will be discussed in terms of sensitivity, applicability and ease of use.

MATERIALS and METHODS

The test design involved 3 vineyards for biodynamic management (A, B and C sites) and 3 for the conventional one (D, E and F sites), located in Monte dei Ragni farm of Fumane (Verona, Italy).

The "Fertimetro" method is based on the observation that soil fertility does not depend solely on the availability of nutrients but also on the promptness at which soil microbes can process organic matter, thus liberating its soluble nutrients that will be absorbed by plant roots. Using buried 'bait' filaments made of cotton (= cellulose) or silk (= proteins) and recording the change in their tensile strength after a week of persistence in top-soil with a dynamometer, one can obtain a direct information on the state of soil microbes potential activity. The faster the soil microorganisms are working, the earlier the threads will break. Recording such data gives the first level information about the soil vitality. In addition, parallel versions of the same threads previously impregnated with nitrogen or phosphorus solutions are monitored. If a soil is defective in nitrogen or phosphorus, the pre-treated threads will break earlier, as the microorganisms, who need the same nutrients as plants do, will find, right on the threads, those limiting nutrients that are scarce in the soil. Being therefore able to multiply thanks to the nutrients, the microbes will work progressively faster and thread break will be anticipated. The extent of anticipation in the break of the Nitrogen-spiked or Phosphorus-spiked threads compared to the rupture time of the untreated threads is proportional to the lack of nitrogen or phosphorus in that soil and provides direct information to plan the extent of fertilization required. The following formula was adopted to determine the thread resistance: R =(Ri / Rni) x 100 (where: R= resistance percentage; Ri = rupture value of the thread buried in the soil; Rni=rupture value of a control filament when new). Resistance was converted into the degradation percentage (D) by subtracting resistance percentage values from 100. Enzyme activity: arylsulfatase (**aryS**), beta-glucosidase (**betaG**), acid- and alkaline-phosphomonoesterase (**acP** and **alkP**), phosphodiesterase (**bisP**), pyrophosphate-phosphodiesterase (**piroP**), chitinase (**chit**), leucine aminopeptidase (**leu**), nonanoate-esterase (**nona**) using a high-throughput, desorption-based enzyme assay in microplates using fluorogenic substrates (Cowie et al., 2013).

Statistical analysis were carried out using STATISTICA 13 software (StatSoft, Padova, Italy).



RESULTS

Figure 1. Sum of the % of degradation obtained from "Fertimetro" method, along a year of experimentation, (c=cotton, cN= cotton with N, cP=cotton with P, s=silk, sN=silk with N, sP=silk with P) related well with the activity of beta-glucosidase and leucine-aminopeptidase (see below).

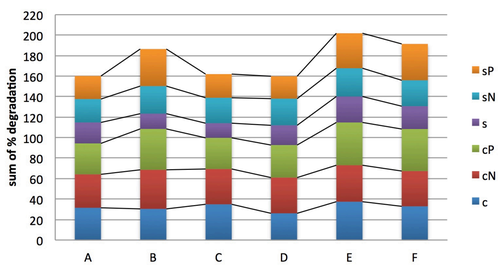
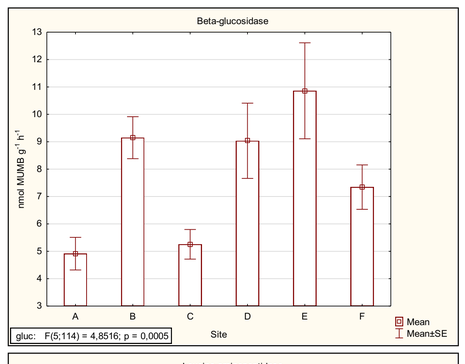
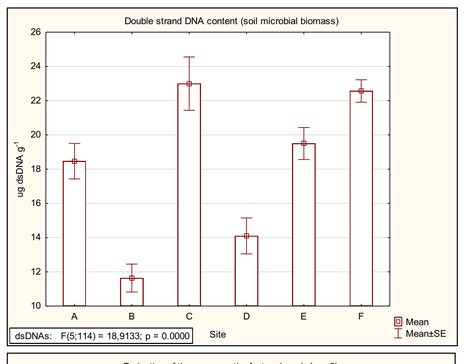
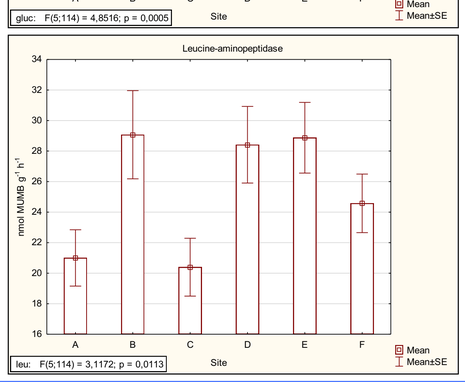


Figure 2. Soil microbial biomass measured as double-strand DNA content of the six vineyards and the PCA data showing the clustering of similar biodynamic vineyards (green circle, sites A and C) and of conventional vineyards (red circle, sites E and F).







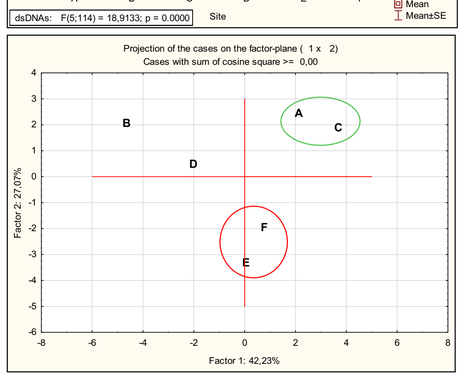


Table 1. Main characteristics of the vineyards' soils.

vineyards			soil analysis							soil classification
code	plantation year	management	texture	lime (g/kg)	pH	EC (5:1)	organic matter (g/kg)	CEC (cmol+/kg)	N (g/kg)	P (mg/kg)	FAO-UNESCO (WRB)
A	2007	biodynamic	sandy clay	480	8,30	179	31,5	24,5	2,8	19,9	Calcaric Regosol
B	2009	biodynamic	clay	575	8,17	87	13,8	10,0	1,4	31,5	Calcaric Regosol
C	1999	biodynamic	clay	540	8,01	265	37,8	36,2	3,1	27,8	Calcaric Regosol
D	1970	conventional	clay	590	8,19	222	21,7	20,7	2,6	18,7	Calcaric Cambisol
E	2001	conventional	clay	570	8,53	208	25,0	18,8	2,4	11,8	Calcaric Cambisol
F	1970	conventional	clay	620	8,43	144	21,9	36,0	3,1	22,9	Calcaric Cambisol

The soils show very peculiar features linked to the place where they evolved, with particular relevance to the limestone and pH (high). The supply of organic matter, especially for vineyards A and C, is high. Site B has recently been impacted by an intense excavation to plant the vineyard (2009) which yielded large amounts of surface skeleton and calcium carbonate which, together with the intensive processing, have dramatically lowered the content of organic matter. With respect to enzymatic analyses and quantification of biomass, some similarities are evident between the A, C and F vineyards. The values of the CSC and N as regards the chemical characteristics, and the dsDNA values for microbiological ones, have proven to be the highest. In contrast for the enzymes β-glucosidase and leucine-aminopeptidase, these sites present the least activity. Almost the opposite situation occurred in the sites less rich in biomass (B and D), where the soils are in less stable conditions and are experiencing the highest rates of enzymatic degradation. In particular, in the PCA data, site B is placed distant from all other sampling sites. The conventional sites and the two oldest vineyards with biodynamic system resulted instead closely ordinated .

Enzymatic activities related to the phosphorus cycle showed no statistically significant differences, while chitinase and arylsulfatase showed the same trend of the beta-glucosidase and leucine-aminopeptidase (data not shown).

The fertility of the vineyards where the soil, given the age of the plant, is more stable, seems to be in the situations of greater microbial balance. In particular biomass, identified by dsDNA value, reaches higher values in stable soils. In those soils even chemical fertility results higher, highlighted in the major values of CSC and N. The degrading activity, assessed by the activity of enzymes such as beta-glucosidase was conversely rather lower, suggesting a situation of greater stability.

CONCLUSION

The areas with less balance, induced by intense recent excavation, show lower biomass content and higher enzyme activities to suggest a higher demand for nutrients from the environment to regain equilibrium and fertility.

Biodynamic agriculture seems to speed this movement toward a balanced situation and higher fertility, as evidenced by the good state of vineyards with different age but under different managements. The methods used in this work have proven effective in showing the differences between soils that differ in management, history and evolution.

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