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A cura di Giuseppe Lo Papa e Anna Benedetti

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Soil functionality assessment in degraded plots of vineyards

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Introduction

Land transformation to adapt fields to mechanization in perennial crop farming is a common practice which includes land levelling, deep ploughing, stone-breakage and clearing, application of fertilizers and amendments. Manipulation of the natural soil profile along its entire depth can severely disturb the naturally existing chemical, physical, biological and hydrological equilibrium (Costantini and Barbetti, 2008; Costantini *et al.*, 2013). The most common effects of the land transformation are mixing of soil horizons and soil truncation, which result in reduction of soil depth and available water, organic matter depletion, enrichment of calcium carbonate content in the topsoil, imbalance of some element ratio, and decline in the activity and diversity of soil biological communities involved in nutrient cycles. A decline in the capacity of soil to accommodate the soil-dwelling organisms causes a strong impact on several ecosystem services, in particular, the growth of the vine, the quality and quantity of the grapes, the production costs and the risk of erosion.

These negative effects of a pre-planting mismanagement can occur simultaneously and interact to decrease soil fertility and grapevine performance (Lanyon *et al.*, 2004; Tagliavini and Rombolà, 2001; Martínez-Casasnovas and Ramos, 2009).

Since soil spatial variability is usually high, soil manipulations frequently result into reduced soil functionality and decline of soil ecosystem services in defined plots of the vineyards. Sometimes soil degradation in these areas is very high and compromises not only vine performance and crop yield, but also disease resistance of plants to diseases and their survival. The impact of improper soil manipulations in vineyards may be of particular concern, because vineyards are frequently located on marginal hillsides, which are sensitive to soil erosion and characterized by shallow soil depth (Ramos, 2006).

This paper wants to show the assessment of soil functionality in degraded areas within two farms in Tuscany. This work reports the results of the first activities in Italian sites of the ReSolVe Core-organic+ project, aimed at restoring optimal Soil functionality in degraded areas within organic European vineyards.

Materials and methods

Italian experimental sites are situated in two commercial farms in Tuscany (Figure 1): i) Fontodi, Panzano in Chianti (FI) and ii) San Disdagio, Roccastrada (GR). Both sites show a Mediterranean suboceanic climate, with long term mean temperaures around 13.5 °C, annual rainfall about 880 mm, and a potential summer water deficit around 160 mm. In each farm, three plots (250 m^2 each) of degraded plots and three relative non-degraded control plots were selected. Delineation of degraded areas was carried out following the indication of the farmer and a soil proximal sensing method, namely passive gamma-ray spectroscopy. The gamma-ray spectrometer measures on-the-go natural gamma-emissions from the radioactive elements or radionuclides of the soils and rocks. Although gamma-ray spectroscopy has been used for years in mineral exploration, in the last decade the method has been successfully used for digital soil mapping (Viscarra Rossell *et al.*, 2007; Dierke and Werban, 2013; Priori *et al.*, 2014). This technique can map the soil spatial variability of several features (clay, carbonates, stoniness, and compaction) in the topsoil (0-30 cm). The experimental plots have been studied trough: i) soil profile description and analysis; ii) topsoil sampling for organic matter, enzymes, and microbiology (bacteria, nematodes and microarthropods) analysis; iii) grapevine water stress; and iv) grape yield and quality.

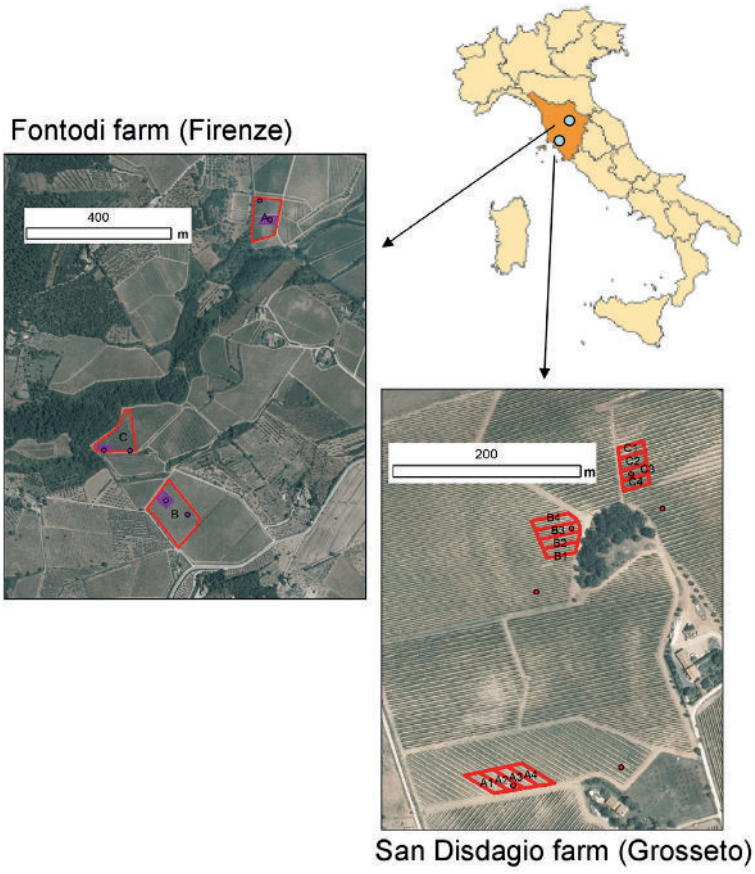

Figure 1 – The experimental farms and the vineyards (in red) with degraded areas and the non-degraded control sites.

Soil profiles were analyzed for the following chemical properties: pH (1:5 soil–water suspension), electrical conductivity (1:5 soil-water extract), total organic carbon (TOC) and total nitrogen (TN) (dry combustion with a Thermo CN soil analyzer), total equivalent $CaCO_3$ (gas-volumetric method), cation exchange capacity (CEC) and exchangeable bases (barium chloride method). Soil classification followed the international WRB system.

Topsoil samplings (0-10 and 10-30 cm) were carried out on April 2015, to analyze bulk density, SOC and total nitrogen, enzymes, microarthropods, nematodes and bacteria.

Bulk density was determined on soil at field capacity conditions using the core method (Blake and Hartge, 1986).

Total organic C (TOC) and total N (TN) contents in the bulk soil were measured by dry combustion on a Thermo Flash 2000 CN soil analyzer. To this aim, 20 to 40 mg soil were weighed into Ag-foil capsules and pre-treated with 10% HCl until complete removal of carbonates.

– *Enzymes*: Enzyme activity was measured according to the methods of Marx *et al.* (2001) and Vepsäläinen *et al.* (2001), based on the use of fluorogenic methylumbelliferyl (MUF)-substrates. Soil samples were analyzed for cellulase, β-glucosidase, acid phosphatase and arylsulphatase activity using methylumbelliferyl (MUF) conjugated surrogate substrates (Sigma, St Louis, MO, USA). Moist soil sample (equivalent to 1 g oven-dry material) was weighed into a sterile jar and 50 mL of distilled water. A homogenous suspension was obtained by homogenising with UltraTurrax at 9600 rev/min for 3 min. Aliquots of 100 μL were withdrawn and dispensed into a 96 well microplate (3 analytical replicates/sample/substrate). Finally, 100 μL of 1 mM substrate solution were added giving a final substrate concentration of 500 μM. Fluorescence was measured after 0, 30, 60, 120, 180 min of incubation at 30 °C. Fluorescence (excitation 360 nm; emission 450 nm) was measured with an automated fluorimetric plate-reader (Fluoroskan Ascent).
– *Soil microarthropods*: For each plot in degraded area, four soil samples (10x10x10cm) were dug from inter-row and compared with controls sampled in non-degraded area; the microarthropods were extracted with Berlese-Tullgren funnels (25 cm diameter, 2 mm mesh, 60 W lamp at 25 cm distance, 5 days extraction time) and observed by a stereomicroscope. The abundance of the biological forms (FB) (Parisi *et al.*, 2005) was determined and analyzed by ANOVA. The biological soil quality and similarity of arthropod communities were evaluated by the Biological Soil Quality index (QBS-ar) (Parisi *et al.*, 2005) and Jaccard coefficient (J) (Krebs, 1989), respectively. The statistical analyses were performed by SPSS software (2004).
– *Soil nematode community analysis:* Soil samples were collected at 0-30 cm depth. For each soil sample, five scores were randomly sampled and then mixed to form one composite sample. Nematodes were isolated from 100 ml of each soil sample using a modified Baerman funnel methods (extraction time 48 h). Nematodes were counted and then mounted on temporary slides for identification at higher magnification to genus or family level using keys from Marinari-Palmisano and Vinciguerra (2014) and taxonomic families were assigned a trophic grouping based on Yeates *et*

al. (1993). Nematode communities were characterized using absolute abundance of individuals, richness determined by counting the number of taxa, Maturity index and Plant Parasitic index by Bongers (1990).

– *Soil eubacterial diversity*: has been investigated by mean of molecular methods involving direct DNA extraction from soil samples collected at two different depth (0-10 and 10-30 cm), 16S rDNA specific amplification and DGGE (Denaturing Gradient Gel Electrophoresis) analysis of the electrophoretic patterns, clustering the different patterns according to their similarity and calculating biodiversity indices evaluated with ANOVA.

– *Viticultural parameters* considered the average production per plant (APP), determined by weighing the grapes from 5 vines in three distinct sections of the rows. For each repetition a grape sample was taken to measure total acidity, sugar content and pH. The analyses were performed according to the official methods of the International Organization of Vine and Wine (http://www.oiv.int/).

Results and discussion

The soil profiles showed a number of significant differences between degraded and non-degraded vineyards, which appeared of larger extent at the San Disdagio site. In particular, the topsoil of degraded plots had a surface enrichment of $CaCO_3$ (FON: $P<0.05$; SD: $P<0.01$), along with higher pH ($P<0.05$), lower TOC (FON: $P<0.05$; SD: $P<0.001$), lower TN (FON: ns; SD: $P<0.001$) and lower CEC (FON: ns; SD: $P<0.001$). There were no significant differences between degraded and non-degraded areas for soil bulk density.

Soil organic matter and enzyme activities showed a similar pattern in the two sites, with higher values in the first 10 cm than in the deeper layer (Table 1). Comparing the two sites, Fontodi showed, on average, a larger amount of soil organic matter and enzyme activities. However, the largest differences between degraded and non-degraded areas were observed in San Disdagio in both soil layers. Differently, Fontodi showed a larger heterogeneity among plots, with lower differences due to degradation.

On the whole, the microarthropod density was higher in Fontodi than in San Disdagio (Figure 2, t test =-3.81; $p<0.001$); in each farm, significant difference was not detected between degraded and non-degraded plots (San Disdagio, t test = -1,29, $P=0.22$; Fontodi, t test = 0.50, $P=0.63$). The Acari group was the most representative in each plot (60-85% of microarthropods), followed by Collembola (5-21%) and other 18 arthropod groups: eu-edaphic (*Proturi, Pauropoda, Symphyla, Pseudoscorpionida, Diplura*), emi-edaphic (*Diptera larvae, holometabolous larvae, Isopoda, Chilopoda, Diplopoda, Coleoptera, Hymenoptera*) and epigeic forms (*Araneida, Rhynchota, Psocoptera, Orthoptera, Thysanoptera, Diptera*). In the two experimental sites, very high similarity in the composition of the communities was detected ($J=0.90$). The QBS-ar values were always high (>100), with good and optimal quality classes – level 3 and 4 (Griselli, 2006); in all plots in Fontodi, the highest level was registered.

Table 1 – Organic carbon, total nitrogen and enzymes of the topsoil in the experimental vineyards.

	TOC (g kg^{-1})	TN (g kg^{-1})	Cellulase	Acid phosphatase	β-glucosidase	Arylsulphatase
FONTODI						
	0-10 cm					
Degr.	10.6 (a)	1.7 (a)	25.9 (a)	146.2 (a)	231.9 (a)	40.5 (a)
Not degr.	9.6 (a)	1.6 (a)	26.0 (a)	147.5 (a)	257.4 (a)	42.5 (a)
	10-30 cm					
Degr.	7.2 (a)	1.5 (a)	15.2 (a)	112.8 (a)	127.0 (a)	28.6 (a)
Not degr.	7.4 (a)	1.4 (a)	15.9 (a)	119.7 (a)	110.6 (a)	32.3 (a)
SAN DISDAGIO						
	TOC (g kg^{-1})	TN (g kg^{-1})	Cellulase	Acid phosphatase	β-glucosidase	Arylsulphatase
	0-10 cm					
Degr.	5.6 (a)	1.0 (a)	11.4 (a)	124.6 (a)	94.7 (a)	16.3 (a)
Not degr.	11.1 (b)	1.5 (b)	26.5 (b)	174.6 (b)	214.4 (b)	33.9 (b)
	10-30 cm					
Degr.	4.5 (a)	0.9 (a)	7.8 (a)	123.5 (ab)	62.2	15.9 (a)
Not degr.	9.9 (b)	1.4 (b)	17.3 (b)	167.6 (ab)	140.5 (b)	31.9 (b)

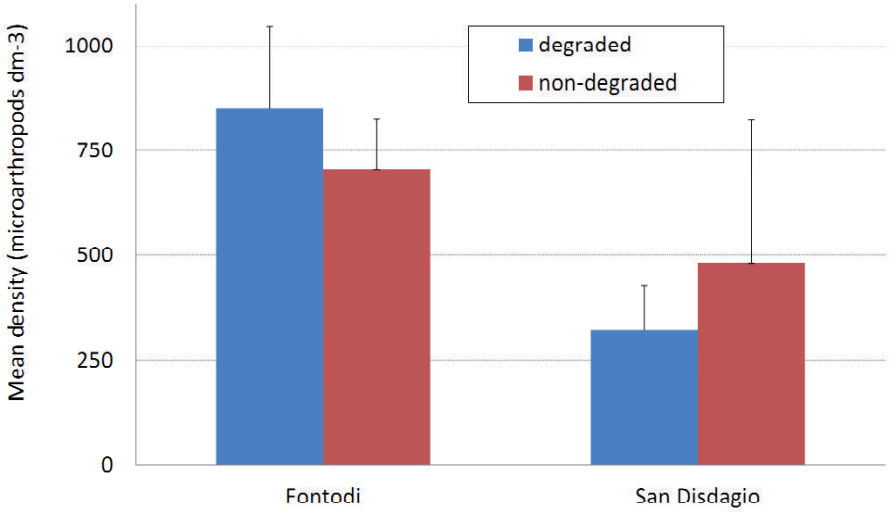

Figure 2 – Abundance of microarthorpods in the two sites (San Disdagio, Fontodi), in degraded and non-degraded plots.

Nematode abundance, taxa richness and maturity (MI) and plant parasitic (PPI) indices were higher in non-degraded than degraded areas, but differences were not significant. In general, MI and PPI values indicated the high presence of general opportunistic and a food web dominated by decomposer bacteria. The proportion of nematodes in

the feeding groups was also similar under both sites: bacterial feeders dominated in degraded areas, while plant parasitic were the most rapresentative group in non-degraded areas.

Table 2 – Soil degradation effect on total abundance, taxa richness (standard error), nematode indicators and rellative abundance of trophic groups extracted by 100 ml soil. Levels of significance are indicated by letters a, b for P<0.05. Bact., bacterial feeders; Fung., fungal feeders; Omni., omnivores; Pred., predators; MI, maturity index; PPI, plant parasitic index.

	Abundance	**Taxa richness**	**Trophic groups**					**Nematode indicators**	
			Bact.	**Fung.**	**Omni.**	**Pred.**	**Pl. Par.**	**MI**	**PPI**
Fontodi									
Degraded	307.2±86.7	4.8±0.3	50.9±3.01	0.03±0.03	10.3±1.2	1.4±0.7	37.5±3.8	1.6±0.1	2.8±0.1
Non-degraded	416.0±16.8	5.3±0.3	40.1±2.3	0	12.3±1.9	0.1±0.1	47.6±0.44	1.7±0.1	2.7±0.2
San Disdagio									
Degraded	102.3±35.8**b**	4.0±0.4	56.6±4.7	0	8.8±1.4	0	34.7±4.0	1.4±0.1	2.5±0.3
Non-degraded	827±134.4 **a**	4.3±0.3	47.3±1.8	3.8±3.8	11.8±5.7	0	37.1±11.1	1.6±0.2	2.8±0.1

Soil eubacterial richness, i.e number of DGGE bands, is slightly higher in the deeper Fontodi soil samples, both degraded and not degraded, while conversely in San Disdagio soil samples from the 0-10 depth had a higher richness; ANOVA pointed out a significant difference ($p \geq 0,05$) only among San Disdagio soil samples.

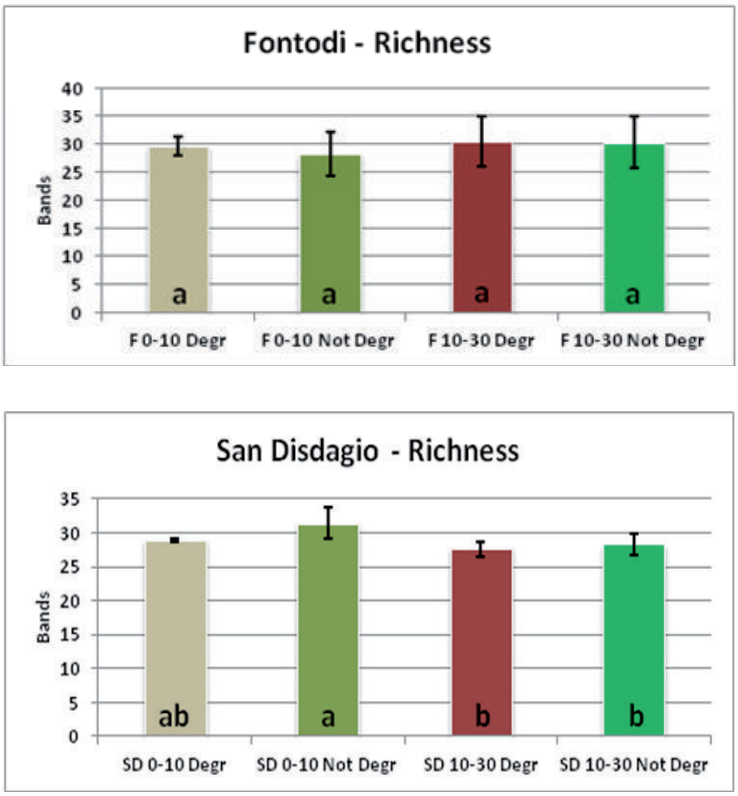

Figure 3 – Soil eubacterial richness, calculated through DGGE bands.

Soil conditions influenced both the quality and quantity of grapes. From the results (Figure 4), it is evident that in the degraded areas, the production was scarce and never reached 1 kg per plant. The reduced productivity also caused an excessive accumulation of sugars in the grapes (> 25° brix). The total acidity, in San Disdagio vineyards, was higher in the grapes from the rows on non-degraded soils, maintaining the pH at lower values. In conclusion, from an oenological point of view, grapes from degraded soils showed an unbalanced maturity that would lead to the production of wines with excessive alcohol concentrations (> 14.5% v/v ethanol) and low acidity.

Conclusions

The differences between degraded and non-degraded plots within vineyards were more evident in San Disdagio farm, which applied organic viticulture since 1 year only. Fontodi farm, which have managed the vineyard soils with compost and cover crops for several years, has almost completely recovered the soil functionality of the degraded areas, originated by land levelling.

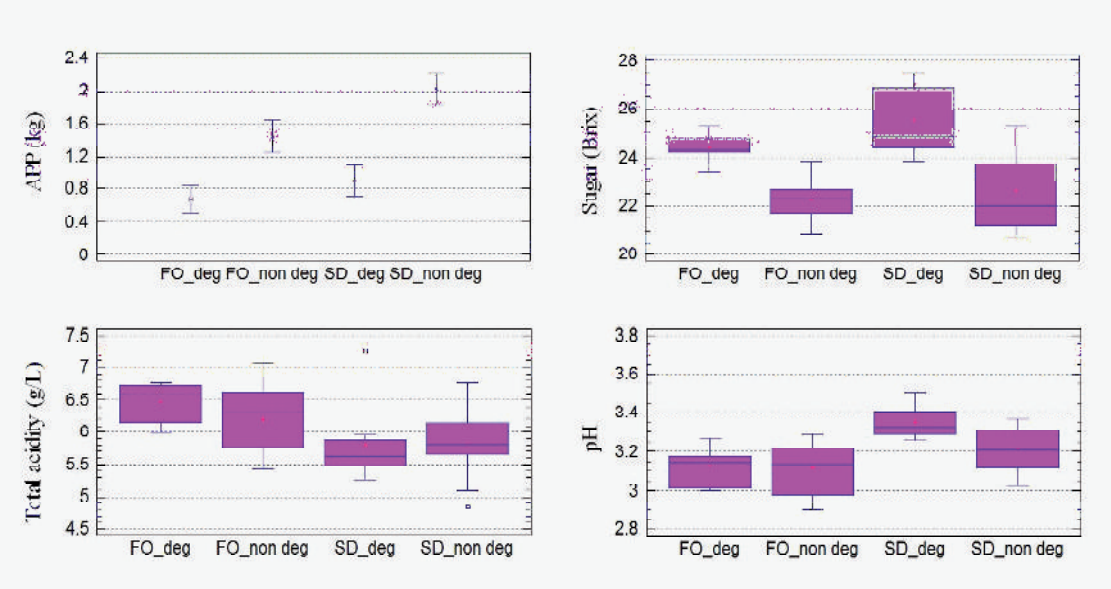

Figure 4 – Average production per plant and technological maturity of grapes (FO: Fontodi farm, SD: San Disdagio farm).

In general, degraded areas of the vineyards showed lower amount of SOC, nitrogen and CEC, whereas the total carbonates and pH showed higher values, particularly in the topsoil.
The enzyme functionality of the topsoil showed significant differences between degraded and non-degraded plots in a new organic farm like San Disdagio, whereas did not show any differences in Fontodi, which has been appyling compost and covercrops for years. This was also confirmed by the biological results, namely microarthropods, nematodes and bacteria.
The results of QBS-ar are according with the evidence acquired in vineyards similarly managed (Gagnarli *et al.*, 2015; Miani *et al.* 2005; Costantini *et al.*, 2015) or undisturbed ecosystems (Menta *et al.*, 2011). The analysis of the overall data did not show differences between degraded soil and respective control, neither in QBS-ar values nor in abundance. Presumably, this is due to the organic management guaranteeing a complex structure of microarthropod community as confirmed by high number of euedaphic biological forms observed in each site.
Overall, in semi-arid conditions, the non-degraded system led to increased nematode populations, especially of bacterial-feeding nematodes. This is probably associated with the increased concentration of soil organic matter and moisture content.

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