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Research article

# Ecological restoration of a copper polluted vineyard: Long-term impact of farmland abandonment on soil bio-chemical properties and microbial communities

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ABSTRACT

This study aimed at investigating the degree of interference of high soil copper (Cu) contamination when an old vineyard is converted into a protected area. This study was performed within an intensive agricultural system; it was organized into a two-factorial nested design to analyze the impact of management (conventional vs re-naturalized orchard) and position within each orchard (tree-rows and strips). Chemical and biochemical properties along with bacterial and fungal communities, evaluated with PCR-DGGE starting from total soil DNA, were analyzed. Total Cu was localized in tree rows in the old vineyard at 1000 mg $kg^{-1}$ of soil, whereas it did not exceed 80 mg $kg^{-1}$ soil in the other treatments. Total organic carbon and all biochemical properties significantly improved in re-naturalized compared to conventionally cultivated site, while no significant differences were observed between tree row and strip. Moreover, a higher extractable carbon-extractable nitrogen ($C_{ext}$-to-$N_{ext}$) ratio in the re-naturalized (19.3) site than in the conventionally managed site (10.2) indicated a shift of soil system from C-limited to N-limited, confirming a successful ecological restoration. Deep improvement of soil biochemical properties exceeded the negative impact of Cu contamination. A shift of bacterial community composition as well as increased bacterial diversity in Cu contaminated treatment indicated a bacterial response to Cu stress; to the contrary, soil fungi were less susceptible than bacteria, though an overall reduction of fungal DNA was detected. Findings suggest that ecological restoration of highly polluted agricultural soils leads to overcoming the reduction of soil functionalities linked to Cu contamination and opens interesting perspectives for mitigating Cu stress in agricultural soils with strategies based on conservative agriculture.



## 1. Introduction

The Po valley in Northern Italy is one of the most important intensive agricultural areas in Italy. It is characterized by a sub-continental climate in the western and central part which is devoted to arable crops (cereals, maize, tomato, sugar beet, potato, etc.) and forage for cattle farming, which is the main cause of high nitrate content in soil and water in this area. On the contrary, the eastern side of the Po valley, close to the Adriatic coast with a Mediterranean climate, is traditionally devoted to fruit tree crops and fresh vegetable production, characterized, therefore, by an organic carbon depletion which has caused a desertification process across these farmlands (Zdruli et al., 2004). For this reason, great attention is paid to any research which serves to highlight the advantages of soil restoration and to increase a general interest in conservative agriculture and any other means of raising public awareness concerning the need to protect agricultural land from degradation.

Within this context a preliminary survey was performed on an old farm located in the coastal area. This farm had been completely abandoned and uncultivated for 35 years. This long-term abandonment determined ecological restoration of this site to transform it into a reserve for *in situ* preservation of indigenous plant and

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animal species, located in the middle of an intensive fruit tree growing area.

A preliminary survey on chemical properties of the above mentioned re-naturalized site showed that soil samples taken from the old vineyard were characterized by a copper (Cu) content largely above 100 mg $kg^{-1}$ which represents the threshold of Cu in soil as established by the European Commission in 1986 (Council Directive, 1986).

Total Cu concentrations in soil of 100–150 mg $kg^{-1}$ is conventionally recognized as the threshold above which it can negatively influence plant growth and soil functioning (Kabata-Pendias and Pendias, 2001; Ippolito et al., 2010). The limits of effective Cu soil pollution and toxicity on plant and microorganisms vary greatly because Cu is a low bioavailable trace element, which can occur in several forms in soil (Adriano, 1986), and soil pH as well as organic matter (OM) content strongly affect Cu immobilization (McBride and Blasiak, 1979). Furthermore, Cu is a common metal pollutant of vineyards, therefore there is still much debate among European countries on its limits content in soils and on the need to adapt those limits to climatic conditions and main agricultural productions in different European agricultural areas. This is actually a "hot topic" especially for Mediterranean countries such as Spain and Italy, where there is an increase of land used for organic agriculture due to a rising trend in organic products required by the European Market. Specifically, grape and tomato are two economically important productions for which organic cultivation cannot be performed independently from Cu-based fungicides for controlling downy mildew.

For a longtime, successive applications of Cu-based fungicides on vineyards has been the only method for controlling downy mildew of vine (*Plasmopara viticola*). The non-biodegradable nature and long-term biological half-life of Cu, in combination with its low mobility in soils, has caused Cu accumulation in upper soil layers of traditional European wine-producing countries such as Portugal (Magalhães et al., 1985), Italy (Deluisa et al., 1996), France (Brun et al., 1998), Spain (Fernández-Calviño et al., 2010) and Serbia (Ninkov et al., 2014).

Some studies, which focus on a number of specific areas, report that Cu contamination can induce a change of bacterial communities in the top soil layer (Dell'Amico et al., 2008; Lejon et al., 2010), on the other hand, wider surveys on long-term Cu polluted agricultural soils have shown that pH and soil management rather than Cu content in soil are the most important factor in determining the composition of bacterial communities (de Boer et al., 2012; Mackie et al., 2013; Fernández-Calviño et al., 2010). Based on these findings, a study was performed to investigate whether land abandonment is sufficient to restore Cu high polluted soils and how Cu pollution can affect biochemical properties and microbial communities.

## 2. Material and methods

### 2.1. Experimental site and sampling

The Pantaleone property (Bagnacavallo, Ravenna Italy, 11°58′E, 44°25′N, 6–11 m asl) (http://www.poderepantaleone.it) was a traditional farm up to the 1950s, which was transformed into a protected area in 1989 and then, in 2006, was included in the EU-wide *Natura 2000* ecological network of protected areas, as a 'Site of Community Importance' in the Mediterranean biogeographical region (Evans, 2012). Although the innovative cropping set of vineyards has been periodically renewed in Italy from the 1950s, the owners of Pantaleone, surprisingly, did not change their traditional agricultural techniques or their tools for plant protection, from the 50s until total abandonment in 1980, when the whole 7 ha property began its ecological restoration, which led local administration to acquire and transform the farm into a protected area in 1989. The original farm setting included a vineyard that was cultivated according to an traditional technique known as "married vine", where one fruit or woody plant acted as a support for one or two vines spaced at 3 m on the planted row. This evolved into a forest system, while strips were about 4 m wide with a permanent vegetative ground cover. Therefore, inorganic Cu-based fungicides, such as the Bordeau mixture ($Ca(OH)_2$+$CuSO_4$), and sulfur were used for controlling downy mildew (*Plasmopara viticola*) and powdery mildew (*Uncinula necator*), respectively, for decades.

The soil was sampled in the re-naturalizated Pantaleone property (RE-NAT) and in a nearby cultivated orchard, representative of the average conventional soil management (CONV) in that area (Supplemental material Fig. 1). The two sites had therefore similar soil origin and vegetation; indeed, both were tree crops with tree-rows alternated to strips with vegetation cover. The cultivated orchard was managed according to practices commonly adopted in that growing area: permanent vegetation cover on strips and periodical tillage for controlling weeds on the tree-rows and chemical control of pests and foliar diseases.

Soil of conventional (CONV) and re-naturalized (RE-NAT) areas was classified as Calcaric Cambisol according to the World Reference Base for soil resources (FAO, 2015); soil texture was silty-loam according texture triangle. Soil sampling was performed in three points for each treatment at a 0–20 cm deep after removing vegetative ground cover. Samples on rows were collected under a canopy of three trees, while those of strips were collected in the middle of corresponding inter-rows. Each sample was mixed by hand to obtain an homogeneous representative sample of 2 kg per each replicate which was divided into several subsamples to be handled and stored in different ways according to technical requirements of relevant analysis.

### 2.2. Soil analysis

#### 2.2.1. Chemical analysis

Soil subsamples (500 g), for each replicate, were air dried, milled, and sieved at 2 mm before chemical analysis.

Chemical properties were determined according to SSSA methods (Sparks, 1996). Briefly, texture was determined by pipet method, pH was determined in water and 1 M KCl (1:2.5, m:v); total carbonates were determined using a volumetric calcimeter; total organic carbon (TOC) was determined by dichromate oxidation with external heat; total nitrogen with the Kjeldahl method; available phosphorous was extracted with 0.5 M sodium bicarbonate at pH 8.5, and determined with ascorbic acid method; total sulfur and metals were extracted with aqua regia and hydrogen peroxide solution using a microwave oven, and determined by atomic emission spectroscopy inductively coupled plasma (EOS-ICP). Bioavailable metals were extracted with DTPA solution in agreement with Lindsay and Norwell (1978) and determined by atomic emission spectroscopy inductively coupled plasma (EOS-ICP). Humic substances (HS) were extracted with 0.1 M sodium hydroxide + 0.1 M sodium pyrophosphate solution (Na + PP), purified and quantified in agreement with Ciavatta and Govi (1993). Copper content was determined in Na + PP, and water (1:10, mass-to-volume, for 30 min at room temperature) extracts by EOS-ICP.

#### 2.2.2. Biochemical analysis

The biochemical analysis was carried out on fresh sieved (2 mm) subsamples. Soil microbial biomass carbon ($C_{mic}$) and nitrogen ($N_{mic}$) were determined through the fumigation–extraction method (Vance et al., 1987). Fumigated and unfumigated soil samples were extracted with 0.5 M $K_2SO_4$, and analyzed for organic