Soil Biology & Biochemistry 65 (2013) 245–253

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Long-term copper application in an organic vineyard modifies spatial distribution of soil micro-organisms

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ARTICLE INFO

Article history:
Received 28 February 2013
Received in revised form
21 May 2013
Accepted 3 June 2013
Available online 19 June 2013

Keywords:
Copper
Vineyard
Enzymes
Spatial distribution
Organic carbon
Tillage

ABSTRACT

Organic viticulturists utilize copper to prevent and reduce downy mildew (*Plasmopara viticola*) within the vineyard. Being a heavy metal, copper either builds up in the soil or is leached into the groundwater or taken up by living organisms. Therefore, its use impacts the environment. In organic farming there are currently no copper substitutes available and, therefore, it is necessary to understand the depth of damage that copper is inflicting on soil microbial communities over the long-term. Here a field-scale grid, 4 m by 5 m, was analyzed within a 17 year practicing organic vineyard in Southwestern Germany. Copper fractions, enzyme analyses (phosphatase, arylsulfatase, invertase, urease, xylanase), fungal analyses (ergosterol, fungal PLFA), bacterial analyses (bacterial PLFA), and microbial biomass were measured and spatial distribution maps were interpolated. Readily available and exchangeable copper fractions were higher within the vine rows and lower between them. Total copper ranged from 43 mg kg^{-1} to 142 mg kg^{-1}, which is above prevention levels for Germany. In areas of high copper, a negative effect on total carbon, ergosterol, as well as phosphatase and invertase enzyme activities was observed. Tillage practices were found to be more important than copper for the distribution of carbon, nitrogen and xylanase activity within the vineyard.

1. Introduction

Viticulture is an important perennial cropping system in Europe. In certain regions, however, these crops are blighted by downy mildew, *Plasmopara viticola*, a mold that in turn stresses the vine and reduces grape quality (Salinari et al., 2006; Jermini et al., 2010). Copper-based fungicides have been used to combat *P. viticola* in different production systems worldwide for more than a century. Today, synthetic fungicides play a major role in conventional farming; these fungicides replace copper products and, thus, reduce this input of the heavy metal in vineyard soils, although copper-based fungicides continue to be applied in restrained amounts. In organic farming, copper-based fungicides are still the only effective method permitted to treat grapes against *P. viticola*. As organic methods are becoming more common, especially in Europe, where 100,000 ha of grapes are under organic management (IFOAM, 2009), the total use of copper-based fungicides remains important even though the maximum amount of copper input per year is limited to a maximum of 6 kg y^{-1} ha^{-1} according to the EU Regulation on Organic Production and Labeling of Organic Products (European Commission, 2007). As copper is known to accumulate within topsoil following fungicidal sprays (Pietrzak and McPhail, 2004; Rusjan et al., 2007) and can never be degraded (McBride et al., 1981), its potential to have adverse eco-toxicological effects on the environment is large. In environments, such as vineyards, where pH is often above neutral, copper is immobile, accumulating over years of use. However, soluble fractions are always present (McBride et al., 1981) and both low organic matter content (OM) and low cation exchange capacity (CEC) often found in vineyards encourage copper mobilization (Maier et al., 2000). When copper becomes mobile and more available to organisms, it stresses macro fauna (such as earthworms), microorganisms and their enzyme activities and, in high amounts, becomes toxic to plants, thereby disturbing essential elemental cycles (McBride et al., 1981; Moolenaar, 1998; Paoletti et al., 1998; Maier et al., 2000; White, 2009; Hinojosa et al., 2010).

The focus of this study was the influence of copper use in grape cultivation on the spatial abundance and function of soil microorganisms. Copper is said to reside in the topsoil and decrease with distance from the vine (Wightwick et al., 2006; Komárek et al., 2010;

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0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.06.003

Mackie et al., 2012). The question remains, how does copper affect soil quality within the vineyard system and where is its impact greatest? Soil microbial abundance and activity are heterogeneously distributed within the soil matrix at different scales ranging from millimeters to meters (Berner et al., 2011). This distribution is affected by the carbon/nitrogen ratio, the chemical composition, the porosity, the location of organic substrates and plant biomass, the grade of humification, the water content, the pH, and the heavy metal content of the soil (Buscot and Varma, 2005). Investigation of meso-scale (field-scale) distribution can be helpful in understanding multivariable interactions, which are difficult to observe on a larger scale (Philippot et al., 2009; Berner et al., 2011; Keil et al., 2011). Heavy metals, particularly, can modify spatial distribution of microbial abundance and activity by altering soil characteristics and reducing microbial biomass (Kandeler et al., 1996). In vineyard soils, copper has been found to diminish the rate of ammonification, which signifies an alteration in bacterial presence and/or functions (McBride et al., 1981). However, field knowledge regarding copper impacts specifically on soil microorganisms is limited (Dell'Amico et al., 2008; Wang et al., 2009; Brandt et al., 2010; Fernández-Calviño et al., 2010) and, as these communities maintain essential processes and support soil fertility, their upkeep is vital for viticultural and other agricultural systems. We expected that even in areas of low to moderate copper pollution, such as vineyards, soil microorganisms and enzymes involved in the nitrogen (N), phosphorus (P) and sulfur (S) cycling will be compromised. Therefore, it was the goal of this study to investigate meso-scale distribution of copper, soil properties and microorganisms within an organic vineyard and to identify the interactions between copper accumulation and the soil eco-system. We hypothesized that the spatial distribution of the activity (i.e. enzyme activity) and abundance of soil microorganisms is correlated with the spatial distribution of copper in the soil, thus, (i) areas of high copper will indicate areas of low soil microbial activity and (ii) copper distribution will influence the spatial distribution of the microbial community composition (i.e. phospholipid fatty acid composition) in the soil.

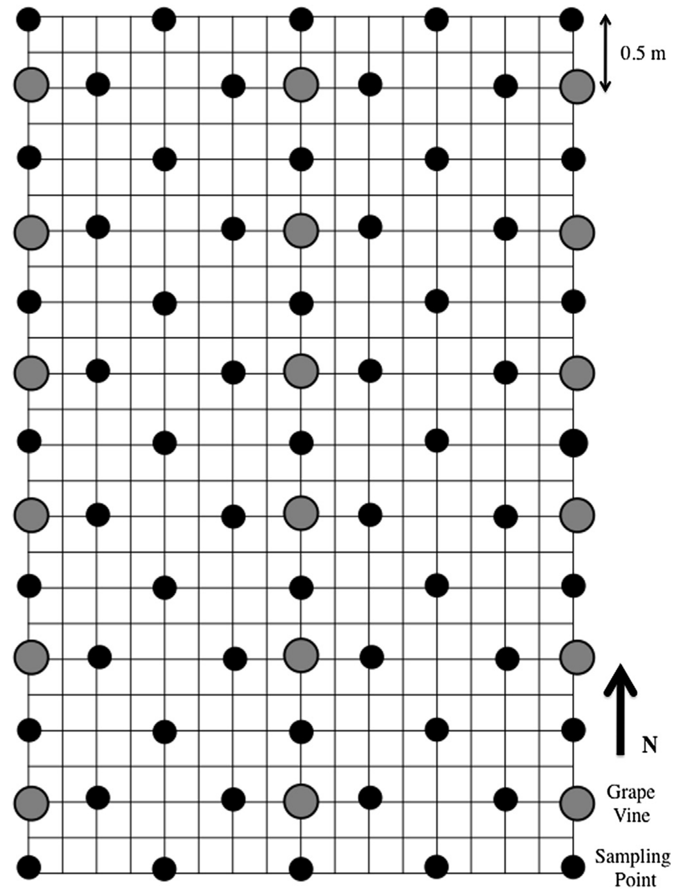

Fig. 1. Sampling grid. Each box within the grid represents 25 cm × 25 cm within a total area of 24 m^2 (4 m × 6 m). Numbers represent sampling points with distinct distances from the vine row (0 cm, 50 cm or 100 cm).

2. Materials & methods

2.1. Study area

Samples were taken from an organic vineyard in Brackenheim, Baden Württemberg, Germany (49°5′43.53″N, 9°2′57.93″E). The site has an elevation of approximately 230 m, an average precipitation of 650 mm and an average temperature between 7 °C and 14 °C. The vineyard was established in 1988 and has been under certified organic management since 1993. The soil type sampled on the site is an Umbric Leptosol. It is planted with Trollinger vines. Various species of grass were growing between each row. The site has a southern exposure and a slope of approximately 35%. Harvest residues (leaves, wood chips, pomace), mixed with an addition of wheat straw, were spread homogenously over the vineyard during a period of five years. Each year every second row is plowed. The last plowing event was in April of 2009, more than one year before soil samples were taken.

Since 1993, based on organic certification standards in Germany, the vineyard manager has sprayed a maximum of 3 kg y^{-1} ha^{-1} of copper solution on the grape vines (BIOLAND, 2011). Both copper oxychloride ($Cu_3Cl_2(OH)_4$) and copper hydroxide ($Cu(OH)_2$) have been used.

2.2. Soil sampling

Soil samples were taken in April 2010 prior to any copper solution treatment for the 2010 season. Fig. 1 illustrates the sampling pattern chosen based on maximal coverage of the plot and statistical accuracy. Each box represents 0.25 m by 0.25 m. There were a total of 59 sampling points, each either 0, 50 or 100 cm from the grape vine row. Within a row grape vines are 1 m apart, while the rows are separated by a distance of 2 m. The right interrow was plowed in 2009.

Soil cores (5.5 cm diameter) were taken to a depth of 10 cm. Samples were kept in a cooler and placed in a 4 °C refrigerator after sampling for a maximum of 5 days. The soil was sieved through 2 mm mesh metal sieves and was stored in a −20 °C freezer until further analyses. Samples for copper analyses were air-dried.

2.3. Plant sampling

Grass samples were taken on the same day as the soil samples. A 30 cm diameter metal ring was used to mark the area directly around the sampling spot. All living aboveground biomass within the ring was harvested and placed in plastic bags. Directly after sampling, the plant material was cut and dried in aluminum foil trays in an oven at 60 °C for 48 h and at 105 °C for 10 h to completely dry the samples.

2.4. Soil analyses

Total copper (Cu_T) was extracted by Aqua regia ($HNO_3 + HCl$) extractant (DIN ISO 11466, 1995). Ammonium nitrate extractable copper ($Cu_{NH_4NO_3}$) was extracted with ammonium nitrate (NH_4NO_3) and was chosen because it may provide a good indication of copper that is not complexed (Ettler et al., 2007; DIN ISO, 19730,