Science of the Total Environment 500–501 (2014) 34–43

Contents lists available at ScienceDirect

Science of the Total Environment

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

Cover crops influence soil microorganisms and phytoextraction of copper from a moderately contaminated vineyard

K.A. Mackie [a,*], H.P. Schmidt [b], T. Müller [c], E. Kandeler [a]

[a] Institute of Soil Science and Land Evaluation, Soil Biology Section, University of Hohenheim, Emil-Wolff-Strasse 27, 70599 Stuttgart, Germany
[b] Ithaka Institute, La Place 92, 1966 Ayent, Switzerland
[c] Institute of Crop Science, University of Hohenheim, Fruwirthstrasse 20, 70599 Stuttgart, Germany

HIGHLIGHTS

- This is one of the few studies used to measure Cu phytoextraction *in situ* in a vineyard.
- Variation of cover crop biomass determined Cu phytoextraction potential.
- Soil microorganisms showed Cu tolerance at moderate pollution levels (135 mg Cu kg^{-1}).
- Nutrient resources and environmental factors regulated soil microorganisms.

ARTICLE INFO

Article history:
Received 1 April 2014
Received in revised form 3 July 2014
Accepted 25 August 2014
Available online xxxx

Editor: Charlotte Poschenrieder

Keywords:
PLFA
Enzyme activity
Microbial biomass
PCA

ABSTRACT

We investigated the ability of summer (*Avena sativa* [oat], *Trifolium incarnatum* [crimson clover], *Chenopodium* [goosefoot]) and winter (*Vicia villosa* [hairy vetch], *Secale Cereale* L. [Rye], *Brassica napus* L. partim [rape]) cover crops, including a mixed species treatment, to extract copper from an organic vineyard soil in situ and the microbial communities that may support it. Clover had the highest copper content (14.3 mg Cu kg^{-1} DM). However, it was the amount of total biomass production that determined which species was most effective at overall copper removal per hectare. The winter crop rye produced significantly higher amounts of biomass (3532 kg DM ha^{-1}) and, therefore, removed significantly higher amounts of copper (14,920 mg Cu ha^{-1}), despite less accumulation of copper in plant shoots. The maximum annual removal rate, a summation of best performing summer and winter crops, would be 0.033 kg Cu ha^{-1} y^{-1}. Due to this low annual extraction efficiency, which is less than the 6 kg Cu ha^{-1} y^{-1} permitted for application, phytoextraction cannot be recommended as a general method of copper extraction from vineyards. Copper concentration did not influence aboveground or belowground properties, as indicated by sampling at two distances from the grapevine row with different soil copper concentrations. Soil microorganisms may have become tolerant to the copper levels at this site. Microbial biomass and soil enzyme activities (arylsulfatase and phosphatase) were instead driven by seasonal fluxes of resource pools. Gram+ bacteria were associated with high soil moisture, while fungi seemed to be driven by extractable carbon, which was linked to high plant biomass. There was no microbial group associated with the increased phytoextraction of copper. Moreover, treatment did not influence the abundance, activity or community structure of soil microorganisms.

1. Introduction

Due to the long history of application and continued use of copper containing fungicides in agriculture, copper (Cu) has accumulated within these topsoils (McBride et al., 1981; Mackie et al., 2012). Moderate levels of Cu have been shown to negatively affect macro-organisms, such as earthworms and plants, specifically in biomass and seed set, as well as organic matter decomposition (Moolenaar, 1998; Paoletti et al., 1998; Brun et al., 2003; Hinojosa et al., 2010). It severely decreases the functional diversity of the soil microbial community, impairs specific pathways of nutrient cycling and impacts soil fertility indicators at amounts as low as 140 mg Cu kg^{-1} (Kandeler et al., 1996; Fernández-Calviño et al., 2010; Hinojosa et al., 2010; Mackie et al., 2013). For these reasons, the European Union has set a limit on the amount of copper fungicide permitted for use in agriculture at 6 kg ha^{-1} y^{-1} (European Commission, 2007). However, as there are currently no viable alternatives in organic agriculture (Heibertshausen et al., 2006; La Torre et al., 2007) and as the potential for infection

* Corresponding author at: Emil-Wolff-Straße 27, 70599 Stuttgart, Germany. Tel.: +49 711 459 22825.
E-mail address: k.mackie@uni-hohenheim.de (K.A. Mackie).

http://dx.doi.org/10.1016/j.scitotenv.2014.08.091
0048-9697/

from plant pathogens increases with climate change (Salinari et al., 2006), Cu fungicides have not been prohibited and may even increase in the future.

In response to these negative effects, one possible solution is Cu removal through *in situ* accumulation by plants. Phytoextraction is the use of (hyper) accumulator plants to remove metals/metalloids from the environment by taking them up into their shoots and subsequently removing them from the contaminated area (Wenzel, 2009). It is a low cost, environmentally sensitive method, which displaces Cu from the environment, but does not require full soil removal impractical in perennial agriculture and/or large tracts of land (Gómez-Sagasti et al., 2012; Meier et al., 2012a).

Particular microorganisms prefer specific plants and plant species support and encourage associated microorganisms (Terry and Bañuelos, 2000; Wardle et al., 2004; Castaldi et al., 2009; Narula et al., 2009; Epelde et al., 2010; Haferburg and Kothe, 2010). The most recent mechanism for enhancing phytoextraction is inoculating the soil with bacteria producing siderophores, which assist in chelating Cu, suggesting that microorganisms may play a significant role in successful phytoextraction (Haferburg and Kothe, 2010; Rajkumar et al., 2010). Phytoextraction, with and without inoculated microbial assistance, has been successfully investigated in laboratories and greenhouses (Poschenrieder et al., 2001; Brun et al., 2003; Kos and Leštan, 2004; Song et al., 2004; Chen et al., 2006; Meier et al., 2012b; Ma et al., 2009; Zeremski-Škorić et al., 2010; Andreazza et al., 2011). However, phytoextraction of Cu has seldom been monitored in the field (Poschenrieder et al., 2001; Clemente et al., 2005; Brej and Fabiszewski, 2006).

The aims of this study were to investigate the *in situ* relationship between microorganisms and plants within Cu contaminated topsoil, and identify the practicability of phytoextraction and monitor ecosystem services, such as soil health and nutrient mineralization, using microbial biomass, enzyme activity and phospholipid fatty acids (PLFAs) (Epelde et al., 2014). Enzyme activities are consistent biological indicators of heavy metal pollution and PLFA patterns have been seen to change quickly with changing soil metal concentration in as little as two weeks (Frostegård et al., 1996; Hinojosa et al., 2010; Ge and Zhang, 2011). Additionally, PLFAs identify microbial groups, which may indicate whether such groups naturally support increased phytoextraction of specific plants *in situ*. This project focused particularly on vineyards, a representative system of fruit production where Cu is most often applied. In vineyards with sufficient water, i.e. central Europe, cover crops grown between the vine rows have been observed to increase desirable properties in soil and vine performance (Morlat and Jacquet, 2003; Guerra and Steenwerth, 2012). Therefore, phytoextraction has the potential to improve grape production and soil fertility in vineyards, while removing Cu from the topsoil. We investigated whether (i) the efficiency of phytoextraction depends on plant species, plant community composition, distance from vine row, and growing season, (ii) if effective phytoextraction is associated with a microbial community structure, and (iii) if diverse plant communities will mitigate the negative influence of Cu on soil microorganisms.

The plants chosen within the present study were a mixture of Cu adapted plant species with high biomass production known from laboratory research as well as common vineyard cover crop species in central Europe not yet researched for Cu removal potential (Poschenrieder et al., 2001; Kos and Leštan, 2004; Andreazza et al., 2010; Haferburg and Kothe, 2010). Moreover, diverse plant systems, in comparison to monoculture systems, have been seen to reduce the impact of pollution by increasing microbial diversity and activity (Yang et al., 2007). Therefore, a treatment consisting of a mixture of plant species has also been added.

2. Materials & methods

2.1. Study site and experimental setup

This field experiment was designed specifically to understand the *in situ* potential of cover crop plant species to accumulate Cu and was established at the Ithaka Institute in Canton Wallis, Switzerland (46°16′N, 7°24′E) in the spring of 2012. The study site is a vineyard planted with Pinot noir (*Vitis vinifera* L.) with a southeastern exposure at an elevation of 760–780 m a.s.l. The site has a mean annual precipitation of 550 mm and an average temperature of 11.4 °C. The soil is predominately calcaric Leptosol with a bulk density of 1.34 g cm^{-3} and 47% gravel (>2 mm). It has a pH_{CaCl_2} of 7.5, C_T of 37 g kg^{-1}, N_T of 4.1 g kg^{-1}, and a total microbial biomass of 694 μg C_{mic} g^{-1} soil. The vines are planted at a distance of 1 m and a row width of 3 m with an adapted Mosel arch training system. At their maximum, vine plant height from July until October averages 2.50 m. The Ithaka Institute manages the vineyard organically; however, for this field experiment the use of copper fungicides was excluded during the trial period so that the samples would not be superficially contaminated. Plant and compost derived solutions (100 L compost tea ha^{-1}, 100 g NU-Film ha^{-1}, 2 kg stinging nettle ha^{-1}, 1 kg horsetail ha^{-1}, and 100 g sage ha^{-1}) and sodium bicarbonate were sprayed to stimulate the plants natural defenses and protect against *Oidium* and *Peronospora*, respectively. The initial total Cu in soil was 135 mg Cu_T kg^{-1} soil (95.9 kg Cu_T ha^{-1}), while the exchangeable Cu fraction was initially 48.6 mg Cu_{DTPA} kg^{-1} soil (34.5 kg Cu_{DTPA} ha^{-1}). Soil mass was calculated using soil depth, bulk density and fraction of coarse material (>2 mm) in order to calculate kg soil Cu ha^{-1}. The site was superficially tilled (8 cm) before seeding in April 2012.

We designed four summer (2012) treatments followed successively by four winter (2012/2013) treatments. The summer crops were seeded in April 2012 and harvested in August 2012; a) *Avena sativa* (oat) 12 g m^{-2}, b) *Trifolium incarnatum* (clover) 3 g m^{-2}, c) *Reseda luteola* (*Reseda*) 0.3 g m^{-2}, and d) a mixture of treatments 1, 2 and 3 (summer mix) at 4, 1 and 0.1 g m^{-2}, respectively. Unfortunately, although a drought tolerant species of *Reseda* was chosen, the species did not germinate well in all plots and *Chenopodium album* L., *Chenopodium hybridum* L. and *Chenopodium fielfolium* Sm. (*Chenopodium*) spontaneously took over. Therefore, *Chenopodium* was harvested and sampled in both treatments c and d. The winter crops were seeded in September 2012 and harvested in May 2013; a) *Vicia villosa* (Hairy vetch) 10 g m^{-2}, b) *Secale Cereale* L. (Rye) 20 g m^{-2}, c) *Brassica napus* L. partim (Rape) 0.6 g m^{-2}, and d) a mixture of treatments 1, 2 and 3 at 3.3, 6.7 and 0.2 g m^{-2}, respectively (winter mix). The treatments were set up in a random block design, where each plot had an area of 36 m^2 and spanned three vine rows. The treatments were replicated five times. Plant and soil samples were taken over three sampling dates: June 2012 (normal mowing date for summer cover crop), August 2012 (extended mowing date and plant senescence), and May 2013 (normal mowing date for winter cover crop). As there is a buffer time between the summer crop harvest and winter crop seeding, two dates were chosen to assess whether the extended time could achieve higher extraction rates. As summer crop seeding has to be done within a specific time window, there was no possibility to extend the winter crop harvest and only one date was chosen. Samples were taken on either side of the middle vine row, reducing edge effects, at both 70 cm and 120 cm away from the vine row in order to represent high Cu areas and high plant biomass areas, respectively (S.1).

2.2. Plant sampling and analyses

Plant samples were taken directly above and surrounding the soil corer with the guidance of a plastic ring (= 30.5 cm) and were removed prior to soil sampling. All living above ground biomass was cut and placed directly into paper bags. The samples were placed in a 60 °C oven for at least 48 h after which they were weighed for dry mass (DM).

Dried plant material was finely milled (SM1 Schneidemühle, Retsch GmbH, Germany) and underwent microwave digestion in nitric acid following the method of VDLUFA (2011a). To determine the Cu content of the plant biomass the method of VDLUFA (2011b) was used and