Copper dynamics and impact on microbial communities in a vineyard soil. Influence of the soil organic status

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Abstract

In a coupled microcosm and lysimeter study, the effect of the soil organic status (SOS) on copper dynamics and impact was investigated in a vineyard soil that had been amended with varying types of organic matter during a previous long-term field experiment. Soil microcosms and lysimeters were contaminated at 240 mg Cu/kg and incubated for 12 months. Copper distribution and dynamics were assessed in the solid matrix by size fractionation and sequential extraction procedures and in the soil solution by measuring total and free exchangeable copper concentrations. Copper bioavailability was also measured with a whole-cell biosensor. Variations in microbial communities were assessed by means of bacteria enumeration and characterization of genetic structure using ARISA (Automated-Ribosomal-Intergenic-Spacer-Analysis). Results showed that copper distribution, speciation and bioavailability are strongly different between organically amended and non-amended soils and largely driven by the SOS. Unexpectedly, in solution, bioavailable copper correlated with total-copper, suggesting that non-free copper remains bioavailable to microorganisms. Similarly the observed differential copper impact on micro-organisms suggested that organic matter controlled copper toxicity through the control of the structure of the microbial communities in the different soils. In the two soils, microorganisms were dominantly distributed in the <20µm fraction, where native and spiked copper also accumulate. This soil smallest size fraction is also the one that more releases free and bioavailable copper, explaining thus the important amount of copper-resistant bacteria inhabiting this fraction. A clear relation between copper speciation, bioavailability, distribution and impact was established in the present study and will permit better predicting the fate and impact of trace elements in complex heterogeneous soils.

Key Word

Copper speciation, soil, organic matter, impact, bioavailability, micro-organism, biodiversity.

Introduction

Since many decades, copper is widely applied on soil through various land use practices (biosolids application, mining, pesticides use, etc.) leading to large increases of total Cu content, i.e., up to 1000 mg /kg of soil. At such high concentrations, copper has detrimental and irreversible effects on soil biocenosis and impacts the population structure of soil microbial communities. The impact of copper depends on its speciation, which controls its bioavailability. Factors such as pH, redox potential, mineralogical, and soil organic matter contents and types are all known to affect copper speciation (Tom-Peterson *et al.* 1999, Sauvé *et al.* 2008, Dumestre *et al.* 1999). Among the soil constituents, soil organic matter (SOM) is one of the more reactive towards copper, as demonstrated by several studies with soil organic matter or with organic ligands (Dumestre *et al.* 1999). Organic ligands interact with trace elements essentially through complexation reactions (Guiné *et al.* 2006). Consequently, the correlation between total and bioavailable copper content was shown to be poor in complex soil systems (Kunito *et al.* 1999). In order to better understand the reasons of this poor correlation, we studied the fate of copper in a soil variably amended with organic matter under different physical chemical conditions and at different scales.

Material and methods

The studied soil is a vineyard soil from Macon (France) variably amended with organic matter for over twenty years After collection and sieving at 4 mm, the control and OM amended soils were incubated in lysimeters at 20°C in the dark, and supplemented with water or copper solution at 240 mg Cu./kg dry soil. At given times the different soils were submitted to granulometric fractionation (Jocteur-Monrozier *et al.* 1991) in order to obtain 4 size fractions (>250µm, 250-63µm, 63-20µm, and <20µm). Copper distribution and dynamic were assessed i) in the solid matrix after size fractionation and sequential extraction procedures and ii) in the soil solution by measuring total and free exchangeable copper concentrations (Lejon *et al.* 2008). Copper bioavailability was also measured with a whole-cell biosensor (Lejon *et al.* 2008). Variations in microbial communities were assessed by means of biomass-C measurements and characterization of genetic structure using the ARISA fingerprinting technique (Lejon *et al.* 2007).

Results and discussion

Mineral and organic distribution in the size fractions of the amended and non amended soils

The mass distribution of the different size fractions confirmed the soil as a loamy clay (Figure 1a). No significant differences between the two soils were observed indicating that the organic amendment did not change the size fraction distribution, i.e. soil structure. The size fractions below 63 µm were dominant and represented up to 80 % of the total soil mass. Clay particles account for more than 50%.

The carbon concentration in the OM amended soil is slightly higher than in the non amended control soil (Figure 1b). This was observed also on the distribution of the organic matter in the different fractions of the two soils (Figure 1b). The differences were observed mostly in the coarser fractions where large fragments of raw organic matter coming from the compost amendment are observed. The micro-aggregated fraction (63-20µm) is OM-poor and does not vary significantly between soils. Similarly the finest size fraction (<20µm) that contains the more evolved OM was only slightly enriched by the compost amendment.

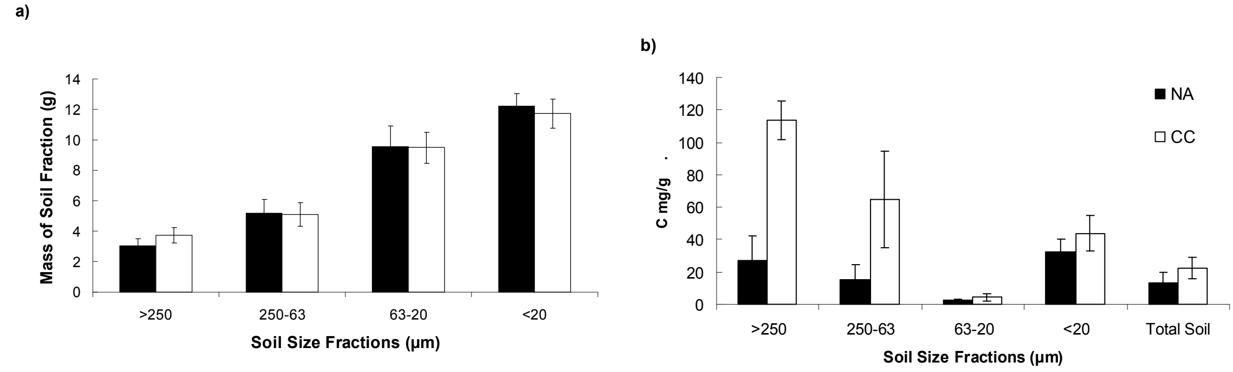

Figure 1. Distribution of the mass (a) and the total organic carbon (b) of the Non-Amended (NA) and compost amended soil size fractions. Error bars correspond to the standard deviations calculated with triplicates.

Distribution of copper in the size fractions

The distribution of copper in the fractions of both soils is presented in Figure 2. The results show that the distribution of native copper (low amount compared to the spiked copper) was shown to be very similar than that of the spiked copper. These two types of copper accumulated preferentially in the finest fraction (<20µm) of the two soils. The solid speciation measurements (sequential extractions) revealed furthermore that copper is firstly bound to OM and then redistributed over time among OM and reactive iron and manganese oxides (Lejon *et al.* 2008), especially in the conifer compost amended soil. Copper speciation measured in solution after exchange with Ca^{2+} showed a fast decrease of free copper concentration during the two first days and then a stabilisation, whatever the organic matter status (Lejon *et al.* 2008). Figure 3 shows that copper released from the soil size fractions (exchanged with Ca^{2+}) is dominated by the smallest fraction (<20µm), that contributes to about 50% of copper in solution. The observed total copper exchange is in agreement with the solid copper distribution measured in the soil size fractions. However the release of free copper varies with the size of the fraction, clearly indicating a different copper reactivity with size fractions constituents of both soils. Especially, copper present in the coarse fractions (>63 µm) appears strongly bound with the poorly-degraded organic matter that is thus less available (free) than copper weakly bound with the constituents of the smallest fractions (<20 µm) (Figure 3). This is also in agreement with the observed enrichment in copper-resistant bacteria in the smallest soil fractions presented below.

Copper impact

The results on the impact of Cu on the distribution of total and Cu-resistant cultivable bacteria in both soils are presented in Figure 4. These results showed that about 99% of bacteria are located in the fine fractions (<20µm) of the two soils with a slight enrichment of bacteria (total and Cu-resistant) over time. Bacteria distribution is well correlated with the distribution of the readily degradable OM in the soil (<20µm). Cu-resistant bacteria represented about 0.1% of the total heterotrophic bacteria in all soil fractions and are the more abundant in the <20µm soil fraction that also accumulates dominantly native and spiked Cu (Figure 2).

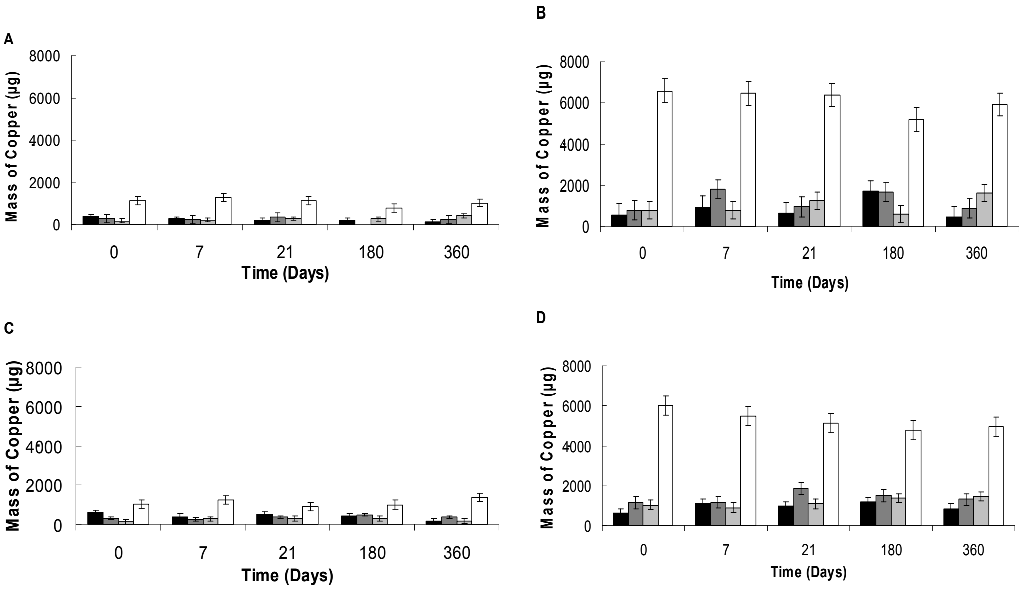

Figure 2. Temporal evolution of the mass of copper in soil fractions (>250µm : black; 250-63µm : dark grey; 63-20 µm : light grey; <20µm : white). A: Non amended and uncontaminated control soil. B: Non amended control soil contaminated at 240 mg/kg of copper. C: Uncontaminated conifer compost amended soil. D: Conifer compost amended soil contaminated at 240 mg/kg of copper.

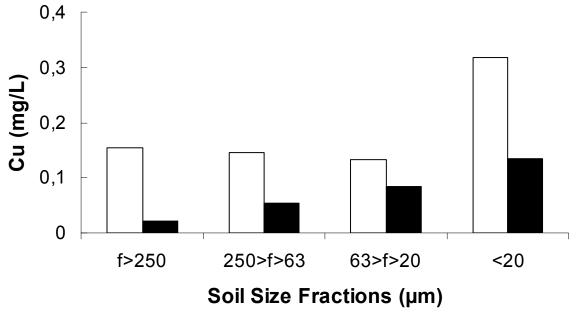

Figure 3. Total (white bars) and free (dark bars) copper concentrations exchanged with Ca^{2+} from the fractions of the compost-amended soil.

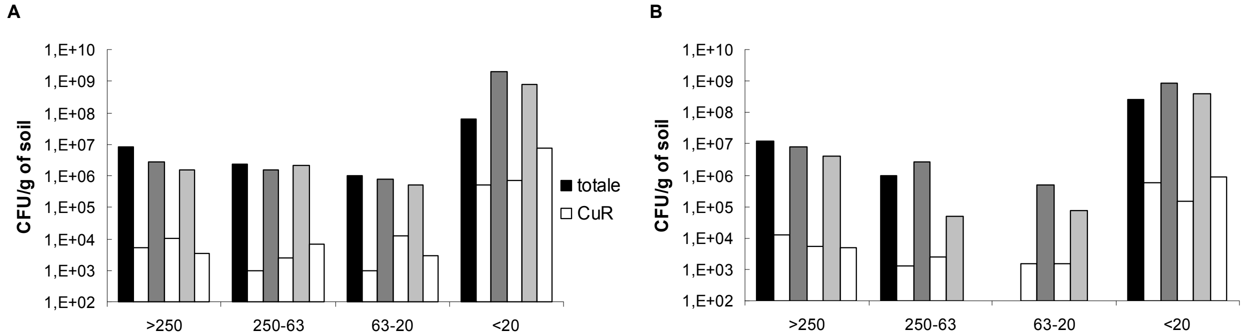

Figure 4. Temporal evolution (T0j : black, T7j : dark grey and T21j : light grey) of the distribution of total heterotrophic (black) and copper resistant (white) bacteria in fractions of the non-amended control soil (A) and the conifer compost amended soil (B).

The results about the toxicity of copper on soil microbial communities (Figure 5) revealed an impact of copper in both soils: the ARISA profiles were always significantly different between the control (water) and the copper (Cu) contaminated soils in the unfractionated soils and in the four size fractions. However, when considering the temporal evolution of the ARISA profiles, the smallest fraction appears clearly as the more impacted by copper spiking (Fig. 5a and b, right), especially in the non amended soil, indicating that the

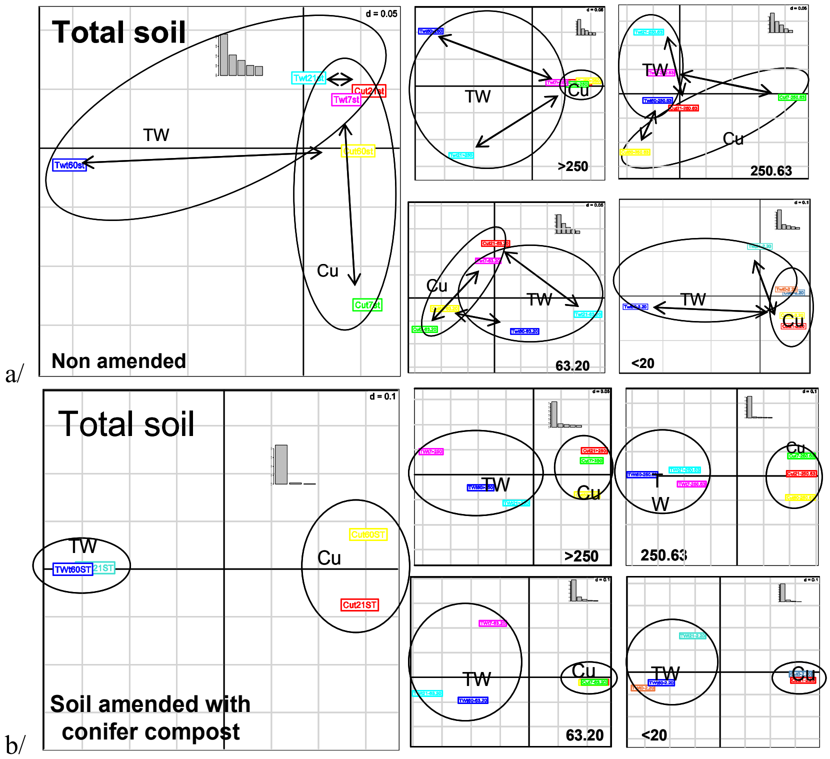

Figure 5. Principal component (PC1 × PC2) factorial maps generated from B-ARISA profiles obtained from the total (left) and size fractions (right) of the non-amended (a) and compost amended (b) soils (at t0d, t7d, t21, and t60d) for the water (W) and copper (Cu) treatments.

compost amendment protected microorganisms from copper toxicity to an extent that still needs to be established. The observed variations consisted mostly of an enrichment of *Actinobacteria*, especially in the non amended soil where copper is the more bioavailable (Figure 2 and 3). These results confirm that the structure and the sensitivity to metal contamination of the microbial community is controlled by the soil organic status (Lejon *et al.* 2008).

Conclusion

In the present study, we showed that the organic status of natural soils controls largely the solid and liquid speciation of copper as well as its availability to microorganisms. The SOS was shown to influence strongly the microbial community structure and its sensitivity to a Cu contamination. Cu was shown to be dominantly distributed in the smallest size fractions of both control and amended soils. This distribution was well correlated with organic matter and microorganisms distributions in these two soils. The preferential impacts of copper observed in the non amended soil and in the smallest size fractions demonstrated that copper toxicity and impact is controlled by the reactivity of the soil fractions (amount of reactive organic matter). This reactivity controls especially the release and the liquid speciation of Cu and thus bacteria-metal contact.

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