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# Copper dynamics in vineyard topsoils as affected by the supply of aerated compost tea: Insights from a batch experiment<sup>☆</sup>

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## ABSTRACT

Aerated compost teas (ACTs) are rich in soluble humic substances (SHS) that have high affinity for metals, notably Cu. Using a batch experiment, we measured the extent to which two ACTs altered Cu dynamics in vineyard topsoils one day and 21 days after their addition. Soils were extracted with 0.01 M KCl, and total Cu concentration, free Cu ion fraction and size distribution of Cu ligands were measured in the extraction solution to assess the impact of ACT on the mobility of Cu. Diffusive gradient in thin film (DGT) measurements were carried out to assess the effect of ACT on Cu bioavailability, and the dissociation rate of Cu-SHS complexes was measured. The results revealed that ACT increased the mobility of Cu from a factor 1.2 to 5.8 depending on the soil, the ACT and the incubation time. Cu mobilization was associated with an increase in absorbance at 254 nm and a decrease in the free Cu ion fraction in the KCl extract. Associated with the strong agreement between the size distribution of SHS and that of Cu ligands in the KCl extract of soils treated with ACT, these results showed that Cu was mobilized through complexation by the SHS present in ACTs. A fraction of the SHS supplied with ACTs sorbed onto the soil constituents, notably in calcareous soils where this fraction reached 86% for ACT B. Between 15% and 50% of the SHS remaining in solution degraded between day one and day 21 under the presumed action of microflora. This explains why the Cu mobilization efficiency of ACTs was on average lower in calcareous soils than in non-calcareous soils, and decreased with time. Lastly, ACT increased the bioavailability of Cu in soils from a factor 1.3 to 4.2, due to the relatively high dissociation rate of Cu-SHS complexes.

## 1. Introduction

Due to its fungicidal properties, copper (Cu) is widely used in winegrowing to prevent downy mildew (*Plasmopara viticola*) of vine. Copper-based fungicides are applied to vine leaves before rainfall and, depending on the fungicide formulation and the duration and intensity of the rain, between 30 and 70% of the copper applied can be leached out and accumulate in the topsoil (Pérez-Rodríguez et al., 2016). For these reasons, accumulation levels of up to several hundred mg per kg of Cu can be found in vineyard topsoils (Komárek et al., 2010; Ballabio et al., 2018), notably in historic vineyards. Although demonstrations of Cu ecotoxicity at plot level are rare (Cornu, 2023), at the concentrations found in vineyard soils, Cu can theoretically harm soil biological communities, from macro- to micro-organisms (Adrees et al., 2015). For example, detrimental effects of Cu were observed from 250 mg Cu kg<sup>-1</sup> on rhizobacteria (Sharaff and Archana, 2015), from 300 mg Cu kg<sup>-1</sup> on

enchytraeids (Maraldo et al., 2006) and from 50 mg Cu kg<sup>-1</sup> on earthworms (Bundy et al., 2008; Zhou et al., 2013). At similar concentrations, excess Cu can also impair plant growth by inhibiting seed germination (Baruah et al., 2019), or by disrupting, for example, photosynthesis and cell structures through the formation of reactive oxygen species (Mir et al., 2021). In contexts prone to soil erosion such as in sloping vineyards, Cu can be partially transferred to adjacent aquatic environments, where it can theoretically reduce diversity and damage the ecosystem functions provided by aquatic organisms (Pesce et al., 2024). The ecotoxicity of Cu in vineyard soil is usually managed by limiting its bioavailability, i.e. the degree to which Cu can be taken up or metabolized by biological receptors (ISO 17402). These management practices generally involve liming and/or adding solid organic matter to enhance Cu binding to the solid phase (Brunetto et al., 2016).

Aerated compost teas (ACTs) are liquids made by steeping solid compost in aerated water for a couple of days (Ingham, 2005), after

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which the teas are used as organic fertilizers, notably in organic farming, as they contain soluble nutrients (De Corato et al., 2020) and phytohormones (Pant et al., 2012) that are readily absorbed by plants. ACTs also contain beneficial fungi and bacteria, which may make them effective in preventing certain plant diseases such as the botrytis bunch rot or the powdery mildew of vine (Palmer, 2009; Evans et al., 2013). Although the plant growth promoting effect likely varies considerably among ACTs depending on the extraction time, the compost/water ratio and the characteristics of the compost, ACTs are an attractive option that is increasingly used in organic viticulture (Pilla et al., 2023), as they can be made by winegrowers themselves. However, ACTs also contain soluble humic substances (SHS) that derive from the extraction and the transformation of dissolved organic matter during the infusion process (De Corato et al., 2020), and have been shown to complex (Li et al., 2021) and mobilize Cu in soil (Borggaard et al., 2019). Indeed, SHS are made of humic acids (HA) and fulvic acids (FA) in proportions that depend on the original organic matter used to make the compost tea (Tahiri et al., 2014), and whose carboxylic and phenolic moieties exhibit high affinity for metals (Milne et al., 2003). This raises the question of the effect ACT may have on the availability and ecotoxicity of Cu in vineyard soils.

A recent study by our team (Eon et al., 2023) revealed that the repeated addition of ACT increased the mobility and the phytoavailability of Cu in a vineyard soil. To gain insight into how ACTs alter the dynamics and the effect of Cu in vineyard topsoils, we needed to investigate how the SHS they contain behave in soils in terms of sorption, complexation of Cu and degradation. In the present study, we used a batch experiment to compare the effect of ACTs made from contrasted composts on the dynamics of Cu in a series of vineyard topsoils with contrasting characteristics. The first objective was to identify the characteristics of soil and of ACT that determine the extent to which ACTs increase the mobility and the bioavailability of Cu in soil. The second objective was to demonstrate the key role played by SHS in mobilizing and increasing the bioavailability of Cu in soils by measuring the size and lability of Cu-SHS complexes that form in pore water after the addition of ACTs. The third objective was to assess the duration of the ACT effect on the mobility of Cu in the soil by monitoring changes in the metal mobilization efficiency of ACTs over time.

## 2. Materials and methods

### 2.1. Experimental design

The four vineyard soils used in this study differed in their Cu contents and in several properties including pH, texture and CaCO<sub>3</sub> content that can alter the dynamics of SHS and Cu. The impact of ACTs on Cu dynamics was therefore of particular interest to compare the four soils. Two aerated compost teas were compared, one extracted from a compost made from green waste (ACT A) and the other one from a compost made from animal manure (ACT B). Three replicates were performed per soil and per treatment (ACT, incubation time). For each soil, six soil disks were supplied with ACT A, six others with ACT B and the last six with ultra-pure water (control), and incubated for one day or 21 days before extraction.

### 2.2. Soils

Four contrasted vineyard topsoils were used to investigate the impact of ACT on the dynamics of Cu. Two of the soils were non-calcareous (Ncalc1 and Ncalc2) and the two others were calcareous (Calc3 and Calc4). Ncalc1, Ncalc2 and Calc3 soils were collected in 2021, 2018 and 2018, respectively, in the AOC (i.e. registered designation of origin) zone of Pessac-Léognan (south of Bordeaux, France), while Calc4 soil was collected in 2018 in the AOC zone of Medoc (north-west of Bordeaux, France). The soil samples were air dried and sieved to 2 mm before analysis. Table 1 summarizes the results of the soil analyses

**Table 1**

Selected physical and chemical properties of the four soils used in this study. The parameters were determined on soils sieved to 2 mm: granulometry (NF X 31–107), organic matter (ISO 10694), cation exchange capacity (NF X 31–130), CaCO<sub>3</sub> (ISO 10693), pH in water (ISO 10390), total Cu (ISO 11466), EDTA-extractable Cu (NF X 31–120) and water holding capacity (Revaillot et al., 2021).

Parameter	Unit	Ncalc1	Ncalc2	Calc3	Calc4
Sand	g kg <sup>-1</sup>	774	870	584	554
Silt	g kg <sup>-1</sup>	145	89	171	218
Clay	g kg <sup>-1</sup>	81	41	245	228
OM	%	3.2	1.8	2.7	1.8
C:N ratio		14.0	18.1	11.2	8.7
CEC	cmol <sub>c</sub> kg <sup>-1</sup>	5.2	4.0	18.6	8.8
CaCO <sub>3</sub>	g kg <sup>-1</sup>	<1	<1	132	20
pH <sub>water</sub>		6.2	7.2	8.1	8.5
Total Cu	mg kg <sup>-1</sup>	90	218	126	476
Cu <sub>EDTA</sub>	mg kg <sup>-1</sup>	47	136	61	107
Cu <sub>EDTA</sub> :OM ratio <sup>a</sup>		15	76	23	59
Water holding capacity (w/w)	%	11.7	5.8	23.1	20

OM: organic matter; CEC: cation exchange capacity; WHC: water holding capacity.

<sup>a</sup> Cu<sub>EDTA</sub> in mg kg<sup>-1</sup> and percentage organic matter.

conducted by a soil-testing laboratory (LAS, INRAE Arras, France), using standard methods. The total concentration of Cu in the four soils ranged between 90 and 476 mg kg<sup>-1</sup>, which is the same as or higher than the mean concentration of Cu found in the topsoil of French vineyards (Ballabio et al., 2018). The two non-calcareous soils had a sandy texture, and differed in their pH, organic matter content and total Cu content such that the concentration of EDTA-extractable Cu (Cu<sub>EDTA</sub>) was 2.9 times higher in Ncalc2 soil than in Ncalc1 soil. The two calcareous soils were characterized by higher clay content (23.6% on average, sand clay loam texture), higher pH (pH > 8) and higher cation exchange capacity (CEC) than the two non-calcareous soils. Despite its calcareous nature, Calc4 was characterized by a high Cu<sub>EDTA</sub> content (>100 mg kg<sup>-1</sup>) due to its very high total Cu content and its rather low OM content. In the Ncalc2 and Calc4 soils, the EDTA-extractable Cu (Cu<sub>EDTA</sub>) to OM ratio was close to the threshold limit of Cu toxicity proposed by Braun (2019) for cereals.

### 2.3. Aerated compost teas (ACTs)

The two aerated compost teas (ACT A and ACT B) used in this study were produced using commercially available composts. ACT A was extracted from a compost made from green waste collected from parks and gardens (TerraKtiv®, Klassman-Deilmann), and ACT B from a compost made from animal manure and grape pomace (Biofumur®, Florentaise). Both ACTs were prepared 3 days before they were added to the soils by adding 3 L of deionized water to 900 g of a compost - for more details see Eon et al. (2023). Table 2 summarizes the physico-chemical characteristics of the ACTs used in this study. Both ACTs had an alkaline pH (pH > 9) and contained high concentrations of organic (TOC >1 g L<sup>-1</sup>), aromatic (SUVA >25 L g<sup>-1</sup> cm<sup>-1</sup>) and humified (HIX >15) carbon. The higher absorbance at 254 nm (A<sup>254</sup>) of ACT B suggests that the concentration of SHS was significantly higher in ACT B than in ACT A (Weishaar et al., 2003). The proportion of humic acids (HA) was also higher in ACT B (72%) than in ACT A (26%). The concentration of macronutrients and micronutrients also differed in the two ACTs. ACT B had higher concentrations of N, P, Ca, Al, Fe and Zn but lower concentrations of Mg and Si than ACT A. The concentration of Cu was 1.5 mg L<sup>-1</sup> in ACT A and 1.0 mg L<sup>-1</sup> in ACT B, meaning the amount of Cu added to the soil through the addition of ACT (see section 2.4) was negligible (<0.05%) compared to the amount of Cu originally present in the four soils. Size-exclusion chromatograms (suppl. Fig. F1) showed that the size distribution of Cu ligands ranged from 5 to 75 kDa in both

**Table 2**

Selected physical and chemical properties of the aerated compost teas (ACT) used in this study, after centrifugation and filtration at 0.2  $\mu\text{m}$ . TOC stands for total organic carbon,  $\text{SUVA}_{254}$  for specific UV-absorbance at 254 nm, and HIX for humification index. pH and  $A^{254}$  were measured using the procedure described in section 2.4. Conductivity was measured using a polycarbonate conductivity electrode (LE703, Mettler Toledo), total concentrations of organic carbon (TOC) and nitrogen (TN) were measured by combustion catalytic oxidation (VCSH, Shimadzu), and total concentrations of Al, Fe, Mn, Cu, Zn, Si, Ca, Mg, P and S were determined by ICP-OES (ICAP 6300, Thermo-Fisher). The distribution of humic and fulvic acids was measured using the extraction scheme described by Thurman and Malcolm (1981) for the isolation and purification of HA and FA from aqueous samples.

Parameter	Unit	ACT A	ACT B
pH		9.3	9.3
Conductivity	$\text{mS cm}^{-1}$	28.9	26.1
TOC	$\text{g L}^{-1}$	1.89	2.62
$\text{SUVA}_{254}$	$\text{L g}^{-1} \text{cm}^{-1}$	28.6	60.3
$A^{254}$		54	158
HIX	$\text{cmol}_+ \text{kg}^{-1}$	17.0	23.7
Humic acids	%	26	72
Fulvic acids	%	74	28
Macronutrients	$\text{mg L}^{-1}$		
N		297	559
P		104	1941
S		287	267
Ca		58	105
Mg		169	40
Micronutrients and trace metals	$\mu\text{g L}^{-1}$		
Al		0.09	0.44
Fe		0.56	7.7
Mn		< LOQ	1.9
Cu		1.5	0.98
Zn		0.06	1.6
Si		65	21

LOQ: limit of quantification.

ACTs, and closely matched the size distribution profile of compounds absorbing at 254 nm, notably in ACT A (see section 2.8 for details on the method).

#### 2.4. Soil incubation

Disks made of dry soil (weight 5 g, thickness 3 mm, diameter 32 mm) were placed in plastic lids, rewetted with water to 70% WHC and equilibrated for 2 weeks at 20 °C before ACT was added. This delay avoided the microbial flush that follows soil rewetting (Birch, 1964) and impacts the dynamics of Cu in soils (Amery et al., 2007). Each soil disk was supplied with 145  $\mu\text{L}$  of either ACT A, ACT B or ultra-pure water (control), after which sufficient ultrapure water was added to reach 100% WHC. Six soil disks per soil and per ACT treatment were incubated in the dark at 20 °C and sampled after one day and after 21 days.

#### 2.5. Soil extraction and analyses of the extraction solution

Soil extraction was performed as follows: 5 g of wet soil were transferred to a 15 mL polypropylene tube, shaken with 12 mL of 0.01 M KCl for 1 h at 20 °C, centrifuged at 4500 rpm for 10 min, and the supernatant was then filtered through 0.2  $\mu\text{m}$  cellulose acetate filters (037042, ClearLine). pH and  $A^{254}$  were measured immediately after extraction. The pH was measured using a combined pH electrode (11786348, Fisher Scientific) and  $A^{254}$  was measured by UV-VIS spectrometry using a microplate reader (Synergy H1, Biotek, quartz glass microplate, path length of 1 cm). The extraction solution was then divided into two subsamples. One subsample was acidified with 2%  $\text{HNO}_3$  (v/v) to measure the total concentration of Cu by ICP-OES (ICAP 6300, Thermo Fischer). The second subsample was stabilized with 1 mM  $\text{NaN}_3$  to measure free Cu ion activity using a cupric ion-selective electrode (ISE, 9629BNWP, Thermo Scientific Orion) following the

procedure described in Cornu et al. (2019). Briefly, each day the ISE was calibrated in the 6.9 to 13.7 range of pCu ( $=-\log_{10} a(\text{Cu}^{2+})$ ) in Cu solutions buffered with iminodiacetic acid and potassium phthalate. The calibration curve ( $r^2 = 0.983$ ,  $n = 27$ ) showed 88% slope efficiency compared with the theoretical Nernstian slope. The concentration of free Cu ion was calculated from pCu and the  $\text{Cu}^{2+}$  activity coefficient. The latter was given by the extended Debye-Hückel equation presented in Ritsema (1993), assuming the ionic strength of all the extraction solutions was fixed by the 0.01 M KCl background electrolyte.

#### 2.6. Dissociation rate of Cu-SHS complexes

The dissociation rate of Cu-SHS complexes was measured using the competitive ligand exchange method of Degryse et al. (2006) with modifications. Briefly, Chelex-100 resin (100–200 mesh, Na form, Bio-Rad) was converted to the K-form by putting it in contact with 1 M KCl + 2 mM MES (pH 6) for 2 h under shaking. Solutions were prepared containing 10 mM KCl, 2 mM MES (pH 6), 0.1 mM ligand and 10  $\mu\text{M}$  Cu ( $\text{NO}_3$ )<sub>2</sub>. The ligands compared were ACT A, ACT B, nitritotriacetate (NTA) and N-(2-hydroxyethyl)ethylenediamine-N,N',N'-triacetate (HEDTA). The NTA and HEDTA were added to check the accuracy of our results and to provide a reference for, respectively, fast and slow dissociation rates of Cu-ligand complexes. Speciation was calculated using Visual MINTEQ ver. 3.1 (Gustafsson, 2020) and showed that Cu was almost completely complexed (>99%) in these solutions. Chelex resin was added to the agitated solution at 20 °C, in a solution with a ratio of 10 g per 50 mL of solution. Subsamples (500  $\mu\text{L}$ ) were removed from the solution at different times depending on the lability of the Cu complex (up to 600 s), directly diluted and filtered at 0.2  $\mu\text{m}$ , then acidified with  $\text{HNO}_3$  and analyzed by ICP-OES (ICAP 6300, Thermo-Fisher). The concentration of the Cu-ligand complex at any time ( $t$ ) is given by eq. (1), where  $C_{\text{CuL}}^0$  is the initial concentration of the complex:

$$C_{\text{CuL}}(t) = C_{\text{CuL}}^0 \exp(-k_d t) \quad (1)$$

The first order dissociation rate constant ( $k_d$ ) was fitted to the measured data using a least-squares nonlinear regression.

#### 2.7. Diffusive gradient in thin film (DGT) measurement

DGT was used to assess the bioavailability of Cu, as it has been reported to be a good indicator of the bioavailability of metals in the soil, e.g. for plants (Zhang et al., 2001; Luo et al., 2014) and for earthworms (Bade et al., 2012). Standard soil DGT units (0.8 mm diffusive gel layer) obtained from DGT Research Ltd (<http://www.dgtresearch.com>) were deployed on the surface of soil disks for 18 days at 20 °C. Copper accumulated in the resin was eluted for 24 h with 1 mL of 1 M  $\text{HNO}_3$  after which the eluates were analyzed by ICP-OES (ICAP 6300, Thermo-Fischer). Each treatment (4 types of soil, 2 different ACTs) was replicated three times. The flux of copper accumulation by DGT ( $F_{\text{DGT}}^{\text{Cu}}$ ) was calculated from the mass ( $M$ ) of Cu in the resin, the surface area of exposed gel ( $A = 3.14 \text{ cm}^2$ ) and the deployment time ( $t$ ), following the equation presented in Zhang et al. (2001).

#### 2.8. Size distribution of Cu ligands

Size exclusion chromatography (SEC) was used to assess the size distribution of Cu ligands in the two ACTs, and in the KCl extract of Ncalcl soil after addition (or not) of ACT B. Samples stored at  $-4$  °C pending analysis were first thawed and centrifuged at 5,000 g for 7 min and the supernatant was filtered through 0.2  $\mu\text{m}$  cellulose acetate filters before 2:1 dilution (sample:buffer solution) was performed using a 5 mM ammonium formate (pH 5.5) buffer solution. Chromatographic analysis was performed using a 1200 series HPLC pump (Agilent, Tokyo, Japan) connected to a G1314A variable wavelength detector (Agilent, Tokyo,

Japan) fixed at 254 nm followed by a 7700cs ICP-MS instrument (Agilent, Tokyo, Japan) fitted with nickel cones, a 1.5 mm i. d. injector torch, and a Scott spray chamber cooled to 2 °C. The H<sub>2</sub> collision cell mode for ICP-MS detector was used to exclude possible interference of the analyzed elements. Twenty µL of the 2:1 diluted solution were injected in a size exclusion column Superdex 75 10/300 GL and elution was carried at a flow rate of 0.7 mL min<sup>-1</sup> using 5 mM ammonium formate buffer at pH 5.5. The column was calibrated with proteins of known molecular weight: conalbumin, ovalbumin, carbonic anhydrase, ribonuclease A and aprotinin.

2.9. Data treatment and statistical analysis

All data treatment and statistical analyses were performed using R software version 4.2.2 (R Core Team, 2020). The data were log

transformed when necessary to achieve normal residual distribution. Tukey's HSD tests were performed at 5% to identify the parameters monitored in the KCl extract that were significantly affected by the addition of ACT. Pearson's correlation tests were performed to test the linear relationship between A<sup>254</sup> and 1/K<sub>d</sub><sup>Cu</sup> in the KCl extract.

3. Results

3.1. Concentrations of total Cu and free Cu ion fraction in the KCl extract

The concentration of total Cu in the KCl extract, which we considered as a good proxy of the mobility of Cu in soil, ranged from 0.5 µM in Calc3 soil to 1.1 µM in Ncalc2 soil in the controls on day 1 (Fig. 1a). ACT significantly increased (p < 0.05) the concentration of total Cu in the KCl extract regardless of the soil, the ACT added, and the length of time after

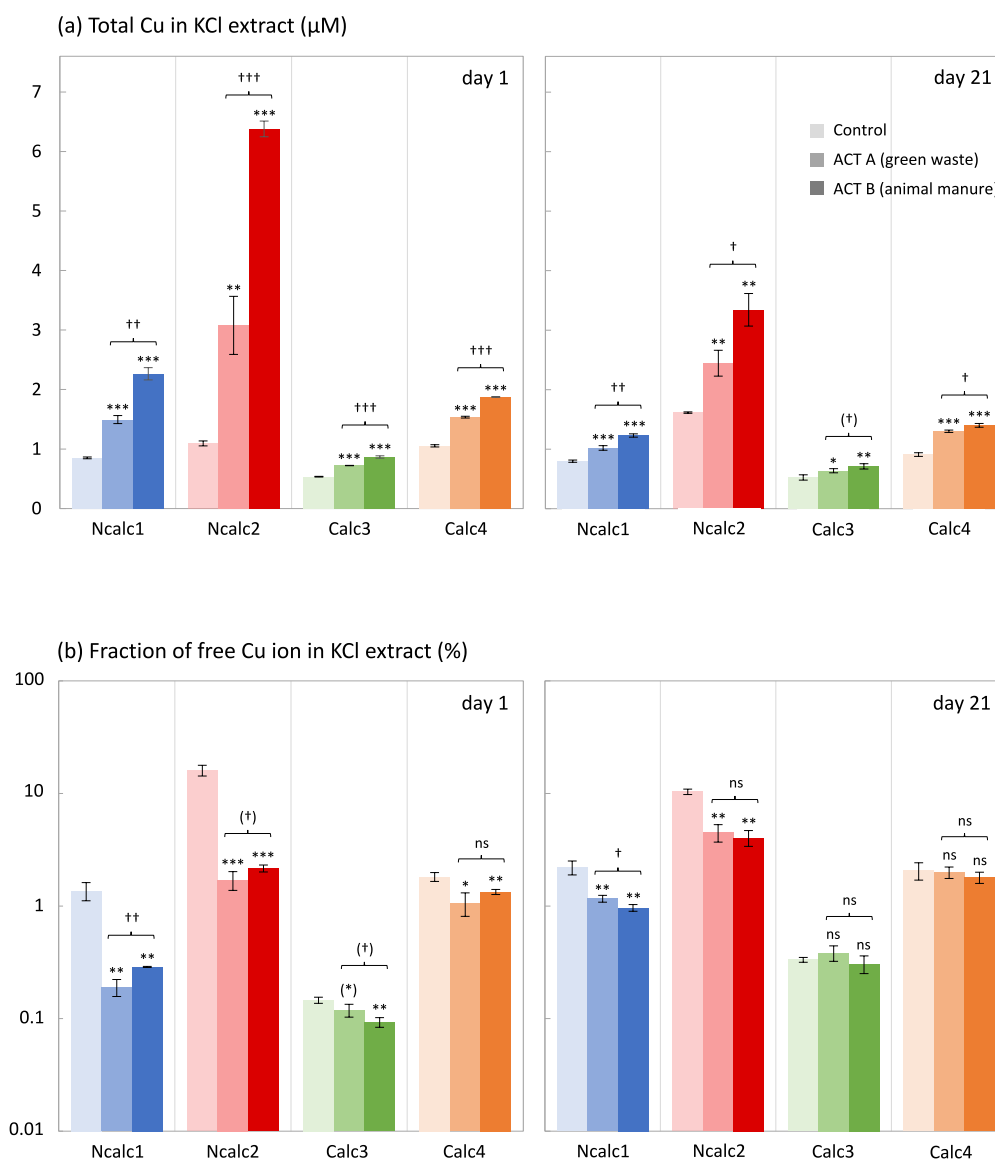


Fig. 1. Concentration of total Cu (a) and free Cu ion fraction (b) in the 0.01 M KCl extract, in four Cu-contaminated vineyard topsoils treated with aerated compost tea A (ACT A, medium color), aerated compost tea B (ACT B, darker color) or ultrapure water (control, lighter color). Soil extraction was performed on day 1 and on day 21. For a given soil and a given soil extraction day, (\*), \*, \*\* and \*\*\* indicate that the total Cu concentration (a) or the free Cu ion fraction (b) measured when ACT A or ACT B was supplied, differed significantly from that measured in the control, at a probability level of p < 0.1, p < 0.05, p < 0.01 and p < 0.001, respectively. For a given soil and a given soil extraction day, (†), †, †† and ††† indicate that the total Cu concentration (a) or the free Cu ion fraction (b) measured when ACT B was supplied differed significantly from that measured when ACT A was supplied, at a probability level of p < 0.1, p < 0.05, p < 0.01 and p < 0.001, respectively. The error bars in (a) and (b) stand for mean standard deviations. In (b), the Y-axis scale is logarithmic. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



ACT addition. However, the extent to which ACT increased the mobility of Cu varied among the soils. The effect both ACTs had on the mobility of Cu was more pronounced in non-calcareous soils than in calcareous soils. For example, the concentration of total Cu in the KCl extract increased by a factor 2.7 in Ncalc1 soil and by 5.8 in Ncalc2 soil, while it increased by a factor 1.6 in Calc3 soil and by 1.8 in Calc4 soil, one day after the addition of ACT B. The extent to which ACT increased the mobility of Cu also varied with the two ACTs. ACT B showed higher Cu mobilization efficiency than ACT A in all four soils and at both incubation times. For example, ACT B increased the concentration of total Cu in the KCl extract two-fold more than ACT A in Ncalc soils on day 1 (Fig. 1a). Lastly, the extent to which ACT increased the mobility of Cu decreased over time after the addition of ACT. Indeed, the concentration of total Cu in the KCl extract in a given soil supplied with a given ACT was always lower ( $p < 0.05$ ) after 21 days than after one day of incubation. For example, the efficiency with which ACT B enhanced the mobility of Cu in Ncalc2 soil decreased from a factor of 5.8 after one day to a factor of 2.1 after 21 days, compared to the control.

The free Cu ion fraction ( $f_{Cu}$ ) in the KCl extract ranged from 0.15% in Calc3 soil to 16% in Ncalc2 soil in the controls on day 1 (Fig. 1b), indicating that the majority of Cu was complexed to ligands in the extraction solution. Both ACTs reduced the free Cu ion fraction ( $p < 0.05$ ) in similar proportions, but the proportions varied among the four soils and with the length of incubation. The decrease in  $f_{Cu}$  induced by ACT was more pronounced in non-calcareous soils than in calcareous soils. For instance,  $f_{Cu}$  decreased by a factor 7.2 in Ncalc1 soil and by 1.7 in Calc4 soil one day after ACT A was added. Like total Cu, the effect of ACT on the free Cu ion fraction was less pronounced after 21 days than after one day of incubation. On day 21, no further difference in  $f_{Cu}$  ( $p > 0.05$ ) was observed in calcareous soils between controls and ACT-treated soils, while in non-calcareous soils, the drop in  $f_{Cu}$  was more limited than on day 1. For instance, ACT A decreased  $f_{Cu}$  by an average factor of 9.4 after one day, and an average factor of 2.3 after 21 days, in Ncalc2 soil.

### 3.2. Flux of Cu accumulation by DGT

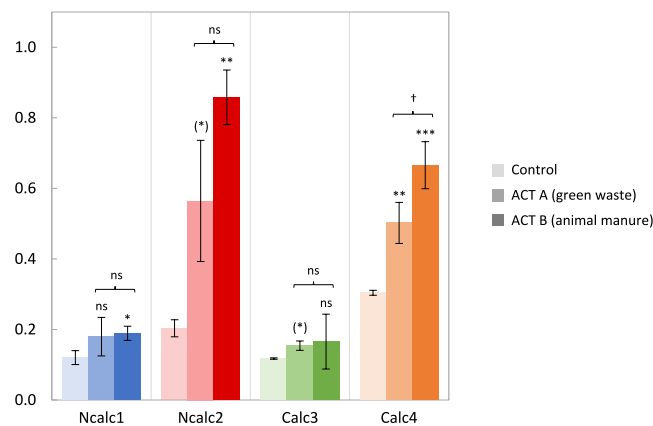
Overall, ACT tended to increase the flux of Cu accumulation by DGT (Fig. 2), suggesting that ACT enhanced the bioavailability of Cu in soils. The effect of ACT on  $F_{DGT}^{Cu}$  was observed notably in Ncalc2 and Calc4 soils, i.e. the two soils with the highest Cu content (Table 1). In these soils, ACT induced a clear and always significant ( $p < 0.1$ ) increase in  $F_{DGT}^{Cu}$ , for example by a factor of 4.2 in Ncalc2 soil and by a factor of 2.2 in Calc4 soil with ACT B. In these soils, ACT B tended to be more efficient than ACT A in promoting the bioavailability of Cu, even if the difference in  $F_{DGT}^{Cu}$  between the two ACTs was only significant ( $p < 0.05$ ) in Calc4 soil. In contrast, the effect of ACT on  $F_{DGT}^{Cu}$  was not always significant in Ncalc1 and Calc3 soils, and even when it was significant ( $p < 0.05$ ), led to an increase in  $F_{DGT}^{Cu}$  by a factor of 1.6 at most.

### 3.3. pH and absorbance at 254 nm of the KCl extract

As expected, the pH of the KCl extract was lower in non-calcareous soils than in calcareous soils (Fig. 3a). In the controls, the pH of the KCl extract was lower than 6.5 in non-calcareous soils and averaged 7.5 in calcareous soils, at both incubation times. ACT did not significantly affect ( $p > 0.05$ ) the pH of the KCl extract in calcareous soils, either on day 1 or on day 21. Conversely, ACT did significantly increase ( $p < 0.05$ ) the pH of the KCl extract in non-calcareous soils, and in similar proportions after one day and 21 days of incubation. At both incubation times, the increase in the pH of the KCl extract of non-calcareous soils was greater ( $p < 0.05$ ) after the addition of ACT A than after the addition of ACT B. On average, ACT A increased the pH of the KCl extract of non-calcareous soils by 0.36 units and ACT B by 0.16 units.

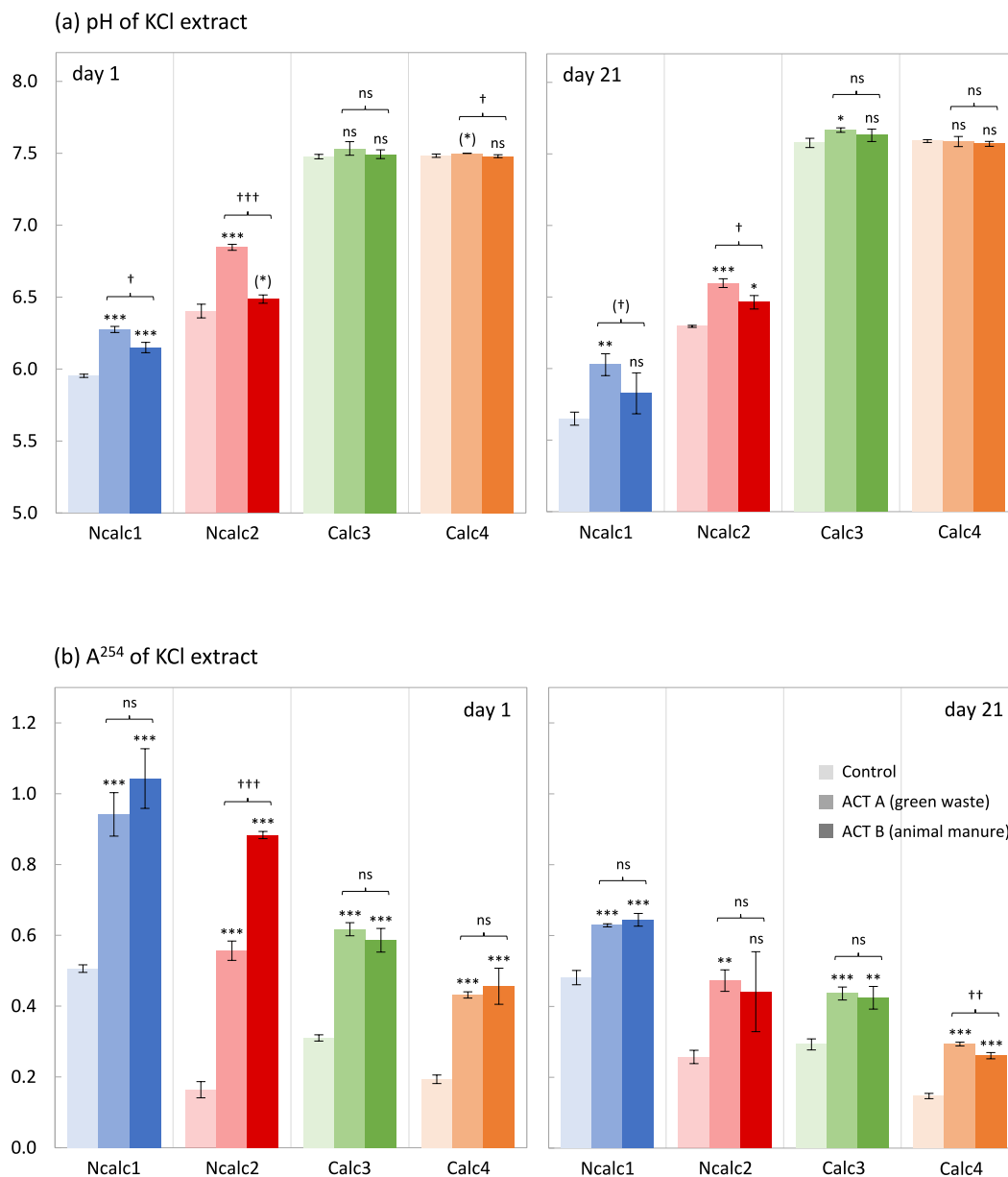
The absorbance at 254 nm ( $A^{254}$ ) of the KCl extract was almost always higher ( $p < 0.01$ ) in soils treated with ACT than in the controls

Flux of Cu accumulation by DGT (nmoles  $m^{-2} s^{-1}$ )



**Fig. 2.** Flux of Cu accumulation by DGT in four Cu-contaminated vineyard topsoils treated with aerated compost tea A (ACT A, medium color), aerated compost B (ACT B, darker color) or ultrapure water (control, lighter color). DGT units were deployed on the surface of soil disks for 18 days. For a given soil, (\*), \*, \*\* and \*\*\* indicate that the flux of Cu accumulation by DGT measured when ACT A or ACT B was supplied differed significantly from that measured in the control, at a probability level of  $p < 0.1$ ,  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively. For a given soil, (†), †, †† and ††† indicate that the flux of Cu accumulation by DGT measured when ACT B was supplied differed significantly from that measured when ACT A was supplied, at a probability level of  $p < 0.1$ ,  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively. Error bars stand for mean standard deviations. ns: non-significant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 3b). This suggests that ACT increased the concentration of SHS in the extraction solution of all four soils. The increase in  $A^{254}$  of the KCl extract induced by ACT was on average larger in Ncalc soils than in Calc soils, notably on day 1. For instance, ACT B increased  $A^{254}$  by 0.54 and 0.72 units, respectively, in Ncalc1 and Ncalc2 soils, whereas it increased  $A^{254}$  by 0.28 and 0.26 units, respectively, in Calc3 and Calc4 soils, one day after its addition. Despite an almost three times higher  $A^{254}$  value than that of ACT A (Table 2), ACT B did not increase the  $A^{254}$  of the KCl extract significantly more ( $p < 0.05$ ) than ACT A, except in Ncalc2 soil on day 1. Lastly, the extent to which ACTs increased the  $A^{254}$  of the KCl extract decreased with time after their addition. For instance, ACT A increased  $A^{254}$  by a factor of 1.9, 3.4, 2.0 and 2.2, in respectively, Ncalc1, Ncalc2, Calc3 and Calc4 soils on day 1, while its effect was reduced to a factor of 1.3, 1.8, 1.5 and 2.0, respectively, in the same soils on day 21. We calculated the fraction of the SHS added to the soil via the supply of ACT that sorbed onto the soil constituents from the  $A^{254}$  of the KCl extract on day 1 (Table 3). The fraction of SHS bound to the solid phase differed significantly ( $p < 0.05$ ) between the two ACTs. Depending on the soil, it ranged from 33% to 63% for ACT A, and from 62% to 86% for ACT B. In addition, for a given ACT, the fraction of SHS bound to the solid phase was significantly higher ( $p < 0.05$ ) in Calc soils than in Ncalc soils, suggesting increased sorption of SHS to the soil constituents in calcareous soils. In the same way, we calculated the fraction of the SHS in pore water on day 1 that was degraded during the incubation period from the decrease in  $A^{254}$  values between day 1 and day 21 (Table 3). Assuming the vast majority of SHS sorbed to the soil constituents within the first 24 h following ACT addition, the fraction of SHS in pore water that was degraded ranged from 15% to 33% for ACT A and from 27% to 50% for ACT B. No significant difference ( $p > 0.05$ ) in the rate of SHS degradation was observed between the two ACTs except in Ncalc2 soil where ACT B was more degraded than ACT A.



**Fig. 3.** pH (a) and UV-absorbance at 254 nm ( $A^{254}$ ) (b) of the 0.01 M KCl extract, in four Cu-contaminated vineyard topsoils treated with aerated compost tea A (ACT A, medium color), aerated compost tea B (ACT B, darker color) or ultrapure water (control, lighter color). Soil extraction was performed on day 1 and on day 21. For a given soil and a given soil extraction day, (\*), \*, \*\* and \*\*\* indicate that pH (a) or  $A^{254}$  (b) measured when ACT A or ACT B was supplied differed significantly from that measured in the control, at a probability level of  $p < 0.1$ ,  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively. For a given soil and a given soil extraction day, (†), †, †† and ††† indicate that pH (a) or  $A^{254}$  (b) measured when ACT B was supplied differed significantly from that measured when ACT A was supplied, at a probability level of  $p < 0.1$ ,  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively. In (a) and (b) the error bars stand for mean standard deviations. ns: non-significant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**3.4. Relationship between Cu mobilization and absorbance of the KCl extract at 254 nm**

To test the relationship between  $A^{254}$  and the solid-liquid partitioning of Cu, the parameter  $1/K_d^{Cu}$  was plotted against  $A^{254}$  (Fig. 4). For a given soil and a given ACT, the two variables were closely and positively correlated ( $p < 0.001$ ). The slope of the relationship differed significantly ( $p < 0.05$ ) among the four soils and between the two ACTs, as shown by the non-overlap of the corresponding 95% confidence intervals. For a given ACT, the slope of the relationship was significantly higher ( $p < 0.05$ ) in Ncalc soils than in Calc soils. For instance, when ACT A was added, the slope was 1.05, 1.39, 0.32 and 0.28 in respectively, Ncalc1, Ncalc2, Calc3 and Calc4 soils. For a given soil, the slope of the relationship was significantly higher ( $p < 0.05$ ) with ACT B than

with ACT A. For instance, in Ncalc1 soil, the slope was 1.05 for ACT A and 1.81 for ACT B, and in Calc3 soil, the slope was 0.32 for ACT A, and 0.61 for ACT B.

**3.5. Dissociation kinetics of Cu-SHS complexes**

Fig. 5 shows representative results of the dissociation rate measurements of Cu complexes. The faster the decrease in the concentration of total Cu, the higher the dissociation rate constant ( $k_d$ ) and the more labile the complex. The decrease in the concentration of total Cu over time fitted the first-order model for the four ligands well. The values of  $\log k_d$  ( $s^{-1}$ ) for the Cu complexes with NTA (-1.29) and HEDTA (-2.59) were in reasonable agreement with the values reported by Degryse et al. (2006). The dissociation rate constant of the Cu complexes with SHS was

**Table 3**

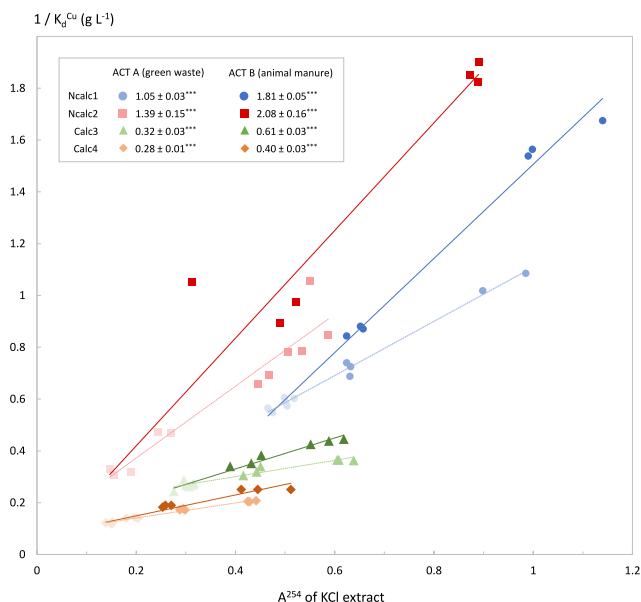
Fraction of the soluble humic substances (SHS) added to the soil via the supply of aerated compost tea (ACT) that sorbed to the solid phase of soils on day 1, and that was degraded in the soil pore water between day 1 and day 21. The fractions were calculated from the UV absorbance of the KCl extract at 254 nm ( $A^{254}$ ) assuming (i) SHS were the main compounds of the KCl extract that absorb at 254 nm, (ii) SHS were not degraded during the first day, and (iii) no SHS sorption to and desorption from the solid phase occurred between day 1 and day 21.

ACT	Soil	SHS sorbed to the solid phase on day 1	SHS degraded in pore water between day 1 and day 21
		%	%
ACT A	Ncalc1	33 ± 9 <sup>a</sup>	33 ± 3 <sup>b</sup>
	Ncalc2	39 ± 4 <sup>a</sup>	15 ± 7 <sup>a</sup>
	Calc3	53 ± 3 <sup>b</sup>	30 ± 4 <sup>b</sup>
	Calc4	63 ± 1 <sup>b</sup>	32 ± 3 <sup>b</sup>
ACT B	Ncalc1	<u>72</u> ± 4 <sup>b</sup>	39 ± 4 <sup>a,b</sup>
	Ncalc2	<u>62</u> ± 1 <sup>a</sup>	50 ± 11 <sup>b</sup>
	Calc3	<u>86</u> ± 2 <sup>c</sup>	27 ± 7 <sup>a</sup>
	Calc4	<u>86</u> ± 3 <sup>c</sup>	43 ± 8 <sup>a,b</sup>

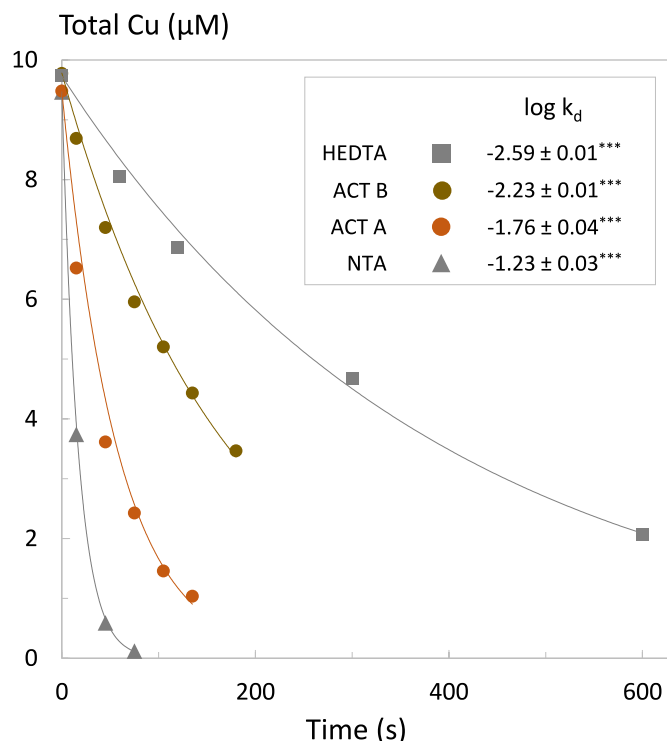
Values are means of three independent replicates.

For a given ACT, different letters in a column indicate significant differences ( $p < 0.05$ ) among soils.

For a given soil, underlined values are significantly higher ( $p < 0.05$ ) than values measured with the other ACT.



**Fig. 4.** Relationship between the variable  $1/K_d^{Cu}$  and the UV-absorbance of the KCl extract at 254 nm ( $A^{254}$ ), for the solutions extracted on day 1 and on day 21 in the four Cu-contaminated vineyard topsoils used in the present study.  $K_d^{Cu}$  stands for the solid-liquid partitioning coefficient of Cu in soil. Each color refers to one soil: blue (Ncalc1), red (Ncalc2), green (Calc3) and orange (Calc4), and each symbol refers to one extraction solution. Light colored symbols represent controls, medium colored represent soils supplied with ACT A, and dark colored symbols represent soils supplied with ACT B. The slope of the linear relationship between the two variables differed between the ACT A and ACT B. For each soil, the dashed line represents the best linear fit obtained between the two variables for the solutions extracted in the controls and in the soils treated with ACT A ( $n = 12$ ), and the solid line represents the best linear fit obtained between the two variables for the solutions extracted in the controls and in the soils treated with ACT B ( $n = 12$ ). The inset shows the slope  $\pm 95\%$  confidence interval of the linear relationship between the two variables for each soil and each ACT. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 5.** Dissociation kinetics of Cu-ligand complexes measured with the competitive ligand exchange method in solutions containing 10 mM KCl and 2 mM MES (pH 6). The lines show the exponential regression (eq. (1)) and the inset shows the dissociation rate constants of the Cu complexes through the  $\log k_d$  value.

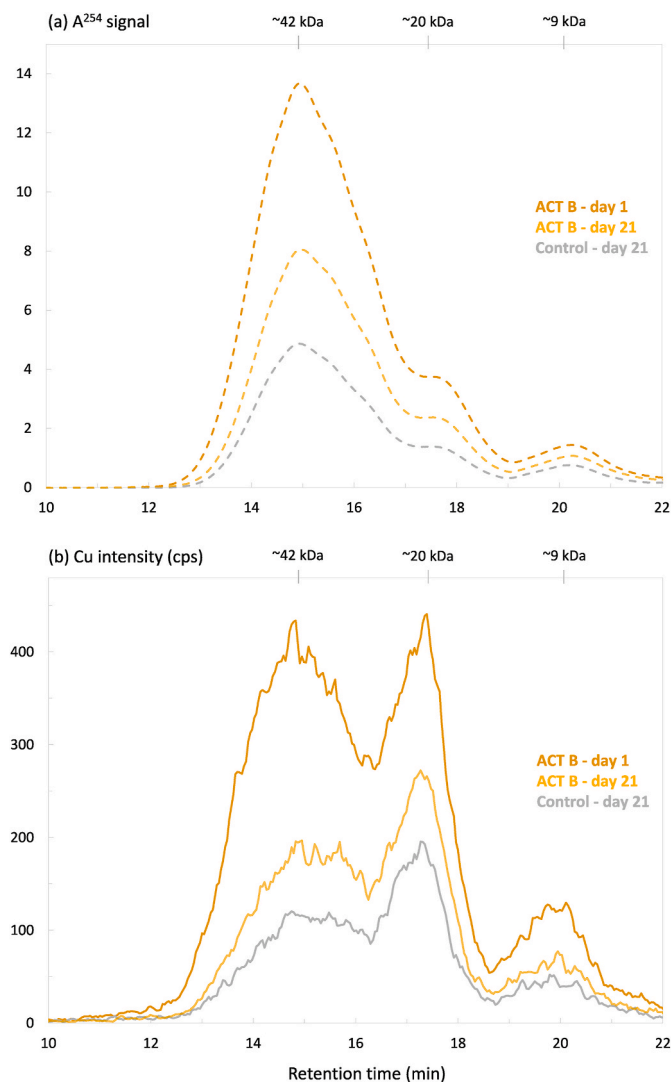
between that with NTA and that with HEDTA for both ACTs. The  $\log k_d$  value for the Cu complexes with SHS from ACT A was higher ( $-1.76$ ) than that for the Cu complexes with SHS from ACT B ( $-2.23$ ). This suggests that the lability of Cu-SHS complexes in soils supplied with ACT A was higher than in soils supplied with ACT B.

### 3.6. Size distribution of Cu ligands in the KCl extract of Ncalc1 soil

SEC-UV chromatograms at 254 nm showed that the molecular weights (MW) of the dissolved organic matter (DOM) ranged between 5 and 65 kDa in the KCl extracts of Ncalc1 soil (Fig. 6a). Three major sub-fractions of DOM were isolated in the control. The dominating peak at around 15 min, which corresponds to MW of approximately 42 kDa, was attributed to SHS. The two less intense peaks at around 18 and 20 min were considered to be breakdown products of SHS (i.e. SHS building blocks) as their MW corresponded to  $1/2$  ( $\sim 20$  kDa) and  $1/4$  ( $\sim 9$  kDa), respectively, of the MW of SHS. ACT enhanced  $A^{254}$  intensity with no notable effect on the MW distribution of DOM in the KCl extract. The same three major sub-fractions were isolated at the same retention times, and in similar relative proportions, in the KCl extract of the control and of the ACT-treated soil. The only difference was the slightly higher contribution of the peak attributed to SHS in the soil treated with ACT, notably on day 1, which is consistent with the DOM composition of ACT B itself (see suppl. Fig. F1).  $A^{254}$  intensity decreased between day 1 and day 21 to a similar extent across the three peaks, suggesting SHS degradation did not substantially alter the MW distribution of DOM in the KCl extract of Ncalc1 soil.

SEC-ICP-MS chromatograms are shown for retention times of less than 22 min since no Cu peaks were detected after that (Fig. 6b). This means that very little Cu was bound to ligands of MW less than 1 kDa, i.e. to low molecular weight organic acids (LMWOA), in the KCl extract of either the control or of the soil treated with ACT. In the control, the MW distribution of Cu ligands closely matched that of DOM, with three peaks





**Fig. 6.** SEC-UV chromatograms (a) and SEC-ICP-MS chromatograms for Cu (b) of the KCl extract of Ncalc1 soil supplied with ACT B or not (control). Chromatograms are shown for the solution extracted on day 1 and on day 21 for the ACT-treated modality. For the control, only the chromatogram for the solution extracted on day 21 is shown since the chromatograms at the two dates were very similar. The molecular weights at the top of the figure, which correspond to those of the three major sub-fractions of dissolved organic matter isolated in the extraction solutions, were determined thanks to the column calibration protocol detailed in section 2.7.

at the same MW as those observed for DOM. The main difference was that the main peak for Cu was not the one at 15 min attributed to SHS, but the one at 18 min attributed to the SHS building blocks of 20 kDa. ACT enhanced Cu intensity without notably affecting the MW distribution of Cu ligands in the KCl extract, the main difference being the larger contribution of the peak at 15 min in the soil treated with ACT. This suggests that more Cu was bound to SHS in the KCl extract in presence of ACT, notably one day after ACT addition.

#### 4. Discussion

This study highlights the fact that the use of ACT can affect the dynamics of Cu in vineyard soils. Indeed, the two ACTs tested in this study led to increased mobility and bioavailability of Cu in four vineyard topsoils with contrasting properties. However, the extent to which ACT altered the dynamics of Cu varied significantly among the four soils and between the two ACTs. For instance, the Cu-mobilizing efficiency of ACT

B on day 1 varied from a factor 1.6 to a factor of 5.8 among the soils. In the same way, the efficiency of ACT to increase the bioavailability of Cu in Calc3 soil varied from a factor 2.8 to a factor of 4.3 between ACTs. The aim of the following discussion is to explain these differences so as to better understand the risks of transfer and ecotoxicity of Cu in vineyard soils associated with the use of ACT.

##### 4.1. Copper mobilization by ACT relied on its complexation by SHS

Several results of this study support the hypothesis formulated by Borggaard et al. (2019) that SHS, such as those contained in ACT (Eon et al., 2023), can complex and mobilize Cu in soils. First, ACT decreased the free Cu ion fraction in the KCl extract of all four soils showing that ACT promoted the complexation of Cu, likely by DOM, in the soil pore water. In non-calcareous soils, where ACT led to a slight alkalization of the KCl extract (Fig. 3) due to its alkaline nature ( $\text{pH} > 9$ , Table 1), the decrease in  $f_{\text{Cu}}$  could also result from an increase in pH since Cu complexation by DOM is usually enhanced at higher pH (Bravin et al., 2009; Cornu et al., 2019). However, this was not the case in calcareous soils where ACT did not alter the pH of the KCl extract, and where its decreasing effect on  $f_{\text{Cu}}$  was solely due to the Cu complexing ability of the DOM it contains. Second, the strong and positive relationship between  $1/K_d^{\text{Cu}}$  and  $A^{254}$  in a given soil and with a given ACT (Fig. 4) shows that Cu was mobilized in soil treated with ACT in direct proportion to the increase in the concentration of soluble ligands that absorb at 254 nm. This suggests that Cu was predominantly mobilized by ligand-controlled dissolution (Kraemer, 2004) in soils treated with ACT, and that the ligands that mobilized Cu in soils treated with ACT were mainly the aromatic compounds (Weishaar et al., 2003) contained in the compost tea. Third, SEC analyses of ACT B (suppl. Fig. F1) and of the KCl extracts of Ncalc1 soil (Fig. 6) revealed strong agreement between (i) the MW distribution of DOM in ACT B and in the KCl extract of the soil treated with ACT B, and between (ii) the MW distribution of DOM and that of Cu-ligands in the KCl extract of the soil treated with ACT B. Thereby, SEC analyses demonstrated that the excess Cu mobilized in soils treated with ACT B was associated with the DOM supplied by ACT in the soil pore water. In ACT B, DOM consisted almost exclusively of SHS, with a mean MW of around 40–50 kDa (suppl. Fig F1). For the sake of comparison, the study by Zhao et al. (2023) reported MWs higher than 20 kDa for humic acids extracted from mature composts, in varying proportions depending on the origin of the compost. The molecular weight of the SHS in ACT B was therefore consistent with those found in the water extracts of mature compost. In the KCl extract of Ncalc1 soil supplied with ACT B, DOM consisted of SHS but also of SHS building blocks. The presence of SHS building blocks in the KCl extract of the soil treated with ACT from day 1 can be explained as follows: the SHS supplied by ACT are macromolecular structures stabilized by weak bonds, such as hydrophobic interactions or hydrogen bonds (Sutton and Spósito, 2005). Once added to the soil, SHS form covalent bonds with the soil components and the weak bonds are broken, thereby releasing building blocks of smaller MW into the solution (Hur and Schlautman, 2003). However, we cannot rule out the possibility that SHS building blocks are of biotic origin linked to an initial phase of SHS decomposition that occurred during the first 24 h.

SEC-ICP-MS analyses showed that Cu was exclusively associated with SHS and with SHS-like compounds (i.e. building blocks) in the KCl extracts of Ncalc1 soil. This confirms the prominent role of SHS in the mobilization of Cu in soils treated with ACT. Surprisingly, the MW distribution of Cu ligands was similar in the control and in the soil treated with ACT B. Notably, ACT induced no shift in Cu ligands towards higher MW than in the control. The main explanation for this result is that the MW of the SHS supplied by ACT B was close to that of the SHS originally present in the Ncalc1 soil, suggesting that the organic matter in the Ncalc1 soil was highly stable and humified from the start of incubation. SEC-ICP-MS analyses also showed that the building blocks of 20 kDa were the main ligands of Cu in the KCl extracts, although they were not

the most concentrated SHS-like compounds. The sulfur peak observed at around 18 min, notably in the soil treated with ACT B (suppl. Fig. F3), suggests that this sub-fraction of DOM was proteinaceous and composed of sulfur-containing amino acids such as cysteine, whose affinity for Cu is high (stability constant of the order of  $10^{10} \text{ M}^{-1}$ , Rigo et al., 2004). It would be interesting to further investigate the reasons for the stronger affinity of Cu for this sub-fraction of DOM to better understand the effect of SHS on the dynamics of Cu in soils, and more generally on the biogeochemical cycle of trace metals in the environment.

#### 4.2. Differences in Cu mobilization by ACT among soils and between ACTs

As discussed in section 4.1 above, the driving force behind the increased mobilization of Cu in soils treated with ACT is complexation of Cu by SHS. Therefore, the Cu mobilization efficiency of ACT depends on the affinity for Cu of the SHS it contains, as well as on the extent to which its supply increases the concentration of SHS in the soil pore water. The differential affinity for Cu of the SHS contained in ACT A and ACT B is one reason for their contrasted ability to mobilize Cu in the different soils tested in the present study. For a given soil, the higher slope of the relationship between  $A^{254}$  and  $1/K_d^{\text{Cu}}$  observed for ACT B than for ACT A (Fig. 4) suggests that the SHS from ACT B had a higher affinity for Cu than the SHS from ACT A. In a way, this result is supported by the lower lability of Cu-SHS complexes measured for SHS from ACT B than for SHS from ACT A (Fig. 5), and could be explained by the higher percentage of humic acids in ACT B than in ACT A (Table 2) since HA have a higher affinity for Cu than FA (Tipping, 1993).

The efficiency of ACT in mobilizing Cu also depends on how it affects the concentration of SHS in the soil pore water. Hence, the extent to which ACT increased the mobility of Cu in soil depends to a great extent on the partitioning between the solid and the liquid phase of the SHS it contains, as well as on the degradation rate of SHS in soil pore water. As observed in chelant-assisted phytoextraction (Evangelou et al., 2007; Cornu et al., 2014), the sorption of chelators (SHS in the present case) to the solid phase is a key factor that influences the efficiency with which they mobilize metals in soils. The more the chelator is sorbed to the solid phase, the less efficiently it mobilizes metals. The present study showed that sorption of SHS to the solid phase was significant but varied considerably among the four soils and between the two ACTs (Table 3). First, the sorption of the SHS from ACT B was higher than the sorption of the SHS from ACT A. As mentioned above regarding their differential affinity for Cu, this might be due to the higher percentage of humic acids in ACT B than in ACT A (Table 2). Indeed, HA have a higher affinity for soil constituents such as phyllosilicates and metal oxyhydroxides than FA due to their higher MW and higher unsaturation degree (Chang et al., 2014). Second, the sorption of SHS was higher in calcareous soils than in non-calcareous soils (Table 3). Bridging with calcium cations has been proposed as a possible sorption mechanism for SHS to clay phases in calcareous soils (Majzik and Tombacz, 2007). In this mechanism,  $\text{Ca}^{2+}$  ions serve as cation bridges linking negative sites on soil constituents and an anionic or polar functional group on SHS. Another mechanism is the sorption of SHS to calcite that has been described as fast and almost irreversible (Lee et al., 2005). Taken together, all these results concerning SHS sorption to the solid phase explain why on day 1 (i) the Cu mobilization efficiency of the two ACTs was not as different as their  $A^{254}$  would suggest, and (ii) the Cu mobilization efficiency of ACTs was greater in Ncalc soils than in Calc soils.

The rate of SHS degradation in pore water assessed based on the difference in  $A^{254}$  between day 1 and day 21 was similar among the four soils and in the two ACTs (Table 3). On average,  $35 \pm 15\%$  of SHS in pore water was degraded during the incubation period likely by soil microorganisms such as saprotrophic fungi and bacteria (Filip & Bielek, 2002; Kulikova & Perminova, 2021). Even if differences in SHS degradation were observed between treatments, our results did not succeed to identify a strong link between composition and degradation rate of SHS

in pore water, or any influence of physico-chemical soil characteristics, such as pH or  $\text{CaCO}_3$  content, on the activity of carbon hydrolytic enzymes. In this respect, our results contrast with the review by Burns et al. (2013) on soil enzymes. In our study, the degradation of SHS in pore water resulted in a decrease over time in the efficiency with which ACT mobilized Cu (Fig. 1). The effect of ACTs on the dynamics of Cu in soils was therefore only transient, and this experiment suggests that their ability to mobilize Cu persisted for at least three weeks after their addition, whereas the study by Ouédraogo et al. (2024) showed that cover crop residues increased the mobility of Cu in vineyard soil for 60–90 days after their incorporation. Adding ACT is consequently not the only OM-related practice that can disrupt the dynamics of Cu in vineyard topsoil.

#### 4.3. ACT increased the bioavailability of Cu in soils due to the high dissociation rate of Cu-SHS complexes

The supply of ACT increased the flux of Cu accumulation by DGT (Fig. 2) whereas it reduced the fraction (Fig. 1) and the concentration (suppl. Fig. F2) of free Cu ion in the KCl extract, notably in the calcareous soils. This deviation from the FIAM model was associated with a relatively high dissociation rate of Cu complexes with SHS, notably of Cu complexes with SHS from ACT A (Fig. 5). Taken together, these results show that Cu-SHS complexes contributed to the flux of Cu accumulation by DGT, and that their contribution was likely due to their dissociation within the diffusion layer. Thereby, this study shows that ACT may increase the bioavailability of Cu in soils, as reported by Eon et al. (2023) for crimson clover, because Cu-SHS complexes are labile enough to significantly contribute to the flux of free Cu ion taken up by soil organisms. However, ACT did not increase the flux Cu accumulation by DGT in all the treatments and when it did, did not increase it to the same extent as it increased the concentration of total Cu in the KCl extract. The contribution of Cu-SHS complexes to the flux of Cu accumulation by DGT was therefore only partial, as is probably their contribution to the flux of Cu taken up by soil organisms. As often reported in chelant-assisted phytoextraction (Evangelou et al., 2007; Cornu et al., 2014), most of the Cu mobilized by ACT likely remains in solution, with the risk of being leached out to groundwater.

This study also shows that the dissociation rate constant of Cu complexes with SHS from ACT A was higher than that of the Cu complexes with SHS from ACT B (Fig. 5) for reasons that remain to be determined, but which could be linked to their lower HA fraction. The higher lability of Cu complexes with SHS from ACT A did not result in a more pronounced effect of ACT A compared to ACT B on the flux of Cu accumulated by DGT (rather the opposite was observed in Ncalc2 and Calc4 soils), underlining the fact that the dissociation of Cu-SHS complexes in the diffusion layer is not the only process that determines the intensity with which ACTs increase the bioavailability of Cu in soils. Finally, we observed a stronger effect of ACTs on the flux of Cu accumulation by DGT in the two soils with the highest Cu content (Ncalc2 and Calc4). Although the underlying process remains to be determined, notably in Calc4 soil where the effect of ACTs on the mobility of Cu was not particularly strong, this result calls for increased vigilance regarding the use of ACTs in the vineyard plots that are most contaminated by Cu.

## 5. Conclusions

This work showed that the addition of ACT systematically increased the mobility and, to a lesser extent, the bioavailability of Cu in vineyard soils. This increase relied on the complexation of Cu by the SHS contained in ACT and by the relatively high dissociation rate of Cu-SHS complexes. This practice therefore needs to be supervised so as not to increase the risk of Cu ecotoxicity and of Cu transfer to groundwater in vineyard soils, notably in those with the highest rates of contamination by Cu. In our opinion, ACT addition should be carefully controlled to avoid significantly increasing the concentration of SHS in soil pore

water. Our first recommendation is therefore to limit the amount of ACT supplied to the soil, notably in soils with a low capacity to bind SHS. Our second recommendation is to use ACT with a “low” level of SHS. Our third recommendation is to split ACT inputs so that part of the SHS added to the soil can be degraded by the soil biota between two inputs. Conversely, the ability of ACT to increase the bioavailability of Cu in soils could be used in phytoextraction to enhance the amount of Cu phytoextracted. For this specific use, the formulation and the way ACT is supplied to the soil should be adapted to maximize its effect on the concentration of SHS, and hence of Cu, in the soil pore water, within the limits of what the accumulating plant can tolerate.

### CRedit authorship contribution statement

**Pierre Eon:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Laurent Ouerdane:** Writing – review & editing, Supervision, Methodology. **Alex Goupil:** Writing – review & editing, Investigation, Data curation. **Agathe Vidal:** Writing – review & editing, Investigation. **Jean-Yves Cornu:** Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2024.124382>.

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