

Article

Aerated Compost Tea Did Not Promote Cu Downward Transfer but Increased Cu Phytoavailability in a Vineyard Soil

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Abstract: Vineyard soils are frequently contaminated with copper due to the use of Cu fungicides to prevent downy mildew. This study investigated the effects of an aerated compost tea (ACT) made from grape pomace and animal manure on the downward transfer of Cu and on the accumulation of Cu in plants in a sandy loam vineyard soil. Crimson clover and pot marigold were grown in a 40 cm soil column with Cu supplied to the surface at loadings representative of those applied in European vineyards, plus additions of ACT. A source of Cu enriched in the stable isotope ⁶⁵Cu was used to distinguish freshly added Cu (fresh Cu) from Cu already present in the soil (aged Cu). ACT increased the concentration of soluble humic substances (SHS) in pore water in the top 7.5 cm of the column, and increased the concentration of Cu, Al, and Fe in pore water in proportion to the concentration of SHS. The transfer of fresh Cu to deeper soil was limited to the top 5 cm, even after the addition of ACT, although fresh Cu reacted slightly more to ACT than aged Cu. ACT had no effect on Cu phytoextraction but increased the concentration of Cu in roots by almost twofold. Relatively more fresh Cu was transferred to plants than aged Cu, primarily due to its preferential accumulation on the surface. The risk associated with the use of ACT on vineyard soils is not that of promoting the downward transfer of Cu, but rather of increasing Cu availability to plants and likely to other living organisms in the topsoil.

Keywords: copper; leaching; soluble humic substances; stable isotope labeling; trace metals; winegrowing



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1. Introduction

Copper fungicides have been used since the end of the nineteenth century to prevent grapevine downy mildew (*Plasmopara viticola*) and are thus responsible for copper (Cu) contamination of vineyard ecosystems [1]. Indeed, Cu concentrations up to several hundred mg kg^{−1} soil have been measured in the topsoil of an old vineyard [2]. The potentially harmful effects of Cu on vineyard ecosystems, including for macro-organisms such as earthworms [3] or on cover crops sown in the inter-row [4], can be prevented in different ways. The most common solutions involve liming the soil and/or adding organic matter to fix Cu on the soil solid phase, thereby reducing its bioavailability and ecotoxicity [5].

The EU Regulatory Recycling Framework introduced by Directive 2008/98/EC requires European countries to implement circular economy policies, in particular to reduce food waste. For example, Directive 2018/851 sets a recycling target of at least 60% for municipal solid waste in every member country by 2030. This regulation requires local

authorities to provide a biowaste recovery solution for private individuals. Increased use of organic fertilizers derived from biowaste is consequently to be expected in the future [6]. Organic fertilizers can take various forms, for example, biowaste can be used to make compost or vermicompost, but also aerated compost tea (ACT). ACT has the advantage of being a direct source of both dissolved organic matter (DOM) and nutrients, and can increase crop yields [7,8]. The use of ACT as fertilizer in vineyard ecosystems could therefore increase over time to a similar extent as its use in horticulture [9]. However, this practice is not devoid of risk, as the DOM added via ACT can complex and solubilize metals that are already present in the soil [10,11]. As a result, the risk of transfer of DOM–metal complexes to deeper soil has been highlighted in several studies, with contrasting risks depending on the DOM added and the metal concerned. For example, in a soil amended with sewage sludge compost, DOM was a critical factor that influenced Cu, Ni, and Zn leaching [12]. Similarly, in a soil column study, Kaschl et al. [13] highlighted the fact that the concentration of DOM in soil pore water following the addition of a compost water extract increased the transfer of Cu and Ni to deeper soil. However, DOM is not the only parameter that can influence metal leaching; pH and the concentration of Ca also play important roles in the process [14,15].

Stable isotope labeling has been widely used to track the fate of metals in the environment [16,17], while stable isotope fractionation has been used to identify metal uptake and accumulation mechanisms in plants [18,19], although rarely in the case of Cu. To our knowledge, only two studies, one by Ostermann et al. [20] and one by Wiggenhauser et al. [21], used isotopic labeling to monitor the kinetics and extent of the downward transfer of Cu in soil. In the present study, we investigated how the addition of ACT impacted the dynamics of metals, particularly of Cu, in a vineyard soil. Crimson clover and pot marigold were grown in a soil column with surface application of Cu and the addition of ACT. A source of Cu enriched in the stable isotope ^{65}Cu was used to distinguish freshly added Cu (fresh Cu) from Cu already present in the soil (aged Cu). Our main objective was to investigate the effect of repeated additions of ACT on Cu solubilization, the downward transfer of Cu, and the accumulation of Cu in plants, with a particular focus on fresh Cu.

2. Materials and Methods

2.1. Soil

The soil used for this study was collected from the upper 20 cm of a hyperskeletal Leptosol (WRB soil classification system) in a vineyard plot in the AOC (i.e., registered designation of origin) of Pessac-Léognan (south of Bordeaux, France). The soil sample was composed of 77% sand, 15% silt, and 8% clay and had an organic matter content of 2.6%, a pH_{water} (pH in water extract) of 6.6, a C/N ratio of 14.4, a CEC of $7.0 \text{ cmolc}_+ \text{ kg}^{-1}$, carbonate content $< 1\%$, and a water holding capacity (WHC) of 11.7%. Total Cu concentration in the soil was 90 mg kg^{-1} , and the concentration of EDTA-extractable Cu was 47 mg kg^{-1} . The WHC was measured in our laboratory (ISPA) using the method detailed by Revaillet et al. [22]. All other analyses were carried out in the INRAE soil-testing laboratory (LAS, Arras, France). Before the start of the experiment, the soil was air dried, sieved to 2 mm, then rewetted with water to 70% WHC and equilibrated for three weeks to avoid the “Birch effect” that can impact metal dynamics in soils after rewetting [23].

2.2. Aerated Compost Tea

The aerated compost tea was prepared in the same way as detailed in Eon et al. [24]. Briefly, 39 kg of a commercial compost made from grape pomace and animal manure (Biofumur[®], Florentaise, Saint-Mars-du-Désert, France) was placed in a ‘tea bag’ made from a non-woven polypropylene wintering fabric (P17, Serres Val de Loire, Longué-

Jumelles, France) and extracted with 130 L of deionized water, in a 210 L polypropylene storage bin. After shaking and aerating the mixture for 48 h at 20 °C, the compost was retrieved, and the compost tea was stored at 4 °C before being used for the experiment. Table 1 details the physico-chemical characteristics of the ACT used in the experiment. Predictably, ACT was characterized by a high SHS content ($A^{254} = 45$), a high concentration of carbon ($TC > 2 \text{ g L}^{-1}$), and was rich in nutrients such as N and K (about 26 mM each). ACT also contained trace metals, but the amount of Cu added by ACT corresponded to less than 0.1% of the amount of Cu originally present in the soil column.

Table 1. Selected physical and chemical properties of the aerated compost tea (ACT) used in this study, after centrifugation and filtration at 0.2 µm; pH and absorbance at 254 nm (A^{254}) were measured using the procedure described in Section 2.6, and conductivity using a graphite conductivity electrode (LE703, Mettler Toledo, Greifensee, Switzerland). Total concentrations of carbon (TC) and nitrogen (TN) were measured by combustion catalytic oxidation (TOC-VSCH, Shimadzu, Kyoto, Japan) and total concentrations of S, K, Ca, Mg, Al, Fe, Mn, Cu, and Zn were measured by ICP-OES (ICPA 6300, ThermoFisher Scientific, Waltham, MA, USA). The distribution of humic and fulvic acids was measured using the extraction scheme described by Swift (1996) [25] for the isolation and purification of HA and FA from aqueous samples.

	ACT
pH	7.52
Conductivity (mS cm^{-1})	78.11
TC (mg L^{-1})	2009
TN (mg L^{-1})	370
A^{254}	45
Humic acids ¹ (%)	26
Fulvic acids ¹ (%)	74
Macronutrients (mM)	
N	26
P	1.4
S	2.2
K	26
Ca	1.2
Mg	1.0
Micronutrients and trace metals (µM)	
Al	119
Fe	75
Mn	16
Cu	44
Zn	10

¹ [25].

2.3. Preparation and Addition of ^{65}Cu -Enriched Solution to the Soil

In order to obtain a stock solution of 16.84 mM $^{65}\text{Cu}(\text{NO}_3)_2$, two hundred milligrams of stable ^{65}Cu isotope-enriched copper metal nugget purchased from Eurisotop (Cambridge, UK) were dissolved with 1 mL of 69% HNO_3 (ppb-trace analysis grade, Scharlab, Barcelona, Spain) and diluted to 183 mL with ultrapure water (Synergy[®] UV, MilliporeSigma, Burlington, MA, USA). The isotopic abundance of the two Cu sources used in this study was the following: 69.15% of ^{63}Cu and 30.85% of ^{65}Cu for the standard (Cu_s) (source IUPAC), and 0.03% of ^{63}Cu and 99.7% of ^{65}Cu for the ^{65}Cu -enriched (Cu_{ei}) (source Eurisotop). Three weeks before sowing, 10 mL of 16.84 mM $^{65}\text{Cu}(\text{NO}_3)_2$ were added to the surface of the column, giving a total of 10.93 mg $^{65}\text{Cu}_{ei}$. This corresponds to approximately 10 kg Cu ha^{−1} and increased the total stock of Cu in the column by 2.3%.

2.4. Experimental Setup

Crimson clover (*Trifolium incarnatum* L. cv. Bolsena) and pot marigold (*Calendula officinalis* L. cv. Ball's Golden Yellow) were chosen because of their ability to accumulate Cu in their shoots [24,26] and their contrasting root systems. The root system of crimson clover mainly spreads in the surface soil [27], in contrast to pot marigold, whose tap root system penetrates deeper. Five crimson clover seeds and three pot marigold seeds were sown in a column 40 cm in height and 11.8 cm in diameter, containing 5300 g dry weight (DW) of soil. Starting five weeks after sowing, half the columns ($n = 5$) were supplied with 40 mL of raw ACT three times a week while the other half was supplied with the same volume of deionized water as a control, representing a total of 28 additions. The plants were grown for 107 days in a greenhouse under LED lamps under a 16 h photoperiod. Before the experiment began, several 1.7 cm diameter holes were drilled in the wall of the soil columns to enable soil to be sampled at depths of 2.5 cm, 5 cm, 7.5 cm, and 36 cm. During the course of the experiment, the holes were covered with duct tape to prevent the irrigation water from leaking. The daily mean air temperature ranged from 18 °C to 25 °C, daily mean air humidity was approximately 50%, and the photosynthetic active radiation (PAR) was above 1000 $\mu\text{mol cm}^{-2} \text{s}^{-1}$ on sunny days.

2.5. Plant Sampling and Analyses

At harvest, i.e., 107 days after sowing (Supplementary Figure S1), the plants were pulled out of the soil by hand to recover part of their root system. The plant's roots and shoots were separated before being washed with deionized water to remove all soil particles, oven-dried at 50 °C for 48 h, and weighed. The shoots were separated according to species, but the roots of the two species were kept mixed. Next, all the dried organs were ground into a fine powder (MM 400, Retsch, Eragry, France) and wet digested using the procedure detailed in Eon et al. [24]. Briefly, 500 mg of dried powder was added to 5 mL of a $\text{HNO}_3/\text{H}_2\text{O}_2$ mix (4:1 v/v) in a 50 mL Teflon[®] tube and digested in a graphite digestion block system (DigiPREP MS, SCP Science, Villebon-sur-Yvette, France). The total concentrations of P, K, S, Ca, Mg, Fe, Mn, Cu, Zn, and Si in the roots and shoots were determined by ICP-OES (ICAP 6300, ThermoFisher Scientific). The concentration of N in the shoots was measured with an elemental analyzer (Flash EA 1112, ThermoFisher Scientific), using the Dumas method.

2.6. Soil Extraction and Analyses

After the plants were harvested, soil was sampled at depths of 0, 2.5, 5, 7.5, and 36 cm, and extracted as follows: 5 g of wet soil were shaken with 12 mL of 0.01 M KCl for 1 h at 20 °C, centrifuged at 4500 g for 10 min, and the supernatant was then filtered through a 0.2 μm cellulose acetate filter [28]. The concentration in the KCl extract was set at 0.01 M to mimic the extraction power of the soil pore water. A^{254} and pH were measured immediately after extraction. A^{254} was measured by UV-VIS spectrometry using a microplate reader (BioTek Synergy H1, Agilent Technologies, Santa Clara, CA, USA—quartz glass microplate, path length of 1 cm), and pH was measured using a combined pH electrode (11786348, Fisher Scientific, Illkirch, France). The extraction solution was then acidified with 2% ultrapure HNO_3 (v/v) before the total concentration of P, S, Ca, Mg, Fe, Al, Mn, Cu, Zn, and Si in the solution was measured by ICP-OES (ICAP 6300, ThermoFisher Scientific).

2.7. Copper Isotope Measurement

The $^{65}\text{Cu}/^{63}\text{Cu}$ isotopic ratio in the 0.01 M KCl extracts and in the plant digests was determined by ICP-MS (NEXion 320D, PerkinElmer, Waltham, MA, USA) at the CRBE laboratory in Toulouse, France. Prior to each measurement sequence, the instrument was

tuned to ensure maximum sensitivity and stability. The samples were diluted in 2% HNO₃ prepared from ultrapure 65% HNO₃ to reach a final total concentration of Cu of between 15 and 25 µg L⁻¹. The instrumental mass bias was checked and corrected by bracketing the replicates with the natural standard solution NIST SRM 976 (Cu concentration of standard solution = 25 µg L⁻¹). During the measurements, the ample time spent rinsing in 2% HNO₃ made it possible to avoid a memory effect. Each isotopic ratio corresponds to the average of 50 runs of 5 scans. The analytical precision of the ⁶⁵Cu/⁶³Cu isotopic ratio measured in the standard NIST solution ranged from 0.2% to 0.5% with no significant drift during the analytical sequence.

For each extraction solution and each plant digest, the ratio of the amount of unlabeled Cu derived from aged Cu (Q_s) and the amount of labeled Cu derived from fresh Cu (Q_{ei}) was calculated from the ⁶⁵Cu/⁶³Cu ratio of the sample (R_m) according to Equation (1) [29]:

$$\frac{Q_s}{Q_{ei}} = \left(\frac{M_s}{M_{ei}} \right) \cdot \left(\frac{{}^{65}A_{ei}}{{}^{63}A_s} \right) \cdot \left(\frac{(1/R_m) - R_{ei(63/65)}}{1 - (1/R_m) \cdot R_{s(65/63)}} \right) \quad (1)$$

where M represents the molar weight ($M_s = 63.546 \text{ g mol}^{-1}$, $M_{ei} = 64.922 \text{ g mol}^{-1}$), A the abundance of the isotope, and R the isotopic ratio of ⁶⁵Cu/⁶³Cu or ⁶³Cu/⁶⁵Cu ($R_{s(65/63)} = 0.4461$ and $R_{ei(63/65)} = 0.003$). The subscript s and ei indicate values derived from Cu with standard (s) versus enriched ⁶⁵Cu isotope (ei) distributions.

From Q_s/Q_{ei} , the contribution of fresh Cu to total Cu in the KCl extract and in the plant digest was calculated as follows:

$$\text{Fresh Cu (\%)} = \left(\frac{1}{1 + \frac{Q_s}{Q_{ei}}} \right) \times 100 \quad (2)$$

2.8. Data Treatment and Statistical Analyses

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 meet normal residual distribution and equal variance. Tukey's HSD tests were performed at 5% to identify the parameters monitored in the KCl extracts and in plant digests that were significantly affected by the addition of ACT. Pearson's and Spearman's correlation tests were conducted to test the linear relationship between the different parameters.

3. Results

3.1. Absorbance at 254 nm and pH of the KCl Extract

In the controls, the absorbance at 254 nm (A^{254}) of the KCl extract remained relatively similar along the depth gradient due to homogenization of the soil before the experiment (Figure 1a). ACT had a significant ($p < 0.05$) positive effect on the A^{254} of the KCl extract down to a depth of 7.5 cm. ACT increased the absorbance at 254 nm at depths of 0, 2.5, 5, and 7.5 cm by a factor of, respectively, 1.7 ($p < 0.01$), 7.0 ($p < 0.001$), 2.6 ($p < 0.01$), and 1.5 ($p < 0.001$). The pH of the KCl extract was acidic ($\text{pH} < 5.3$) and remained constant with depth in the controls, except at the soil surface where the pH was 6.8 (Figure 1b). Like for A^{254} , ACT increased ($p < 0.05$) the pH of the KCl extract down to a depth of 7.5 cm. Apart from the samples collected at the surface of the column, the pH was closely ($p < 0.05$) and positively correlated with A^{254} , suggesting that ACT increased the pH of the KCl extract (Supplementary Figure S2).

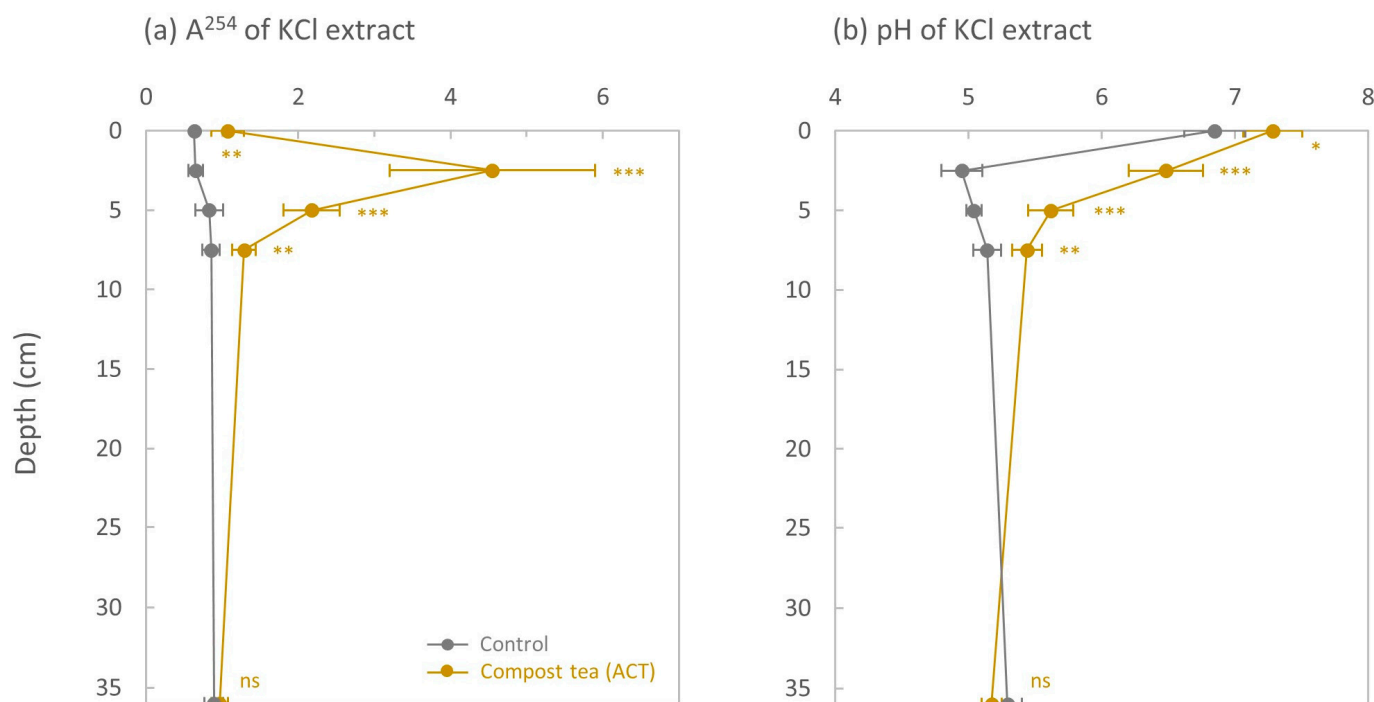


Figure 1. Distribution profile of A^{254} (a) and pH (b) in the 0.01 M KCl extract as a function of depth in a vineyard soil supplied with aerated compost tea (ACT) or not (control). For a given depth, *, **, and *** indicate that A^{254} (a) or pH (b) in the KCl extract when ACT was supplied differed significantly from that measured in the control at a probability level of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns: not significant.

3.2. Total Concentrations of Cu, Fe, and Al in the KCl Extract

The total concentrations of Cu, Fe, and Al in the KCl extract were not significantly affected ($p > 0.05$) by the downward transfer of water in the controls (Supplementary Table S1). The average total concentration of Cu in the controls was $1.5 \mu\text{M}$ (Figure 2a). ACT significantly increased ($p < 0.05$) the total concentration of Cu in the KCl extract at depths of 2.5, 5, and 7.5 cm, and the maximum concentration of Cu ($11.8 \mu\text{M}$) was measured at a depth of 2.5 cm in ACT-treated soils. Conversely, the total concentration of Cu in the solution extracted at the surface and at the bottom of the column did not vary ($p > 0.05$) between the two ACT treatments. The total concentrations of Fe and Al in the KCl extract increased significantly ($p < 0.05$) at depths of 2.5 cm and 5 cm following the addition of ACT (Figure 2b,c). Like for Cu, the maximum concentrations of Fe and Al ($33 \mu\text{M}$ and $52 \mu\text{M}$, respectively) were measured at a depth of 2.5 cm in ACT-treated soils. A positive linear relationship ($p < 0.05$) was observed between A^{254} and the total concentrations of Cu, Fe, and Al in the KCl extract, with a higher slope for Al (10.05) and Fe (6.46) compared to Cu (2.50) (Supplementary Figure S3).

3.3. Total Concentrations of Ca and Zn in the KCl Extract

In the controls, the total concentration of Ca in the KCl extract remained almost unchanged with depth, averaging 1 mM (Figure 2d). Following the addition of ACT, the total concentration of Ca in the KCl extract varied significantly with depth and was negatively correlated with A^{254} (Supplementary Figure S4). ACT decreased the concentration of total Ca by a factor of 1.3 at the soil surface ($p < 0.01$) and by a factor of 5.5 at a depth of 2.5 cm ($p < 0.001$), again highlighting the fact that the effect of ACT was more pronounced at 2.5 cm, the depth at which it induced the biggest increase in A^{254} in the KCl extract. The total concentration of Zn in the KCl extract was lower at the surface than in the rest of the

column (Figure 2e). At depths of 2.5, 5, and 7.5 cm, ACT reduced the total concentration of Zn in the KCl extract by a factor of, respectively, 1.8 ($p < 0.001$), 1.4 ($p < 0.05$), and 1.3 ($p < 0.01$). The close ($p < 0.05$) negative relationship between pH and the log concentration of Zn in the KCl extract (Supplementary Figure S5) suggests that the decrease in the solubility of Zn in ACT-treated soils was mainly due to the increase in pH (e.g., from pH 5 to pH 6.5 at a depth of 2.5 cm) induced by ACT.

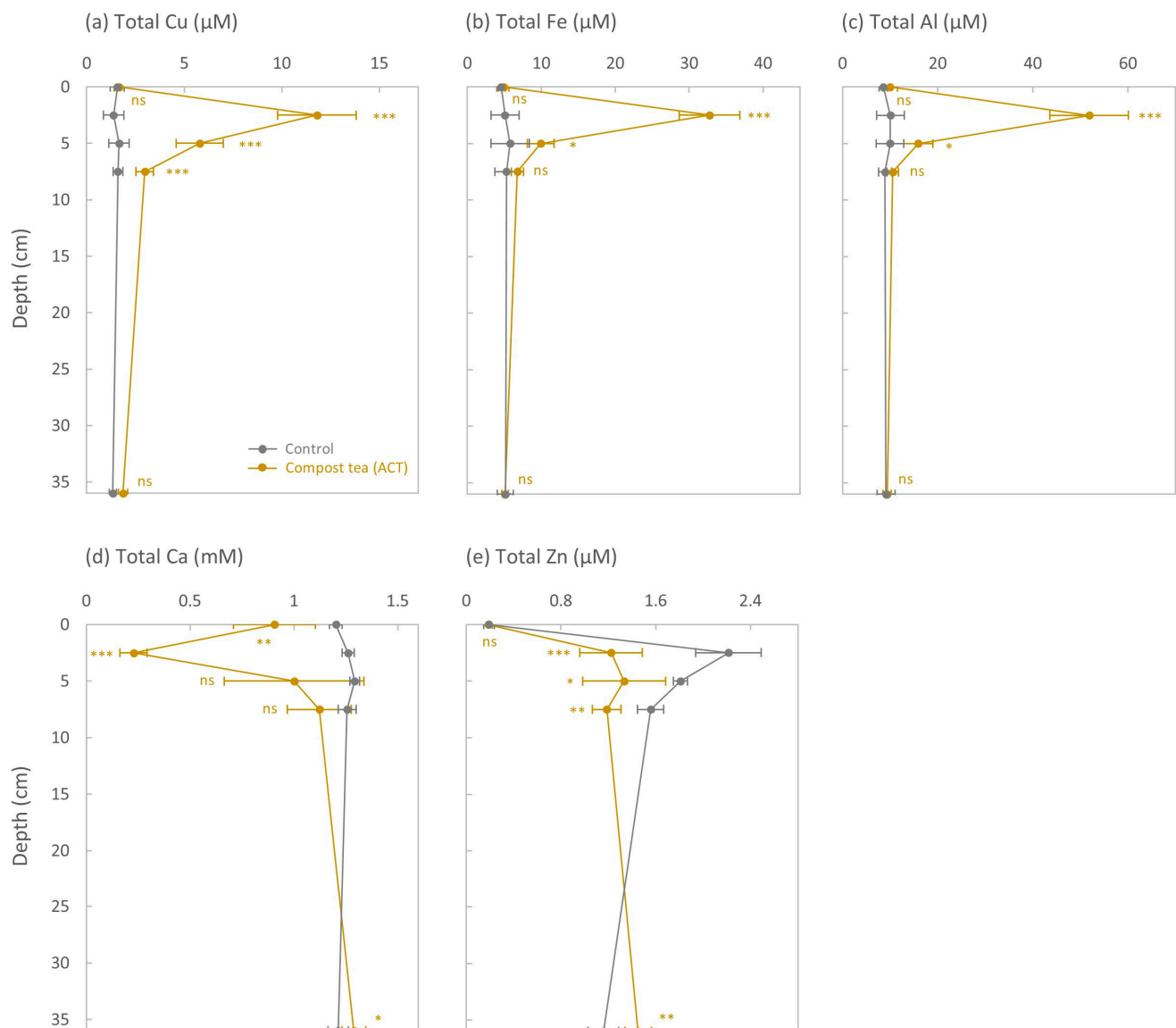


Figure 2. Distribution profile of total Cu (a), total Fe (b), total Al (c), total Ca (d), and total Zn (e) in the 0.01 M KCl extract as a function of depth in a vineyard soil supplied with aerated compost tea (ACT) or not (control). For a given depth, *, **, and *** indicate that total Cu (a), total Fe (b), total Al (c), total Ca (d), or total Zn (e) in the KCl extract when ACT was supplied differed significantly from that measured in the control at a probability level of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns: not significant.

3.4. Plant Growth

Plant growth was assessed by the dry matter content of shoots, as we did not collect the entire root system of the plants. ACT increased the overall dry weight of shoots ($p < 0.05$), on average, by 35% compared to the controls (Figure 3a). The addition of ACT enhanced the growth of pot marigold, whose dry matter content increased threefold, on average

($p < 0.001$), from 2.2 g DW per column in the controls to 8.0 g DW per column in ACT-treated soils. Conversely, the dry matter of crimson clover was not affected by the supply of ACT ($p > 0.05$), and averaged 8.7 g DW per column in both ACT treatments. As a result, the total biomass was predominantly composed of crimson clover in the controls, where it represented an average of 81% of the total biomass, whereas the distribution of biomass was around 50% for both species in ACT-treated soils.

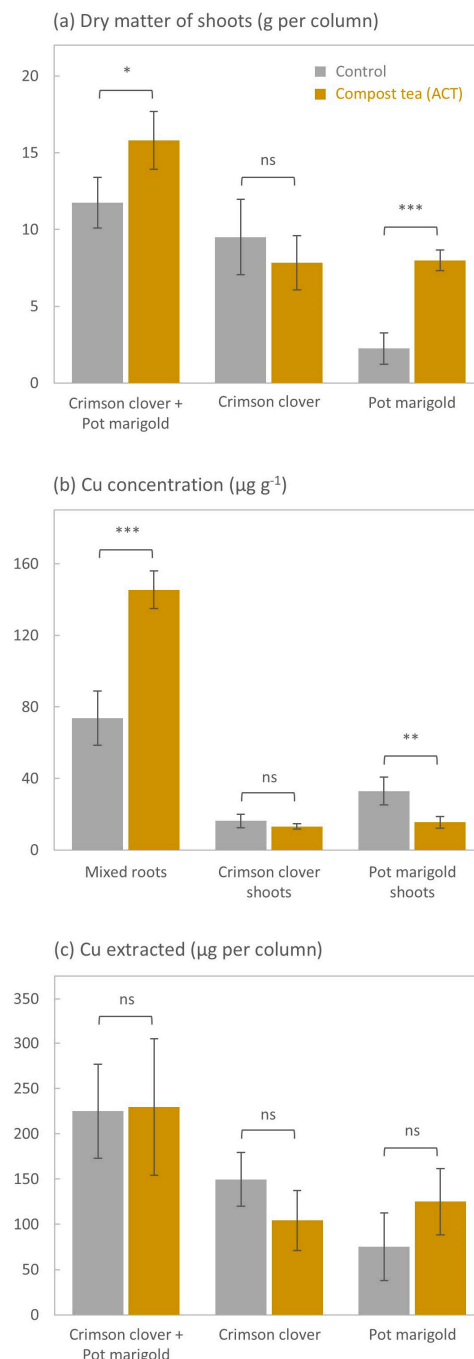


Figure 3. Dry matter of shoots (a), concentration of Cu in shoots and roots (mixed) (b), and amount of Cu extracted by shoots (c) in crimson clover and pot marigold grown in a vineyard soil supplied with aerated compost tea (ACT) or not (control) and harvested at flowering. For a given plant species, *, **, and *** indicate that dry matter (a) or Cu concentration (b) when ACT was supplied differed significantly from that measured in the control at a probability level of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns: not significant.

3.5. Plant Elemental Composition

Both plant species tended to accumulate Cu mainly in the roots (Figure 3b). In the controls, the concentration of Cu in the mixed roots (74 mg Cu kg^{-1}) was 2.2 times higher than in the shoots of pot marigold alone (i.e., 33 mg Cu kg^{-1}), and 4.6 times higher than in the shoots of crimson clover alone (16 mg Cu kg^{-1}). ACT increased the concentration of Cu in roots ($p < 0.05$); the concentration of Cu in the mixed roots was, on average, two times higher in ACT-treated soils than in the controls. Conversely, ACT did not increase the concentration of Cu in shoots; ACT had no significant effect ($p > 0.05$) on the concentration of Cu in the shoots of crimson clover, and decreased the concentration of Cu in the shoots of pot marigold by a factor of 2.1. As a result, the amount of Cu extracted per column averaged $227 \mu\text{g}$ and was not increased ($p > 0.05$) by the supply of ACT (Figure 3c). The concentration of several elements other than Cu in the plants (shoots, mixed roots) was affected by the addition of ACT (Table 2). ACT increased ($p < 0.05$) the concentration of K in the roots and shoots of both plant species by a factor of more than 5. ACT decreased ($p < 0.05$) the concentrations of Ca, Mg, Mn, and Zn in the shoots of both plant species by a factor of 1.5 to 3.4, depending on the species and on the element concerned. In contrast, there was no significant difference ($p > 0.05$) in the concentrations of N, Fe, and Si in the shoots between the plants supplied with ACT and the controls.

Table 2. Elemental composition of shoots of crimson clover, pot marigold, and their mixed roots at harvest. Plants were grown in a vineyard soil supplied with aerated compost tea (ACT) or not (control) and harvested at flowering.

		Dry Matter	N	P	K	S	Ca	Mg	Fe	Mn	Cu	Zn	Si
		g	%	mg g^{-1}				$\mu\text{g g}^{-1}$					
Crimson clover	Control	9.5	1.8	2.2	4.3	1.1	<u>15</u>	<u>3.9</u>	88	<u>109</u>	16	<u>88</u>	30
	ACT	7.8	1.6	1.9	<u>45</u>	<u>2.6</u>	7.5	1.9	141	71	13	53	32
Pot marigold	Control	2.2	1.9	<u>2.4</u>	6.3	3.6	<u>30</u>	<u>5.0</u>	91	<u>239</u>	<u>33</u>	<u>163</u>	26
	ACT	<u>8.0</u>	1.9	1.9	<u>42</u>	2.7	11	1.7	73	92	15	48	26
Mixed roots	Control	/	/	1.6	4.5	1.4	3.7	<u>1.8</u>	558	58	74	<u>74</u>	33
	ACT	/	/	1.6	<u>25</u>	<u>2.5</u>	3.4	1.1	806	65	<u>145</u>	47	25

Values are means of five independent replicates. For a given plant species or for mixed roots, underlined values are significantly higher ($p < 0.05$) than values obtained under the other ACT treatment.

3.6. $^{65}\text{Cu}/^{63}\text{Cu}$ Isotopic Ratio and Concentration of Fresh Cu in the KCl Extract

The $^{65}\text{Cu}/^{63}\text{Cu}$ isotopic ratio in the KCl extract decreased with soil depth (Figure 4a). In the controls, the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio of the KCl extract averaged 2.002 at the soil surface, 0.524 at a depth of 2.5 cm, and at depths of 5, 7.5, and 36 cm, was the same as that in nature (i.e., 0.446, source IUPAC). Hence, fresh Cu accumulated exclusively in the uppermost 2.5 cm of the soil, where its contribution to Cu extracted by KCl ranged from 52% at the surface to 5% at a depth of 2.5 cm (Figure 4a), and its concentration in the KCl extract from $0.83 \mu\text{M}$ at the surface to $0.07 \mu\text{M}$ at a depth of 2.5 cm (Figure 4b). ACT significantly increased ($p < 0.05$) the $^{65}\text{Cu}/^{63}\text{Cu}$ isotopic ratio in the KCl extract at depths of 2.5 ($p < 0.05$) and 5 cm ($p < 0.1$). The increase in the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio was the most pronounced at 2.5 cm, i.e., at the depth at which ACT produced the biggest increase in the concentration of SHS in the KCl extract. At this depth, fresh Cu accounted for 14% of the Cu extracted by KCl, and its concentration in the KCl extract reached $1.61 \mu\text{M}$ in ACT-treated soils. Conversely, there was no significant difference ($p > 0.05$) in the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio of the solutions extracted at depths of 7.5 cm and 36 cm between the controls and ACT-treated soils.

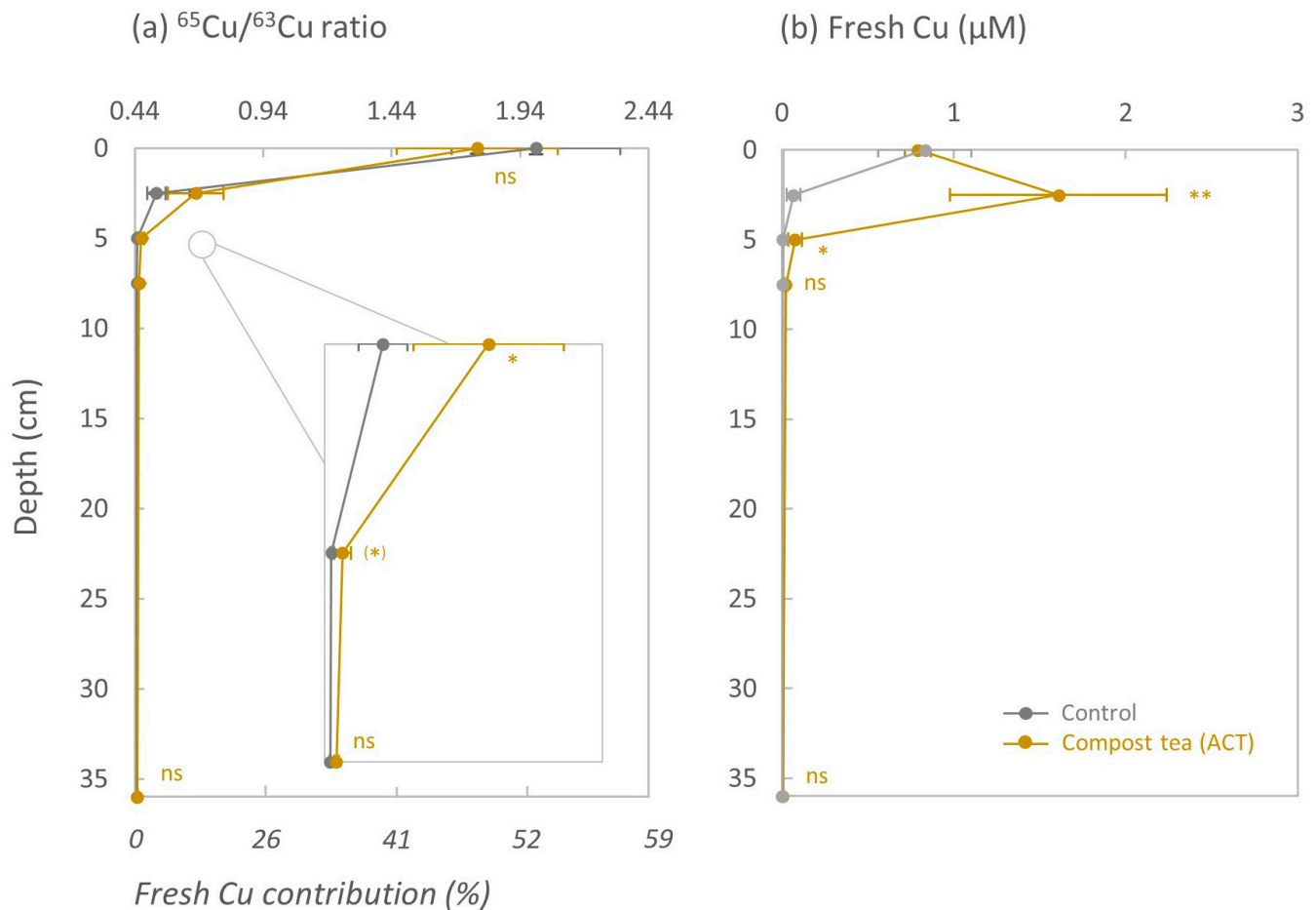


Figure 4. Distribution profile of the $^{65}\text{Cu}/^{63}\text{Cu}$ isotopic ratio (a) and of the concentration of fresh Cu (b) in the 0.01 M KCl extract as a function of depth in a vineyard soil supplied with aerated compost tea (ACT) or not (control). In (a), the inset is a zoom on the distribution profile between 2.5 and 7.5 cm depth, and the scale at the bottom of the figure is the relative contribution of fresh Cu to total Cu in the KCl extract. For a given depth, (*), *, and ** indicate that the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio in the KCl extract when ACT was supplied differed significantly from that measured in the control at a probability level of $p < 0.1$, $p < 0.05$, and $p < 0.01$, respectively; ns: not significant.

3.7. $^{65}\text{Cu}/^{63}\text{Cu}$ Isotopic Ratio in Plants

The $^{65}\text{Cu}/^{63}\text{Cu}$ isotopic ratio in plants was above 0.48, which is the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio of the soil assuming a homogeneous distribution of the ^{65}Cu spike within the column (Figure 5). In the controls, the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio was 0.88 in the mixed roots, which means that fresh Cu accounted for 23% of the amount of Cu accumulated in the roots. The $^{65}\text{Cu}/^{63}\text{Cu}$ ratio in shoots was significantly higher ($p < 0.05$) in crimson clover than in pot marigold. In crimson clover, fresh Cu accounted for 20% of the amount of Cu accumulated in shoots, whereas it only accounted for 6% in pot marigold. ACT had no significant impact ($p > 0.05$) on the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio in the mixed roots or in the shoots of crimson clover (Figure 5). Conversely, ACT increased the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio in the shoots of pot marigold ($p < 0.05$), where the proportion of fresh Cu in the amount of Cu accumulated in its shoots increased from 6% in the controls to 14% in ACT-treated soils.

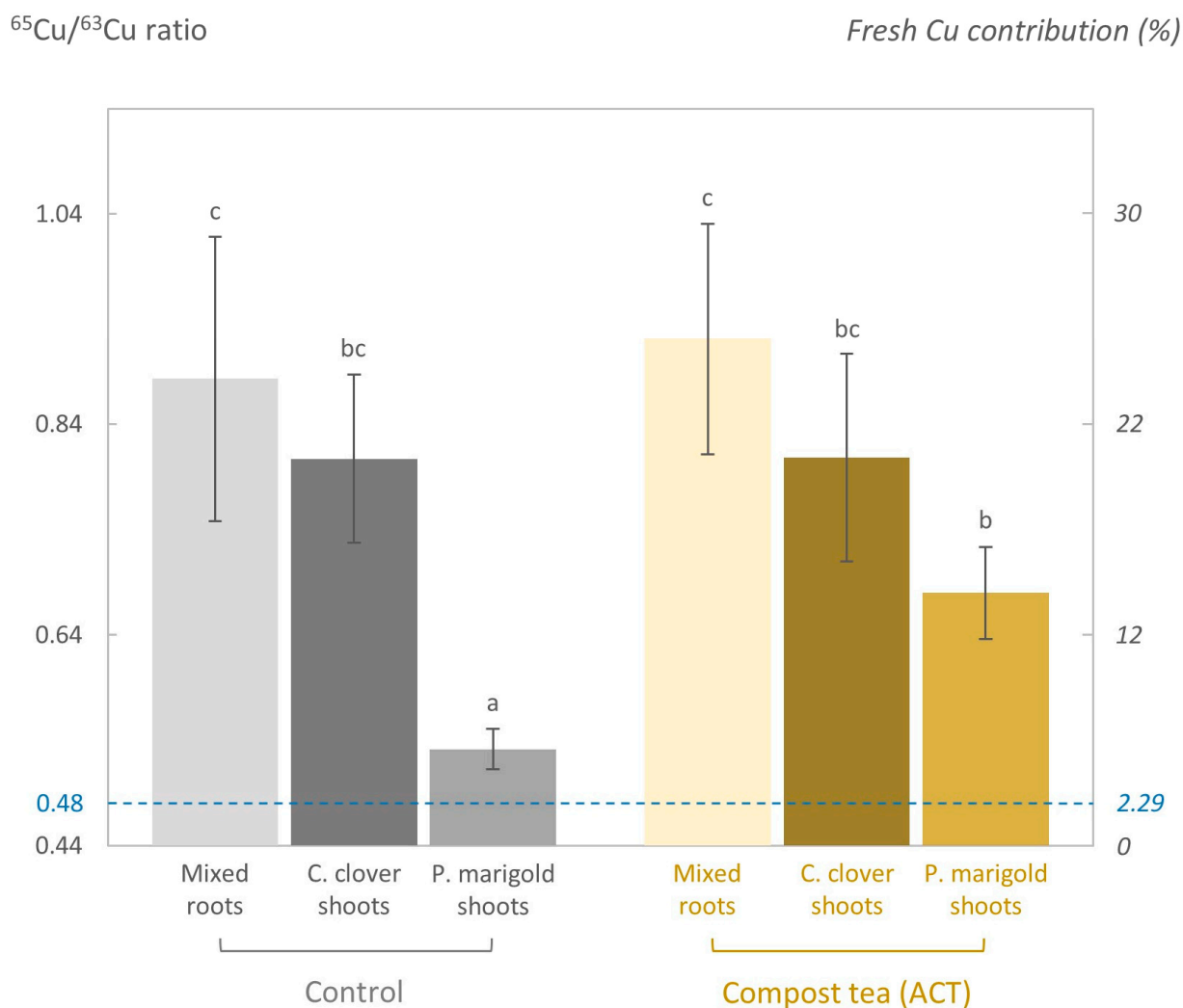


Figure 5. $^{65}\text{Cu}/^{63}\text{Cu}$ isotopic ratio in shoots of crimson clover, pot marigold, and in their mixed roots at harvest. Plants were grown in a vineyard soil supplied with aerated compost tea (ACT) or not (control) and harvested at flowering. The dotted blue line represents the $^{65}\text{Cu}/^{63}\text{Cu}$ isotopic ratio of the soil, assuming a homogeneous distribution of the ^{65}Cu spike within the column. The scale at the right of the figure is the relative contribution of fresh Cu to total Cu in plant tissues. For a given ACT treatment, mean values with different letters are significantly different ($p < 0.05$).

4. Discussion

4.1. The Effect of ACT on the Downward Transfer of Cu Was Limited to the Top 7.5 cm

The downward transfer of ACT and of metals in soil was assessed using A^{254} and the concentrations of Al, Fe, Cu, and Zn in the KCl extract. Results showed that, in the KCl extract, ACT increased the absorbance at 254 nm at depths of 0, 2.5, 5, and 7.5 cm, but not at a depth of 36 cm. The downward transfer of ACT was therefore limited to the top 7.5 cm, likely due to the sorption of the SHS it contains onto soil constituents such as metal oxyhydroxides, clays, and/or organic matter [30,31], and/or to the flocculation of SHS with Ca (see below). As SHS are known to complex and increase the solubility of Al, Fe, and Cu in soil [10,11], the concentration of these three elements was expected to increase with the increase in A^{254} in the KCl extract. The distribution profiles of Al, Fe, and Cu in the KCl extract were indeed very similar to that of A^{254} in ACT-treated soils, with concentrations higher than in the controls at depths of 2.5 and 5 cm. The close and positive linear relationships between A^{254} and the concentrations of Al, Fe, and Cu in the KCl extract suggest that the enhanced solubility of these three metals in ACT-treated

soils was due to complexation by SHS. The different slopes observed for Al, Fe, and Cu can be interpreted as differences in SHS affinity for these three metals. According to this hypothesis, the affinity of SHS for Al(III) and Fe(III) (slopes of 10.1 and 6.5, respectively) is higher than for Cu(II) (slope of 2.5), which is consistent with the higher affinity of SHS reported for trivalent metals than for divalent metals [32].

Conversely, the addition of ACT reduced the concentration of Ca in the KCl extract at depths of 0 and 2.5 cm, and that of Zn at depths of 2.5, 5, and 7.5 cm. A possible explanation for the reduction in the concentration of Ca is coagulation of Ca with the humic substances supplied by ACT [33] since the concentration of Ca measured in the KCl extract was above the flocculation threshold of 1–10 mM reported by Römken and Dolfing [34]. This hypothesis is supported by the negative relationship between A^{254} and the concentration of Ca in the KCl extract, although the relationship is not close, perhaps because some of the Ca that coagulated with humic substances was in colloidal form, and therefore not quantified by A^{254} . The increase in pH is the most likely reason for the drop in the concentration of Zn in the KCl extract of ACT-treated soils. Indeed, ACT had a higher pH than the soil used in this study (Table 1), and its addition increased the pH of the KCl extract in the top 7.5 cm layer. The close negative relationship between pH and the log concentration of Zn in the KCl extract supports this hypothesis, and more generally, the idea that Zn solubility in soil is strongly pH-dependent [35]. Interestingly, the relationship between the two variables did not apply to the solutions extracted at a depth of 2.5 cm. This can be interpreted as a side effect of SHS, whose affinity for Zn(II) is not null despite being lower than its affinity for Al(III), Fe(III), and Cu(II), and may increase the solubility of Zn when the concentration of SHS in pore water is particularly high, as it was the case here at a depth of 2.5 cm.

Taken together, these results show that the addition of ACT altered the solubility of metals in soil through its effect on the concentration of SHS and on the pH of the soil pore water. However, the effect of ACT on metal solubility was limited to the top 7.5 cm, which is the maximum depth to which the SHS it contained were transferred. The risk of metal leaching, particularly of Cu, associated with the use of ACT in vineyard soils, therefore appears to be limited, as shown by the similar composition of the KCl extract at the bottom of the soil column in both controls and in ACT-treated soils.

4.2. ACT Had No Effect on Cu Phytoextraction but Enhanced the Growth of Pot Marigold and the Accumulation of Cu in Roots

Surprisingly, ACT had no visible effect on the growth of crimson clover, in contrast to the results of a recent study by our team [24] in which the same ACT was applied to almost the same soil. Conversely, in the present study, ACT did enhance the growth of pot marigold, increasing its aboveground biomass by a factor of 3. Among the elements analyzed in plant tissues, only the concentration of K was higher in the shoots of pot marigold grown on ACT-treated soils. Does this mean that ACT promoted the growth of pot marigold by correcting a K deficiency? The fact that the concentration of K was among the highest in the compost tea (Table 1) tends to support this hypothesis. However, the concentration of K in the shoots of controls (Table 2) suggests that this hypothesis would be credible only if pot marigold were a K-demanding plant with a critical leaf concentration for K deficiency higher than 0.63%. ACT enhanced the accumulation of Cu in roots. The concentration of Cu in the mixed roots was, on average, two times higher in ACT-treated soils than in the controls, likely due to a contribution of Cu-SHS complexes to root uptake of Cu. Indeed, a recent study by our team [11] showed 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. Therefore, we suspect that Cu-SHS complexes are sufficiently labile to significantly contribute to the flux of free Cu ions taken up by roots. However, most (if not all) the excess Cu taken up by plants in ACT-treated soils was stored

in the roots, as evidenced by the twofold higher concentration of Cu in the roots of plants grown on ACT-treated soils than in the roots of the controls. Indeed, plants usually increase sequestration of Cu in roots in response to increased phytoavailability of Cu in the soil to prevent the accumulation of Cu in their green tissues, since excess Cu is detrimental to photosynthesis [36,37]. Aboveground, the concentration of Cu was no higher in plants grown on ACT-treated soils than in controls, and even lower in pot marigold. This explains why ACT did not increase the total amount of Cu accumulated in shoots, i.e., the amount of Cu phytoextracted, although it did enhance the growth of pot marigold. As reported for nitrogen [38], Cu can be diluted in aboveground parts of the plant due to the fact that structural tissues (e.g., stem, veins) often contain lower concentrations of Cu than metabolic tissues (e.g., leaf blades), and that their relative contribution to aboveground biomass increases with plant age and/or biomass. Hence, the lower concentration of Cu measured in the shoots of pot marigold grown in ACT-treated soils may have resulted from more intense dilution of Cu in leaves that were larger than the leaves of the control plants.

4.3. Fresh Cu Was Barely Transferred Downward and Was Slightly More Sensitive than Aged Cu to the Addition of ACT

Isotopic labeling made it possible to trace the downward transfer of fresh Cu in the soil via the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio in the KCl extract. In the controls, the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio in the KCl extract decreased with soil depth, highlighting the fact that fresh Cu accumulated preferentially in the topsoil. The $^{65}\text{Cu}/^{63}\text{Cu}$ ratio in solutions extracted at depths of 5, 7.5, and 36 cm showed no isotopic enrichment in ^{65}Cu , evidence that fresh Cu was barely transferred downward but remained in the top 5 cm of the soil. Plants may have limited the vertical transfer of fresh Cu through sorption of fresh Cu on their roots [39] rather than through their uptake of fresh Cu, since less than 0.5% of the amount of ^{65}Cu added to the soil was recovered in plant shoots. The limited transfer of fresh Cu to deeper soil evidenced by this study is in agreement with the results of the few studies that have investigated the downward transfer of Cu in soil using stable isotope labeling [21], as well as with vineyard soil profiles showing Cu accumulation in the topsoil [40], and can be explained by strong affinity of Cu for (and rapid sorption of Cu onto) soil constituents, particularly organic matter present in the surface layer [41]. Retention of fresh Cu in the top centimeters of the soil was observed whether Cu was applied as free Cu ion, as in the $\text{Cu}(\text{NO}_3)_2$ form used in this study, or as pig manure [20]. This is therefore a feature of Cu behavior in soil that seems to be observed regardless of the form in which Cu is applied to the soil.

It can be assumed that the downward transfer of fresh Cu is affected by soil characteristics and agricultural practices. Here, we tested the effect of ACT while assuming that, like for total Cu (see above), the SHS it contains would promote the transfer of fresh Cu to deeper soil. Contrary to our expectations, ACT had only a small, although still detectable, effect on the downward transfer of fresh Cu. Like total Cu, ACT only increased the solubility of fresh Cu at the depths at which it increased the concentration of SHS in the KCl extract, i.e., mainly at a depth of 2.5 cm and to a much lesser extent at a depth of 5 cm. The fact that ACT increased the solubility of total Cu but not that of fresh Cu at a depth of 7.5 cm even suggests that the downward transfer of fresh Cu was more restricted than that of SHS in the vineyard soil used for the present study. The higher $^{65}\text{Cu}/^{63}\text{Cu}$ isotopic ratio in the solution extracted at a depth of 2.5 cm in ACT-treated soils suggests that fresh Cu was a little more available for complexation and solubilization by SHS than aged Cu, for reasons that likely have to do with aging. This interpretation is in line with the fact that Cu aging in soils is a slow process [42], and implies that Cu binds to soil constituents through reactions, some of which require interaction times of more than eight weeks (i.e., the aging time in our experiment, which corresponds to the delay between the soil labeling

with $^{65}\text{Cu}_{\text{ei}}$ and the first addition of ACT) such as diffusion into micropores, changes in the forms of surface complexes, and occlusion within soil particles [43,44].

4.4. Fresh Cu Was More Available to Plants than Aged Cu Due to Its Preferential Accumulation in the Topsoil

The $^{65}\text{Cu}/^{63}\text{Cu}$ isotopic ratio in plants was systematically higher than the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio in soil, assuming the distribution of the ^{65}Cu spike within the column is homogeneous. This highlights the fact that relatively more fresh Cu was transferred to plants than aged Cu, and that consequently fresh Cu was more available to plants than aged Cu in the vineyard soil used in our study. Combined with the fact that fresh Cu was barely transferred downwards (as explained above), this result suggests that the higher phytoavailability of fresh Cu than that of aged Cu was due to its preferential accumulation in the topsoil. In the controls, marked differences in the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio were observed between the shoots of crimson clover and the shoots of pot marigold, suggesting that the two species were not exposed to exactly the same concentrations of fresh Cu. The higher enrichment in ^{65}Cu in crimson clover can be interpreted as a consequence of its root system, which is composed of fibrous lateral roots [45], which likely spread out more in the surface soil, and are hence more exposed to fresh Cu than the taproot system of pot marigold. This interpretation would imply that the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio in the mixed roots was intermediate between that in the shoots of crimson clover and pot marigold, which, however, was not the case. The enrichment in ^{65}Cu was just as high in the mixed roots as in the shoots of crimson clover (Figure 5) for reasons that may be linked to our root harvesting method, which consisted in simply pulling the roots out of the soil at the surface of the column, and resulted primarily in the collection of surface roots, i.e., those most exposed to fresh Cu.

ACT increased the $^{65}\text{Cu}/^{63}\text{Cu}$ ratio in the shoots of pot marigold. In our view, this increase was not linked to the greater availability of fresh Cu for solubilization by SHS compared to aged Cu in the top 5 cm of the soil, since the difference between the availability of the two Cu pools was small and was not associated with a higher concentration of Cu in shoots in ACT-treated soils. In the same way as we interpreted the difference in the enrichment in ^{65}Cu between the two species (see above), we believe this increase is rather the result of the expansion of the roots of pot marigold in the surface layer of ACT-treated soils, i.e., where ACT and the nutrients it contains accumulated most. Although this hypothesis was not confirmed in the present study, in which root exploration in the soil column was not investigated, it is consistent with the plant's strategy for acquiring soil nutrients, directing roots toward nutrient hotspots [46].

5. Conclusions

This study provided new insights into the dynamics of Cu in vineyard soil and how these dynamics can be influenced by adding aerated compost tea to the soil. Firstly, this study confirmed that the SHS contained in ACT can bind Cu as well as Al and Fe, thereby increasing their solubility in soil, likely including the rhizosphere. However, the increase in Cu solubility induced by ACT was restricted to the topsoil, as the vast majority of the SHS contained in ACT was still found in the top 7.5 cm of the column at the end of the experiment. The risk of promoting the downward transfer of Cu in the soil by adding ACT thus appears to be limited. Secondly, the study showed that even if ACT increased plant growth and Cu phytoavailability, it did not increase Cu phytoextraction since excess Cu taken up by plants in ACT-treated soils was retained in the roots. Thirdly, this study showed that the downward transfer of fresh Cu added to the surface was limited to the top 5 cm of the column, even after the addition of ACT, and that fresh Cu was more likely to be transferred to plants than aged Cu due to its preferential accumulation in the topsoil.

The risk associated with the use of ACT on vineyard soils is thus more of increasing Cu availability to living organisms in the topsoil. These results will now be verified at field scale, as several factors that were not (or only poorly) reproduced in our experimental design are likely to influence the dynamics of both fresh and aged Cu in the soil, such as the existence of a gradient of aged Cu as a function of soil depth, and the role played by soil fauna in structuring soil porosity, notably via the bioturbation activity of earthworms.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/su17104414/s1>, Figure S1: Photograph of a soil column at harvest; Figure S2: Relationship between A^{254} and pH in the KCl extract; Figure S3: Relationship between total Cu, total Al, total Fe, and A^{254} in the KCl extract; Figure S4: Relationship between total Ca and A^{254} in the KCl extract; Figure S5: Relationship between total Zn and pH in the KCl extract; Table S1: Selected physico-chemical properties and elemental composition of the KCl extract.

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Abbreviations

The following abbreviations are used in this manuscript:

ACT	Aerated compost tea
SHS	Soluble humic substances
DOM	Dissolved organic matter
CEC	Cation exchange capacity
WHC	Water holding capacity
A^{254}	Absorbance at 254 nm
DW	Dry weight
DGT	Diffusive gradients in thin-films

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