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Changes in Cu accumulation and fractionation along soil depth in acid soils of vineyards and abandoned vineyards (now forests)

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ABSTRACT

This study investigated changes in copper accumulation and fractionation based on the depth of soils obtained from active and abandoned vineyards. Soil samples were collected at different depths in three areas with active and abandoned vineyards, and the concentrations of total copper and copper fractions were measured in these soil samples. Results revealed that in active vineyards, total copper accumulation was the highest in the first layer of soil (depth = 0–2 cm), with concentrations (193–892 mg kg^{-1}) considerably higher than those in the deepest soil layer (depth = 40–50 cm; concentration = 63–71 mg kg^{-1}). This accumulation was significantly correlated with the total carbon concentration in the soil. However, the total copper concentration in abandoned vineyards (84–374 mg kg^{-1}) was the highest in the subsurface layers (depth = 10–15 cm) and was not significantly correlated with the total carbon content. Moreover, the percentage of available copper was lower in abandoned vineyards than in active vineyards. In terms of copper fractionation, the main fraction in active vineyard soil was copper bound to organic matter, whereas the main fraction in abandoned vineyard soil was residual copper. Therefore, vineyard abandonment and its transformation into forest changed the distribution and fractionation of copper based on soil depth, thus decreasing the amount of available copper and improving the soil quality, which could enable new ecosystems.

1. Introduction

Copper-based fungicides have been used in vineyards for decades (Pavlovic, 2011). Therefore, high amounts of copper (Cu) have accumulated in such soils worldwide (Komárek et al., 2010). Cu may enter vineyard soils through the use of fertilizers, manure, or Cu-polluted irrigation water (Hölzel et al., 2012; Kelepertzis et al., 2018). The accumulation of Cu in vineyard soils can be toxic to plants (Miotto et al., 2014) and soil biota (Karimi et al., 2021). Moreover, high Cu concentrations in soil pose the risk of polluting surface and groundwater in the areas surrounding vineyards (Cornu et al., 2019). These risks that negatively affect soil biota and freshwater rely on potential Cu bioavailability and mobility, both highly depend on the physicochemical characteristics of the soil (Brun et al., 2001) such as pH (Álvarez-Puebla et al., 2004), the organic matter content (Fernández-Calviño et al., 2010), the clay content (Wäldchen et al., 2012), and the cation exchange capacity (Rashidi and Seilsepour, 2008). Moreover, Cu bioavailability and mobility in soil are not directly related to the total Cu (Cu_T) concentration (Wightwick et al., 2008) but dependent on metal fractionation between different soil components (Arias et al., 2004) and the energy and strength with which Cu is bound to them. Therefore, examining Cu fractionation in soil is essential to improve knowledge of its potential bioavailability and mobility.

The Cu concentrations in vineyard soils have a heterogeneous spatial distribution within topsoil, and areas with great phytotoxic potential can thus develop (Jacobson et al., 2007). The degree of Cu accumulation varies between and within vineyards, ranging from values close to those found in natural soils (approximately 30 mg kg^{-1}) (Fernández-Calviño et al., 2008), to > 3000 mg kg^{-1} (Mirlean et al., 2007). This variability depends on the age of the vineyard, the climate area, or heterogeneous management practices. Moreover, Cu concentrations may differ in the same vineyard soil based on surface and depth. For example, Fernández-Calviño et al. (2013) observed variations in the Cu concentrations (ranging from 235 to 1438 mg kg^{-1}) in the soil surface layers (depth = 0–5 cm) within a 240-m^2 vineyard. Although total Cu concentrations in vineyard soil can vary between the soil layers and they

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generally decrease with soil depth, few studies have examined the changes in Cu accumulation in active vineyard soils based on depth (Pietrzak and McPhail, 2004; Beygi and Jalali, 2019; Sonoda et al., 2019).

Vineyard land use changes when the enterprise is abandoned, and it is thus important to understand changes in Cu within such soils. The Regulatory Council of Ribeira Sacra Wine estimates that approximately 70 % of vineyards have been abandoned since the beginning of the 19th century. This has led to the establishment of autochthonous forest systems and an evident change in land use. There are thus regions where forest ecosystems have naturally been established on abandoned vineyards with highly Cu-polluted soils. However, such land use transformations likely alter Cu fractionation and its potential availability (Fernández-Calviño et al., 2008). Under these circumstances, Cu aging becomes an important factor that determines Cu bioavailability and makes Cu less available through its transfer from labile fractions to more recalcitrant ones (Arias-Estévez et al., 2007). This effect is crucial and must be considered before abandoning vineyards and allowing the establishment of forest ecosystems. Therefore, this study aimed to investigate the changes in total Cu accumulation and Cu fractionation with respect to soil depth and land use (active vs. abandoned vineyards) in acidic soils that were highly polluted with Cu.

Herein, the following hypotheses are presented: (1) Cu accumulation in active acidic vineyard soil is the highest in the uppermost layers, and it then decreases with soil depth; (2) a similar Cu accumulation pattern with depth is expected to occur in abandoned vineyards that have become forests; and (3) abandoned vineyard soils have a higher proportion of nonlabile Cu fractions than active vineyard soils.

2. Material and methods

2.1. Study area and soil sampling

Three active vineyards (v) were selected as a function of the Cu_T concentration in the 0–20 cm–deep soil layer (>150 mg kg^{-1}) by using vineyard soils data-base: Portotide-O Mato (PM), Portotide (PT), and A Raña (AR). All vineyards are located on steep slopes landscape within the Miño river valley (Galicia, NW Spain) and belong to the Ribeira Sacra Designation of Origin, a well-known vineyard area where vines have been cultivated for several centuries. Furthermore, the oceanic Mediterranean transition climate serves as a characteristic of the study area, and the study area has a mean annual temperature of 15 °C and annual rainfall ranging from 800 to 1300 mm. It is therefore necessary to use Cu-based fungicides in such climatological conditions. Schist with small patches of granite dominates the local lithology, and the soil in all vineyards had a sandy loam texture.

In addition, many vineyards have been abandoned for over 35 years for socioeconomic reasons, leading to their transformation into forests and thus changing in the purpose of land use. Consequently, forests adjacent to the active vineyards were also sampled to be used as samples from abandoned vineyards that have been transformed into an Atlantic forest (f). The dominant plant species in these forests are *Quercus robur* and *Quercus pyrenaica*. In all the vineyards (active and abandoned), soil samples were collected up to a depth of 50 cm and one composite sample was taken from each selected depth. Soil samples were collected from layers that were 2-cm apart at a depth of 0–10 cm, 5-cm apart at a depth of 10–20 cm, and 10-cm apart at a depth of 20–50 cm. A total of 10 samples were thus obtained per sampled vineyard area and within forest areas (60 soil samples). Each soil sample was air-dried, sieved using a 2-mm mesh, and homogenized prior to analysis.

2.2. Soil characterization

Soil pH in distilled water (pH_w) and 0.1-N KCl (pH_{KCl}) was measured in 1:2.5 (w:v) suspensions using a combined glass electrode (Crison, Barcelona, Spain). The soil samples were milled using an agate mortar RM200 Retsch (Haan, Germany), and the total organic carbon (TOC) and nitrogen (N) contents in the samples were determined using a Thermo Finnigan 1112 Series NC autoanalyzer. The effective cation exchange capacity (eCEC) of the soil samples was obtained after measuring major basic cations (Na^+, K^+, Ca^{+2}, and Mg^{+2}) during soil extraction using 1-N NH_4Cl and Al^{+3} obtained from 1-N KCl–based soil extraction (Fernández-Calviño et al., 2009). Sodium (Na) and potassium (K) concentrations were measured using atomic emission spectrometry, whereas calcium (Ca), magnesium (Mg), and aluminum (Al) concentrations were measured through atomic absorption spectrometry using a Thermo Solaar M Series spectrophotometer (Thermo Fisher Scientific Inc., Massachusetts, USA). The bacterial community activity was estimated by extracting bacterial communities using homogenization/centrifugation and a method for incorporating leucine (Leu) into bacteria (Bååth et al., 2001). This methodology estimates bacterial protein synthesis, which is used as a proxy for bacterial activity, by estimating the amount of ^{3}H-Leu incorporated into the extracted bacteria over time (expressed in DPM/h).

2.3. Total and potentially available Cu

Cu_T was extracted from soil by digesting 0.5 g of soil with 5 mL of HNO_3, 4 mL of HF, and 1 mL of HCl in a microwave oven at 6.9 10^5 Pa (Fernández-Calviño et al., 2009). Potentially available Cu (Cu_{ED}) was extracted from soil by shaking 5 g of soil in a 50-mL solution containing 0.02 M of Na_2EDTA and 0.5 M of NH_4OAc at a pH of 4.65 for 1 h (Arias et al., 2004). The Cu concentrations of extracts were measured using atomic absorption spectrometry with the Thermo Solaar M Series spectrophotometer.

2.4. Cu fractionation

Cu fractions bound to different soil components indicate their potential mobility in soil. These values were obtained after several different single selective extractions (Arias et al., 2004). Subsequently, Cu concentrations were measured after soil extraction using 1-N NH_4Ac (pH 7) (Cu_ac), 0.1-N Na-pyrophosphate (Cu_p), 0.2-N oxalic acid–ammonium oxalate (pH 3) (Cu_o), and 0.2-N oxalic acid–ammonium oxalate–ascorbic acid (pH 3.25) (Cu_ao). The Cu concentrations in the extracts were then measured via atomic absorption spectrometry using the Thermo Solaar M Series spectrophotometer.

The following fractions were obtained according to their bioavailability: i) the exchangeable Cu fraction (Cu_{EX}) coinciding with the concentration in the Cu_ac extraction, ii) the metal fraction bound to soil organic matter (Cu_{OM}) obtained by subtracting Cu_ac from Cu_p, iii) the metal bound to iron (Fe) and Al inorganic amorphous (non-crystalline) oxyhydroxides (Cu_{IA}) obtained from Cu_o minus Cu_p, iv) the metal bound to Fe and Al crystalline oxyhydroxides (Cu_C) obtained from Cu_ao minus Cu_o, and v) the residual metal fraction (Cu_R) obtained from Cu_T minus Cu_ao. The Cu bioavailability sequence was $Cu_{EX} > Cu_{OM} > Cu_{IA} > Cu_C > Cu_R$ (Fernández-Calviño et al., 2009).

2.5. Statistical analyses

A two-way ANOVA test was used to compare the results of the samples obtained from the different soil depths of the active and abandoned vineyard soils. Pearson's correlation analyses were used to test the correlation between Cu_T and carbon accumulation in different soil layer samples from active and abandoned vineyard soils. All the statistical analyses were conducted using IBM SPSS Statistics 25.

3. Results

3.1. Variations in general soil characteristics with depth

In all active vineyard soil samples, pH_w and pH_{KCl} were the highest in the samples obtained from the 0–2 cm–deep surface layer, and the values decreased progressively with depth up to 10 cm. Thereafter (depth = 10–50 cm), pH_W and pH_{KCl} remained relatively constant with increasing depth (Figs. 1A and 1C). In the samples obtained from the abandoned vineyards, the general trend and magnitude of pH changes with depth were similar to those in active vineyards (Figs. 1B and 1D). Furthermore, TOC significantly ($p < 0.001$; Table 1) decreased with depth from 0–2 to 10–15cm in active vineyard soils; in deeper horizons, the TOC remained constant (Fig. 2A). In abandoned vineyards, although the TOC showed a similar trend with respect to depth as that in active vineyards (Fig. 2B), significantly ($p = 0.001$; Table 1) TOC concentrations were observed in all the soil layers compared with the concentrations in layers within active vineyards. The trend of eCEC was similar to that of TOC, i.e., eCEC values were significantly higher ($p < 0.001$; Table 1) in the surface layers than in the deeper layers in both active and abandoned vineyard soils (Figs. 2C and 2D). The trend and magnitude of eCEC variation with respect to depth were similar in active and abandoned vineyards (Table 1). The results of other soil characteristics, such as nutrients and bacterial activity, are presented in the Supplementary material (Tables S1–S8).

3.2. Variations in total and potentially bioavailable Cu with soil depth

Very high Cu_T concentrations were found in the uppermost soil layer (depth = 0–2 cm) in active vineyards (193–892 mg kg^{-1}), but they decreased significantly ($p = 0.024$; Table 1) with increasing depth. In the deeper layers, Cu_T concentrations decreased up to a depth of 6–20 cm, reaching a concentration of approximately 63–71 mg kg^{-1} in the deepest sampled layer (Fig. 3A). By contrast, the general trend differed in abandoned vineyard soil layer samples. Intermediate soil layers had more Cu_T concentrations than the surface layers (Fig. 3B). Although the Cu_T concentration was 46.0–155 mg kg^{-1} at a depth of 0–2 cm, it was approximately two-fold higher (84–374 mg kg^{-1}) at a depth of 10–15 cm. In deeper soil layers (depth =15–50 cm), Cu_T concentrations decreased, reaching the values between 28.1 and 69.8 mg kg^{-1}. Moreover, the Cu_T concentrations in abandoned

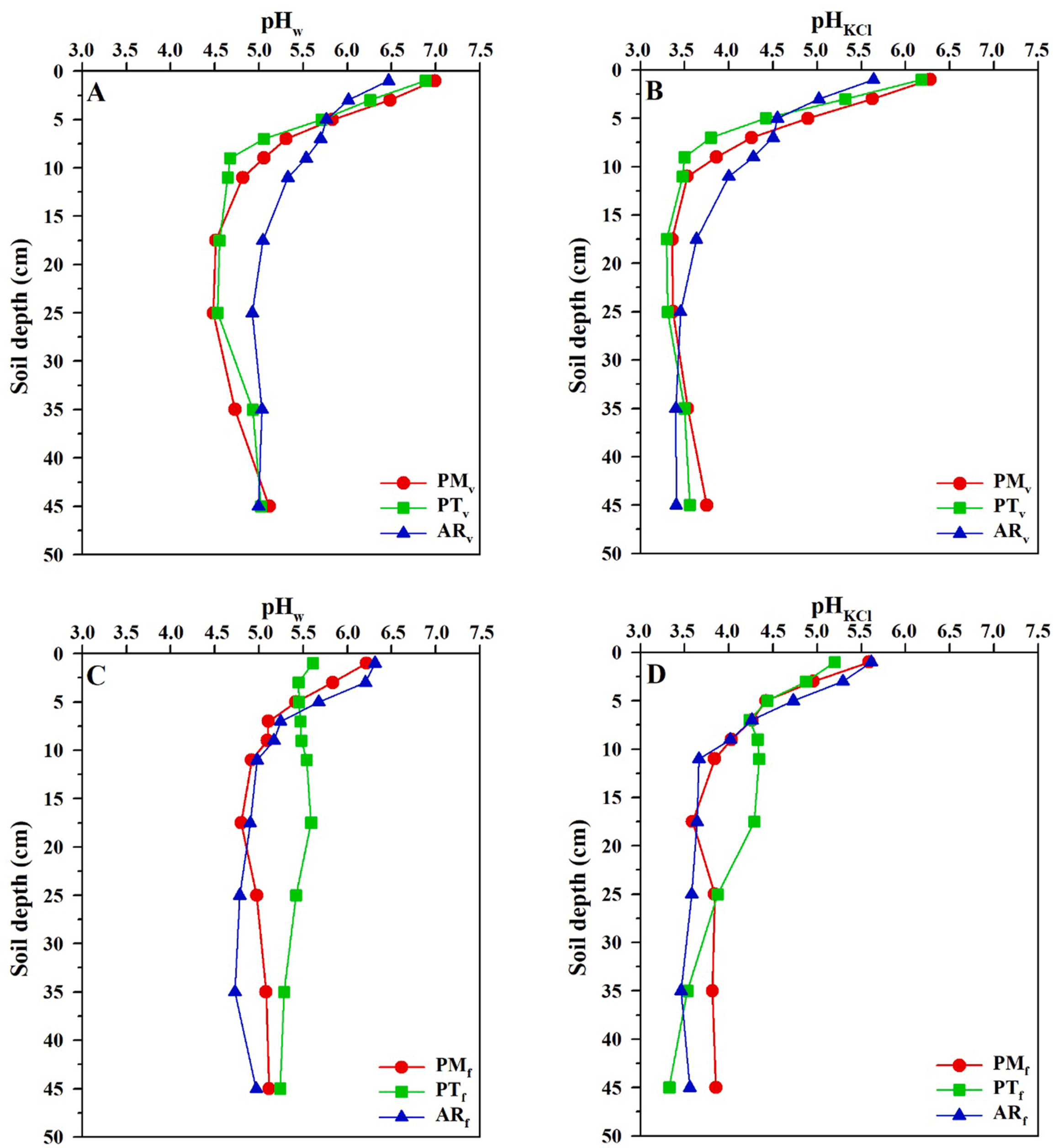

Fig. 1. Distribution of pH in water (pH_w) and KCl (pH_{KCl}) with increasing depth in samples from active (A and B) and abandoned (C and D) vineyards.

Table 1
Two-way ANOVA results of the analyses of changes in soil characteristics and copper fractions using soil depth and land use as factors.

	Factors	DF	F	P
pH_W	Use	1	0.021	0.885
	Depth	9	20.649	< 0.001
	Use × Depth	9	2.488	0.023
pH_{KCl}	Use	1	0.784	0.381
	Depth	9	54.867	< 0.001
	Use × Depth	9	2.230	0.040
TOC	Use	1	12.883	0.001
	Depth	9	13.180	< 0.001
	Use × Depth	9	2.113	0.051
eCEC	Use	1	1.847	0.182
	Depth	9	11.226	< 0.001
	Use × Depth	9	0.466	0.888
Cu_T	Use	1	16.657	< 0.001
	Depth	9	2.476	0.024
	Use × Depth	9	1.487	0.186
Cu_{ED}	Use	1	34.887	< 0.001
	Depth	9	3.940	0.001
	Use × Depth	9	3.934	0.001
% Cu_{ED}	Use	1	58.242	< 0.001
	Depth	9	1.773	0.104
	Use × Depth	9	1.828	0.093
Cu_{EX}	Use	1	52.987	< 0.001
	Depth	9	6.288	< 0.001
	Use × Depth	9	2.426	0.026
Cu_{OM}	Use	1	66.523	< 0.001
	Depth	9	4.660	< 0.001
	Use × Depth	9	2.952	0.009
Cu_{IA}	Use	1	17.467	< 0.001
	Depth	9	2.666	0.016
	Use × Depth	9	2.623	0.017
Cu_C	Use	1	5.018	0.031
	Depth	9	1.438	0.205
	Use × Depth	9	0.794	0.623
Cu_R	Use	1	0.559	0.459
	Depth	9	1.473	0.104
	Use × Depth	9	0.463	0.890
% Cu_{EX}	Use	1	36.967	< 0.001
	Depth	9	6.154	< 0.001
	Use × Depth	9	2.012	0.063
% Cu_{OM}	Use	1	8.149	0.007
	Depth	9	0.229	0.988
	Use × Depth	9	0.129	0.999
% Cu_{IA}	Use	1	22.442	< 0.001
	Depth	9	2.703	0.015
	Use × Depth	9	1.637	0.138
% Cu_C	Use	1	4.940	0.032
	Depth	9	1.277	0.279
	Use × Depth	9	1.302	0.266
% Cu_R	Use	1	29.280	< 0.001
	Depth	9	0.604	0.786
	Use × Depth	9	0.576	0.808

vineyards were significantly lower than those in active vineyards ($p < 0.001$; Table 1).

Cu_{ED} values ranged between 110 and 496 mg kg^{-1} in the uppermost soil layers (depth = 0–2 cm) of active vineyards (Fig. 3C). Generally, Cu_{ED} decreased up to a depth of 4 cm (73–189 mg kg^{-1}). Although Cu_{ED} generally showed a decreasing trend, an increase in Cu_{ED} was observed in the intermediate soil layers (from 10–15 to 35–40 (Fig. 3C). Further, significant decreases in Cu_{ED} ($p = 0.001$; Table 1) to 14–30 mg kg^{-1} in the deepest soil layers (depth =40–50 cm) of active vineyards were observed. Abandoned vineyard soils showed a different trend from active vineyards (Fig. 3D). In the surface layers (depth = 0–2 cm), although the range of Cu_{ED} was similar to that in the deep layers (10–31 and 5–8 mg kg^{-1}, respectively), an increase in Cu_{ED} in the intermediate layers was recorded at a depth of 15–20 cm, with a range of 42–97 mg kg^{-1} in all the soil samples. This trend observed for Cu_{ED} in abandoned vineyards (Fig. 3D) was the same as that of Cu_T (Fig. 3B). The total Cu_{ED} concentration was significantly higher ($p < 0.001$; Table 1) in active vineyards than that in abandoned vineyards for all the soil layers.

In addition, the percentage of Cu_{ED} significantly decreased ($p < 0.001$; Table 1) in abandoned compared to active vineyard soils, but no change was observed with soil depth (Table 1).

3.3. Variation in Cu fractionation with soil depth

Cu_{EX} in the samples of active vineyard soils ranged from 0.9 to 5.4 mg kg^{-1} in the uppermost soil layer (depth = 0–2 cm). Then, it increased with depth, reaching a maximum concentration (12.1–18.1 mg kg^{-1}) at a depth of 15–30 cm (Fig. 4A). For deeper layers (depth = 40–50 cm), Cu_{EX} decreased, reaching the values of 0.7–0.8 mg kg^{-1}. By contrast, the abandoned vineyard soil samples showed two trends (Fig. 4F). First, the Cu_{EX} values in all AR_f soil layers were similar, with a range of 0.7–1.2 mg kg^{-1}. Second, PM_f and PT_f soils had higher values of Cu_{EX} at a depth of 6–30 cm than the surface soil layer. All abandoned vineyard soils exhibited similar Cu_{EX} values in the surface (depth = 2 cm) layer (0.7–0.8 mg kg^{-1}), and the values were similar to those found in the deepest soil layer (0.7 mg kg^{-1} at a depth of 40–50 cm). Furthermore, Cu_{EX} values were significantly higher in the active vineyard soils than in abandoned vineyard soils ($p < 0.001$; Table 1).

As a general trend, in active vineyard soils, Cu_{OM} decreased significantly ($p < 0.001$; Table 1) from the maximum values at a depth of 0–2 cm to the minimum values at a depth of 40–50 cm, although these values were not homogeneous. The trends of Cu_{OM} values in abandoned vineyard soils (Fig. 4G) were similar to those of Cu_{ED} (Fig. 3D) or Cu_{EX} (Fig. 4F), i.e., showed maximum values at a depth of 10–15 cm, except in AR_f soils, where the Cu_{OM} values were similar in all the soil layers. On comparing active vineyard soils and abandoned vineyard soils from the same area, Cu_{OM} was significantly higher in the active vineyards ($p < 0.001$; Table 1).

For active vineyards, Cu_{IA} attained maximum values at a depth of 0–2 cm (Fig. 4C). As observed in the figure, Cu_{IA} levels considerably decreased in PM_v and PT_v soils with an increase in soil layer depth. However, AR_v soils had similar Cu_{IA} values in all the soil layers. Abandoned vineyards also showed similar Cu_{IA} values in all the soil layers, except for PT_f, where the Cu_{IA} values were higher in the intermediate soil layers (Fig. 4H). A comparison of active and abandoned vineyard soils from the same area revealed that Cu_{IA} was significantly higher in active vineyards ($p < 0.001$; Table 1).

As a general trend, Cu_C decreased with increasing depth in active vineyards, although it increased (depth = 4–10 cm) in AR_v soils (Fig. 4D). The Cu_C trend in abandoned vineyard soils was similar to that of other Cu fractions (Fig. 4I), such as Cu_{ED}, Cu_{EX}, or Cu_{OM} (Figs. 3B, 4F, and 4G, respectively). In this study, although the AR_f soils showed similar Cu_C values in all the soil layers, PT_f and PM_f soils showed an increase in Cu_C in the intermediate layers (depth = 2–20 cm). On comparing active and abandoned vineyard soils, Cu_C was found to significantly differ between active and abandoned vineyards ($p = 0.031$; Table 1).

For active vineyard soils, the general trend was that Cu_R decreased with increasing depth. However, PT_v showed a different behavior because, although Cu_R decreased in the first soil layer (depth = 2–4 cm) (Fig. 4E), it present similar values for the intermediate layers (depth = 6–15 cm) as in surface layer (depth = 0–2 cm). In abandoned vineyards, although the AR_f soil had similar Cu_R values in all the soil layers, the PT_f and PM_f soils had higher Cu_R values in the intermediate layers (depth = 6–20 cm) (Fig. 4J). No significant differences in Cu_R were observed between soil profiles of active and abandoned vineyard soils in the same area (Table 1).

The percentage fractionation (Fig. 5) revealed that although most mobile fractions (Cu_{EX}, Cu_{OM}, and Cu_{IA}) significantly decreased in abandoned vineyards, most recalcitrant fractions (Cu_C and Cu_R) significantly increased (Table 1). Additionally, the Cu_{EX} percentages significantly increased ($p < 0.001$; Table 1) with increasing depth, while that of Cu_{IA} significantly decreased ($p = 0.015$; Table 1), and the percentages

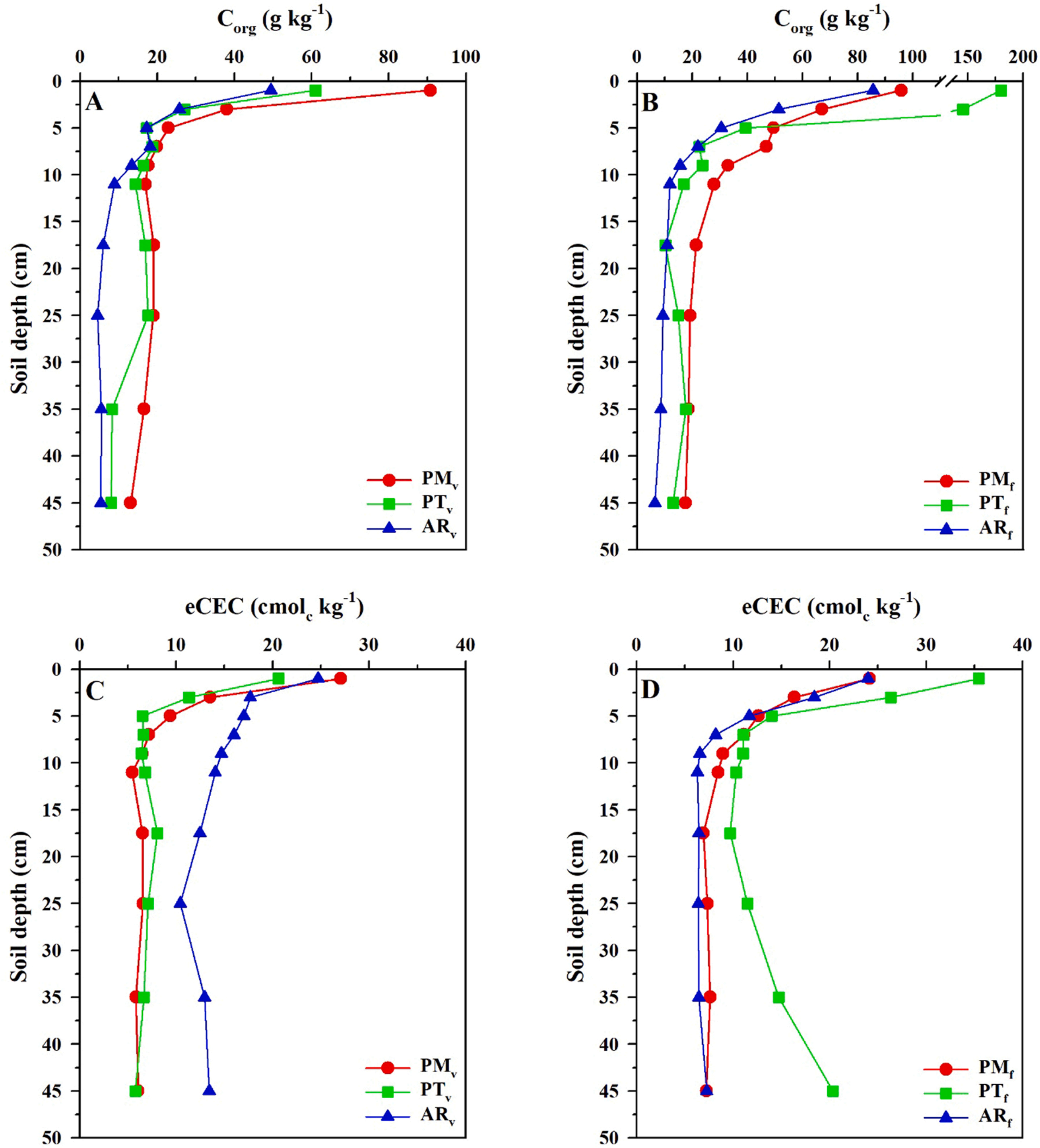

Fig. 2. Distribution of total organic carbon (C_{org}) and effective cation exchange capacity (eCEC) with respect to increasing depth in active (A and B) and abandoned (C and D) vineyards.

of the other fractions changed insignificantly with depth (Table 1).

4. Discussion

High Cu concentrations (maximum of 400–900-mg kg^{-1} Cu_T) were detected in active vineyards, and these values can be attributed to the application of Cu-based fungicides over years (Komárek et al., 2010). These concentrations are much higher than the baseline values of soils with similar the parent material, which were established as 12 and 25 mg Cu_T kg^{-1} in soils developed over granite and schist, respectively (Macías and Calvo, 2009). Abandoned vineyards have lower Cu_T concentrations than active vineyards; however, they exhibit higher Cu_T concentrations than the traditionally accepted phytotoxicity limit of 100 mg kg^{-1} (Kabata-Pendias and Pendias, 2001). The Cu_T concentrations in both active and abandoned vineyards were in the range previously obtained for vineyards on the NW Iberian Peninsula (25–666 mg kg^{-1}), reported by Fernández-Calviño et al., (2008, 2009).

Two types of trends were observed for Cu_T with respect to depth in active vineyard soils: (i) high Cu_T concentrations were observed close to the surface and then a considerable decrease was observed up to a depth of ~5 cm (PM and PT soils), followed by a less pronounced decrease and (ii) similar concentrations of Cu_T were observed between 0 and 30 of depth, albeit with a decreasing value with increasing depth (AR soil). Both groups of patterns support the first hypothesis, Cu accumulation in active acidic vineyard soils is higher in the uppermost layers and decreases with increasing soil depth, but with different strengths. This behavior has been reported for soils from vineyards in France (Besnard et al., 2001; Duplay et al., 2014), Australia (Pietrzak and McPhail, 2004), and the NW Iberian Peninsula (Nóvoa-Muñoz et al., 2007). However, Provenzano et al. (2010) reported a contrasting result: a slight increase of Cu_T with depth. The differences between these results may be related to the sampling methods used (specifically to a sampling

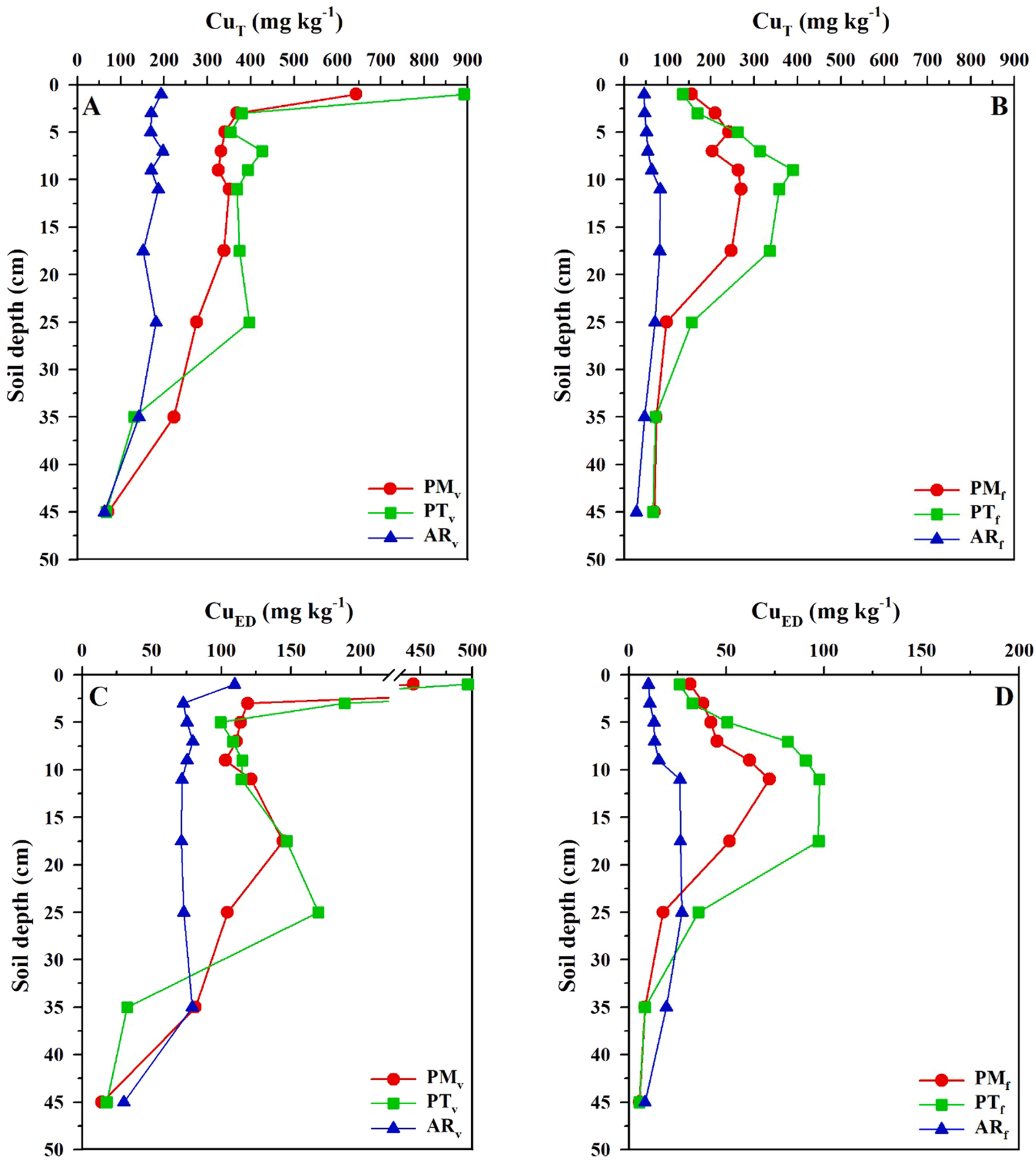

Fig. 3. Distribution of total Cu (Cu_T) and bioavailable Cu (Cu_{ED}) with respect to increasing depth in active (A and B) and abandoned (C and D) vineyards.

resolution of 0–20 and 20–40 cm), including deep soil tillage applied in vineyards from the Apulia region (Provenzano et al., 2010) compared with the low deep tillage frequency applied in the study area. Sonoda et al. (2019) sampled vineyard and fruit farm soils in depth (at a mm detail) and showed that Cu_T increased in the subsurface horizons, indicating the depthwise mobility of Cu. In the present study, the deepest layers (depth = 40–50) exhibited a Cu concentration of 63–71 mg kg^{-1}, which exceeds that of nonpolluted soils with similar parent materials (12–25 mg kg^{-1}; Macías and Calvo, 2009). This suggests the depthwise mobility of Cu, and it is proposed that such mobility is associated with Cu leaching via preferential flow in solution or with soil colloids (Filipović et al., 2020).

High Cu concentrations in the uppermost soil layers of active vineyards may negatively affect vineyard sustainability. Although the mean Cu concentrations at depths of 0–20 cm are approximately 50–200 mg kg^{-1} (Fernández-Calviño et al., 2009), the results from this study indicate that the uppermost soil layers (depth = 0–2 cm) can become hotspots for Cu accumulation reaching 900 mg kg^{-1}, where toxicity to soil biota (Karimi et al., 2021) or phytotoxicity (Verdejo et al., 2015) may occur. In fact, bacterial activity (Table S8, Supplementary material) in the uppermost layers (0–4 cm) was lower than that in the subsuperficial soil layers (4–10 cm). In addition, Cu concentrations of 500–1000 mg kg^{-1} can result in toxicity to soil bacterial communities and even increase bacterial community tolerance to antibiotics (Fernández-Calviño and Bååth, 2013; Santás-Miguel et al., 2020). Therefore, such concentrations may also affect public health because this bacterial resistance may be transferred to human pathogenic bacteria (Forsberg et al., 2012).

The high phytotoxicity of these Cu hotspots in the uppermost soil layers (depth = 0–2 cm) may also hinder the introduction of cover crops in vineyards, a practice recommended to increase vineyard sustainability (Gattullo et al., 2020), or crop diversification via intercropping,

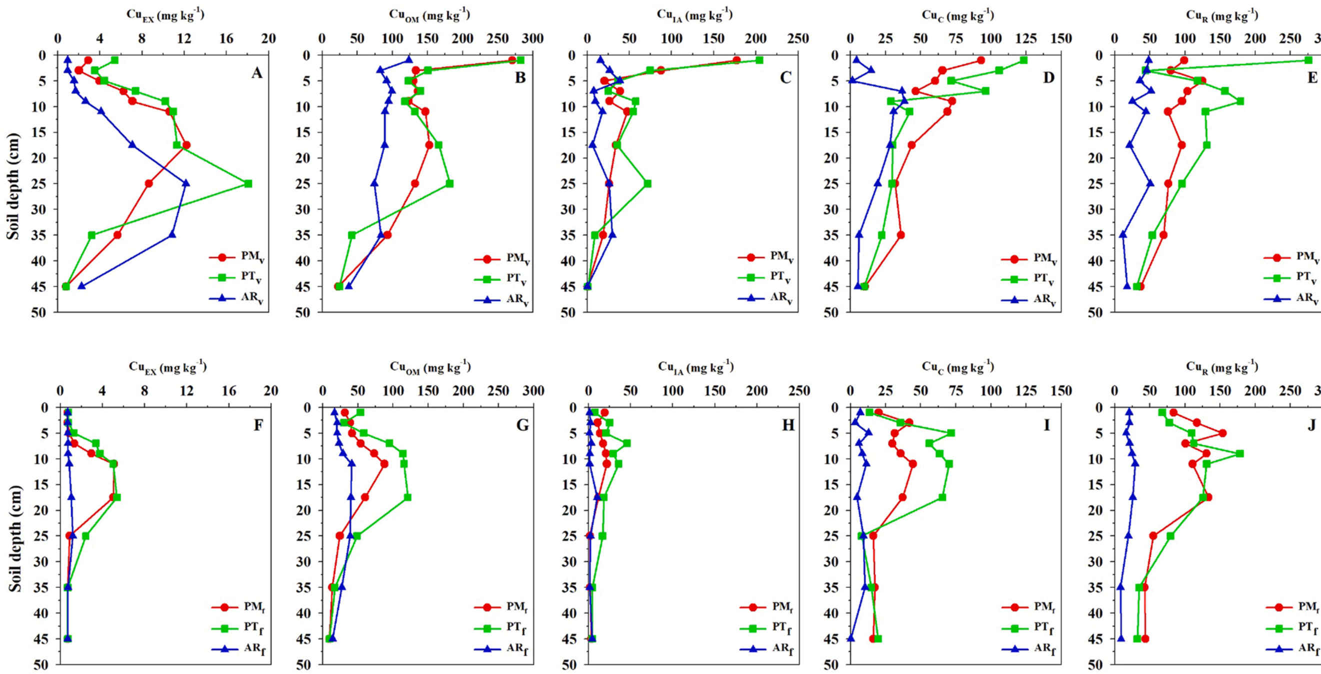

Fig. 4. Distribution of exchangeable Cu (Cu$_{EX}$), Cu associated with soil organic matter (Cu$_{OM}$), Cu associated with inorganic amorphous oxides (Cu$_{IA}$), Cu associated with crystalline oxides (Cu$_{C}$), and residual Cu (Cu$_{R}$) with respect to increasing depth in active (A, B, C, D, and E) and abandoned vineyards (F, G, H, I, and J).

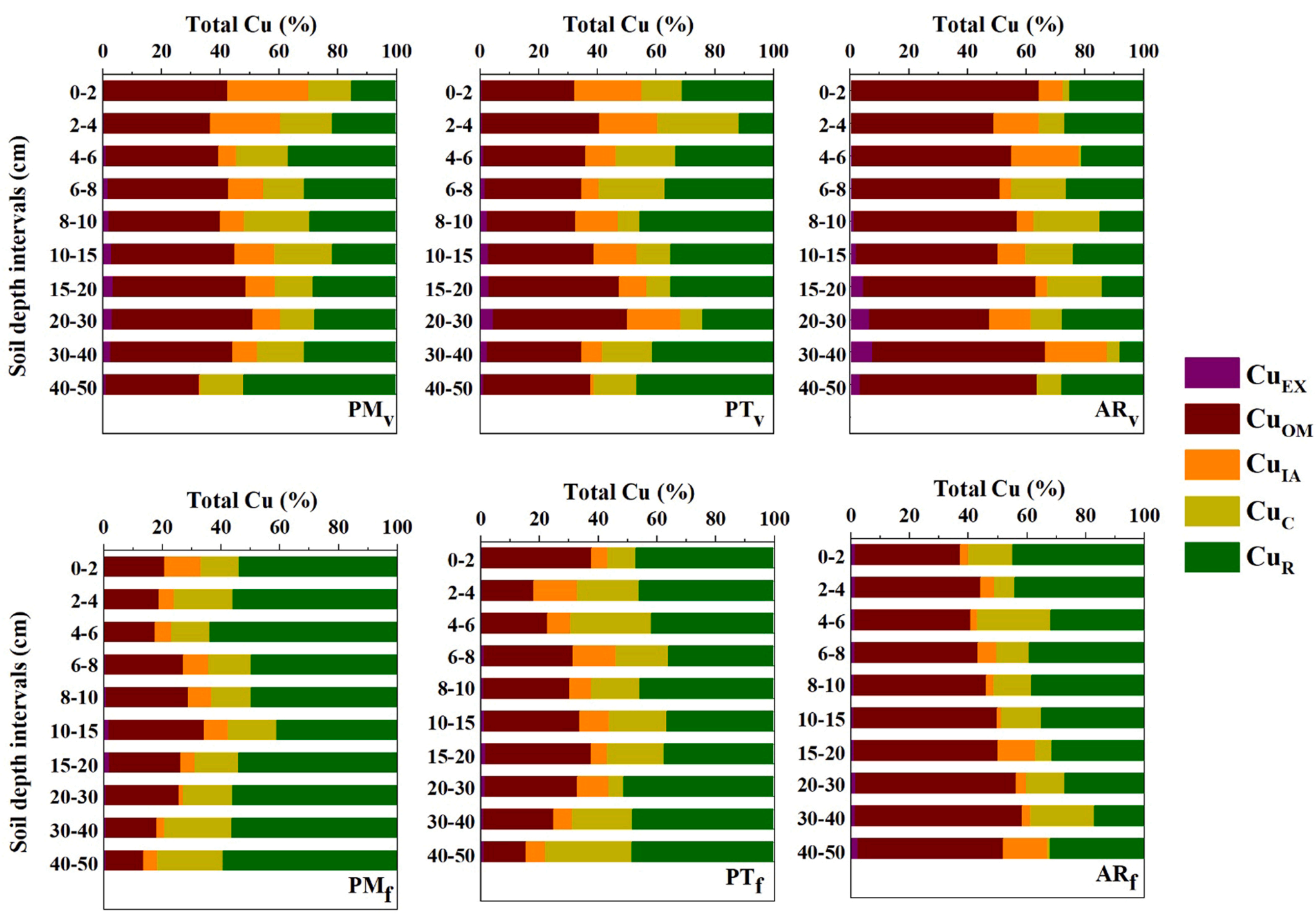

Fig. 5. Average vertical distribution of Cu fractions in active vineyards (upper row) and abandoned vineyards (lower row) expressed as percentages of the corresponding total copper content. EX, exchangeable; OM, bound to soil organic matter; IA, bound to Fe and Al inorganic amorphous oxyhydroxides; C, bound to crystalline oxyhydroxides; R, residual copper.

which is another sustainable option for vineyards (Ripoche et al., 2010). These potential problems are relevant in vineyards where zero-tillage practices are adopted. Thus, mixing the first 20 cm of soil using a rotavator (not frequently) may be a recommended practice to avoid those Cu hotspots in vineyards. Other sustainable options are phytoextraction and phytostabilization (Cornu et al., 2019, 2022), which allow the removal or immobilization of Cu in soils, and both the options result in reduced Cu bioavailability.

In this study, abandoned vineyards exhibited the maximum Cu_T levels at an approximate depth of 10 cm, leading to reject the second hypothesis – the Cu distribution with respect to the depth in abandoned vineyards is similar to that in active vineyards. This trend is possibly related to the incorporation of superficial materials, especially organic ones, which may have a diluting effect on Cu concentrations. This assumption is supported by the organic carbon data obtained. Although there were significant correlations between Cu_T and TOC in active vineyards (Fig. 6A; $r = 0.707$; $p < 0.001$), the same correlation was insignificant in abandoned vineyards (Fig. 6B; $r = 0.025$; $p = 0.898$). This difference in land use may be because new materials incorporated from plants in the uppermost layers had low Cu concentrations owing to the absence of Cu-based applications and low Cu transfer from soil to plants. In this regard, the analysis of annual and biannual plants, which grow spontaneously in these soils, had Cu concentrations ranging from 66 to 88 mg kg^{-1} in aerial parts and roots (Campillo-Cora et al., 2019); these concentrations were lower than those in the soil, which ranged from 50 to 400 mg kg^{-1}. Therefore, the potential transport of Cu from soil to plants and subsequently to the topsoil is limited. Other mechanisms could also be the reason underlying the higher Cu concentration in the subsurface layer than in the surface soil layer, such as the transport of Cu through the soil (Sonoda et al., 2019) along with a reduction in Cu input because of vineyard abandonment that resulted in stopping the use of Cu-based fungicides. A reduction in Cu concentrations at the surface is also proposed to favor the establishment of plants in soils compared with active vineyards and a healthier microbiome.

The Cu_{ED} values showed a similar pattern with respect to depth as that of Cu_T, in the active and abandoned vineyards. These results are consistent with those of other study that have reported a high correlation between these two variables (Brunetto et al., 2018). However, the Cu_{ED} percentage was significantly higher in active vineyards than that in abandoned vineyards. This indicates a reduction in bioavailable Cu when active vineyards are abandoned (Fernández-Calviño et al., 2009), which is supported by the lower percentages of most Cu labile fractions (Cu_{EX}, Cu_{OM}, and Cu_{IA}) in abandoned vineyards compared with the active ones. By contrast, the most recalcitrant fractions (Cu_C and Cu_R) with less potential toxicity showed an opposite trend. Therefore, results of Cu fractionation support the third hypothesis that higher proportions of nonlabile Cu fractions are present in abandoned vineyards than those in active vineyard soils, confirming that Cu becomes more recalcitrant with abandonment. These results are in agreement with those reported by other authors (Arias-Estévez et al., 2007; Liu et al., 2020) that have shown reductions in Cu bioavailability with time. This result is crucial as it indicates that Cu becomes less soluble with time, implying that its phytotoxic effect will deteriorate. Consequently, it is possible that in addition to allowing spontaneous forest growth, abandoned vineyards could be used to plant different crop types, and as such the results of this study are of both ecological and agricultural importance.

The percentage of Cu fractionations in the active vineyard soils did not change significantly, except for Cu_{EX} and Cu_{IA}, which increased and decreased with increasing depth, respectively. In addition, the more labile fractions (Cu_{EX}, Cu_{OM}, and Cu_{IA}) were lower in abandoned vineyards than those in active vineyards, whereas the more recalcitrant fractions presented the opposite trend (Cu_C and Cu_R). Therefore, in abandoned vineyards, the total amount of Cu was lower than that in active vineyards and Cu was present in more recalcitrant fractions at all soil depths. Therefore, this result indicates that Cu toxicity on soil biota (Karimi et al., 2021) became much lower in abandoned vineyards than in active ones in all the soil layers.

5. Conclusions

This study revealed that in active vineyard soils, Cu accumulated in highest concentrations at the surface layer (depth = 0–2 cm), whereas in abandoned vineyards transformed into forests, the highest Cu concentrations were observed in subsurface soil layers (depth = 10–15 cm). However, although the Cu concentration decreased with the increasing depth, reaching much lower values at a depth of 50 cm than at surface layer (depth = 0–2 cm), the concentrations at a depth of 50 cm were higher than background values observed in the nonpolluted soils with a similar parent materials. Obtained results also showed that Cu_T and Cu fractionation changed with soil depth after the land use changed. The Cu concentrations in abandoned vineyards were found to be lower than those in active ones for all the soil layers. Further, Cu accumulates in less mobile fractions in abandoned vineyards (resulting in higher proportions of residual Cu). Therefore, the change in land use after vineyard abandonment may contribute to the development of new ecosystems by reducing the total amounts of Cu and its associated toxicity in all soil layers. Hotspots with high Cu concentrations observed in the uppermost soil layers (depth = 0–2 cm) of active vineyard soils are also of concern, suggesting the need for performing further research on the effect of Cu on these hotspots and developing sustainable alternatives to soil tillage.

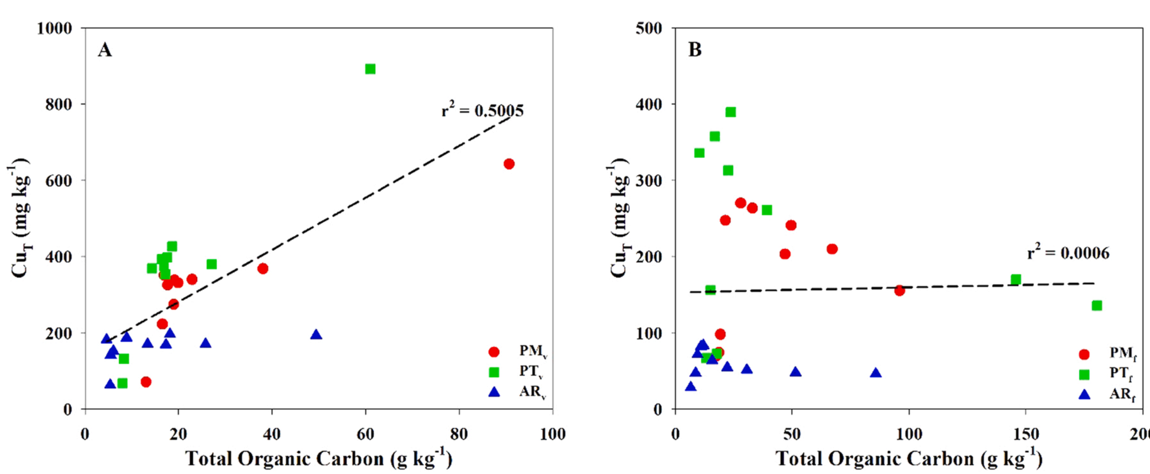

Fig. 6. Relationship between total Cu (Cu_T) and soil organic carbon content in active (A) and abandoned (B) vineyards.

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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2022.108146.

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