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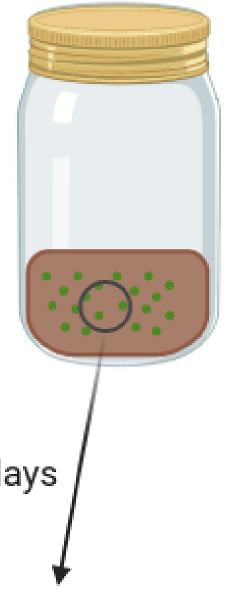
Cover crops residues:
*Faba bean*
or *Oat*

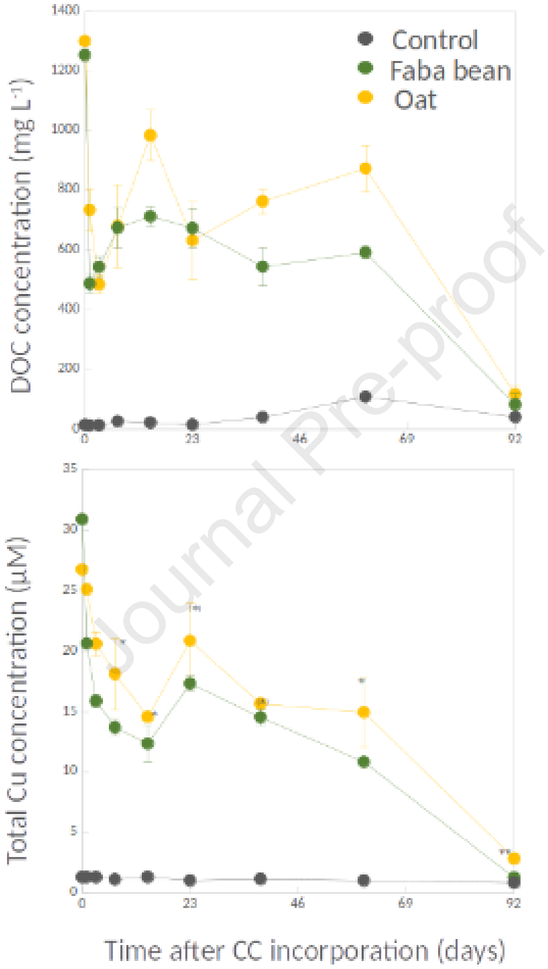
Cu-contaminated vineyard soil

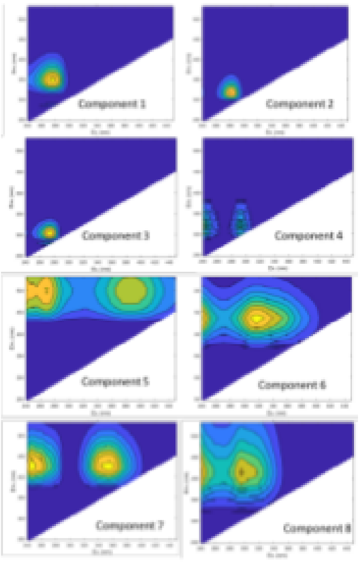


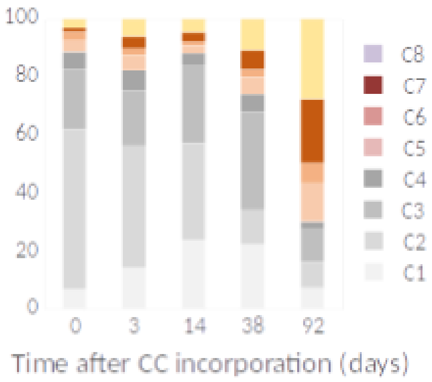
3-92 days

*0.01 M KCl extraction*

- Concentration and fluorescent properties of disolved organic matter
- Total and free Cu ion concentration







# Changes over time in organic matter dynamics and copper solubility in a vineyard soil after incorporation of cover crop residues: insights from a batch experiment

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## Abstract

Cover crops (CCs) are increasingly used in viticulture because they benefit the soil and the environment in many ways. This study investigated the extent to which the incorporation of CC residues altered organic matter (OM) and Cu dynamics in a Cu-contaminated vineyard topsoil. A 92-day incubation period was used to monitor changes over time in carbon mineralization, carbon hydrolytic enzyme activity, concentration and optical properties of dissolved organic matter (DOM), and Cu solubility after the addition (or not) of two CC residues, oat or faba bean. The results revealed that adding CCs transitorily increased the concentration of DOM in soil solution, as well as the activity of C hydrolytic enzymes and C mineralization rates. DOM content was approximately two orders of magnitude higher in CC-amended soils than in the control soil on day 0, after which it gradually decreased to reach concentrations similar to those measured in the control soil on day 92. Analyses of DOM optical properties showed that its molecular weight and degree of humification increased over time with a decrease in its concentration. The close relationship between DOM and Cu concentrations in the soil solution suggests that degradation of CCs releases soluble forms of C capable of complexing and solubilizing Cu, and hence that incorporating CC residues can transitorily increase the solubility of Cu in vineyard topsoils. Despite their different C:N ratios, oat and faba bean had almost the same effect on Cu dynamics, implying that C inputs played a prominent role in explaining the interactions between OM and Cu within the timeframe of our experiment. In conclusion, this study enabled recommendations on how to mitigate the risk of Cu ecotoxicity associated with incorporating CCs in Cu-contaminated vineyard soils.

**Keywords**

Dissolved organic matter; enzyme assay; ion-selective electrode; metals; PARAFAC; viticulture

## 1. Introduction

The long-term use of copper-based fungicides in vineyards to prevent the downy mildew of vine led to the accumulation of Cu in vineyard soils. Concentrations as high as several hundred mg Cu $kg^{-1}$ soil have been measured in the topsoil of old vineyards around the world (see Komarek et al., 2010 and Mackie et al., 2012, and references therein). Although the average application rate of Cu-based fungicides is now limited, for instance to 4 kg Cu $ha^{-1}$ $year^{-1}$ in Europe (EU 2018/1981), Cu continues to accumulate in vineyard soils, especially in organic viticulture where the use of synthetic fungicides is prohibited. A recent meta-analysis by Karimi et al. (2021) of Cu ecotoxicity suggests that Cu can reduce the biomass, the activity and/or the diversity of soil microorganisms at a dose of 200 kg Cu $ha^{-1}$, which corresponds to a concentration in the topsoil (0-20 cm) of about 70 mg Cu $kg^{-1}$ soil, compared with 91 mg Cu $kg^{-1}$, which is the mean concentration of Cu in French vineyard soils as assessed based on a Land Use/Cover Area frame Survey (LUCAS) survey (Ballabio et al., 2018). Soil Cu contamination is thus suspected to be detrimental to the biological quality of at least some vineyard soils, as suggested by Dequiedt et al. (2011), who showed that the average soil microbial biomass was lower in vineyards (and orchards) than in grasslands, forests and in other cropping systems.

Copper bioavailability, i.e. the degree to which Cu is absorbed or metabolized by ecological receptors (ISO 17402), depends to a great extent on the pH and organic matter content of the soil as both affect the concentration of free Cu ion ($Cu^{2+}$) in the soil solution (Sauvé et al., 2000; Bravin et al., 2009; Ouédraogo et al., 2022), which is the main (and usually the only) form in which Cu is taken up by plant roots (FIAM; Campbell, 1995). Therefore, to manage the risk of Cu ecotoxicity in vineyard plots contaminated by Cu, particular attention must be paid to agronomic practices that affect the pH and organic matter dynamics in soil.

Cover crops (CCs) are increasingly used in agriculture because of the benefits they provide to the soil and the environment. Incorporating CCs in agricultural rotations is one way of increasing soil organic carbon in agroecosystems (Poeplau and Don, 2015) thereby also improving soil structure, biological activity and mineral fertility (Blanco-Canqui et al., 2015). In addition, CCs can limit the growth of weeds, provide habitats for beneficial insects and predators, and protect the soil against erosion (Blanco-Canqui et al., 2015). Mixtures of CCs of different functional types, legume, grass and brassica, are sown in the vineyard inter-rows in the fall (Abad et al., 2021), and incorporated in the soil in spring, soon after the vine bud break. The use of CCs in Cu-contaminated vineyards raises the question of how incorporating their residues affects the solubility and bioavailability of Cu in the topsoil. The few studies carried out on this topic (Merrit and Erich, 2003; Cui et al., 2008) showed that CC incorporation increased the solubility of Cu in soil, likely due to the Cu-mobilization potential of dissolved organic matter (DOM) released during aerobic degradation of their residues (Wershaw et al., 1996). As described in the context of the "Birch effect" (Birch, 1958), soil microbial activity is likely to alter the characteristics of DOM over time, thereby also altering the effect of CCs on Cu dynamics. However, knowledge is still lacking on temporal changes in the quantity and the quality of DOM after CC incorporation, as well as on the relationship between DOM quality, Cu complexation and Cu solubility in this context. Another pending question is the effect of CCs of different functional types on DOM release and Cu dynamics. Incorporating CCs with a low C:N ratio, such as legumes, is suspected to release more DOM more rapidly, and consequently to have a distinct effect on the timing and magnitude of Cu solubilisation than incorporating CCs with a high C:N ratio such as grasses (Cotrufo et al., 2013). However, this hypothesis remains to be tested, notably for the CC species used in viticulture.

In this study, we investigated the effect of incorporating CC residues on OM and Cu dynamics in a vineyard topsoil using a batch experiment. One original feature of our study is our detailed characterization of the quality of DOM based on its optical properties to better understand how the changes it undergoes over time induced by the incorporation of contrasted CCs affects the solubility of Cu. Our specific objectives were (i) to monitor over time any changes in the quantity and quality of DOM as well as in the solubility and the complexation of Cu in soil solution after incorporation of the cover crop residues, (ii) to compare the effect of two CC residues with distinct C:N ratios: oat and faba bean, and (iii) to investigate the relationship between the quality and the Cu-mobilization potential of DOM in this context.

## 2. Materials and Methods

### 2.1 Soil

The soil used in the present study was collected in 2021 from the uppermost 15 cm of a Podzol (WRB, 2006) in an organic vineyard plot in the “Graves” AOC (i.e. registered designation of origin) zone immediately south of Bordeaux, France. The soil sample is a non-calcareous soil characterized by 89% sand, 8% silt and 3% clay (ISO 11277), 2.9% organic matter (OM) (NF ISO 10694), a pH ($H_2O$) of 6.5 (NF ISO 10390), a C:N ratio of 15.3, and a cation exchange capacity (CEC) of 5.6 $cmol_+ kg^{-1}$ (NF ISO 23470). The total concentration of Cu was 114 mg $kg^{-1}$ soil DW (NF X 31-147) and the EDTAextractable concentration of Cu was 75 mg kg$^{-1}$ DW (NF X 31-120). After sampling, the soil was sieved to 4 mm and stored at its original humidity, i.e. 8% (w/w) at 4 °C until the start of the experiment.

## 2.2 Cover crop residues

The two cover crop (CC) species used in this study, oat (*Avena sativa* L.) and faba bean (*Vicia faba* L.), are often grown in the inter-rows of French vineyards (source French Wine and Vine Institute). The CCs were sampled in 2021 in a vineyard plot close to Bordeaux and treated as follows: since only the shoots were harvested, the plants were cut at ground level, washed with deionized water to remove soil particles, oven-dried at 40 °C for 3 days, then ground to a fine powder (< 0.5 mm, Retsch MM 400) and stored in a dry place. The concentrations of C and N in the CC residues were measured using an elemental analyser (Flash EA 1112, Thermo Fisher), and their concentration of Cu by ICP-OES (ICAP 6300, Thermo Fischer) after digestion in a $HNO_3/H_2O_2$ mix (Linero et al., 2018). As expected, the concentration of N was higher and the C:N ratio was lower in faba bean residues (N: 4.3%, C/N: 10.5) than in oat residues (N: 2.8%, C/N: 14.9). The concentration of Cu was 14.7 mg kg$^{-1}$ in faba bean and 6.3 mg kg$^{-1}$ in oat, meaning the amount of Cu added to the soil through the incorporation of CC residues (see section 2.3) was negligible (< 0.2%) compared to the amount of Cu initially present in the soil.

## 2.3 Microcosm incubation

One hundred grams of dry soil equivalent were placed in a 1 L glass jar and CC residues were added (or not) at a rate of 1 g $C_{residue}$ per 100 g soil DW (Fanin and Bertrand, 2006). The cover crops were incorporated in the soil by mixing, the mixture was then rewetted to 15% humidity (w/w) using deionised water, after which the jar was hermetically sealed and left for 48 hours at 4 °C. Sampling on day 0 corresponds to the soil extraction performed at the end of this pre-incubation at 4 °C. Microcosms were then incubated at 20 °C in the dark for 1, 3, 7, 14, 23, 38, 60 or 92 days. A temperature of 20 °C is suitable to study microbial activity during OM decomposition (McDowell et al., 2006; De Troyer et al., 2011), and corresponds to the mean temperature of vineyard topsoils, as measured from May to September 2019 in the "Graves" AOC zone (Pierdet, 2020). Four repetitions were performed on each sampling date (n= 9) and per CC treatment (n= 3) giving a total of 108 microcosms. Four additional jars with no soil or CC residues were used as controls for the measurement of C mineralization (see section 2.4 below).

### 2.4 Carbon mineralization

To trap the $CO_2$ produced by C mineralization, a scintillation vial containing 2 M NaOH was placed in the microcosms sampled on day 92 and renewed after each measurement (n= 14). Carbon mineralization was measured on days 2, 4, 8, 11, 15, 18, 22, 30, 37, 44, 51, 65, 79 and 92 by back-titration of the unreacted NaOH. For each measurement $i$, the cumulative amount of C mineralized ($Q_{CO_{2,i}}$, in g) was estimated using eq. 1:

$$Q_{CO_{2,i}} = \sum_{i=1}^{14}(V_0 - V_i)/2 \times C \times {}^{V_t}/_{V_s} \times M_c \tag{1}$$

where $V_i$ is the volume of HCl used for NaOH titration for measurement $i$, $V_0$ the mean volume of HCl used to titrate NaOH in the control jars (0.004 L), $C$ the molar concentration of HCl used for NaOH titration (0.5 M), $V_t$ the total volume of NaOH in the scintillation vial (0.015 L), $V_s$ the volume of NaOH sampled in each vial for titration (0.001 L), and $M_c$ the molecular weight of carbon (12 g $mol^{-1}$).

## 2.5 Enzyme assays

We measured the activity of three enzymes that degrade cellulose (β-1,4-glucosidase, 1,4-β-D-cellobiohydrolase and α-1,4-glucosidase) and one enzyme that degrades hemicellulose (β-xylosidase) on days 1, 3, 38 and 92, following the procedure described in Bell et al. (2013). Briefly, 2.5 g dry soil equivalent were mixed with 91 mL of 50 mM sodium acetate buffer in a blender for 1 min. The resulting slurry was added to a 96-deepwell microplate using an eight-channel electronic pipette. Additional quench control replicates of soil slurry with 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (AMC) standard curves (0 - 100 µM concentrations) and controls without substrate addition (soil slurry + water) were included with each sample. Soil slurries were then incubated at 25 °C for 3 h with fluorescent substrates corresponding to each enzyme measured. After the incubation step, the plates were centrifuged for 3 min at 3000 rpm and 250 µL of supernatant was transferred from each well into a black flat-bottomed 96-well plate. Next the samples were scanned on a Synergy H1 microplate reader (Biotek, Winooski, USA) using fluorescence with excitation at 365 nm and emission at 450 nm. The enzyme activities were integrated over time to derive the cumulative enzyme activities expressed in nmoles kg$^{-1}$ soil (Fanin and Bertrand, 2016).

## 2.6 Soil extraction and analyses of the extraction solution

The soil was extracted on days 0, 1, 3, 7, 14, 23, 38, 60 and 92. Soil extraction was performed as follows: 15 g dry soil equivalent was shaken with 30 mL of 0.01 M KCl at 35 rpm for 2 h at 20 °C, centrifuged at 4600 g for 10 min, and the supernatant was filtered through 0.2 µm cellulose acetate filters. pH was measured immediately after the solution was extracted using a combined pH microelectrode (E16M331, Radiometer

Analytical). The extraction solution was then divided into two subsamples. One subsample was acidified with 2% $HNO_3$ (v/v) to measure the total concentration of Cu by ICP-OES (ICAP 6300, Thermo Fischer). The other subsample was stored at 4 °C in the dark to measure free Cu ion activity and to characterize dissolved organic matter on the five days following soil extraction. Free Cu ion activity was measured using a cupric ion-selective electrode (ISE, 9629BNWP, Thermo Scientific Orion) according to the procedure described in Cornu et al. (2019), and the concentration of dissolved organic carbon (DOC) was measured using a TOC analyzer (TOC V-CPH, Shimadzu). The concentration of free Cu ion was calculated from free Cu ion activity using the $Cu^{2+}$ activity coefficient given by the extended Debye-Hückel equation. The free Cu ion fraction ($f_{Cu}$) was determined by calculating the ratio of the concentration of free Cu ion to the concentration of total Cu.

## 2.7 Optical properties of dissolved organic matter

Absorbance and fluorescence of the extraction solutions were measured in a 1-cm quartz cuvette thermostated at 20 °C, using an Aqualog spectrofluorometer (Horiba Scientific). Absorbance spectra were recorded from 239 to 800 nm using a 0.01 M KCl solution as a blank. The spectral slope $S_{275-295}$ used as a proxy of the DOM molecular weight (Helms et al., 2008), was calculated from the slope of the natural logarithm-transformed absorbance spectra in the 275-295 nm wavelength range. To obtain excitation-emission matrix (EEM) fluorescence spectra, excitation scans were performed using a double-grating monochromator (150 Watt Xenon lamp) at wavelengths 240-800 nm at 5 nm intervals. Emission spectra were collected with high CCD detector gain at approximately 0.58 nm (1 pixel) intervals at wavelengths ranging from 245 to 830 nm. When the maximum intensity of the absorbance spectrum was higher than 0.1, the sample was diluted with 0.01 M KCl to avoid internal filter effects. Fluorescence data are expressed in Raman units (RU) after normalization to the area under the Raman peak of an ultrapure water blank at an excitation wavelength of 350 nm.

EEM spectra were modeled using parallel factor analysis (PARAFAC) in MATLAB R2019a using the DOMFluor toolbox 1.7 (Stedmon and Bro, 2008; Murphy et al., 2014). PARAFAC is a multiway mathematical tool that statistically decomposes the EEM spectra into individual fluorescence components. The PARAFAC model was run for three to eight components with non-negativity constraints. The optimal number of PARAFAC components was determined after split-half analysis, random initialization and visual inspection of the spectral shape of each component and residual from models. For each sample, the relative abundance of each component was expressed through its contribution to the maximum fluorescence intensity ($F_{max}$) (Stedmon and Bro, 2008; Murphy et al., 2014). The molecular description of the fluorophore behind each component was made by comparison with the literature and with the "OpenFluor database" (http://www.openfluor.org/) (Murphy et al., 2014).

### 2.8 Data treatment and statistical analyses

Data processing and statistical analyses were performed in R version 4.0.3 (R core Team, 2020). When necessary, the data were log transformed to achieve normal residual distribution. Tukey’s HSD tests at 5% were used to identify the parameters that differed significantly among CC treatments. Pearson’s correlation tests were used to test the linear relationship between the concentration of Cu and DOC (Fig. 5a), Cu and $FI_{C5\text{-}C7}$ (Fig. 5b), free Cu ion and pH (Fig. 5c) and of the free Cu ion fraction and pH (Fig. 5d), in the extraction solution.

## 3. Results

### 3.1 Soil biological activity

Soil biological activity was assessed by analyzing carbon mineralization and the activity of carbon hydrolytic enzymes. As expected, more C was mineralized in the soil supplied with CC residues (i.e. in CC-amended soils) than in the control soil. In CC amended-soils, C mineralization reached maximum within the first three days following CC incorporation, and was slightly higher in the soil amended with oat residues than in the soil amended with faba bean residues (Fig. 1a). The difference in C mineralization between the two CC residues became significant ($p < 0.05$) 22 days after incubation started. At the end of the incubation period, the cumulative amount of C mineralized in CC-amended soils was about 15 times higher than in the control soil, and corresponded to about 20% of the amount of C supplied by the CC residues (24% for oat and 22% for faba bean).

On days 3, 38 and 92, the activity of C hydrolytic enzymes was significantly higher ($p < 0.05$) in CC-amended soils than in the control soil (Fig. 1b). In CC-amended soils, C hydrolytic enzyme activity increased between day 1 and day 3 ($p < 0.05$) and then decreased continuously between day 3 and day 92 ($p < 0.05$) in the soil amended with faba bean residues, whereas it decreased between day 3 and 38 ($p < 0.05$) but then increased between day 38 and day 92 ($p < 0.05$) in the soil amended with oat residues. On day 92, the residual activity of C hydrolytic enzymes was on average 4- to 5-fold higher in CC-amended soils than in the control soil.

### 3.2 Characteristics of dissolved organic matter

#### 3.2.1 Concentration of DOC and absorbance properties

On day 0, i.e. at the end of the pre-incubation period, the concentration of DOC in the KCl extract was almost 100 times higher in CC-amended soils (> 1000 mg C $L^{-1}$) than in the control soil (~ 15 mg C $L^{-1}$), and there was no significant difference ($p > 0.05$) between the soil amended with oat residues and the soil amended with faba bean residues (Fig. 2a). The DOC concentration in CC-amended soils decreased by more than 50% during the first three days of incubation, and then increased between day 2 and day 14, notably in the soil amended with oat residues. From day 14 to day 92, the concentration of DOC gradually decreased in CC-amended soils, with some variability among sampling dates in the soil amended with oat residues, and on day 92 had reached a value close to that measured in the control soil. On day 0, the absorbance spectral slope $S_{275-295}$ of the KCl extract was higher in CC-amended soils than in the control soil (Fig. 2b), meaning that the incorporation of CC residues initially decreased the average molecular weight of DOM. The $S_{275-295}$ value followed the same trend over time as the DOC concentration: it decreased sharply over the first three days of incubation, increased temporarily between day 3 and day 14, and then decreased from day 14 to day 92 when it reached values close to that measured in the control soil (Fig. 2b).

### 3.2.2 PARAFAC components

PARAFAC analysis identified eight components whose spectral characteristics and chemical description are detailed in Table 1 and Fig. F1 (suppl. data). In the control soil, DOM was a balanced mixture of components C3, C4, C5, C7 and C8, and its fluorescent properties were relatively well conserved over time (Fig. 3). In CC-amended soils, the fluorescent properties of DOM differed from those of the control, notably during the first 14 days of the incubation period. On day 0, components C2 (55%) and C3 (21%) contributed the most to the DOM fluorescence properties in the soil amended with faba bean residues, while components C1 (36%) and C3 (34%) contributed most to the DOM fluorescence properties in the soil amended with oat residues. DOM fluorescence properties changed over time in both types of CC-amended soils. From day 14 to day 92, the contribution of components C5, C7 and C8 increased over time to the detriment of the contribution of components C1 and C3 (and C2 in the soil amended with faba bean residues) in what could be interpreted as a process of DOM humification. As a result, the fluorescence properties of DOM in CC-amended soils differed less from those in the control soil at the end (day 92) than at the beginning (day 0) of incubation. Figure 2c shows changes in the cumulative fluorescent intensity of the PARAFAC components C5, C6 and C7 ($FI_{C5-C7}$) over time, which we used as a proxy of the concentration of soluble humic substances (see Table 1) in the KCl extract. $FI_{C5-C7}$ was higher in CC-amended soils than in the control soil and followed approximately the same trend over time as the concentration of DOC. The two noteworthy differences between the two variables were that (i) the decrease in $FI_{C5-C7}$ during the first three days was less marked than that of DOC, and (ii) the difference between the two CC residues was larger for $FI_{C5-C7}$ than for DOC.

### 3.3 Concentration of total Cu and free Cu ion in the KCl extract

On day 0, the concentration of total Cu in the KCl extract was 20-fold higher in CC-amended soils (~ 29 μM) than in the control soil (~ 1.3 μM) and did not differ significantly ($p > 0.05$) between the soil amended with oat residues and the soil amended with faba bean residues (Fig. 4a). Except between day 14 and day 23, the concentration of total Cu in the KCl extract decreased over time in CC-amended soils, whereas it remained fairly constant in the control soil. Except on day 0, the concentration of total Cu in the KCl extract was significantly higher ($p < 0.05$) in the soil amended with oat residues than in the soil amended with faba bean residues (24% on average between day 1 and day 60). As also observed for several variables relative to DOM, on day 92, the concentration of total Cu in the KCl extract was similar in the control soil and in CC-amended soils.

In contrast, the concentration of free Cu ion in the KCl extract was significantly lower ($p < 0.05$) in CC-amended soils than in the control soil throughout the incubation period (Fig. 4b). The concentration of free Cu ion in the control soil did not change much over time, i.e. it averaged 5.3 ± 2.9 nM, whereas in CC-amended soils, it varied over time from 0.02 nM to 3 nM, i.e. by more than a factor of 100. On days 3, 7, 14 and 23, the concentration of free Cu ion was significantly higher ($p < 0.05$) in the soil amended with faba bean residues than in the soil amended with oat residues. The free Cu ion fraction ($f_{Cu}$) was closely ($p < 0.001$) and positively ($r = 0.96$) correlated with the concentration of free Cu ion (data not shown). As observed for the concentration of free Cu ion, $f_{Cu}$ was lower in CC-amended soils than in the control soil, and, in the control soil, did not change much over time, average 0.7 ± 0.3%, whereas in CC-amended soils, it varied over time from 0.0002% to 0.2%, i.e. by a factor of 1000 (suppl. Fig. F2).

### 3.4 Relationships between Cu concentration and DOM characteristics in the KCl extract

The concentration of total Cu in the KCl extract of CC-amended soils was positively correlated with the concentration of DOC ($r = 0.75$, $p < 0.001$) but was more closely correlated with $FI_{C5\text{-}C7}$ ($r = 0.95$, $p < 0.001$) (Fig. 5), notably in the solutions extracted during the period of incubation, i.e. when the quality of the DOM changed most. Figure 5b shows that the higher concentration of total Cu in the soil amended with oat residues than in the soil amended with faba bean residues was associated with a higher value of $FI_{C5\text{-}C7}$, i.e., with a higher concentration of humic-like substances (Table 1). In contrast, neither the concentration of free Cu ion nor the fraction of free Cu ion were significantly correlated with DOM characteristics ($p > 0.05$, data not shown) in the KCl extract of CC-amended soils, but were closely ($p < 0.001$) and negatively ($r = -0.94$ and $-0.87$, respectively) correlated with pH (Fig. 5c, Fig. 5d).

## 4. Discussion

### 4.1 Effect of the incorporation of cover crop (CC) residues on the dynamics of organic matter (OM).

On day 0, i.e. before the start of OM degradation, the concentration of dissolved organic carbon (DOC) in the KCl extract increased from 14 mg C $L^{-1}$ in the control to 1300 mg C $L^{-1}$ in CC-amended soils (Fig. 2a). For each CC-amended microcosm, this corresponded to a 250 mg increase in the soluble organic C pool. Assuming that all this DOC originated from CC residues, the soluble fraction of C was around 25% DW in both CC residues, which is in the range of values reported in the literature for different types of aboveground litter (Fanin and Bertrand, 2006) and different cover crops (CCs) (Weiler et al., 2022). The soluble fraction of C in CC residues is usually composed of hydrophilic compounds such as sugars and amino acids (Marstop, 1996; De Troyer et al., 2011). To some extent, this theoretical composition is in agreement with the optical properties of DOM measured on day 0, which showed a lower molecular weight (Fig. 2b) and a lower degree of humification (Fig. 3) of DOM in CC-amended soils than in the control soil. The dynamics of organic matter in CC-amended soils occurred in three stages. The first stage, which spanned the first three days, was characterized by high C mineralization rates (Fig. 1a) and a marked decrease in the concentration of DOC in the KCl extract over time (Fig. 2a). This early stage of OM decomposition likely resulted from the rapid mineralization of soluble and readily degradable C forms in CC residues, which stimulated a copiotrophic community due to a marked increase in C availability (Fanin et al., 2014). This hypothesis is supported by the fact that during this stage (i) the average amount of C mineralized (i.e. 150 mg C per microcosm) was the same as the decrease in DOC in the KCl extract, and (ii) the average molecular weight of DOM increased with a decrease in the concentration of DOC.

The second stage extended from day 3 to day 60 and was characterized by a shift in the resource-use strategy to target decaying more structural compounds, as indicated by the gradual decrease in C mineralization rates. This second stage is in agreement with that reported in several other studies showing that the rapid increase in $CO_2$ fluxes after CC incorporation was quickly followed by a slowing down of mineralization rates (Lynch et al., 2016; Ghimire et al., 2017; De Notaris et al., 2020; Li et al., 2021). This is usually due to the fact that microbial communities produce more enzymes to acquire energy and nutrients from the organic residues (Fig. 1b), i.e., they shift from exploitative to acquisitive resource strategies with a high enzyme regeneration cost (Malik et al., 2019). During this stage, the production of $CO_2$ was not associated with a decrease in the concentration of DOC in the KCl extract. In our opinion, the most likely explanation is that the drop in the concentration of DOM caused by its mineralization was counterbalanced by production of DOM resulting from the degradation of organic carbon originating from the soil and/or CC residues.

The last stage extended from day 60 to day 92 and was characterized by a sharp decrease in C mineralization rates due to the depletion of the readily-decomposable compounds coupled with an increase in the proportion of recalcitrant organic compounds. At this stage, the C mineralization rates in CC-amended soils were similar to those in the control soil (Fig. 1a), suggesting that the microbial communities returned to their original status characterized by a low metabolic activity and low respiration rates (Fanin and Bertrand, 2016). The concentration of DOC in the KCl extract decreased considerably during this stage due to a process that excluded DOM mineralization. The two processes we propose are (i) the incorporation of C from DOM decomposition into the soil microbial biomass (Hu et al., 2023), and (ii) the sorption of DOM to the soil solid phase linked to changes in its characteristics, e.g. increased molecular weight and hydrophobicity (Zhou and Wong, 2000) that enhanced its affinity for the soil constituents. The resulting DOM on day 92 was characterized by similar quantity and quality (average molecular weight, humification degree) in CC-amended soils and the control soil. Taken together, these results show that the incorporation of CC residues transiently altered the dynamics of OM and led to significant changes in the quantity and quality of DOM that in turn are capable of altering Cu dynamics.

### 4.2 Effect of the incorporation of CC residues on the dynamics of Cu

As observed for DOM, the incorporation of CC residues transiently increased the solubility of Cu in the soil. On day 0, the concentration of Cu was higher in the KCl extract of CC-amended soils than in the control soil (Fig. 2a), suggesting that the soluble organic C released by CC residues was able to complex and solubilize Cu. However, the lower Cu:DOC ratio in the KCl extract of CC-amended soils during the initial stage (i.e. from day 0 to day 3, suppl. Fig. F3) may suggest that the affinity for Cu of the ligands released by the CCs was on average lower than that of the soil DOM. This is in line with the low degree of humification of the DOM measured on day 0 in CC-amended soils (Fig. 3) since aromatic substances usually have a higher Cu-mobilizing potential than aliphatic ones (Amery et al., 2008; Borggaard et al., 2019), as well as with the presumed composition of soluble ligands released by CCs based on hydrophilic compounds (e.g. sugars) whose affinity for Cu is rather low (Laurent et al., 2020). The changes over time in Cu solubility in CC-amended soils closely mirror that of DOM, and particularly of humic-like DOM, as evidenced by the close and positive relationship between the concentration of Cu and $FI_{C5\text{-}C7}$ in the KCl extract (Fig. 5b). As observed for $FI_{C5\text{-}C7}$, the decrease in the concentration of Cu during the first stage was less pronounced than that of DOC, thus supporting the hypothesis of preferential degradation of aliphatic DOM by copiotrophic microorganisms during the initial stage of decomposition. The subsequent changes in Cu solubility can be interpreted as resulting from the changes in the dynamics of humic-like DOM (Eon et al., 2023), notably, the decrease in Cu solubility during the last stage (i.e. between day 60 and day 92) could result from the sorption of Cu-humic DOM complexes onto the soil constituents.

The free Cu ion fraction in the KCl extract provides further information regarding the interaction between Cu and DOM. First, the fact that $f_{Cu}$ never exceeded 1.3% in the control soil, and 0.3% in CC-amended soils, confirms that the vast majority of Cu is bound to DOM in the soil solution (Filipovic et al., 2023). Second, the lower $f_{Cu}$ in CC-amended soils compared to in the control soil supports the hypothesis that the soluble organic ligands supplied by CC residues were able to complex Cu. Third, the negative relationship between $f_{Cu}$ and pH (Fig. 5d) is evidence that the complexation of Cu by DOM is highly sensitive to pH, and that the affinity of DOM to bind Cu is higher at high pH, i.e. when more functional groups at the DOM molecules are deprotonated (Nierop et al., 2002), as shown in previous studies by our group (Cornu et al., 2019; Ouédraogo et al., 2021). This last point also explains why the changes in the concentration of free Cu ion over time (Fig. 4b) were not related to the dynamics of DOM but rather to changes in pH in CC-amended soils. During the incubation period, pH varied from 5.5 to 7.0 in the KCl extract of CC-amended soils (suppl. Fig. F4), for reasons linked to CC decomposition, possibly the production / decarboxylation of organic anions (Yan et al., 1996), which in turn made the free Cu ion concentration vary from 0.03 to 3 nM, i.e. by a factor of 100. However, the lower concentration of free Cu ion in CC-amended soils had nothing to do with pH itself, but was rather the result of an increase in the concentration of Cu-complexing DOM, as evidenced by the distinct relationships between the two variables observed in the control soil and in CC-amended soils (Fig. 5c). In this respect, our results contrast with those of a study performed by Cui et al. (2008) in which the decrease in the concentration of free Cu ion in the soil solution after the addition of rice straw was mainly attributed to an increase in pH.

Taken together, these results highlight that the incorporation of CC residues transiently altered the dynamics of Cu through its impact on the dynamics of DOM and pH, and led to significant changes in the solubility and the speciation of Cu in soil that may alter its ecotoxicity and leaching, as suggested by Merrit and Erich (2003).

### 4.3 Differences between the two CC residues

One objective of this study was to assess whether the effect of CC incorporation on the dynamics of OM and Cu in soil differed between oat and faba bean, i.e. between CC residues with contrasted C:N ratios. Our results do not support the hypothesis that the lower C:N ratio of faba bean makes it more degradable by soil microorganisms than oat and, hence, has more impact on the dynamics of OM and Cu. Indeed, the effect of CC incorporation on the dynamics of OM and Cu was similar in magnitude and timing in the two species (Figs. 1, 2, 4). The absence of an effect of the C:N ratio during the first stage could be due to two factors: (i) soil microorganisms primarily relying on accessible energy from the soluble C fraction, and (ii) non-limiting availability of N in the local environment (Fanin et al., 2014). These results are evidence that the composition of C forms in CCs plays a crucial role in explaining microbial activity in the early stages of CC decomposition (Maxwell et al., 2020), while the C:N ratios only play a secondary role, especially when nutrients are readily available in the soil environment (Fanin et al., 2020). Furthermore, the incorporation of dried ground CC residues into the soil likely facilitated the rapid release of significant quantity of soluble forms of C, thereby potentially reducing differences in mineralization rates between the two types of residues in our experiment.

During the second stage of decomposition, the degradation of oat residues was even slightly more pronounced than that of faba bean residues, for reasons that could be linked to the fact that the organic carbon in grasses is less protected by association with minerals than organic carbon in legumes (Zhang et al., 2022). This was evidenced by a higher C mineralization rate in the soil amended with oat residues (Fig. 1a), coupled with a higher concentration of DOC (Fig. 2a) and of humic-like DOM (Fig. 2c) from day 3 to day 60. During this stage, the higher concentration of humic-like DOM in the soil amended with oat residues was associated with a higher concentration of Cu (Fig. 4a) and a lower fraction of free Cu ion (Fig. 4b) in the KCl extract. Taken together, these results support the hypothesis that the CC residues with the greatest potential to increase the solubility of Cu in soil are those whose degradation most increases the concentration of DOM, notably that of humic-like DOM. Hence, the incorporation of CCs with a high fraction of soluble C such as *Robinia pseudoacacia* (Fanin and Bertrand, 2006) is suspected of having a particularly strong impact on the dynamics of Cu, and likely on those of other trace metals such as Pb (Borggaard et al., 2010), in soil.

## Conclusion

This work highlights the fact that incorporating cover crop (CC) residues can transiently disrupt the dynamics of Cu in vineyard topsoil. CC residues were shown to increase the solubility of Cu in the soil for 60 to 90 days after their incorporation, mainly due to the dissolved organic matter released by their decomposition. Provided the Cu-DOM complexes behind Cu solubilization are sufficiently labile to contribute to Cu uptake by soil organisms, the incorporation of CC residues could increase the bioavailability of Cu and consequently the risk of Cu ecotoxicity in vineyard topsoil. However, the rates and forms of incorporation of CCs tested in our experiment, which were chosen and set to enable us to disentangle their impact on the dynamics on DOM and Cu, are not the same as the rates and forms of CC incorporation normally used at field scale. The effect of incorporating CCs on the dynamics of DOM and Cu in soil is therefore likely less pronounced and spread out over a longer period of time in real vineyard plots than in the present study, due to presumably lower and slower release of soluble organic C. Taken together, these results underline the need to ensure the stability of the organic matter added to Cu-contaminated vineyard soils, as its stability will determine its effect on Cu dynamics and ecotoxicity. In this respect, composting CC residues before their incorporation could mitigate their detrimental effect on Cu solubility in vineyard topsoil, while simultaneously helping control primary inoculum of downy mildew.

## Acknowledgements

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## References

Abad, J., Hermoso De Mendoza, I., Marín, D., Orcaray, L., Santesteban, L.G., 2021. Cover crops in viticulture. A systematic review (1): Implications on soil characteristics and biodiversity in vineyard. OENO One 55, 295-312.

Amery, F., Degryse, F., Cheyns, K., De Troyer, I., Mertens, J., Merckx, R., Smolders, E., 2008. The UV-absorbance of dissolved organic matter predicts the fivefold variation in its affinity for mobilizing Cu in an agricultural soil horizon. Eur. J. Soil Sci. 59, 1087-1095.

Ballabio, C., Panagos, P., Lugato, E., Huang, J.-H., Orgiazzi, A., Jones, A., Fernández-Ugalde, O., Borrelli, P., Montanarella, L., 2018. Copper distribution in European topsoils: An assessment based on LUCAS soil survey. Sci. Tot. Environ. 636, 282-298.

Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K., Wallenstein, M.D., 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. JoVE 50961.

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil 10, 9-31.

Blanco‐Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A., Hergert, G.W., 2015. Cover crops and ecosystem services: Insights from studies in temperate soils. Agron. J. 107, 2449-2474.

Borggaard, O.K., Holm, P.E., Strobel, B.W., 2019. Potential of dissolved organic matter (DOM) to extract As, Cd, Co, Cr, Cu, Ni, Pb and Zn from polluted soils: A review. Geoderma 343, 235-246.

Bravin, M.N., Garnier, C., Lenoble, V., Gérard, F., Dudal, Y., Hinsinger, P., 2012. Root-induced changes in pH and dissolved organic matter binding capacity affect copper dynamic speciation in the rhizosphere. Geochim Cosmochim Acta 84, 256-268.

Campbell, P.G.C., 1995. Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: Tessier, A., Turner, D.R. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. Wiley, Chichester, p. 45e102.

Cornu, J.Y., Randriamamonjy, S., Gutierrez, M., Rocco, K., Gaudin, P., Ouerdane, L., Lebeau, T., 2019. Copper phytoavailability in vineyard topsoils as affected by pyoverdine supply. Chemosphere 236, 124347.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob. Change Biol. 19, 988-995.

Cui, Y.S., Du, X., Weng, L.P., Zhu, Y.G., 2008. Effects of rice straw on the speciation of cadmium (Cd) and copper (Cu) in soils. Geoderma 146, 370-377.

De Notaris, C., Olesen, J.E., Sørensen, P., Rasmussen, J., 2020. Input and mineralization of carbon and nitrogen in soil from legume-based cover crops. Nutr. Cycl. Agroecosyst. 116, 1-18.

De Troyer, I., Amery, F., Van Moorleghem, C., Smolders, E., Merckx, R., 2011. Tracing the source and fate of dissolved organic matter in soil after incorporation of a 13C labelled residue: A batch incubation study. Soil Biol. Biochem. 43, 513-519.

Dequiedt, S., Saby, N.P.A., Lelievre, M., Jolivet, C., Thioulouse, J., Toutain, B., Arrouays, D., Bispo, A., Lemanceau, P., Ranjard, L., 2011. Biogeographical patterns of soil molecular microbial biomass as influenced by soil characteristics and management: Biogeography of soil microbial biomass. Glob. Ecol. Biogeogr. 20, 641-652.

Eon, P., Deogratias, J.-M., Robert, T., Coriou, C., Bussiere, S., Sappin-Didier, V., Denaix, L., Cornu, J.Y., 2023. Ability of aerated compost tea to increase the mobility and phytoextraction of copper in vineyard soil. J. Environ. Manage. 325, 116560.

Fanin, N., Alavoine, G., Bertrand, I., 2020. Temporal dynamics of litter quality, soil properties and microbial strategies as main drivers of the priming effect. Geoderma 377, 114576.

Fanin, N., Bertrand, I., 2016. Aboveground litter quality is a better predictor than belowground microbial communities when estimating carbon mineralization along a land-use gradient. Soil Biol. Biochem. 94, 48-60

Fanin, N., Hättenschwiler, S., Fromin, N., 2014. Litter fingerprint on microbial biomass, activity, and community structure in the underlying soil. Plant Soil 379, 79-91.

Filipović, L., Defterdarović, J., Chen, R., Krevh, V., Gerke, H.H., Baumgartl, T., Kovač, Z., Ondrašek, G., Ružičić, S., He, H., Dusek, J., Filipović, V., 2023. Leached Copper Correlation with Dissolved Organic Carbon in Sloped Vineyard Soil. Water 15, 800.

Ghimire, B., Ghimire, R., VanLeeuwen, D., Mesbah, A., 2017. Cover crop residue amount and quality effects on soil organic carbon mineralization. Sustainability 9, 2316.

Helms, J.R., Stubbins, A., Ritchie, J.D., Minor, E.C., Kieber, D.J., Mopper, K., 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnol. Oceanogr. 53, 955-969.

Karimi, B., Masson, V., Guilland, C., Leroy, E., Pellegrinelli, S., Giboulot, E., Maron, P.-A., Ranjard, L., 2021. Ecotoxicity of copper input and accumulation for soil biodiversity in vineyards. Environ. Chem. Lett. 19, 2013-2030.

Komárek, M., Čadková, E., Chrastný, V., Bordas, F., Bollinger, J.-C., 2010. Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. Environ. Int. 36, 138-151.

Laurent, C., Bravin, M.N., Crouzet, O., Pelosi, C., Tillard, E., Lecomte, P., Lamy, I., 2020. Increased soil pH and dissolved organic matter after a decade of organic fertilizer application mitigates copper and zinc availability despite contamination. Sci. Tot. Environ. 709, 135927.

Li, X., Tan, A., Chen, K., Pan, Y., Gentry, T., Dou, F., 2021. Effect of Cover Crop Type and Application Rate on Soil Nitrogen Mineralization and Availability in Organic Rice Production. Sustainability 13, 2866.

Liñero, O., Cornu, J.Y., De Diego, A., Bussière, S., Coriou, C., Thunot, S., Robert, T., Nguyen, C., 2018. Source of Ca, Cd, Cu, Fe, K, Mg, Mn, Mo and Zn in grains of sunflower (Helianthus annuus) grown in nutrient solution: root uptake or remobilization from vegetative organs? Plant Soil 424, 435-450.

Lynch, M.J., Mulvaney, M.J., Hodges, S.C., Thompson, T.L., Thomason, W.E., 2016. Decomposition, nitrogen and carbon mineralization from food and cover crop residues in the central plateau of Haiti. SpringerPlus 5, 973.

Mackie, K.A., Schmidt, H.P., Müller, T., Kandeler, E., 2014. Cover crops influence soil microorganisms and phytoextraction of copper from a moderately contaminated vineyard. Sci. Tot. Environ. 500–501, 34-43.

Malik, A.A., Puissant, J., Goodall, T., Allison, S.D., Griffiths, R.I., 2019. Soil microbial communities with greater investment in resource acquisition have lower growth yield. Soil Biol. Biochem. 132, 36-39.

Marstorp, H., 1996. Influence of soluble carbohydrates, free amino acids, and protein content on the decomposition of Lolium multiflorum shoots. Biol. Fert. Soils 21, 257-263.

Maxwell, T.L., Augusto, L., Bon, L., Courbineau, A., Altinalmazis-Kondylis, A., Milin, S., Bakker, M.R., Jactel, H., Fanin, N., 2020. Effect of a tree mixture and water availability on soil nutrients and extracellular enzyme activities along the soil profile in an experimental forest. Soil Biol. Biochem. 148, 107864.

McDowell, W.H., Zsolnay, A., Aitkenhead-Peterson, J.A., Gregorich, E.G., Jones, D.L., Jödemann, D., Kalbitz, K., Marschner, B., Schwesig, D., 2006. A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. Soil Biol. Biochem. 38, 1933-1942.

Merritt, K.A., Erich, M.S., 2003. Influence of Organic Matter Decomposition on Soluble Carbon and Its Copper‐Binding Capacity. J. Env. Qual. 32, 2122-2131.

Murphy, K.R., Stedmon, C.A., Wenig, P., Bro, R., 2014. OpenFluor– an online spectral library of auto-fluorescence by organic compounds in the environment. Anal. Methods 6, 658-661.

Nierop, K.G.J., Jansen, B., Vrugt, J.A., Verstraten, J.M., 2002. Copper complexation by dissolved organic matter and uncertainty assessment of their stability constants. Chemosphere 49, 1191-1200.

Ouédraogo, F., Cornu, J.Y., Janot, N., Nguyen, C., Sourzac, M., Parlanti, E., Denaix, L., 2022. Do DOM optical parameters improve the prediction of copper availability in vineyard soils? Environ. Sci. Pollut. Res. 29, 29268-29284.

Pierdet, M., 2020. Evolution spatiale et temporelle de la mobilité des contaminants organiques et inorganiques dans des sols viticoles contrastés. Thèse de doctorat, Université de Bordeaux (France). https://www.theses.fr/2020BORD0316

Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. Agr. Ecosyst. Environ. 200, 33-41.

Sauvé, S., Hendershot, W., Allen, H.E., 2000. Solid-Solution Partitioning of Metals in Contaminated Soils: Dependence on pH, Total Metal Burden, and Organic Matter. Environ. Sci. Technol. 34, 1125-1131.

Stedmon, C.A., Bro, R., 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial: Fluorescence-PARAFAC analysis of DOM. Limnol. Oceanogr. Methods 6, 572-579.

Weiler, D.A., Bastos, L.M., Schirmann, J., Aita, C., Giacomini, S.J., 2022. Changes in chemical composition of cover crops residue during decomposition. Cienc. Rural 52, e20210357.

Wershaw, R.L., Leenheer, J.A., Kennedy, K.R., Noyes, T.I., 1996. Use of 13C NMR and FTIR for elucidation of degradation pathways during natural litter decomposition and composting I. Early stage leaf degradation. Soil Sci. 161, 667-679.

Yan, F., Schubert, S., Mengel, K., 1996. Soil pH increase due to biological decarboxylation of organic anions. Soil Biol. Biochem. 28, 617-624.

Zhang, Z., Kaye, J.P., Bradley, B.A., Amsili, J.P., Suseela, V., 2022. Cover crop functional types differentially alter the content and composition of soil organic carbon in particulate and mineral‐associated fractions. Glob. Change Biol. 28, 5831-5848.

Zhou, L.X., Wong, J.W.C., 2000. Microbial Decomposition of Dissolved Organic Matter and Its Control during a Sorption Experiment. J. Env. Qual. 29, 1852-1856.

## Figure captions

**Figure 1.** Changes in the cumulative amount of C mineralized (a) and the activity of C hydrolytic enzymes (b) over the 92-day incubation period, in a vineyard soil after incorporation of faba bean (green) or oat (yellow) residues. No cover crop residue was incorporated in the control soil (gray). The data presented are average values ± one standard deviation (shown as bars in the figure) calculated from four replicates per treatment. For a given sampling day, (*), *, ** and *** indicate that the amount of C mineralized (a) or the activity of C hydrolytic enzymes (b) in the soil amended with oat differed significantly from that measured in the soil amended with faba bean at a probability level of $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

**Figure 2.** Changes in the concentration of dissolved organic carbon (DOC) (a), the slope of the absorbance spectra in the 275-295 nm wavelength range ($S_{275\text{-}295}$) (b), and the cumulative fluorescent intensity of the PARAFAC components C5, C6 and C7 ($FI_{C5\text{-}C7}$) (c) over the 92-day incubation period in a 0.01 M KCl extract of a vineyard soil after incorporation of faba bean (green) or oat (yellow) residues. No cover crop residue was incorporated in the control soil (gray). The data presented are average values ± one standard deviation (shown as bars in the figure) calculated from four replicates per treatment. For a given sampling day, (*), *, ** and *** indicate that the concentration of DOC (a), $S_{275\text{-}295}$ (b) or $FI_{C5\text{-}C7}$ (c) in the soil amended with oat differed significantly from that measured in the soil amended with faba bean, at a probability level of $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

**Figure 3.** Changes in the contribution of the eight components identified by PARAFAC

analysis to the maximum fluorescence intensity of dissolved organic matter (DOM) over the 92-day incubation period, in the control soil (a), in soil amended with faba bean (b), and in soil amended with oat (c) residues.

**Figure 4.** Changes in the concentration of total Cu (a) and free Cu ion (b) over the 92-day incubation period in a 0.01 M KCl extract of a vineyard soil after incorporation of faba bean (green) or oat (yellow) residues. No cover crop residue was incorporated in the control soil (gray). The data presented are average values ± one standard deviation (shown as bars in the figure) calculated from four replicates per treatment. In (b) the Y-axis scale is logarithmic. For a given sampling day, (*), *, ** and *** indicate that the concentration of total Cu (a), or free Cu ion (b) in the soil amended with oat residues differed significantly from that measured in the soil amended with faba bean residues at a probability level of $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

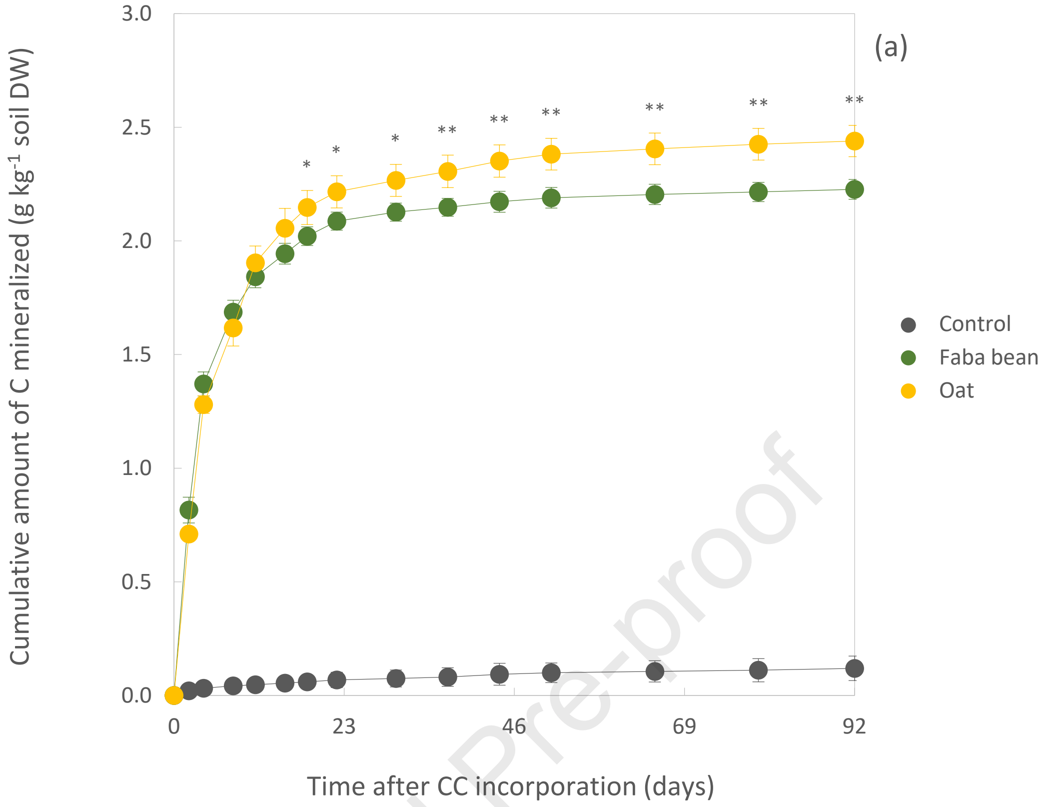
**Figure 5.** Total Cu concentration as a function of the concentration of dissolved organic carbon (DOC) (a), total Cu concentration as a function of cumulative fluorescence intensity of the PARAFAC components C5, C6 and C7 ($FI_{C5-C7}$) (b), free Cu ion concentration as a function of pH (c) and free Cu ion fraction as a function of pH (d), in a 0.01 M KCl extract of a vineyard soil after incorporation of faba bean (green) or oat (yellow) residues. No cover crop residue was incorporated in the control soil (gray). The correlation coefficient (r) is for the two treatments in which cover crops were incorporated. In (c) and (d), the Y-axis scale is logarithmic and the correlation coefficient (r) is for the log-log relationship.

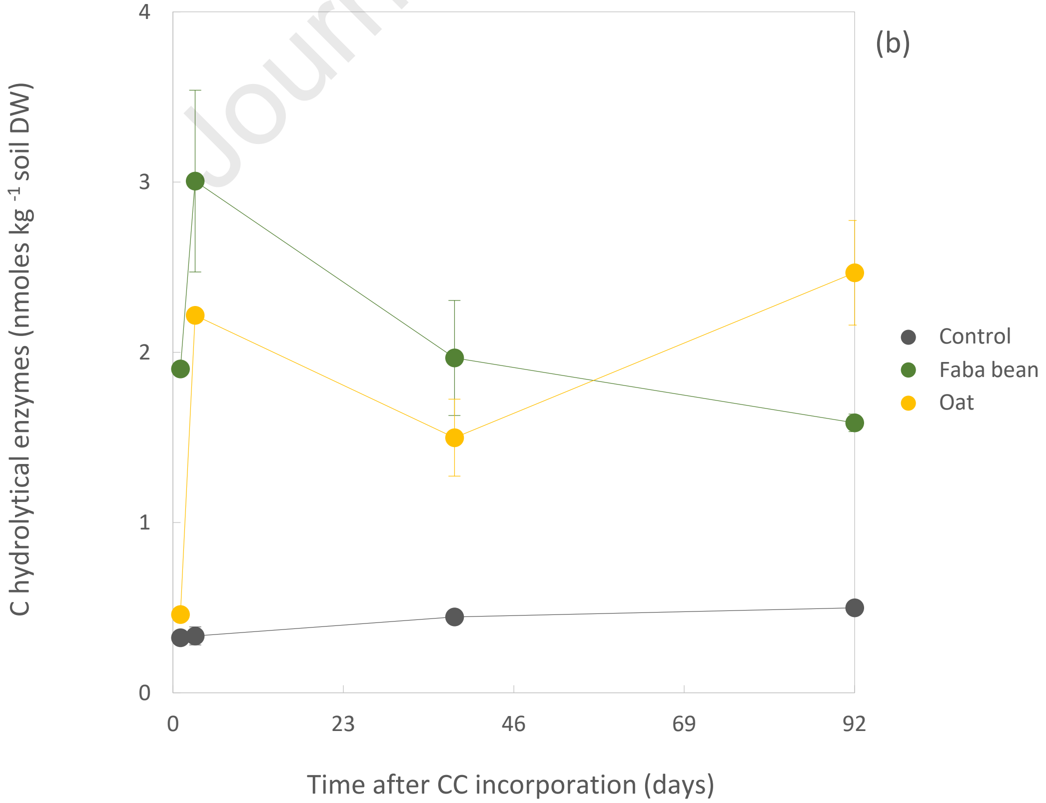
**Table 1.** Characteristics of the eight components identified by PARAFAC analysis

| Component | Ex / Em (nm) | Fluorophores | DOM description | References |
|---|---|---|---|---|
| **C1** | 275 / 345 | Protein-like,<br>Tryptophan-like | Low humified DOM | [1-3] |
| **C2** | 280 / 315 | Free amino acids,<br>Amino acids bound to proteins | Low humified DOM | [4, 5] |
| **C3** | 275 / 305 | Protein-like,<br>Tyrosine-like | Low humified DOM | [6, 7] |
| **C4** | 240-290 / 330 | Protein-like,<br>Tryptophan-like | Low humified DOM | [8] |
| **C5** | 270-380 / 500 | Salicylic acid,<br>Conjugated fluorophores | Recalcitrant DOM associated with microbial reduction reaction | [8-10] |
| **C6** | 240-315 / 435 | Lignin-like | Humified DOM degraded from lignin | [8] |
| **C7** | 245-355 / 435 | Lignin-like | Aromatic DOM with low N content | [11, 12] |
| **C8** | 240-305 / 410 | Oxidized quinone-like | Microbial-based DOM with low molecular weight and low aromaticity | [1, 13, 14] |

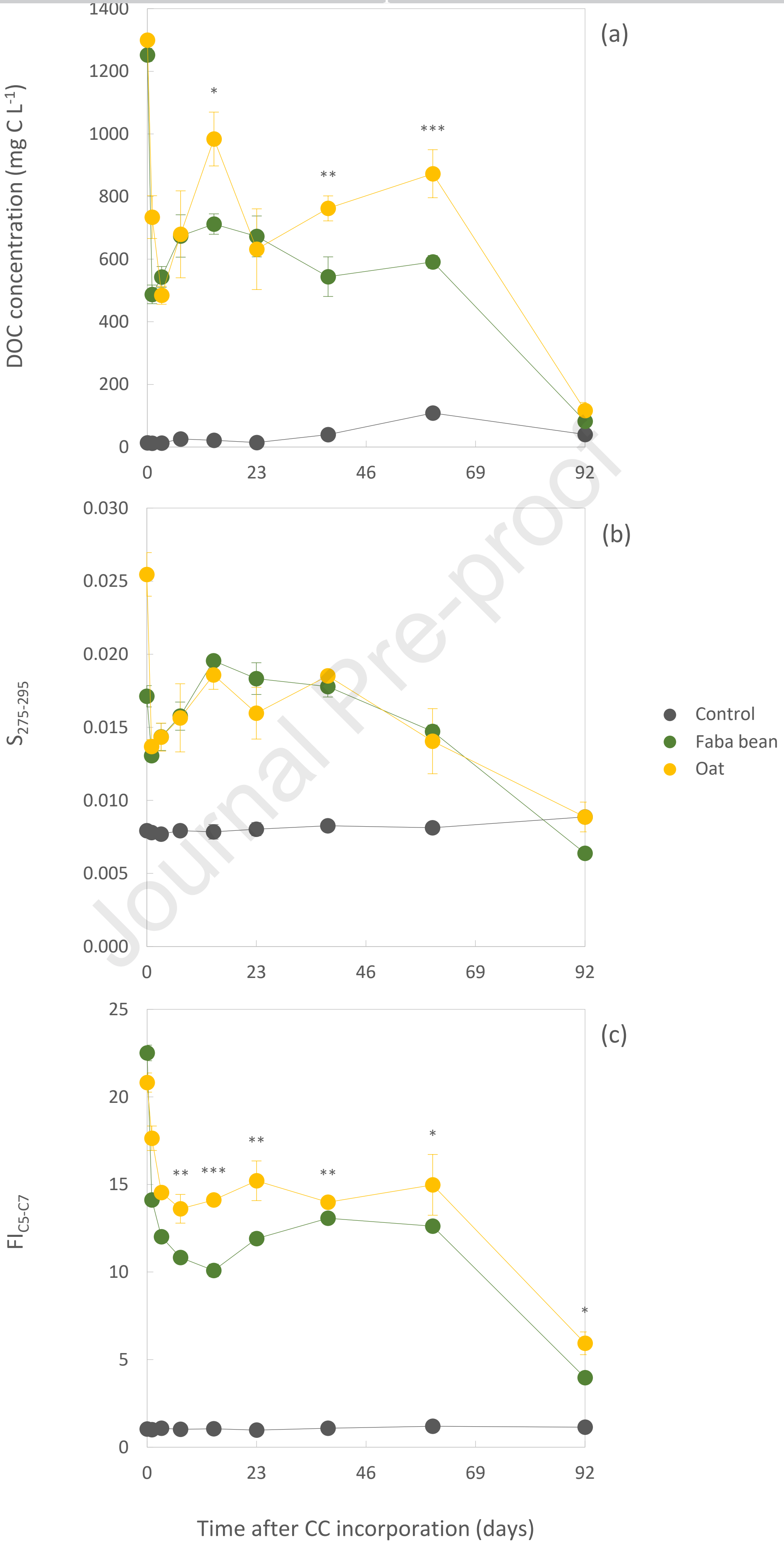
[1] Cory and McKnight (2005). doi.org/10.1021/es0506962
[2] Derrien et al. (2019). doi.org/10.1016/j.scitotenv.2019.02.258
[3] Osburn and Stedmon (2011). doi.org/10.1016/j.marchem.2011.06.007
[4] Stedmon et al. (2007). doi.org/10.1016/j.marchem.2006.11.005
[5] Murphy et al. (2008). doi.org/10.1016/j.marchem.2007.10.003
[6] Paerl et al. (2020). doi.org/10.1002/lol2.10154
[7] DeFrancesco and Guéguen (2020). doi.org/10.1029/2020JC016578
[8] Caldero-Pascual et al. (2022). doi.org/10.1007/s10750-021-04757-w
[9] Wünsch et al. (2015). doi.org/10.3389/fmars.2015.00098
[10] Zhou et al. (2019). doi.org/10.5194/bg-16-2733-2019
[11] Stubbins et al. (2014). doi.org/10.1021/es502086e
[12] Retelletti Brogi et al. (2020). doi.org/10.1016/j.scitotenv.2020.139212
[13] Singh et al. (2013). doi.org/10.1155/2013/532424
[14] Harjung et al. (2018). doi.org/10.1016/j.limno.2017.02.007

# Figure 1


Cumulative amount of C mineralized (g kg$^{-1}$ soil DW)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
23
46
69
92
Time after CC incorporation (days)
(a)
*
*
*
**
**
**
**
**
**
Control
Faba bean
Oat


C hydrolytical enzymes (nmoles kg$^{-1}$ soil DW)
4
3
2
1
0
0
23
46
69
92
Time after CC incorporation (days)
(b)
Control
Faba bean
Oat

# Figure 2


(a)
DOC concentration (mg C L-1)
1400
1200
1000
800
600
400
200
0
*
**
***
0
23
46
69
92
(b)
S275-295
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0
23
46
69
92
(c)
FIC5-C7
25
20
15
10
5
0
**
***
**
**
*
*
0
23
46
69
92
Time after CC incorporation (days)
● Control
● Faba bean
● Oat

# Figure 3

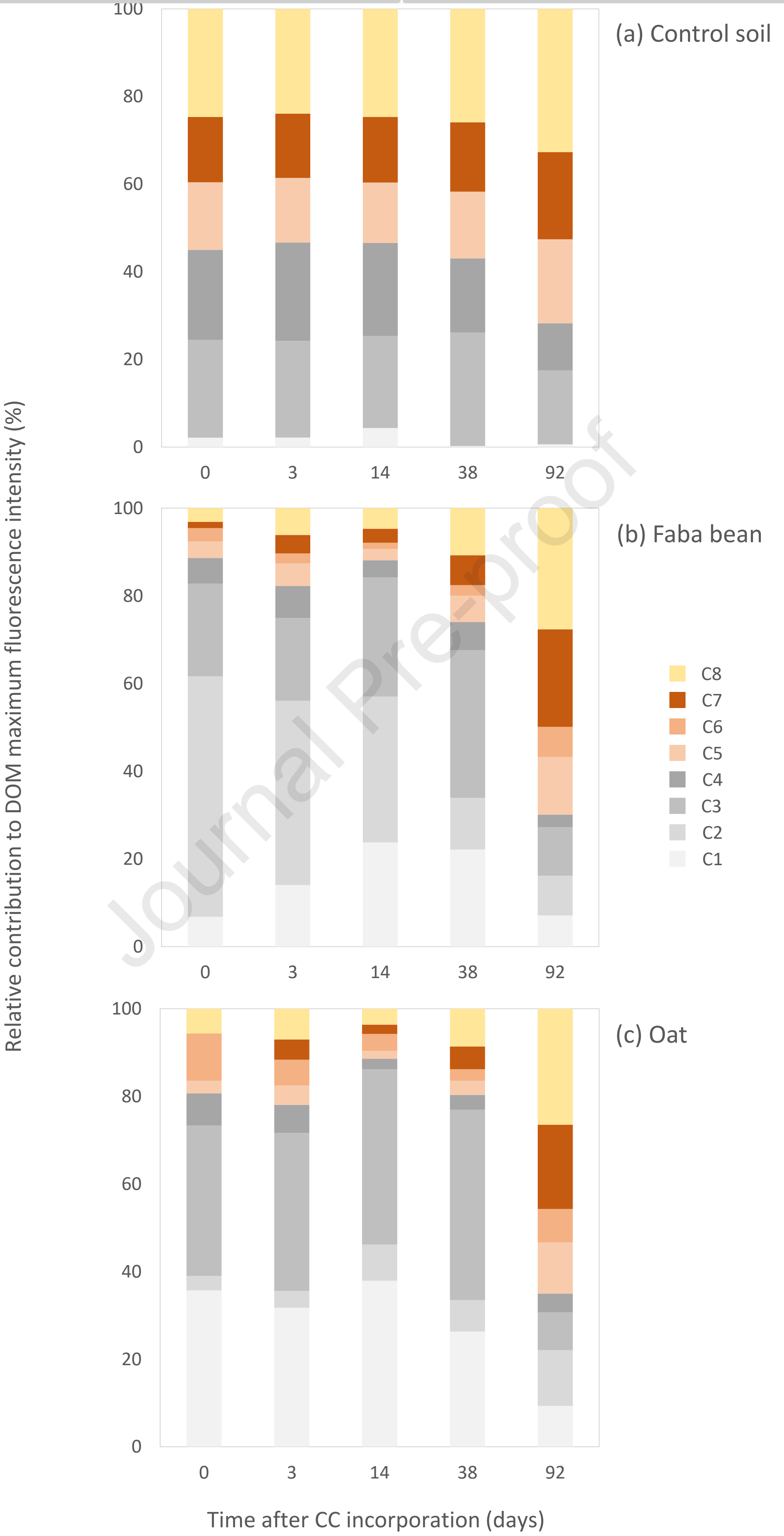
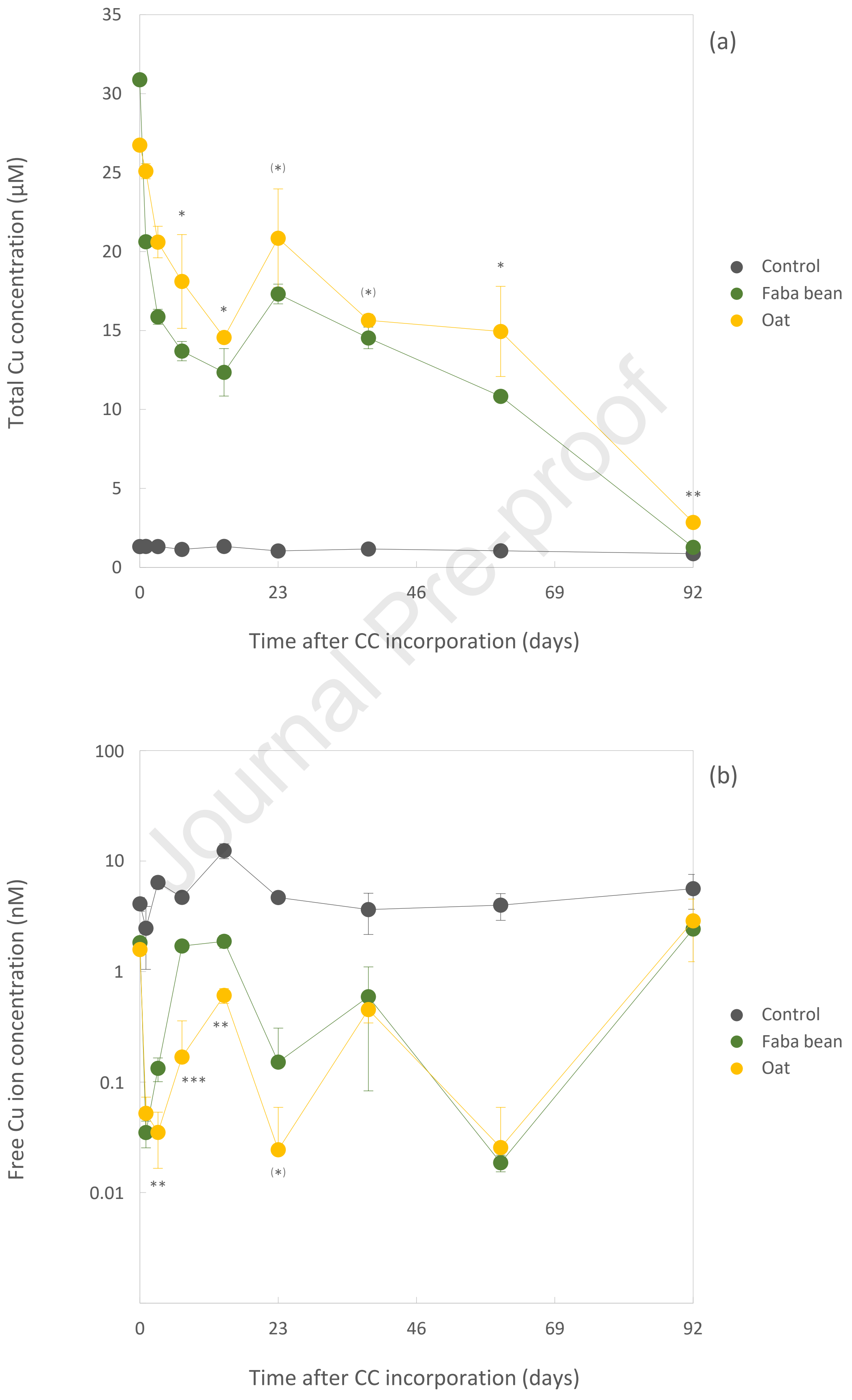
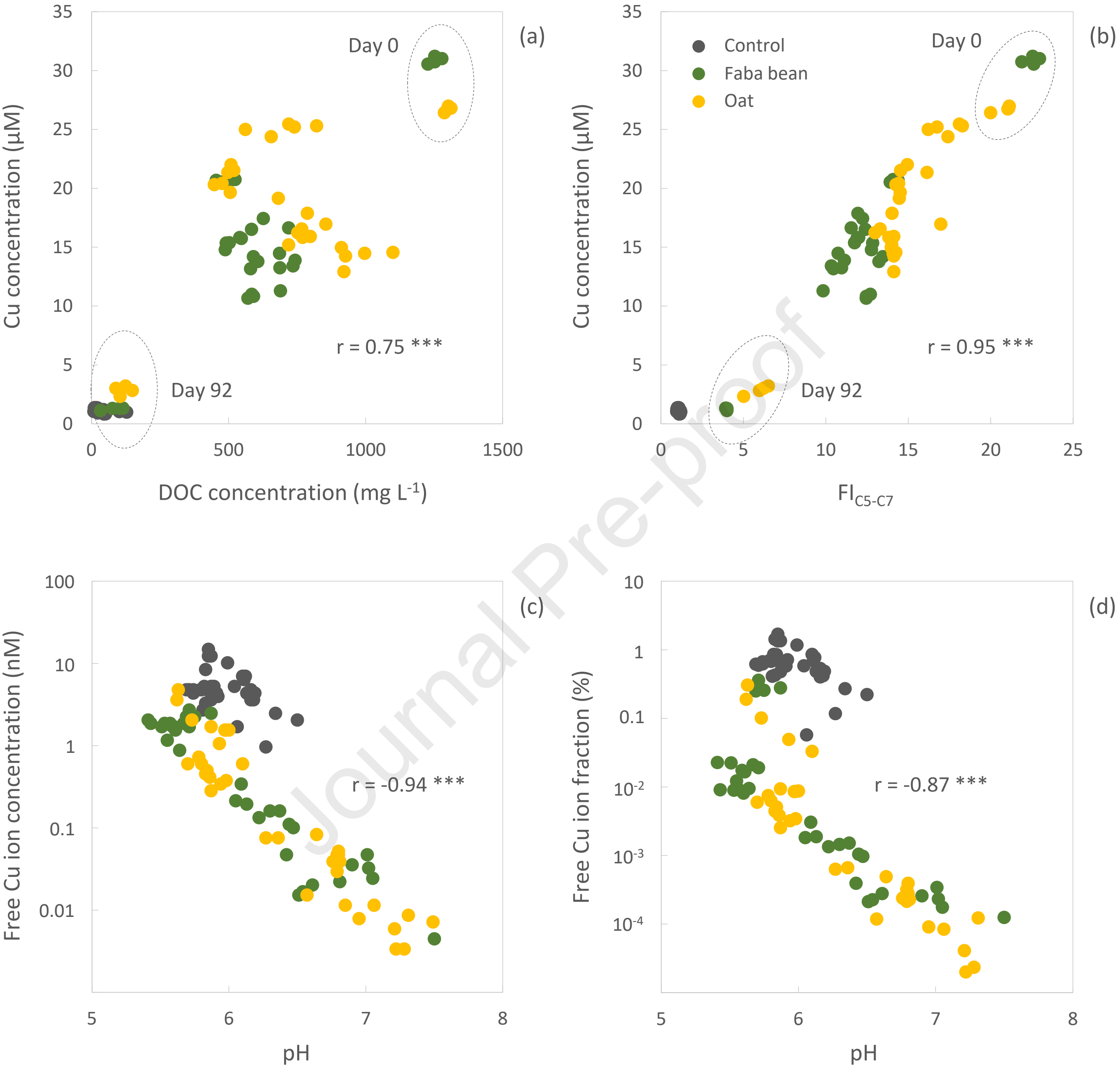

Relative contribution to DOM maximum fluorescence intensity (%)
100
80
60
40
20
0
0
3
14
38
92
(a) Control soil
(b) Faba bean
(c) Oat
C8
C7
C6
C5
C4
C3
C2
C1
Time after CC incorporation (days)

Figure 4


(a)
Total Cu concentration (µM)
35
30
25
20
15
10
5
0
0
23
46
69
92
Time after CC incorporation (days)
*
*
(*)
(*)
*
**
Control
Faba bean
Oat
(b)
Free Cu ion concentration (nM)
100
10
1
0.1
0.01
0
23
46
69
92
Time after CC incorporation (days)
**
***
**
(*)
Control
Faba bean
Oat

# Figure 5


(a)
Day 0
Day 92
r = 0.75 ***
Cu concentration (µM)
35
30
25
20
15
10
5
0
0
500
1000
1500
DOC concentration (mg L$^{-1}$)
(b)
Control
Faba bean
Oat
Day 0
Day 92
r = 0.95 ***
Cu concentration (µM)
0
5
10
15
20
25
FI$_{C5-C7}$
(c)
r = -0.94 ***
Free Cu ion concentration (nM)
100
10
1
0.1
0.01
5
6
7
8
pH
(d)
r = -0.87 ***
Free Cu ion fraction (%)
10
1
0.1
10$^{-2}$
10$^{-3}$
10$^{-4}$
5
6
7
8
pH

## Highlights

Adding cover crops (CCs) transitorily altered the dynamics of Cu in vineyard soil

Adding CCs transitorily increased the concentration of dissolved organic matter

Optical properties of dissolved organic matter changed over time after adding CCs

Soluble forms of C released by cover crop residues are capable of solubilizing Cu

Oat and faba bean had almost the same effect on the dynamics of DOM and Cu

## Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: