Universidade de Aveiro Departamento de Ambiente e Ordenamento
2017

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Utilização de *biochar* na remediação de solos vitícolas contaminados por cobre

Use of biochar in the remediation of copper-contaminated vineyard soils

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Use of biochar in the remediation of copper-contaminated vineyard soils

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Engenharia do Ambiente, realizada sob a orientação científica do Doutor Nelson José Cabaços Abrantes, Investigador Auxiliar do Centro de Estudos do Ambiente e do Mar (CESAM) da Universidade de Aveiro, e coorientação do Doutor Jan Jacob Keizer, Investigador Auxiliar do Centro de Estudos do Ambiente e do Mar (CESAM) da Universidade de Aveiro e da Doutora Isabel Maria Alves Natividade Campos, bolseira do Centro de Estudos do Ambiente e do Mar (CESAM) da Universidade de Aveiro

Este trabalho foi financiado por fundos europeus através da ARIMNet2, e por fundos nacionais através da Fundação para a Ciência e Tecnologia I.P. (FCT, I.P.), no âmbito do projecto MASCC – *Mediterranean Agricultural Soils Conservation under global Change* (ARIMNET2/0006/2015).

o júri

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agradecimentos

Começo por agradecer aos meus orientadores, Nelson e Jacob, que muito me ajudaram ao longo deste trabalho, pela orientação e pelos ensinamentos.

Um obrigada muito especial à Isabel, que me acompanhou no laboratório, e ao João, que me acompanhou no campo, que sempre me apoiaram, e por quem sinto imensa amizade e gratidão. Assim como ao Óscar, que também se mostrou sempre disponível a qualquer esclarecimento na área do solo, e ao Frank, na área do *biochar*.

A toda a equipa, que sempre se mostrou pronta a ajudar no que fosse preciso.

Aos meus pais e à minha prima, que são o meu porto seguro desde sempre.

Por fim, aos meus amigos.

Muito obrigada por tudo.

palavras-chave

Fungicidas, cobre, vinha, escorrências superficiais, matéria particulada suspensa, *biochar*, remediação de solo.

resumo

Na viticultura, o tratamento com fungicidas à base de cobre resulta muitas vezes em elevados teores de cobre nos solos. Sabendo que o cobre em determinadas concentrações se pode assumir como um composto tóxico, a sua acumulação nos solos pode ter impactos nos organismos terrestres, comprometendo as funções ecossistémicas asseguradas pelo solo. Além disso, considerando que as áreas de vinha sofrem frequentemente processos de erosão acentuados, o seu transporte e entrada em sistemas aquáticos adjacentes pode igualmente impactar o biota aquático.

Na tentativa de melhorar as propriedades do solo e garantir uma produção agrícola efetiva, têm sido propostas várias medidas, nomeadamente o uso de *biochar*. O *biochar* é um carvão obtido através do processo de pirólise que é utilizado na remediação de solos devido aos seus benefícios na qualidade do solo. Com o objetivo de avaliar as concentrações de cobre no solo de uma vinha e compreender o papel do *biochar* na qualidade do solo e na redução do transporte de sedimentos ricos em cobre através do escoamento superficial, este trabalho testou a utilização de dois tratamentos de *biochar*, com aplicações distintas (5 kg/m^2 e 10 kg/m^2) e a sua influência nas propriedades do solo, numa vinha na região demarcada da Bairrada.

Pela comparação dos dois tratamentos de *biochar*, verificou-se que o tratamento com menor taxa de aplicação de *biochar* melhorou a qualidade do solo em geral e a sua capacidade de infiltração. Em particular, neste tratamento observou-se uma redução de 69% no transporte de sedimentos e de 76% no transporte de cobre particulado por escoamento superficial. O efeito do *biochar* na imobilização do cobre foi evidenciado sobretudo após a aplicação de cobre, observando um comportamento visivelmente diferente entre os tratamentos com e sem adição de *biochar*.

A aplicação de *biochar* em baixas concentrações mostrou ser efetiva na redução da exportação de sedimentos e consequentemente na exportação de cobre. Não obstante, será importante compreender as implicações que o cobre continuará a exercer nos organismos terrestres e na sua potencial lixiviação para águas subterrâneas.

keywords

Fungicides, copper, vineyard, surface runoff, particulate suspended matter, biochar, soil amendment.

abstract

In viticulture, treatment with copper-based fungicides often results in high levels of copper in vineyard soils. Knowing that copper in certain concentrations can be assumed as a toxic compound, its accumulation in soils can have impacts on terrestrial organisms, compromising the ecosystem functions assured by the soil. In addition, considering that vineyards often suffer from severe erosion processes, copper transport and input into adjacent aquatic systems may also impact the aquatic biota.

To improve soil properties and ensure effective agricultural production, several measures have been proposed, such as the use of biochar. Biochar is a coal obtained through the pyrolysis process that is used in soil remediation due to its benefits in soil quality. In order to assess de concentration of copper in a vineyard soil and understand the role of biochar in soil quality and in reducing the transport of copper enriched sediments through surface runoff, this work tested the use of two biochar treatments with different applications (5 kg/m^2 and 10 kg/m^2) and its influence on soil properties in a vineyard located in the demarcated region of Bairrada.

By comparing the two biochar treatments, it was verified that the treatment with lower application rate of biochar improved the soil quality in general and its infiltration capacity. A reduction of 69% in sediment transport and a 76% reduction in the transport of particulate copper from surface runoff was observed in this treatment. The effect of biochar on the immobilization of copper was particularly evident after the application of copper, with a noticeably different behaviour between the treatments with and without addition of biochar.

The application of biochar in low rates has shown to be effective in the reduction of the export of sediments and consequently in the export of copper. Nonetheless, it is important to understand the implications that copper will continue to exert on terrestrial organisms and its potential leaching into groundwater.

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Acronyms

BH – Biochar high

BL – Biochar low

Cu_D – Dissolved copper

Cu_P – Particulate copper

EC – Electrical conductivity

NB – No biochar

Cu – Copper

SC – Soil carbon

SIC – Soil inorganic carbon

SOC – Soil organic carbon

SOM – Soil organic matter

SPM – Suspended particulate matter

TC_P – Total particulate carbon

TCu– Total copper

TSS – Total suspended solids

VSS – Volatile suspended solids

Chapter 1

General introduction

1.1. Wine sector in Portugal

The wine sector is one of the most dynamic sectors in the Portuguese agriculture (Simões, 2008). The production of wine is distributed throughout the country, with prevalence in the coastal and central area north of the Tagus river (MADRP, 2007). Despite being a small country, the sector occupies a total area of 198 586 ha (Azores and Madeira not included) (Eurostat, 2017). Also, there is a considerable difference between the various regions of Portugal. This differences have a significant impact on grapevine varieties, which are able to successfully acclimatise to the prevailing environmental conditions in each region (Climaco et al., 2012).

Investment in the wine sector made it one of the best adapted sectors to global competition, as a result of Portugal access to European funds (Alberto, 2008). After the Portuguese integration within the European community, a quality policy in wine production was undertaken, by reorganizing the sector institutionally, creating new denominations of origin (Figure 1) and massively supporting investments in the production of quality wines (Simões, 2008). As a result, Portugal has now three different categories of wine: i) wine; ii) protected geographical indication (PGI) wine; and iii) protected designation of origin (PDO) wine (Climaco et al., 2012).

Currently, the wine sector is extremely relevant and of great economic value within the Portuguese context (Afonso, Cruz, & Azevedo, 2012), making them very appealing for trading (AGRO.GES, 2012). On account of this internal and external dynamism of the wine sector, vineyard and wine were combined with other complementary activities, particularly the tourism sector (Simões, 2008).

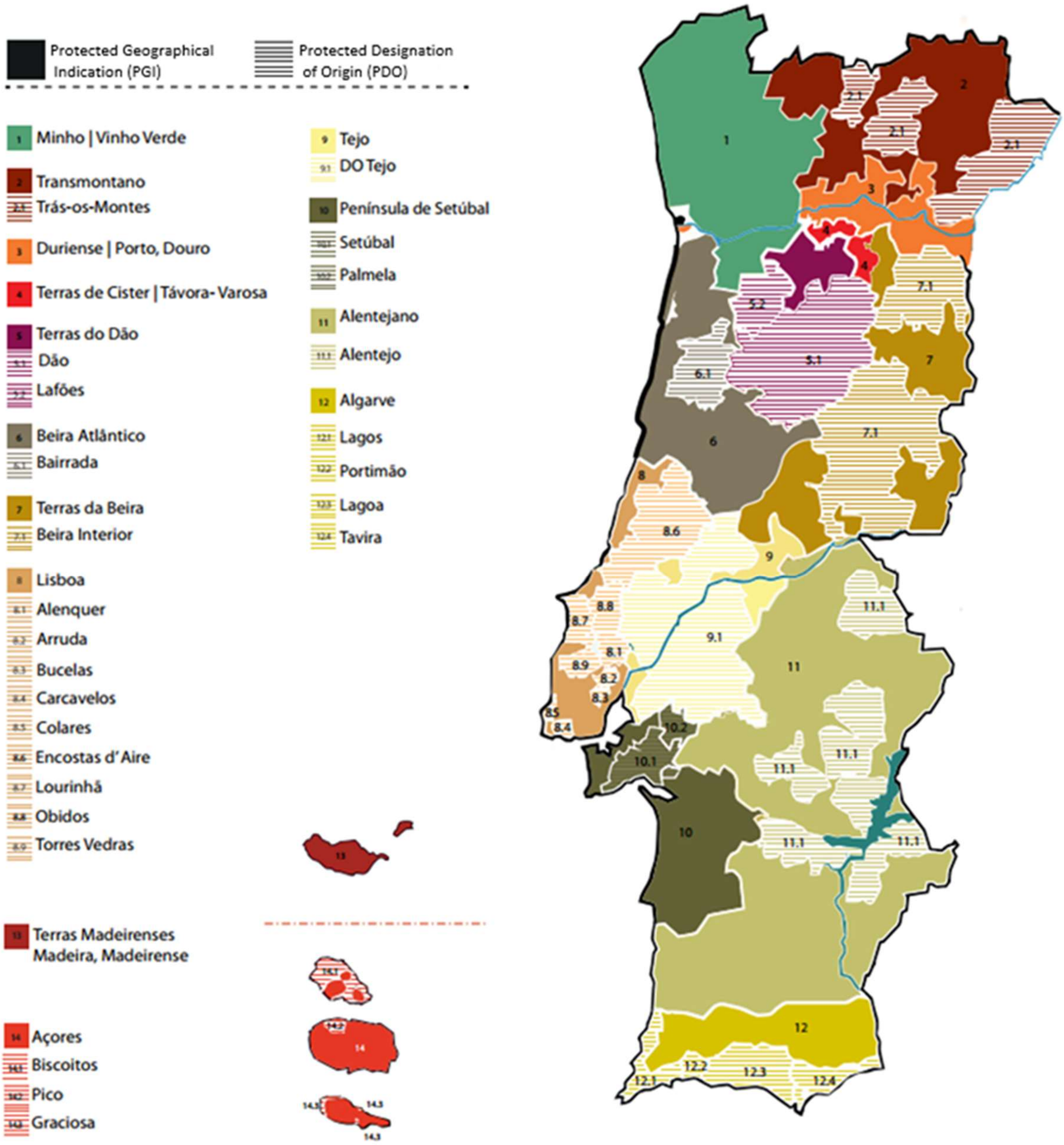

Figure 1: Wine Regions in Portugal according to the quality labels PGI and PDO (IVV, 2012) stablished in EC regulation 607/2009 of 14 July of 2009 (European Commission, 2009).

At the internal level, there has been a clear preference of Portuguese consumers for national wines. Concerning the international trade, Portuguese wines also play a prominent role, both in European and worldwide terms. Inside the European Union (EU), Portugal is a renowned wine producer, even though at a considerable distance from the world's largest producers and exporters, such as Italy, France, and Spain. In consumption, however, Portugal belongs to the group of major consumers, close to countries like France and Italy (Simões, 2008).

1.2. Cu-based plant-protection products (PPPs) used in viticulture

The wine sector faces many problems related with the vines health. In the XIX century, pests and diseases were brought to Europe along with imported vine varieties from north America. The most devastating pest was phylloxera. Entire vineyards perished as consequence. A small bug would attack the roots of the vines, and consequently killing them. As a solution, European varieties were grafted onto phylloxera-resistant varieties from north America and phylloxera was put under control. Nevertheless, the fungal diseases introduced in Europe still represent a real threat to the European vine varieties. The more common are powdery mildew, or oidium, and downy mildew. The application of PPPs, more specifically fungicides, proved itself to be very effective. Among them, Sulphur (S) is worldwide used to fight oidium, and copper (Cu) to fight downy mildew (Robinson, Harding, & Vouillamoz, 2013).

The climate has great influence in the development of fungi (Komárek et al., 2010). Amount of precipitation and relative air humidity are climate conditions that promote fungal diseases (Climaco et al., 2012), as well as the wind, depending on its speed, duration, and frequency. For instance, light winds haste the drying of the leaf, diminishing the period of wetness and lessening the chances of a fungus infection (Roberto, Colombo, & Assis, 2011). The more humidity and water availability, the more it is required of the winegrower to carry out a considerable number of phytosanitary treatments, in order to preserve the quantity and quality of wine production (Climaco et al., 2012). Portugal, as a coastal country in southwestern Europe, has a humid and warm climate, which are good conditions for the proliferation of fungi (Ricce, Caramori, & Roberto, 2013).

Currently, fungicides are the most used PPPs in Portugal. The most popular fungicides are the inorganic ones, which includes sulphur (S) and copper (Cu) compounds. Sulphur is by far the most used, even though there is a great variety of fungicides. According to the most recent report of the DGAV (2017) on PPPs sales in Portugal, S made 80.4% of the inorganic fungicides sales, contributed 49% to the sales of all fungicides and accounted for 25.4% of the total sales of PPPs. Copper has been widely used in agriculture, where it serves as both an essential element and as a toxin for the control of fungus and diseases in plants (Joseph, 1999). Even now, it is still very popular despite the availability of other options (DGAV, 2017).

Cu-based fungicides have been intensively applied in Europe to control vine fungal diseases (Besnard, Chenu, & Robert, 2001; Komárek et al., 2010) for over 100 years. First through the Bordeaux mixture ($CuSO_4$+$Ca(OH)_2$) and later through other Cu compounds (Komárek et al., 2010), e.g. Cu oxychloride ($CuCl_2 \cdot 3Cu(OH)_2$) (Brunetto et al., 2016). Copper sulphate ($CuSO_4$) is a non-mobile compound, very persistent on soils, with low solubility and low leachability (University of Hertfordshire, 2016). Eventually, the intensive use of $CuSO_4$ and subsequent wash-off from the treated vines (Flores-Vélez et al.,1996) led to a widespread and long-term accumulation of Cu in vineyard soils (Besnard et al., 2001; Brun et al., 1998; Komárek et al., 2010; Magalhães, Sequeira, & Lucas, 1985).

1.3. Soil and water Cu contamination by PPPs

Copper content in the soil has three different sources: i) minerals in the soil parent material (e.g. weathered rock, decayed vegetation); ii) anthropogenic inputs (e.g. fungicides); iii) deposition from the atmosphere (e.g. mining dust, volcanic ash). Many factors are responsible for variations in Cu contents of soils, both total and available (Joseph, 1999). Any anthropogenic addition to the soils surface, such as PPPs application, causes a gradual accumulation in soils (IPCS, 1998), which represents a major environmental and toxicological concern (Brunetto et al., 2016).

Soil physicochemical properties, erosion, and tillage are three major processes which contribute to lateral and vertical transport of anthropic Cu in the soil (Besnard et al., 2001; Brun et al., 1998; Fernández-Calviño et al., 2008; Flores-Vélez et al., 1996; Komárek et al., 2008; Nóvoa-Muñoz et al., 2007). Copper in soil is divided in dissolved and particulate phases (Bradl, 2004). The portions of each phase differ depending on the soil properties. Bradl (2004) and Nachtigall et al., (2007) observed that pH has a considerable effect on the amount of Cu adsorbed, as of other metals. In fact, soil pH is a parameter of great importance due to its influence on metal-solution and soil-surface chemistry (Bradl, 2004). In their research, the authors observed an increase of Cu solubility at low pH levels. Fernández-Calviño et al. (2008) also concluded pH strongly affects Cu mobility. The authors observed Cu release enhanced at pH below 5.5, as result of the increasing solubility of this metal under acidic conditions, and at pH above 7.5, when Cu is mobilized due to solubilisation of soil organic matter. Copper immobilization is positively correlated with organic matter, and clay minerals content, from which depends cation exchange capacity (CEC) (Vega et al., 2011) as well as iron (Fe) and manganese (Mn) oxides. Adsorption maxima for soil constituents follows the order Mn oxides>organic matter>Fe oxides>clay minerals (Bradl, 2004; McLaren & Crawford, 1973). Yet, soil organic matter dominates Cu adsorption and it is the main responsible for Cu retention (Bradl, 2004; Fernández-Calviño et al., 2008; Gómez-Armesto et al., 2015; McLaren & Crawford, 1973). Other factors can influence the fate of Cu in the soil besides its nature, such as the climate and the vegetation at the site (IPCS, 1998).

Magalhães et al. (1985) have confirmed the use of Cu fungicides throughout the vineyards lifetime as a significant source of Cu contamination to the soils. High levels of Cu were detected mainly in the surface layers, specially the surface horizon. In European vineyard soils, concentrations of Cu in the upper layers range between 14-945 mg/kg (Komárek et al., 2010). However, vineyards are located on steep slopes, 10% to 35% on average (Besnard et al., 2001), ending up subjected to extensive erosion processes (Tropeano, 1984). As a consequence, particulate Cu is only partially trapped in the surface horizons (Ribolzi et al., 2002), as there exists the possibility of lateral transport by suspended matter through runoff water (Besnard et al., 2001; Fernández-Calviño et al., 2008; Flores-Vélez et al., 1996; Ribolzi et al., 2002). Sediments, which act like reservoirs of Cu (IPCS, 1998), eventually reach nearby surface waters (Bereswill et al., 2012) and cause contamination (El Azzi et al., 2013; Xue, Sigg, & Gächter, 2000). The process of soil and water contamination by Cu is summarised in Figure 2.

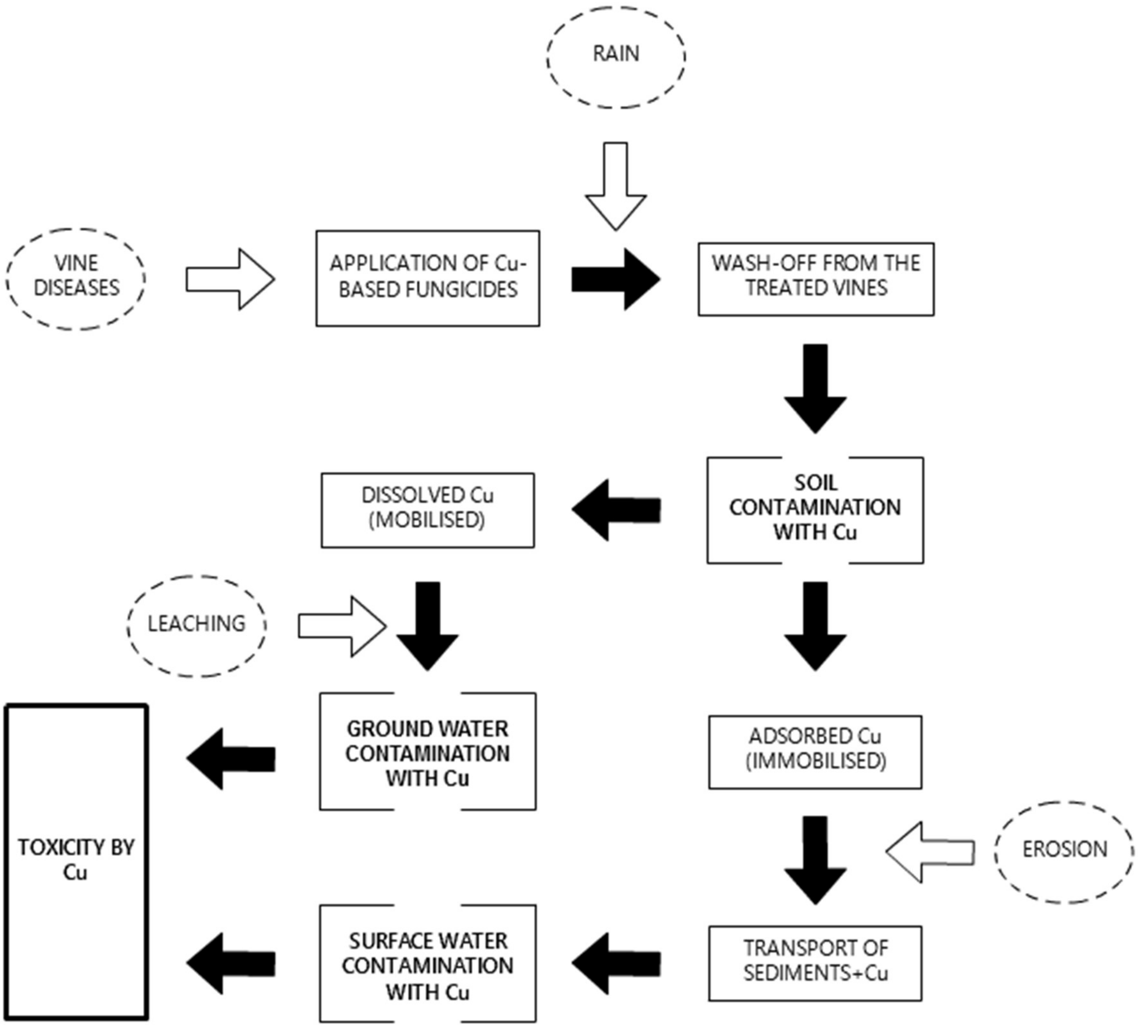

Figure 2: Simplified representation of the contamination process by Cu.

1.4. Consequences on ecosystems and human health

Copper is a crucial element to life, required to essential functions (IPCS, 1998; Xue et al., 2003). Hence, when there is Cu deficiency on soils, Cu is used in agriculture as a fertilizer as well, significantly increasing crop yield depending on crop type (Malhi & Karamanos, 2006).

However, anthropogenic inputs and long-term accumulation of Cu in vineyard soils due to the use of Cu fungicides raised the issue of Cu bioavailability (Brun et al., 2001), and thus, bioaccumulation or toxicity potential to terrestrial organisms (IPCS, 1998; Zyadah & Abdel-Baky, 2000), which varies considerably depending on the organisms sensitivity (Oorts, 2013). Soluble Cu species are specially available and thus more worrying (Lejon et al., 2008). Most of dissolved Cu is available, only a small percentage reacts with dissolved organic matter by complexation (IPCS, 1998).

Since the highest concentrations of Cu are present in the superficial soil horizons (Brun et al., 1998; Flores-Vélez et al., 1996) and vines are deeply rooted to the soil, only in rare occasions vines suffer from Cu toxicity (Komárek et al., 2010). Plants with shallow roots and young vines are more vulnerable to increased Cu concentrations and thus, Komárek et al. (2010) concluded that agricultural practices alterations, such as replacing the vines with young ones, or any other shallow rooting crops or pasture plants, could lead to phytotoxic effects and contamination of the newly grown plants.

Even so, with time, Cu availability decreases. Because of long-term processes, called aging, metal availability decreases with time, resulting in a reduction of easily extracted Cu and an increase in strongly bounded forms, mostly associated with organic matter and mineral oxides. The desorption is much slower and the vegetation uptake grows smaller (Oorts, 2012).

Similarly, the behaviour of Cu in biological processes, its bioavailability, and its toxicity to aquatic organisms from sediments also depends deeply on the chemical form of Cu (Suedel, Deaver, & Rodgers, 1996). Aquatic organisms acquire the Cu they need from soluble Cu in water and in sediments interstitial water, from adsorbed Cu on suspended particles or sediments, and from Cu in animals they ingest (Georgopoulos et al., 2001). Decrease of abundance and diversity of crustaceans and other macroinvertebrates have been reported at high levels of particulate Cu (Fernández et al., 2015; Kraft & Sypniewski, 1981), and Cu contamination of both water and food also reduced significantly the survival and growth of shredder species (Silva et al., 2018).

For humans, Cu compounds are dangerous if inhaled due to its deposition on the lungs. When high concentrations are available in the air, breathing in Cu powder or Cu-containing fume can cause “metal fever”. Symptoms consist of headache, sweating, nausea, and exhaustion (Joseph, 1999). Some vineyard workers show symptoms of pulmonary Cu deposition and fibrosis related with Cu inhalation after spraying fungicidal $CuSO_4$ for years (Salgare, 1991), sometimes reported as fatal (Joseph, 1999). Except for occasional severe incidents of Cu poisoning, few effects stand out in standard populations. Dermal exposure may induce allergic responses, but in sensitive individuals only (IPCS, 1998).

1.5. Biochar applications to soil

Biochar is the product of a process called pyrolysis, where biomass is thermochemically converted in an oxygen-limited environment (IBI, 2015), also used in the production of charcoal (Shackley, Schmidt, & Glaser, 2016). However, biochar is considered a separate category of product, as are charcoal and activated carbon, even though all three of these categories are related (Mašek, Ronsse, & Dickinson, 2016). The material distinguishes itself from other carbon (C) products due to its purpose. Biochar is produced to be added to soil, where it remains more stable and resistant to deterioration (Glaser et al., 2009; Shackley et al., 2016) (Shackley et al., 2016) than other organic amendments or its feedstock. Also, biochar can be produced from a much wider range of biomass feedstock than regular charcoal (Shackley et al., 2016), having the advantage of being much more flexible in terms of feedstock sourcing and thus a greater variety of biochar properties (Mašek et al., 2016).

It can be used for soil application and environmental management (Beesley et al., 2011; Lehmann & Joseph, 2015). Its beneficial effects on agriculture and environmental systems are related with increasing crop yields (higher availability of nutrients and water), soil quality improvement (enhanced soils structure, organic matter content, drainage), soil remediation (sorption of pollutants, reduction of nitrates (NO_3^-) and phosphates (PO_4^{3-}) leaching), and C storage (long term C stability) (Beesley et al., 2011; Lehmann & Joseph, 2015; Lopez-Capel et al., 2016). Hence, biochar is added to soils with the intent to improve soil functions as a low-cost remediation strategy (Thangarajan et al., 2016). The adsorbent characteristic of biochar is imperative to nutrient retention and stabilisation of contaminated soils (Beesley et al., 2011; Pignatello et al., 2015), and the reactions which control the mobility and bioavailability of inorganic contaminants are several, such as redox, sorption and complexation (Thangarajan et al., 2016).

Biochar is characteristically enriched in C, but it is also very rich in phosphorus (P), potassium (K) and nitrogen (N) (Lehmann & Joseph, 2015; Lopez-Capel et al., 2016), which increases these soil nutrients amounts. However, these nutrients within the biochar structure are usually unavailable. Biochar does improve the availability of K and P through soil liming and by reducing leaching losses, but has limited effect on N availability (Biederman & Harpole, 2013). Even though declining over time, this liming effect on the receiving soil (depending on biochar ash content, application rate and the soil buffering capacity) also increases soil organic matter (SOM) (Tian et al., 2016; Zimmerman, Gao, & Ahn, 2011), which is important in the formation of stable soil aggregates, enhancing soil structure and stability, and thus greater water and air provision to plants. This suggests biochar can have beneficial effects on soil aggregation (Liu et al., 2014) and stability (Sun & Lu, 2014), protecting soils from water erosion (Abrol et al., 2016; Cross et al., 2016).

1.6. Aims and thesis structure

The main goal of this thesis was to study the long-term accumulation of Cu-based fungicides on soil and study how biochar could contribute on lessening the Cu and sediment losses by surface runoff at an intensive viticulture area.

More specifically, the goals of the thesis were to:

1) Assess the concentration of Cu in vineyard soils;
2) Assess the quantity of sediments and Cu (particulate and soluble) mobilised by surface runoff;
3) Understand the effects of biochar application on sediment and Cu losses and on soil characteristics.

The thesis' structure is divided in two chapters: Chapter 1 gives a contextualisation, it introduces the wine-growing sector in Portugal and summarises environmental problems associated with the application of Cu-based fungicides in vineyards, as well as the known benefits of applying biochar to the soil; Chapter 2 was written in manuscript format. It includes the methods applied, the results, and the discussion, as well as some final considerations of the study.

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Chapter 2

Article

2.1. Introduction

The wine sector is of great economic value to Portugal (Afonso et al., 2012), with a production very superior to other agricultural sectors (AGRO.GES, 2012). However, pests and diseases pose a threat to the wine production, and PPPs are frequently applied to prevent losses of productivity or quality. Powdery mildew, or oidium, and downy mildew are the most common vine fungal diseases, and the application of PPPs, specifically fungicides, is the most effective way to fight them. Due to their broad-spectrum action, sulphur (S) and copper (Cu) were for over a century the most used in fungicides (Robinson et al., 2013).

In particular, Cu-based fungicides were heavily applied in Europe (Besnard et al., 2001; Komárek et al., 2010), mostly through the Bordeaux mixture ($CuSO_4$+$Ca(OH)_2$), and later through other Cu compounds (Komárek et al., 2010). Copper sulphate ($CuSO_4$) is a non-mobile compound, very persistent on soils, with low solubility and low leachability (University of Hertfordshire, 2016). Eventually, the intensive use of $CuSO_4$ and the subsequent wash-off from the treated vines (Flores-Vélez et al., 1996) led to a widespread, long-term accumulation of Cu in vineyard soils (Besnard et al., 2001; Brun et al., 1998; Komárek et al., 2010; Magalhães et al., 1985). These anthropogenic inputs and long-term accumulation of Cu in vineyard soils raised the issue of Cu bioavailability (Brun et al., 2001) and, thus, bioaccumulation or toxicity potential to terrestrial organisms (IPCS, 1998; Zyadah & Abdel-Baky, 2000).

Copper in soil is divided in dissolved and solid phases (Bradl, 2004). The portions of each phase depend on soil properties. Copper mobilisation can be vertical or lateral and its immobilization is strongly and positively correlated with soil pH. The higher the pH the more

Cu reacts through complexation, reducing dissolved copper concentrations. Also, adsorption maxima for soil constituents follows the order Mn oxides>organic matter>Fe oxides>clay minerals, but organic matter is the one of greatest importance (Bradl, 2004; McLaren & Crawford, 1973).

High levels of Cu have been detected in the surface layers of vineyard soils (Komárek et al., 2010; Magalhães et al., 1985). Considering that vineyards are generally located on steep slopes (Besnard et al., 2001), and are subjected to extensive erosion processes (Tropeano, 1984), Cu could be transported to surface water by surface runoff (Besnard et al., 2001; Fernández-Calviño et al., 2008; Flores-Vélez et al., 1996; Ribolzi et al., 2002). Hence, Cu-contaminated vineyard soils can impair water quality and their ecological status (El Azzi et al., 2013; Bereswill et al., 2012; Xue et al., 2000). Among the distinct Cu species, soluble Cu species are specially available and thus more worrying (Lejon et al., 2008). For instance, aquatic organisms acquire the Cu they need from soluble Cu in water and in sediments interstitial water, from adsorbed Cu on suspended particles or sediments, and from Cu in animals they ingest (Georgopoulos et al., 2001). It has been reported a decrease of abundance and diversity of crustaceans and other macroinvertebrates at high levels of particulate Cu (Fernández et al., 2015; Kraft & Sypniewski, 1981), and Cu contamination of both water and food has also been observed to reduce significantly the survival and growth of shredder species (Silva et al., 2018).

Recent studies have pointed out the benefits of biochar on soil quality in general. Its application can have positive effects on soil aggregation (Liu et al., 2014) and stability (Sun & Lu, 2014), protecting soils from water erosion (Abrol et al., 2016; Cross et al., 2016). The adsorbent characteristic of biochar can decrease bioavailability of heavy metals, and thus be very important to stabilise contaminated soils (Beesley et al., 2011; Pignatello et al., 2015; Thangarajan et al., 2016). Additionally, as biochar is characteristically enriched in C, P, K and N (Lehmann & Joseph, 2015; Lopez-Capel et al., 2016), it contributes to increase these soil nutrients concentrations. Biochar also has a liming effect of the receiving soil and increases SOM (Tian et al., 2016; Zimmerman et al., 2011). Hence, biochar is added to soils with the intent to improve soil functions as a low-cost remediation strategy (Thangarajan et al., 2016).

In this paper, the specific goals were to: (i) assess the concentration of Cu in vineyard soils, (ii) assess the quantity of sediments and Cu (particulate and soluble) mobilised by surface runoff, and (iii) understand the effects of biochar application on sediment and Cu losses and on soil characteristics.

2.2. Material and methods

2.2.1. Study area and site

This study was carried out in a vineyard located in Quinta do Ribeirinho (40°28'16.2"N 8°32'59.9"W), municipality of Anadia, in the central region of Portugal (Figure 3). This vineyard is included in the protected designation of origin (PDO) wine region of Bairrada, which accounts for approximately 5% of the Portuguese wine production (IVV, 2017). The vineyard belongs to the winegrower Adega Luís Pato.

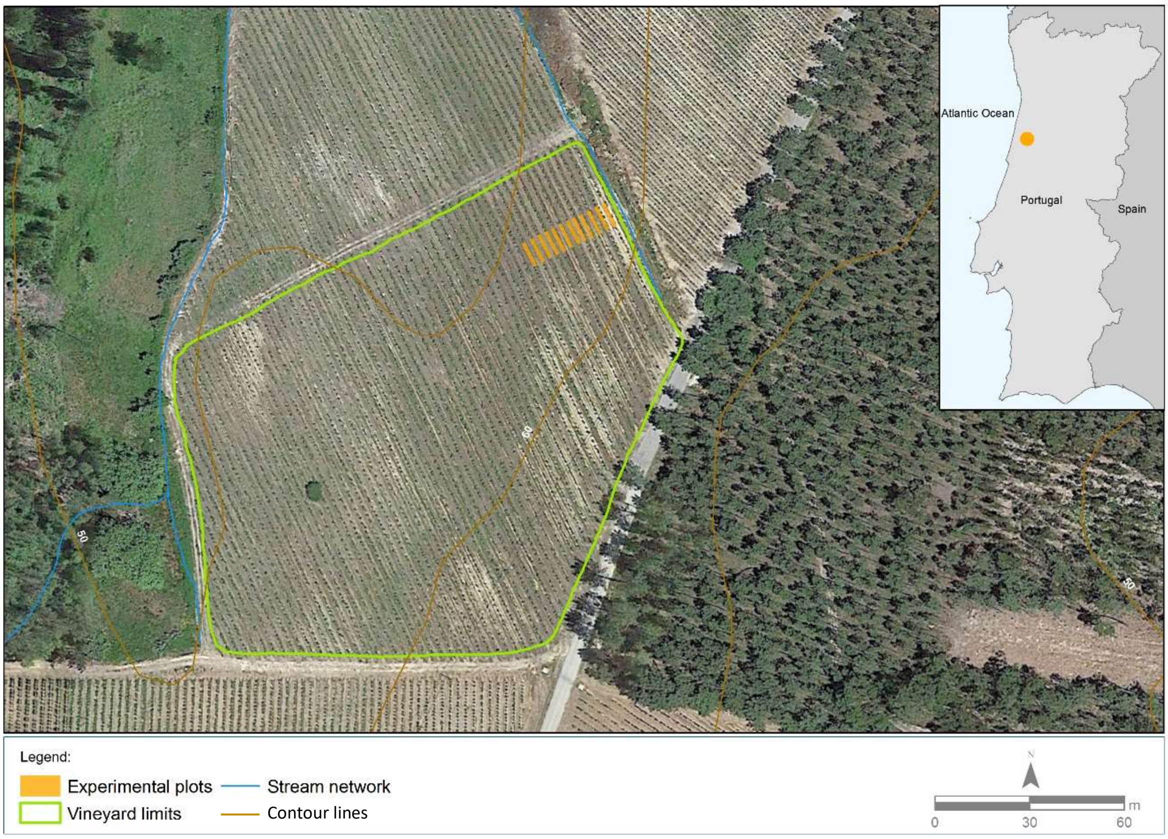

Figure 3: Location of the vineyard studied in the «Bairrada» region, Municipality of Anadia.

The Bairrada wine region is constituted by approximately 12,000 ha of vineyards (Climaco et al., 2012), which bordered in the north by the river Vouga and in the south by the river Mondego, in the east by the mountains of Buçaco and Caramulo and in the west by the Atlantic Ocean (IVV, 2011).

The climate of the Bairrada wine region is classified as temperate, with abundant precipitation (total 1000-1200 mm/year) and moderate temperatures (day average 12.5-15 °C) (Climaco et al., 2012; CVB, 2017; IVV, 2016). It is classified as Csb in the Köppen-Geiger Climate Classification system, with dry and temperate summers with cold nights and moderate drought (Chazarra et al., 2011; IPMA, 2016; Köppen, 1936). The summers are only moderately dry due to the proximity to the ocean, the same reason why winters are cool (IVV, 2016).

The Bairrada vineyards are located between 40 and 120 meters above sea level, meaning the maritime influence is strongly felt throughout the wine-growing region, which has noticeable effects on precipitation, wind and relative humidity (Climaco et al., 2012; IVV, 2016). The soils in the region vary in their majority from sandy to clay-calcareous (IVV, 2016), where main geology goes from the Jurassic period (clay calcareous soils) to the early Quaternary period (sandy and silt deposits) (LNEG, 2017).

The studied vineyard is approximately 60 years old, with vine rows planted 2 m apart. The soil was classified as a dystric Regosol (IUSS Working Group WRB, 2015), characterized by a ten centimetres depth Ap layer, and a more than 70 centimetres depth C layer, both with a sandy loam texture. According to the manager of the vineyard, the top soil was extracted in the beginning of the 20th century for trade purposes. Consequently, the site topography was altered, and the present topsoil of the vineyard has a more recent, less evolved soil than its surroundings.

This vineyard has been managed according to the integrated production systems since 2008/2009. Tillage is alternated between vine inter-rows every year. Weeds in the vine rows are controlled mechanically, avoiding the need for herbicides. This approach, however, is very recent, and until 2015 herbicides were still applied. During the study period (November 2016 to May 2017), sulphur was applied two times, on April 11th and May 16th, and copper was applied one time on April 11th.

2.2.2. Experimental design

In the studied vineyard, four blocks (A, B, C, D) were installed approximately half way the slope (Figure 4). Each block comprised three plots selected randomly, each of which corresponded to one of the three different treatments: i) No Biochar (NB) – treatment without the biochar application, functioning as a control; ii) Biochar Low (BL) – biochar was applied at a rate of 5 kg/m^2; and iii) Biochar High (BH) – biochar was applied at a rate of 10 kg/m^2. These rate applications were chosen based on the previous study conducted by Abrol et al. (2016). In BL and BH treatments, biochar was mixed to the soil by ploughing it at depth of 20 cm. The NB treatment was also ploughed to allow the comparison among the three treatments.

A total of 12 plots were set up, each with an area of 16 m^2 (2 m wide by 8 m long). At the bottom of the plots of blocks A, B, and C (plots 1 to 9), a drainage system was installed to collect and store surface runoff (Figure 4a). The drainage systems consisted of a drainage grid inserted into the soil till the soil surface to collect the surface runoff that was connected to PVC tubing and, at the end, garden hose, to drain the surface runoff into a 500 L tank. At the upper part of plots 1 to 9, a simple diversion system was installed to avoid run-on from upper slope parts (Figure 4b). A rain gauge of 7.5 cm radius was installed nearby the plots. Figures 5-8 display pictures of the experimental site. The plots of block D (plots 10 to 12) were used for soil sampling at repeated occasions.

The biochar used in this study was supplied by Ibero Massa Florestal (Aveiro, Portugal). The characteristics of the biochar are presented in Table 1.

Table 1: Physical and chemical properties of the biochar applied in the study area.

Granulometry cm	**Fixed C** %	**Ash** %	**Volatiles** %	**pH**
≤6	≥90	≤5	≤5	8

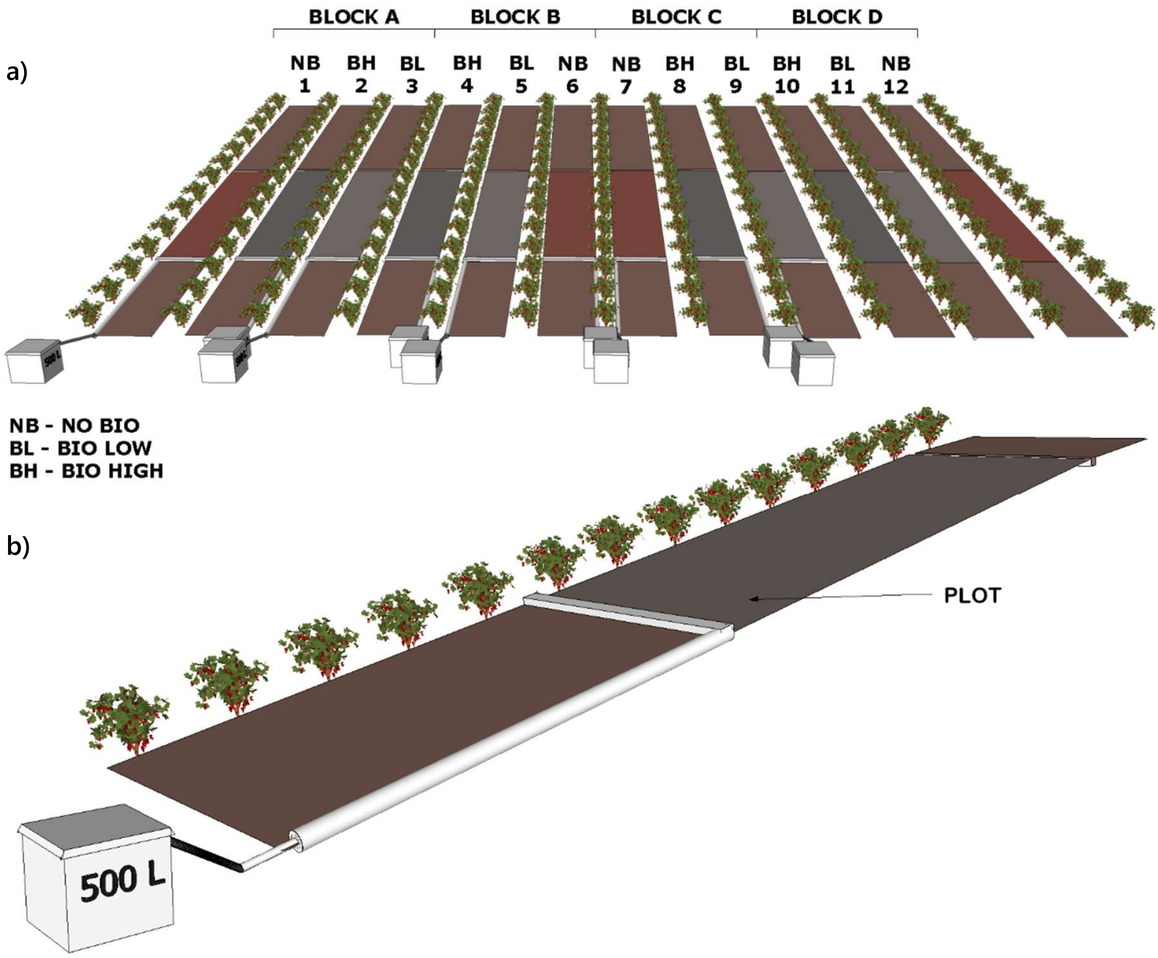

Figure 4: Experimental design of the a) study area, and of the b) surface runoff collection system in plots 1 to 9.

Figure 5: Drainage grids which collected the surface runoff of the plots.

Figure 6: a) 500 L tanks where the surface runoff was collected and b) plot 1 at the beginning of the experiment (Nov/2016).

Figure 7: Application of biochar to the soil.

Figure 8: General view of the plots and study area.

2.2.3. Field data and sample collection

Soil sample collection was performed in the first metre of the plots of block D at the beginning of the experimental period, on November. Three samples were collected at 0-10 cm depth in each of two sub-areas that were identified in each plot, i.e. the tracks of the tractor wheels (compacted soil) and the area in between these tracks (uncompacted soil). Samples were placed in plastic bags and frozen (-18°C) until their analysis could be performed.

During the study period, from November 2016 to May 2017, surface runoff collected in the tanks was measured at 1- to 2-weekly intervals, depending on rainfall, and, whenever possible, runoff samples were taken in 0,5 L plastic bottles. After collection, samples were preserved in the cold (4°C) and handled within a week.

2.2.4. Analytical methods

2.2.4.1. Soil samples

Prior to analysis, soil samples were air-dried at room temperature and sieved (2 mm).

Soil pH was determined following ISO 10390:2005 (International Organization for Standardization, 2005) methodology, while soil electrical conductivity (EC) was determined by following ISO 11265:1994 (International Organization for Standardization, 1994) methodology.

Soil organic matter (SOM) content was determined based on the methodology of Périé & Ouimet (2008). Three g of soil dried at 105°C were heated at 500°C for 4 hours. The mass lost during calcination represents the organic matter.

Soil carbon (SC) content and soil organic carbon (SOC) were measured after milling the soil samples. Soil carbon was measured directly using the multi N/C® 3100 accessory TOC solids module HT 1300. Soil organic carbon content was determined by first adding drops of HCL (10%) until no reaction was observed. Afterwards, 2 drops of concentrated HCL (37%) were added. Samples were dried at 105°C for 3 hours and measured by the same instrument used for SC.

Finally, copper (Cu) content was determined based on Pereira et al. (2008). One g of milled soil dried at 40°C was digested with *aqua regia* (3 HCl: 1 HNO_3) in covered Teflon beakers. The mixture was heated on a hotplate at 100°C, until dryness. Ten mL of HNO_3 were then added to the Teflon beakers and the solution was filtered through 0.45 µm Whatman® ME 25/21 ST filters and transferred into plastic volumetric tubes. Each solution was diluted until completing a volume of 25 mL and its concentration was measured by FLAA analysis using Thermo Scientific® iCE 3000 Series.

2.2.4.2. Surface runoff samples

Electrical conductivity and pH were measured according to the methods 2510 and 4500-H^+, respectively, established by APHA (1995).

Total suspended solids (TSS) and volatile suspended solids (VSS) concentrations were respectively determined based on 2540 D and 2440 E methods defined by APHA (1999). Triplicates of each sample were filtered through 1.5 µm VWR® Glass Fiber Filters, dried at 105°C and then ignited at 550°C.

Dissolved Cu (Cu_D) concentration was determined based on EPA (1994), by filtering first the runoff samples through 0.4 µm Whatman® Nuclepore Track-Etched Membranes, and then filtering 10 mL of the filtrates through a 0.45 µm Whatman® FP 30/0.45 CA-S syringe filter unit. 1 µL of HNO_3 (>68%) was added to the final filtrate, and Thermo Scientific® iCE 3000 Series was used to perform flame atomic absorption spectroscopy (FLAA) analysis.

Particulate Cu (Cu_P) was measured through an adapted version of the method developed by Caetano et al. (2007) to the suspended particulate matter trapped in the same filters used to measure Cu_D. Each filter was digested with 1 mL of *aqua regia* (3 HCl: 1 HNO_3) and 1 mL of HF in Teflon beakers with screw caps at 100°C for 1 hour. The suspended particulate matter (SPM) in the filter were washed off with milli-Q water and the content was transferred into a covered Teflon beaker and heated on a hotplate at 90°C, until dryness. One mL of HNO_3 plus 5 mL of milli-Q water was then added to the Teflon beaker and the mixture was heated at 75°C for 20 minutes. More 25 mL of milli-Q water were added, and the mixture was heated at 90°C for another 20 minutes. The content was filtered through a 0.45 µm Whatman® ME 25/21 ST filter and transferred into a plastic volumetric tube. Each solution was diluted until completing 25 mL. Its concentration was measured by FLAA analysis using Thermo Scientific® iCE 3000 Series.

The particulate total carbon (TC_P) concentration was determined by first filtering the samples through 0.7 µm Whatman® glass microfiber filters GF/F and then measuring the filters using the same equipment used on the measurement of SC and SOC.

Total runoff and total losses of TSS, VSS, TC_P, and Cu_P were estimated by addition of the respective amounts of all events.

2.2.5. Statistical Analysis

Statistical analysis was performed with the statistical software SigmaPlot, using a significance level of 0.05 (p<0.05). Two-Way Analysis of Variance (ANOVA) was applied to infer the overall statistical significance differences in the mean values for soil samples among the different treatments (NB, BL, and BH) and the sub-area (compacted by tractor wheels and uncompacted) (treatment vs. sub-area). Specific contrasts between these factors were analysed *ad posteriori* using the Tukey multiple comparison test.

The Spearman rank-order correlation coefficient was used to determine significant correlations between the different parameters measured in the three treatments (NB, BL, and BH) for both soil and runoff samples.

2.3. Results

2.3.1. The vineyard soil

Figure 9 shows the values obtained in November 2016 for the different soil parameters in the two sub-areas (compacted by tractor wheels and uncompacted) of each of the three treatments (without biochar (NB) and with low (BL) and high (BH) biochar application rates). It is important to stress that these values corresponded to the situation immediately after application of the biochar.

Except for Cu, the different application rates of biochar had a strong effect in all parameters, with significant differences ($p<0.05$) between the control (NB) and treatments with low (BL) and high (BH) biochar application rates (Table 2). Significant differences between the compacted and uncompacted soil were limited to one parameter, SOM, but having the effect of different levels of treatment depending on the level of sub-area present was observed in three parameters, SOM, SC, and SOC.

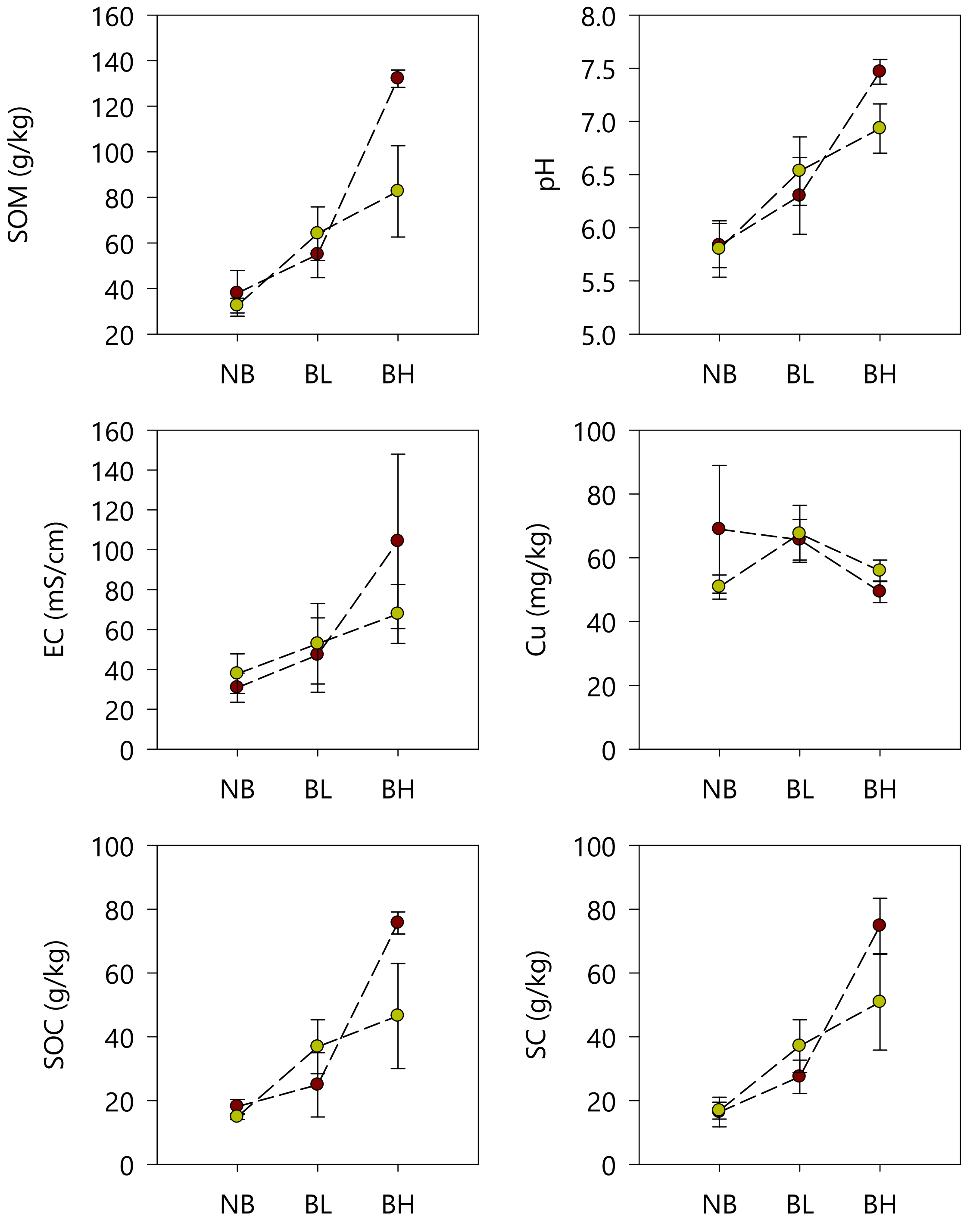

Figure 9: Mean values of organic matter (SOM), pH, electrical conductivity (EC), copper (Cu), carbon (SC), and organic carbon (SOC) in soil compacted (—●—), and uncompacted (—●—), at 0-10 cm depth, in the control (NB) and in soil treated with low (BL) and high (BH) biochar application rates. The error bars indicate the standard deviation (n=3).

Table 2: Two-way analysis of variance (ANOVA) testing the effect of treatment (control, biochar low, biochar high) and the sub-area influence (compacted, uncompacted) in the different soil parameters. Significant interactions (p<0.05) are marked in bold.

Parameter	Source of variation	*df*	Mean square	F	*p*
SOM	Treatment	2	0.00808	63.089	**<0.001**
	Sub-area	1	0.00105	8.170	**0.014**
	Treatment × sub-area	2	0.00139	10.883	**0.002**
pH	Treatment	2	2.887	41.911	**<0.001**
	Sub-area	1	0.0556	0.806	0.387
	Treatment × sub-area	2	0.227	3.298	0.072
EC	Treatment	2	4211.782	8.320	**0.005**
	Sub-area	1	282.427	0.558	0.469
	Treatment × sub-area	2	917.334	1.812	0.205
Cu	Treatment	2	292.177	3.141	0.080
	Sub-area	1	46.853	0.504	0.491
	Treatment × sub-area	2	256.635	2.759	0.103
SOC	Treatment	2	3110.781	40.411	**<0.001**
	Sub-area	1	209.878	2.726	0.125
	Treatment × sub-area	2	647.384	8.410	**0.005**
SC	Treatment	2	3310.588	46.812	**<0.001**
	Sub-area	1	95.866	1.356	0.267
	Treatment × sub-area	2	451.822	6.389	**0.013**

Df – degrees of freedom; F – F statistic; *p* – p value

The higher the biochar rate application in the treatments, the higher was the SOM percentage. The same behaviour was observed for pH, EC, SOC and SC. The application of biochar raised the SOC to more regular levels (Table 3). Also, SOC and SC concentrations were very similar, meaning that soil inorganic carbon (SIC) concentrations at the site were minimum, and apparently below the equipment limit of quantification. Soil inorganic carbon content remained low with increasing biochar rate applications, leading to the conclusion that biochar doesn't influence SIC as it does SOC. Finally, apart from Cu, all parameters are correlated to each other (Table 4).

Table 3: Soil organic carbon (SOC) contribution on SOM, and SIC content of the studied soil at 10 cm depth, per sub-area (compacted, uncompacted) in each treatment (NB, BL, BH). Values shown are means with the respective standard deviations (SD) (n=3).

		SOC/SOM		**SIC**	
		%	SD	g/kg	SD
NB	Compacted	49.2	12.5	-1.72	2.94
	Uncompacted	45.8	3.7	2.01	3.38
BL	Compacted	44.2	9.3	2.51	4.87
	Uncompacted	57.2	2.7	0.22	0.94
BH	Compacted	57.3	1.2	-0.90	10.64
	Uncompacted	56.3	13.8	4.31	3.97

Table 4: Spearman rank order correlation between parameters measured to the soil. Correlation coefficients (r) with p<0.05 (*) or p<0.01 (**) are marked in bold. Some parameters cannot be correlated (□) since their values depend on one another.

		SOC g/kg	**SC** g/kg	**Cu** g/kg	**pH**	**EC** µS/cm
SOM	%	☐	**0.971****	-0.123	**0.948****	**0.913****
SOC	g/kg		☐	-0.00722	**0.931****	**0.880****
SC	g/kg			-0.115	**0.954****	**0.905****
Cu	mg/kg				-0.124	-0.0691
pH						**0.898****

2.3.2. Precipitation and characteristics of the surface runoff

The runoff response of the block A, especially plot 1, was very different from that of the other plots with the same treatment. Field observations suggested this could be attributed to lateral sub-surface flow from the neighbouring vineyard, with a more elevated topographic position, as its topsoil had not been removed. Due to this situation, block A had to be excluded from the study since its surface and sub-surface runoff was affected by a source other than the rainfall.

The early months of 2017 were particularly dry, with a total rainfall of 237.1 mm throughout the study period. Looking at total runoff and losses, during the study period, there was a clear difference comparing the control (NB) to both treatments with biochar application (BL, BH) (Table 5). Treatments with biochar application showed lesser losses compared to NB, but the behaviour between losses and biochar application rates was not linear, with BH showing greater losses of TSS, VSS, TC and Cu_P than BL treatment.

Table 5: Total runoff and total mobilisation of fine particulate matter (TSS), organic matter (VSS), total carbon (TC) and particulate copper (Cu_P) in each type of treatment (control (NB), low (BL) and high (BH) biochar application rates) during the study period.

		NB	**BL**	**BH**
Runoff	mm	13.9	9.9	9.8
TSS	kg/ha	68.7	21.6	34.4
VSS	kg/ha	19.1	7.0	9.2
TC_P	kg/ha	7.9	2.7	4.1
Cu_P	g/ha	34.3	8.2	11.7

Figure 10 shows the rainfall, and the surface runoff observed in each treatment (NB, BL, and BH), throughout the study period. Rainfall (mm) and runoff (mm) had a strong and positive correlation (r= 0.821, p<0.01).

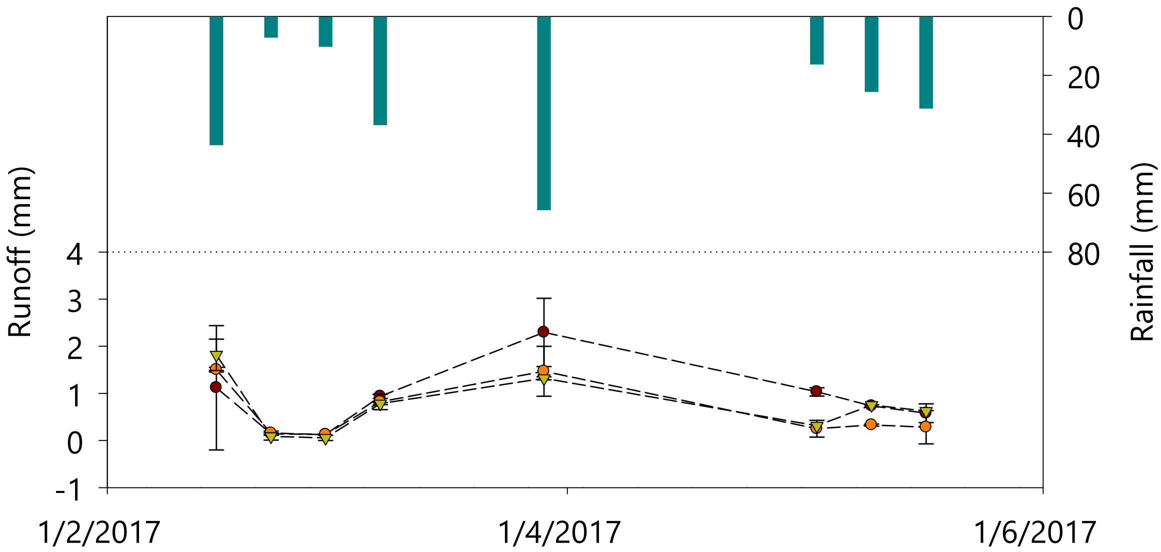

Figure 10: Weekly events of rainfall ( ) (n=1) and surface runoff (n=2) on the different treatments, NB ( ), BL ( ), BH ( ). The error bars indicate the standard deviation.

Runoff coefficient (C) is shown in Figure 11, providing a better view on how biochar affects water infiltration on soil, with lower values in the treatments with biochar (BL and BH) compared to the control (NB). This was especially visible after a time without rainfall, as is the case of the 6th event (3/May).

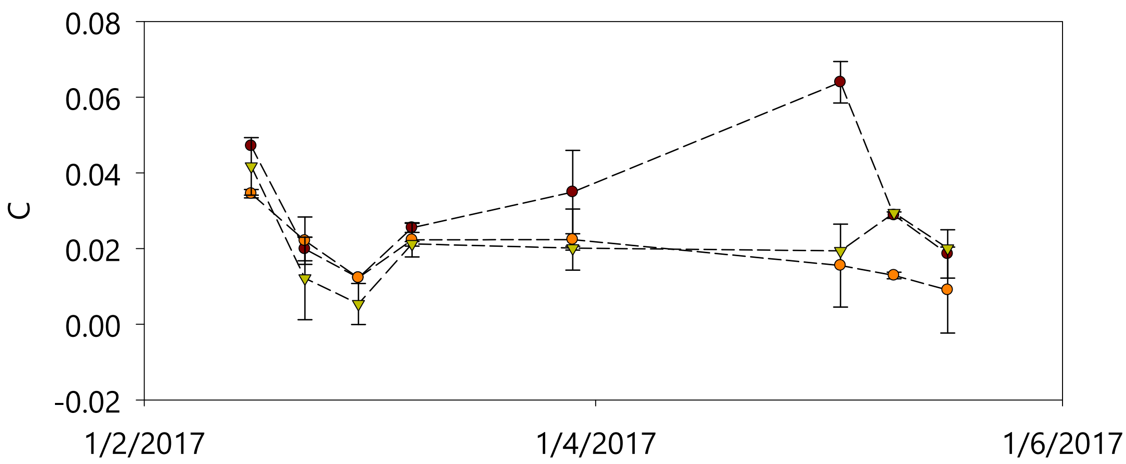

Figure 11: Runoff coefficient (C) in each event on the different treatments, NB ( ), BL ( ), BH ( ), over the study period. The error bars indicate the standard deviation (n=2).

Figure 12 shows the rainfall, and the losses of TSS, VSS, TC_P, and Cu_P, in the runoff, in each treatment, along the study period. Overall, the greatest losses occurred during the first precipitation event after the biochar application, on February 25th. There were also important losses on the 6th event (3/May), which followed the pattern of the runoff coefficient. The behaviour among the losses of TSS, VSS, TC_P, and Cu_P was very similar throughout the study period.

After Cu application, on April 11th, the concentration of Cu in runoff increased considerably, and the different application rates of biochar had a visible influence on the behaviour of Cu_D concentration (Figure 13). The VSS and TC_P concentrations showed a similar pattern in most events. The EC was also affected by the fungicide application, which exhibited a peak after the applications, and had a positive correlation with Cu concentrations (Figure 14 and Table 6).

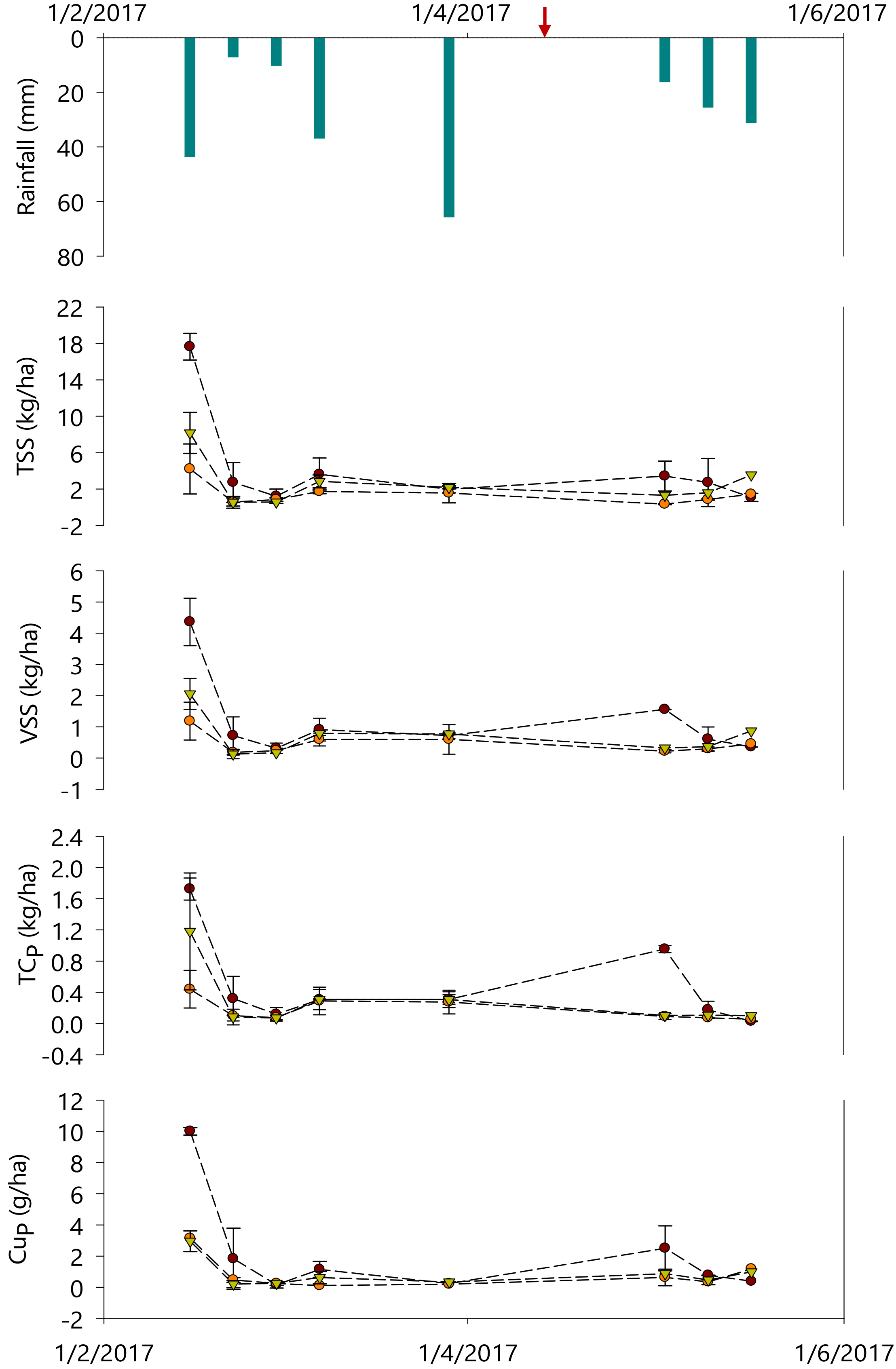

Figure 12: Rainfall ( ) (n=1), and exports (n=2) of total suspended solids (TSS), volatile suspended solids (VSS), total particulate carbon (TC_P), and particulate copper (Cu_P) per treatment, NB

(—●—), BL (—●—), BH (—▼—), over the study period. The error bars indicate the standard deviation. The arrow on the top indicates Cu-based fungicide application.

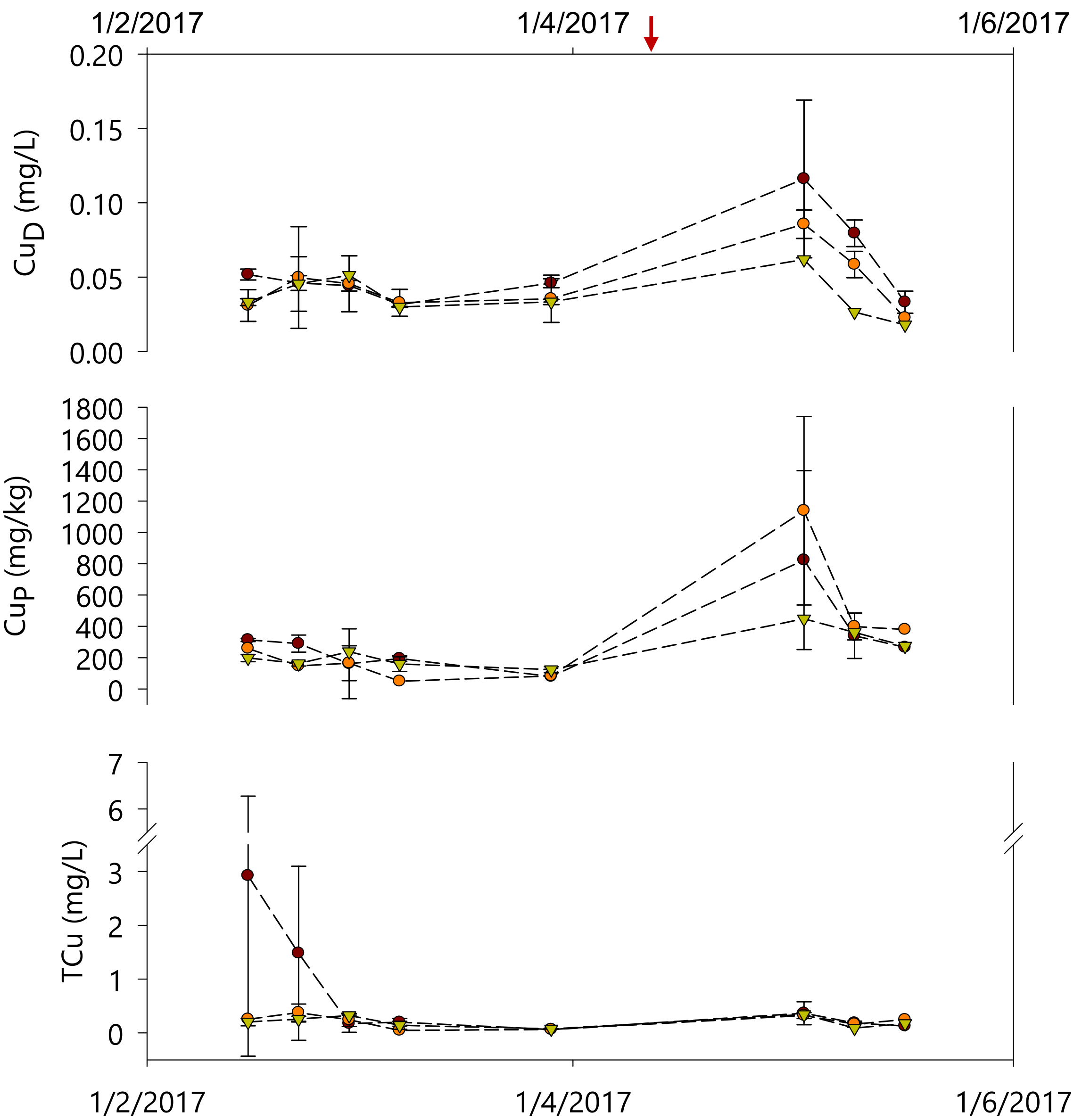

Figure 13: Temporal changes of dissolved, particulate, and total copper (Cu_D, Cu_P, and Cu_T) on runoff, per treatment, NB (—●—), BL (—●—), BH (—▼—). The error bars indicate the standard deviation (n=2). The arrow on the top indicates Cu-based fungicide application.

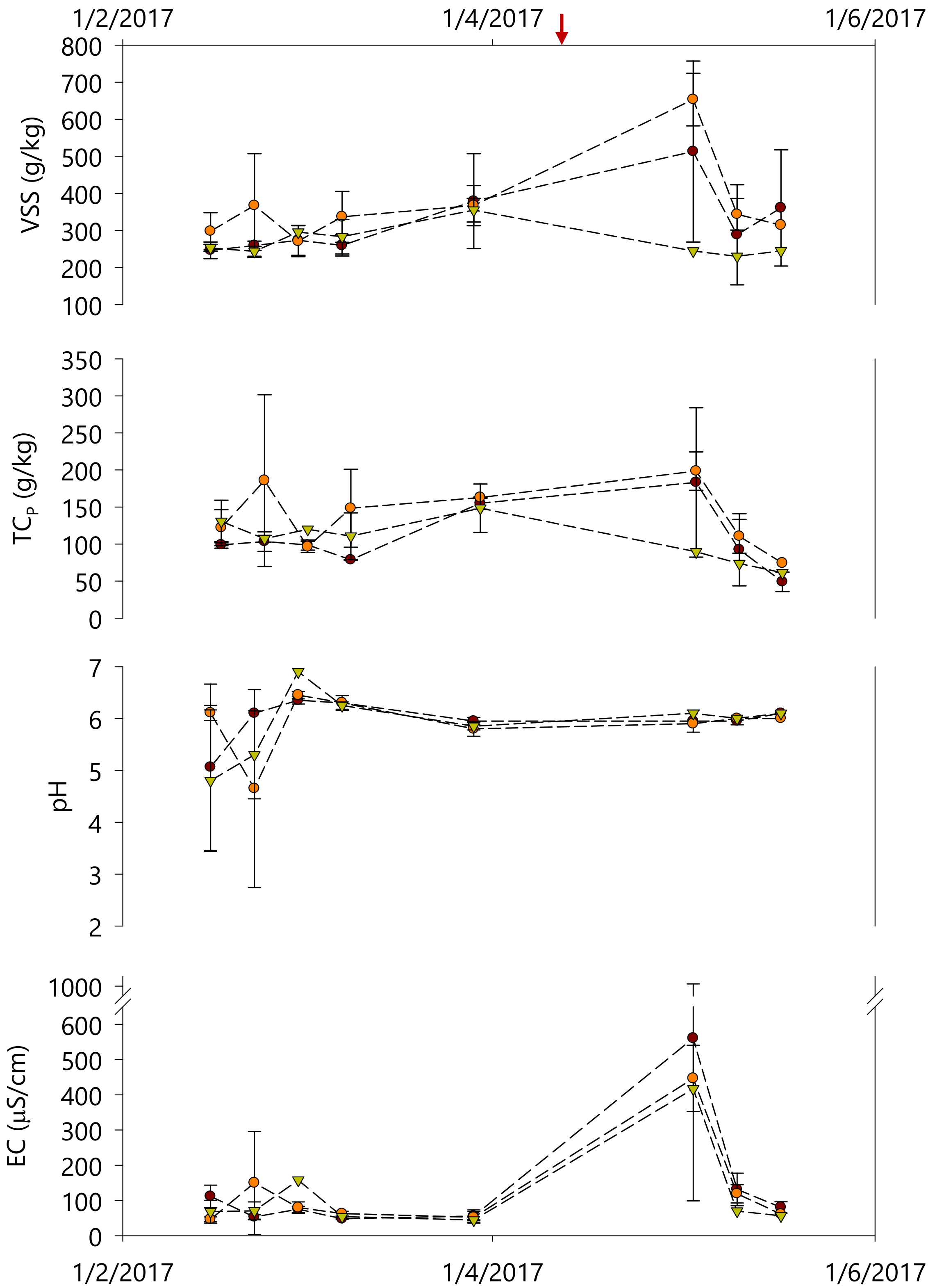

Figure 14: Temporal changes of volatile suspended solids (VSS) and total particulate carbon (TC_P) on runoff, and the runoff pH and EC, per treatment, NB ( ), BL ( ), and BH ( ). The error

bars indicate the standard deviation (n=2). The arrow on the top indicates Cu-based fungicide application.

Table 6 shows the correlations between the measured parameters, confirming higher precipitation resulted in greater losses of the fine particles of the soil (TSS), organic matter (VSS), carbon (TC_P) and copper (Cu_P). As expected, there is also a positive correlation between SOM and TC_P, as organic carbon is a fraction of both, and a positive correlation between dissolved and particulate copper.

Table 6: Spearmen correlation coefficients (r) between the measured parameters. Significant correlations with *p*<0.05 (*) or *p*<0.01 (**) are marked in bold. Some parameters cannot be correlated (□) since their values depend on one another.

		TC_P kg/ha	**Cu_P** g/ha	**pH**	**EC** µS/cm	**VSS** g/kg	**TC_P** g/kg	**Cu_P** g/kg	**Cu_D** mg/L	**TCu** mg/L	**Precipitation** mm	**C** %
Runoff	mm	□	□	**-0.344***	**-0.343***	0.196	0.217	-0.137	-0.220	**-0.370***	**0.821****	□
TSS	kg/ha	**0.844****	**0.692****	0.0182	-0.283	□	-0.146	-0.163	-0.160	-0.106	**0.546****	□
VSS	kg/ha	**0.856****	**0.742****	-0.0678	-0.205	□	-0.0301	-0.0379	-0.107	-0.0857	**0.616****	□
TC_P	kg/ha		**0.659****	-0.136	-0.246	-0.0640	□	-0.154	0.0115	0.0329	**0.521****	□
Cu_P	g/ha			-0.210	-0.0627	-0.0839	-0.0137	□	-0.00733	□	**0.362***	□
pH					-0.0913	-0.240	**-0.400****	-0.135	-0.213	-0.0658	-0.184	**-0.341***
EC	µS/cm					0.114	-0.0547	**0.578****	**0.732****	**0.353***	**-0.441****	-0.0697
VSS	g/kg						**0.685****	0.204	0.120	-0.0489	0.146	0.117
TC_P	g/kg							-0.00284	0.195	0.107	0.173	0.137
Cu_P	g/kg								**0.482****	□	-0.246	0.0449
Cu_D	mg/L									□	**-0.362***	0.0175
TCu	mg/L										**-0.467****	-0.0886

2.4. Discussion

2.4.1. The vineyard soil and the influence of biochar on its characteristics

Biochar treatments significantly influenced soil chemical and physical properties comparing with the control (NB), showing higher values in soil pH, EC, SOM, SC, and SOC content. This was expected, since the biochar is rich in C, has a high pH, and there are studies referring to biochar's many effects on soil, such as increasing the EC, depending on what temperature the biochar was produced (DeLuca et. al, 2009; Tian et al., 2016) and the feedstock used (Mukome & Parikh, 2016). The liming effect can be favourable to the microorganisms which mediate SOM cycling, increasing their activity (Whitman et al., 2015) and thus contributing to a higher SOM as well. Overall, considering the current land use there was an improvement of the chemical and physical properties of the soil.

A low SOM means decreased soil fertility, and increased soil erosion in some areas (Baldock & Nelson, 2000). The application of biochar resulted in a higher SOM content. Soil organic matter in BL and BH treatments was respectively, 1 to 2-fold and 2 to 4-fold higher than in the control (NB). However, decomposition rate of biochar decreases with time and the readily decomposing pool is rather insignificant (≈3%) (Wang et al., 2016). Biochar seems then a good short-term solution for SOM management, but there are still reservations about its effects on SOM, as its priming effect has been reported to shift from positive to negative on long-term periods (Wang et al., 2016; Zimmerman et al., 2011).

Likewise, SOC which is one of the fractions of SOM, seemed to have its fraction improved with biochar application on soil (Table 3). In general, SOM usually comprises 50% to 58% of

C (Baldock & Nelson, 2000). The low concentrations of SIC were predictable once its presence is more common in arid and semi-arid regions (Lal, 2004).

Soil pH affects the solubility of macronutrients (N, P, K, Ca, Mg, S) and micronutrients (B, Cu, Fe, Mn, Zn, Cl) (Mortvedt, 2000), as well as the activity of enzymes and microorganisms (Whitman, Singh, & Zimmerman, 2015). A high rate application of biochar (BH) resulted in a soil pH that could be considered too high, specifically in the soil compacted by the tractor wheels. In fact, when pH is very high, some nutrients lose their solubility, and leave the soil nutrients unavailable for plant uptake. On the other hand, the pH achieved by the lower biochar application (BL) appeared to be more suitable, as many crops grow best if pH range is 6 to 7 (NRCS-USDA, 1998).

Since the soil texture is sandy loam, and because smaller soil particles such as clay conduct more current than larger particles like sand, the low soil EC measured at the control was expected at the study area. Its higher values with biochar application was likely due to the usual presence of salts at the biochar feedstock (Mukome & Parikh, 2016).

Overall, biochar has shown many benefits to the soil on a short-term period. Considering what was previously discussed and the cost of applying bigger quantities of biochar, the application of low rates of biochar seemed to be a better option than applying higher rates or not applying biochar at all.

2.4.2. Effect of the application of biochar on surface runoff and soil losses

The results confirmed a positive relation between soil loss and rainfall intensity, which is in accordance with the study of Prosdocimi, Cerdà, & Tarolli (2016). Major soil losses meant higher quantities of organic matter and nutrients were exported as well, illustrated by the VSS and Cu_P. The mean erosion rate on Mediterranean vineyards is around 8.62 t/ha/year (Cerdan et al., 2010), and the soil lost at the study site in months of data was not even close to a third of this value. In the present study, soil losses consist mainly of fine particles, since TSS measurement often excludes larger suspended particles, like sand, due to the rapid settling rate of sand-size material (Galloway, Evans, & Green, 2005).

The exportations of TSS and Cu_P are correlated to each other, and these exportations are correlated to the precipitation, which means the loss of sediments lead to the loss of Cu, and thus its input on aquatic systems (Serpa et al., 2017). Also, the strong correlation between precipitation and runoff means intense rainfall can lead to larger contaminations by particulate Cu when Cu-based fungicide treatments are applied in vineyards.

This study clearly emphasizes the role of biochar in the formation of runoff and in the losses of TSS, VSS, TC and Cu_P. The low application rate of biochar (BL) had less 28.8% total runoff, and less 68.6% of TSS, 63.4% of VSS, 65.8% of TC, and 76.1% of Cu_P total losses than the control. Whereas the high application rate of biochar (BH) had less 29.5% total runoff, and less 49.9% of TSS, 51.8% of VSS, 48.1% of TC, and 65.9% of Cu_P total losses than the control (Table 4). Hence, the application of biochar at low concentration (5 kg/m^2) showed to be effective in reducing erosion.

According to Corti et al. (2011), the slope gradient is one of the factors that predisposes soil to water erosion. Moreover, they observed that grass cover play an important role in reducing erosion namely at slopes with at least 15%. Prosdocimi et al. (2016) also highlighted the importance of the slope on soil loss and runoff. However, even though the study area is a vineyard, the slope of the studied vineyard is not very accentuated (≈7%). This can justify why the runoff coefficient observed in all treatments (0.1-6.8%) was rather low when comparing to the results of other authors in Mediterranean vineyards, ranging from 2.4 to 36.8% (Biddoccu et al., 2016; Comino et al., 2016; Napoli et al., 2017). After a month with no considerable rainfall, the runoff coefficient on the control was clearly higher than the runoff coefficient on treatments with biochar. This suggests biochar improved infiltration, which can be related to soil water repellency, a common problem especially after long periods of no rainfall. If a soil prone to water repellency dries to less than a critical water content, its behaviour can shift abruptly from wettable to non-wettable. Prolonged wetting is the usual approach to correct it, as it can reverse this shift so that water repellent soils can regain wettability (Hallett, 2007). Hence, applying biochar might be a useful and preferable approach, as it saves water. However, water repellency data would be required to verify this.

By comparing the two treatments, LB and HB, the differences were faint, indicating that there is no advantage in applying high rates over low rates of biochar.

2.4.3. Copper on soil and surface runoff and effect of biochar application

Cu concentrations found in the first 10 cm of the soil were below soil Cu limit values of 100 mg/kg (pH>5.5) for application of sludge on soil (*Decreto-Lei n.º 276/2009 de 2 de Outubro do Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional*, 2009), and below the concentrations reported by Nóvoa-Muñoz et al. (2007) on Gallize, ranging 125-603 mg/kg at a depth of 0-10 cm. Usually at this depth range, concentrations in vineyards are much higher (Komárek et al., 2010). Still, the Cu concentration obtained

(49.3 – 68.9 mg/kg) is high when compared with the mean level of 15.5 mg/kg for agricultural soils in Europe (Oorts, 2013). Soil sampling was shortly after the study area set-up, before fungicides application, which can explain why there isn't any clear difference on Cu content between treatments, only on the wheeled soil sample at the control (NB) which is more compact and thus suffers less erosion, creating better conditions for particulate Cu retention. The Cu concentrations were quite low considering the site is an old vineyard. This is probably due to a combination of factors, namely: the soil texture, which is very sandy; its acidic properties and low organic matter content; and because the soil was ploughed up to 20 cm depth at the beginning of the experiment. The closer it is to the surface, the richer the soil is in Cu (Ribolzi et al., 2002), as the Cu applied as fungicide has a low leachability potential (University of Hertfordshire, 2016). Bioavailability of micronutrients, in which Cu is included, is significantly affected by soil pH, decreasing with increasing pH (Bradl, 2004; Brunetto et al., 2016). Considering the characteristics of the soil, Cu could be easily mobilised by runoff in its soluble form. All these factors could explain the concentrations of Cu at a site where they were expected to be higher.

As described before, a part of the Cu remains in the soil, and another is lost by soil leaching or by physical erosion and can reach nearby watercourses where it can be detected dissolved in water or adsorbed onto suspended particulate matter (SPM) and bed sediments (El Azzi et al., 2013). The SPM collected in the events were highly concentrated in Cu, many times higher than the Cu contents measured in the 0-10 cm soil layer. This is because SPM is the result of the easier mobilisation of the fine particles of the soil, which are more concentrated in Cu. Ribolzi et al. (2002) had a similar result.

It was possible to see how biochar influenced the behaviour of Cu after the fungicides were applied. Greater application rates of biochar resulted in lower concentrations of dissolved Cu, and in lower EC in runoff. This means a greater immobilisation of Cu with the application of biochar, since EC increases with higher concentrations of $CuSO_4$ (Haynes, Lide, & Bruno, 2013), and since Cu strongly reacts with SOM by complexation and is less soluble with higher pH. Because Cu is also a micronutrient, too much biochar and it could result in nutrient deficiency to the plants, as Cu would not be in its soluble form.

Overall, biochar created the desirable conditions to decrease Cu availability and retain it in its particulate form, therefore reducing its exportation as it was observed in this study. Nonetheless, the soil amendment with biochar must be conducted carefully, as it can have negative effects on the environment (Bastos et al., 2014). Toxic substances such as heavy metals present in feedstock, and polycyclic aromatic hydrocarbons and dioxins formed during biochar production must be measured and controlled (Lopez-Capel et al., 2016).

2.5. Final considerations

The application of Cu-based fungicides has been intensively applied in vineyards in Portugal, enhancing levels of cooper in soils and aquatic systems. Notwithstanding the importance of Cu as essential element for growth, development and biological life, it became toxic to the terrestrial and aquatic organisms in certain concentrations.

The key-issue of this study was to assess the long-term accumulation of Cu on soils as a consequence of the application of Cu-based fungicides to vineyards, as well as to evaluate how biochar could contribute on lessening the Cu and sediment losses by surface runoff at an intensive viticulture area.

The present study emphasizes the contribution of viticulture to the mobilisation of dissolved and particulate Cu through surface runoff. Additionally, it also demonstrated the importance of surface runoff as a process for the exportation of cooper enriched sediments from viticulture areas to downstream water bodies. However, it would have been important to know the concentrations of the PPPs applied and the average number of treatments during a year on the studied vineyard, specially the later applications, as it would have been important to clarify the results obtained. Furthermore, information about the past application practices could also represent interesting data. Unfortunately, the manager of the vineyard did not provide this information.

The present study pointed out some of the benefits of applying biochar for the remediation of cooper-contaminated vineyard soils. In fact, biochar was successfully applied as soil amendment, reducing erosion and immobilising copper. Furthermore, it also allowed to confirm the contribution of biochar to the quality of the soil and surface runoff on a shorttime period, as well as the option of using it to reduce soil erosion. Regarding the two biochar treatments, it was found that the treatment with lower application rate of biochar showed to be the most effective treatment in the studied parameters and therefore recommended over the treatment of biochar in higher rates.

This study provides relevant information that could be helpful for the implementation of measures that promote a more efficient management of viticulture. However, additional studies will be required. In this respect, some recommendation are presented for future works:

- Assess the temporal effectiveness of biochar in the retention of copper and the need of a second application;
- Evaluation of the benefits and drawbacks of a new application of biochar in terms to the many soil quality parameters. However, this would probably demand a long monitoring period, which could end up being unreasonable;
- Assess potential impacts of biochar on the soil ecosystem functions.

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