A Study of Grapevine Growth and Performance in Vineyard and Non-Agricultural Portuguese Soils

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Dissertation to obtain the degree of
European Master of Science in Viticulture and Enology

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2022

Acknowledgements

Thank you to Vinifera Euromaster for accepting me into the European Master's program and managing all aspects of the program during these past two years. I thank the associate partner Instituto Superior de Agronomia (ISA) for hosting me as a student and facilitating my education. I thank all professors from the consortium who gave guest lectures throughout the curriculum, whether in-person or online.

I would like to express my sincerest appreciation to the main investigator and co-investigator of this study, Amaia Nogales and Erika S. Santos, for their guidance and insight throughout the duration of my master's thesis.

To Paula, Maria-João, and Anna Maria at ISA, I am grateful for their guidance through soil enzymatic and nutrient analysis.

To Amandine Salunier, I am thankful for the countless hours your spent helping me with soil enzymatic, nutrient analyses, and work in the greenhouse.

Finally, I thank my parents and brothers for their support from thousands of miles away, and my boyfriend Mathieu; without their support this could not have been possible.

Abstract

Studies of vineyard soil show a deterioration of its nutrient content, structure, and microorganism communities. This study's objective was to compare physical-chemical and biological properties of conventionally managed vineyard soils and surrounding non-agricultural soils in three Portuguese winegrowing locations and to evaluate the performance of Aragónez *cv.* x 110 Richter and Aragónez *cv.* x 1103 Paulsen grapevines planted in soils from different land uses, in a controlled greenhouse experiment. Soil physical-chemical analysis (pH, electrical conductivity, nutrients in the available fraction, organic C, total N, cation exchangeable capacity), and soil biological analysis (enzyme activities, density of mycorrhizal infective propagules) were measured. Ten Aragónez *cv.* x 110 Richter and 1103 Paulsen were planted in each soil sample. Grapevine performance was monitored via Photochemical Reflectance Index (PRI) and Normalized Difference Vegetation Index (NDVI) for three months. Root biomass, shoot length, root mycorrhizal colonization was analyzed. Conventionally managed vineyards on average had 55% lower concentrations of organic C and 46% lower concentrations of total N. ß-glucosidase activity in Lisbon was 76% higher in non-agricultural soil. Soil mycorrhizal infective propagule density of non-agricultural soils in Bombarral and Lisbon were over 22 and 10 times greater than the corresponding vineyard soil. Grapevines grown in vineyard soils from Lisbon and Pegões showed the lowest NDVI values. Aragónez x 110 Richter had higher shoot length (44.5, 53, 56, 62 cm) in Bombarral and Lisbon compared to Aragónez x 1103 Paulsen (35.5, 48, 45, 58 cm). In Lisbon and Pegões, Aragónez x 1103 Paulsen had higher root mycorrhizal colonization. This work indicates that agricultural practices conducted in vineyard soils can contribute to a negative effect on some soil physical-chemical and biological parameters, which leads to differences in grapevine performance when compared to non-agricultural soils. Thus, it seems important to include more sustainable soil management practices in these vineyards to obtain a better plant fitness.

Keywords

Grapevine, arbuscular mycorrhizal fungi, vineyard soils, non-agricultural soils

Resumo

Estudos de solos de vinhas mostram uma deterioração dos seus conteúdo de nutrientes, estrutura e comunidades microbianas superior a solos não agrícolas. O objetivo deste estudo foi comparar propriedades físico-químicas e biológicas de solos em diferentes vinhas em modo de produção convencional e solos não agrícolas nas proximidades destas mesmas vinhas. O estudo foi feito em três diferentes regiões Portuguesas produtoras de vinho. Neste trabalho foi avaliado, em condições controladas, o desempenho de videiras da casta Aragonês, enxertada com o porta enxerto 110 Richter (110 R) e a mesma variedade enxertada com o porta enxerto 1103 Paulsen (1103 P). Foram realizadas análises físico-químicas às varias amostras de solo (pH, condutividade elétrica, nutrientes na fração disponível, C orgânico, N total, capacidade de troca catiónica) e análises biológicas do solo (atividades enzimáticas, densidade de propágulos micorrízicos infetados). Em cada amostra de solo foram plantadas dez videiras da casta Aragonês enxertadas com o porta enxerto 110 R e dez videiras da mesma casta com o porta-enxerto 1103 P. O desempenho da videira foi monitorizado através do Índice de Refletância Fotoquímica (PRI) e Índice de Vegetação por Diferença Normalizada (NDVI) durante três meses. Foi analisada a biomassa radicular, comprimento da parte aérea e a colonização micorrízica radicular. As vinhas em modo de produção convencional apresentavam em média 55% menos concentrações de C orgânico e uma concentração total de N inferior em 46%. A atividade ß-glucosidase em Lisboa foi 76% mais elevada em solo não agrícola comparativamente com solo de uma vinha. A densidade de propagação de micorrizas do solo em Bombarral e Lisboa com ocupação não agrícola foi respetivamente 22 e 10 vezes superior comparativamente com o solo com ocupação agrícola correspondente. As vinhas do solo com vinhas em Lisboa e Pegões apresentava os valores mais baixos de NDVI. Relativamente ao desenvolvimento vegetativo, a casta Aragonês x 110 R apresentou o a maior comprimento de sarmento (44,5, 53, 56, 62 cm) em Bombarral e Lisboa, em comparação com Aragonês x 1103 P (35,5, 48, 45, 58 cm). Por outro lado, em Lisboa e Pegões, as videiras Aragonês x 1103 P apresentaram uma colonização micorrízica da raiz mais elevada. Este trabalho mostra que as práticas agrícolas conduzidas em solos de vinha podem contribuir para um efeito negativo em alguns dos parâmetros físico-químicos e biológicos do solo, o que terá um efeito negativo e consequentemente levará a diferenças no desempenho da videira quando comparada com solos não agrícolas. Assim, tendo em conta os resultados seria relevante implementar medidas mais sustentáveis de gestão do solo em vinhas, de forma a obter um melhor desenvolvimento vegetativo por parte da videira.

Palavras-chave

Videira, fungos arbusculares micorrízicos, solos de vinha, solos não agrícolas

Resumo Alargado

Nos últimos anos, tem aumentado a atenção dada à saúde e fertilidade do solo no setor vitivinícola. A literatura afirma que, em geral, os solos de vinhas sujeitos a práticas agrícolas convencionais são mais dependentes da fertilização para manter um solo saudável e contém uma população microbiana mais pobre, em comparação com solos não agrícolas. A viticultura portuguesa tem uma contribuição relevante para este setor a nível mundial, representando 2,7% da área ocupada por vinha a nível mundial. As mudanças que se antecipam no clima representarão ameaças ao desempenho da videira, rendimento e qualidade das uvas. O objetivo deste estudo foi efetuar uma avaliação comparativa das propriedades físico-químicas e biológicas de solos vitícolas com solos não agrícolas, provenientes de três áreas vitivinícolas em Portugal, e determinar o impacto de diferentes caraterísticas do solo no desempenho da videira *Vitis vinifera* L. Foram recolhidas amostras de solo de vinha e de solos não agrícolas em três regiões vitícolas Portuguesas (Lisboa, Bombarral e Pegões). O ensaio utilizou sessenta videiras Aragónez cv. x 110 Richter e Aragonez cv. x 1103 Paulsen adquiridos nos Viveiros Vitioeste, localizados no Bombarral, Portugal. A análise físico-química do solo incluiu pH, condutividade elétrica, capacidade de troca catiónica, C orgânico, N total, e macro e micronutrientes na fração disponível no solo (P, Mg, K, Na, Ca, Fe, Mn, Zn e Cu). A análise biológica do solo incluiu a atividade enzimática da desidrogenase, ß-glucosidase, fosfatase ácida, celulase e urease, que estão associadas à atividade global da comunidade microbiana e aos ciclos de nutrientes. Após a caracterização das amostras de solo, estas foram utilizadas num ensaio em vaso, em estufa, plantando dez videiras Aragónez *cv*. x 110 Richter e Aragonez *cv*. x 1103 Paulsen em cada tipo de solo, fazendo a sua monitorização durante três meses. O desempenho da videira foi avaliado através do Índice de Refletância Fotoquímica (PRI) e do Índice de Vegetação por Diferença Normalizada (NDVI). No final da época de colheita, em setembro, foi feita a amostragem de cinco plantas por tratamento. Os solos de vinha sujeitos a práticas culturais convencionais apresentaram maiores concentrações de P disponível no solo, mas menores concentrações de C orgânico e N total, menores atividades de fosfatase ácida e de ß-glucosidase e menor densidade de propágulos infetantes micorrízicos em comparação com solos não agrícolas. As videiras

cultivadas em solos de vinha de Lisboa e de Pegões apresentaram valores de NDVI mais baixos do que as de solos não agrícolas. Plantas enxertadas com porta-enxertos 110 Richter e cultivadas nos solos de Bombarral e de Lisboa apresentaram maior comprimento de parte aérea. Em contraste, aquelas plantadas em solos de Lisboa e Pegões e enxertadas com porta-enxertos 1103 Paulsen foram as que apresentaram maior colonização micorrízica radicular. Este estudo evidência os menores teores de C orgânico e de N total e, em geral, as características biológicas gerais mais pobres dos solos de vinha, o que parece ter provocado, indiretamente, um menor desempenho das videiras cultivadas nesses solos em termos de NDVI. Portanto, uma melhoria nas práticas de manejo do solo das vinhas, como as práticas de mobilização mínima, redução na fertilização mineral/sintética, e incorporação de mutualistas biológicos, como fungos arbusculares micorrízicos, pode ser fundamental para a melhoria da qualidade do solo e desempenho da videira.

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Abbreviations

AMF	Arbuscular Mycorrhizal Fungi
BD	Biodynamic
CEC	Cation Exchange Capacity
CFU	Colony Forming Units
Mhl	Million hectoliters
NDVI	Normalized Difference Vegetation Index
ORG	Organic
OTU	Operational Taxonomic Unit
PRI	Photochemical Reflectance Index

1. Introduction

1.1 Grapevine Cultivation

Grapevine (*Vitis vinifera* L.) is a prominent cultivated crop worldwide; grapevines cover 7.3 million hectares (ha) of area around the world (OIV, 2020). Grapevines are one of the most significant crops in the Mediterranean basin, as well as in Portugal (OIV, 2020). This country represents 2.7% of the global vineyard surface area, with a total of 194 thousand ha of vines (OIV, 2020). It consumes less than five million hectoliters (mhl) of wine, which is two percent of the world wine consumption. Portugal is a sizable country of wine export in the European Union, exporting about three mhl in 2020. It had an increase in export volume by over five percent in 2020 compared to 2019 (OIV, 2020).

Vitis vinifera is the dominant species of cultivated grapevines around the world (Keller, 2015) and is distributed throughout Europe, North America, South America, Asia, Africa, and Oceania. The grapevine was domesticated in between the years 7 and 4 B.C in the broad area that is presently Iran, Iraq, Syria, Lebanon, Turkey, Georgia (Zohary & Hopf, 2000). It was cultivated seven to eight thousand years ago for wine production as well as consumption as a fresh or dried fruit (Keller, 2015).

Although grafting has been used in agriculture dating back to at least the 5th century B.C. (Melnyk & Meyerowitz, 2015), rootstocks became of marked importance in the realm of viticulture in the mid-19th century, when phylloxera (*Daktulosphaira vitifoliae*, order: hemiptera), an insect native to the United States, was transported overseas and devastated vineyards, most notably in France. European vineyards eventually survived this epidemic once it was discovered that grafting resistant American *Vitis* rootstocks to the Eurasian species *Vitis vinifera* prevents the pest from attacking the roots and killing grapevines via cutting off the transport of water and nutrients to the vine. Currently, most grapevines (>80%) are grafted (Ollat et al. 2016) and the most common rootstocks used are hybrids of *V. berlandieri*, *V. riparia*, and *V. rupestris* (Gerós et al., 2015). The most commonly planted rootstocks are hybrids of *V. berlandieri* x *V. rupestris* (1103 P, 99 R, 110 R and 140 Ru), of *V. berlandieri* x *V. riparia* (420 A, 161-49 C, SO4, 5 BB, 5 C and 125 AA), of *V. riparia* x *V. rupestris* (3309 C), of (*V. vinifera* x *V. rupestris*) x *V. riparia* (196-17 Cl), and of *V. vinifera* x *V. berlandieri* (41 B) (Infovini, 2021).

The process of grafting involves the intact cells remaining from the rootstock and vine after the cut to fuse together, and eventually form a callus as well as xylem and phloem (Melnyk & Meyerowitz, 2015). This union allows for the rootstock to function as a root system and trunk for the grapevine, facilitate water and nutrient uptake, influence stomatal conductance,

productivity, yield, fruit composition, and contribute to drought and nematode resistance of the vine (Keller, 2015; Zhang et al., 2016). Many combinations of scions and rootstocks grown in the field and potted conditions have been undertaken, each combination with its own outcomes on abiotic stress and vigor (Zhang et al., 2016). Furthermore, a study of two different varieties- Cabernet Sauvignon and Chenin Blanc, grafted onto various rootstocks, resulted in differences in shoot growth rate among the experimental groups (Grant & Matthews, 1996).

1.2 Viticulture in Portugal

There are 14 winegrowing regions in Portugal, including Madeira and Azores islands (Figure 1), and 45 appellations of origin or geographic indications (Fraga et al., 2017; OIV, 2016). This country is known for producing a variety of wines, with fortified wines being of exceptional importance to its winemaking image. Not to mention, the rise of enotourism is a factor on the Portuguese wine industry itself, offering tourists the Douro valley, a UNESCO world heritage site, and Alentejo region, among others.

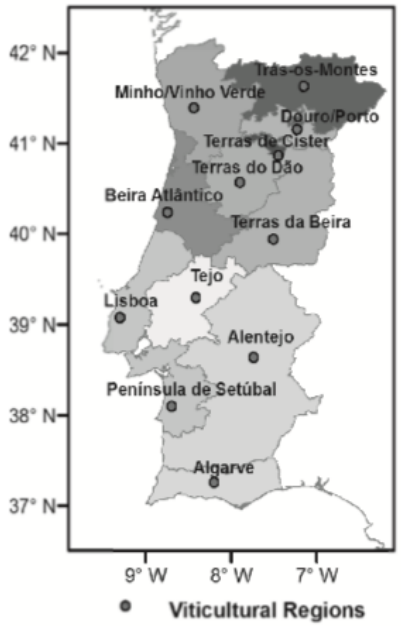

Figure 1. Map of the viticulture regions in Portugal, excluding Madeira and Azores islands. Taken from Fraga et al., (2017).

Portuguese vineyards have over 250 autochthonous grapevine varieties and 344 total varieties grown in the country (Graça, 2011; OIV, 2016). The most commonly planted autochthonous red varieties are Aragónez/Tinta Roriz/Tempranillo (with 20884 ha of planted vineyard area, representing 11% of the total vineyard area), Touriga Franca and Touriga Nacional (7% of vineyard surface in both cases), Castelão/João de Santarém/Periquita (occupying 5% of vineyard area) and Trincadeira/Tinta Amarela/Trincadeira Preta (4% of planted vineyard area) (Instituto da Vinha e do Vinho, 2018). Regarding white varieties, the most planted autochthonous varieties are Fernão Pires / Maria Gomes (6% of vineyard area), Loureiro (3% of vineyard area), Arinto/Pedernã (3% of vineyard area), Síria/Roupeiro/Códega (3% of vineyard area) and Alvarinho (2% of vineyard area).

Among international grape varieties, the most common in Portugal are Syrah and Alicante Bouschet. Each occupies 3% of Portugal's vineyard surface area (Instituto da Vinha e do Vinho, 2018).

The geology of Portuguese wine regions is vastly diverse, with various types of bedrock within a single wine-producing region. For example, the vast geographical area that covers the Douro superior wine region has many geological characterizations, including: (1) bedrock composed of metamorphic units of the Paleozoic to the Neoproterozoic age, (2) typical lithographic succession of the autochthonous rocks of the central Iberian zone, formed between the Edicarian and Devonian age, (3) granite and granodiorite of the Carboniferous to Permian age, and (4) detrital sedimentary rocks from the Cenozoic age (Moreira & Romão, 2018). Another example is the wine region of Bairrada, whose soil was formed on metamorphic rocks (pelitic and siliceous) and sedimentary rocks (siliciclastic and carbonate) (Dinis et al., 2012).

1.3 Climate Change and Portuguese Viticulture

The climatic conditions of the mainland Portuguese winegrowing regions overall is Mediterranean, with warm and dry summers and cool and wet winters (Fraga et al., 2017). Overall, the south of the country experiences a milder winter than the north. On the other hand, coastal vineyards have a high Atlantic influence, and as a result, they have high levels of annual precipitation (>1000 mm) (Fraga et al., 2017). The islands of Azores and Madeira have their own climate, which is characterized by mild to warm yearly temperatures, with low rainfall in Madeira, and variable rain on the Azores islands (ranging around 29 mm in the driest months, and 224 mm in the wettest months).

However, a different climatic scenario is expected for the next years. Global temperature is expected to rise in between one and five degrees Celsius in the 21st century (IPCC, 2013). The threats of increasing average temperatures, less seasonal rainfall, and higher pest and disease pressure primarily due to climate change pose a challenge to the viticulture and enology sector (Fraga et al., 2017). Bioclimatic zoning of Portuguese wine regions are projecting more homogeneity in climatic conditions, most notably (very) warm, dry with warm nights (Fraga et al., 2014).

Climate change and effects on annual average temperatures will impact grape composition, quality, and wine style. Temperature is a major factor impacting the development of grapevines. High temperatures between véraison and harvest will result in a decrease in acidity, a

higher perception of overripe fruity notes (Pons et al., 2017), an unbalanced berry composition, and high levels of sugar (Lopes, 2020a). Besides, stresses such as light, drought, and temperature can alter berry secondary metabolites, such as polyphenols. Furthermore, a surge in severity and frequency of heat stress is expected and can result in sunburnt grapes (Lopes, 2020a). On the other hand, since extreme water stress will be more frequent and intense, this will probably lead to a decrease in bud fertility and berry size, and eventually losses in yield (van Leeuwen et al., 2019).

These estimated impacts have been well-researched in the viticultural sector. However, the indirect impacts of the loss of ecosystem functions from macro and microorganisms in vineyards will be of equal importance to grapevine health and performance. For instance, the rise in temperature impacts insect and arachnid pre-copulatory mating activity, as well as the communication of potential mates (Leith et al., 2021), which poses a threat to the future of these arthropods. A study of cold winter temperatures followed by intense warming in the summer showed that the small soil fauna such as eu-edaphic Collembola and Prostigmata were impacted by these unprecedented drastic shifts in temperatures (Bokhorst et al., 2012). These shifts can cause changes in the soil microfauna and impact plant productivity and mineralization rates within the cycling of nutrients.

1.4 Microbial Diversity in Vineyard Soils

There is an immense diversity of microorganisms within the soil rhizosphere, including, but not limited to, bacteria and fungi. Richardson et al., (2009) defines this specific space within the soil as “the zone around plant roots whereby soil properties are influenced by the presence and activity of the root.” The diversity discovered within the rhizosphere of numerous studied plants was between 10 to more that 55,000 Operational Taxonomic Units (OTUs) (Mendes et al., 2013). In fact, it is known that there are more microbial genes than plant genes within the plant rhizosphere (Mendes et al., 2013).

Vineyard soils possess a complex microbiome that hosts a specific set of microorganisms. Their presence and abundance greatly depend on soil location, soil/vineyard conditions, and soil management practices (Burns et al., 2016; Canfora et al., 2018). Within the cool-climate setting, bacterial genera *Pseudomonas*, *Niastella*, and *Rhizobium*, and fungal genera *Plectosphaerella*, *Trichnosporon*, and *Ilyonectria* were the most commonly observed soil microorganisms (Wright et al., 2021). Bona (2018) found that the *V. vinifera* rhizosphere’s most

active bacteria included genera *Streptomyces*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, and *Pseudomonas*, who were involved in phosphorus (P) and nitrogen (N) metabolism. Two novel species of lactic acid bacteria (genus *Sporolactobacillus*) were discovered in soils in Korea, and were found to ferment glucose, fructose, sucrose, mannose, and sorbitol (Chang et al., 2008). High and medium concentration of rotundone grape metabolite in soils resulted in a lower abundance, but a higher diversity of soil bacteria in Australian vineyards (Gupta et al., 2019). On the other hand, there was no difference in fungi of *Glomeromycota* phylum among all treatments (Gupta et al., 2019). Furthermore, up to 160 different bacterial species were found in soils, depending on the herbicide treatment (e.g. glyphosate, glufosinate, or flazasulfuron) (Mandl et al., 2018). To summarize, there is a vast diversity of microbes which inhabit the vineyard soil environment, which is unique to the vineyard based on its specific soil properties.

Important groups of microorganisms in vineyards include the ones that that make N available to plants, by the fixation of this element and by mineralization of organic N, which makes them crucial for grapevine health and nutrition (Richardson et al., 2009), given that N is thought of as one of the most limiting soil nutrients for plant growth (Franche et al., 2009). Nitrogen fixation is carried out mostly by *Bacteria* and methanogenic *Archaea* (Young, 1992), who assimilate molecular N and via the nitrogenase enzyme, reduce it into ammonia which is then used by plants for amino acid synthesis (Franche et al., 2009).

Vineyard soils possess rhizospheric bacteria that fix N, among which are *Actinobacteria, Proteobacteria, Gemmatimonadetes, and Bacteroidetes* (Bona, 2018). Other N-fixing bacteria include *Rhizobium,* which forms nodules on legumes, which may be present within vineyard vegetation and bacteria genus *Frankia* (Kandeler et al., 2005).

Soil microorganisms also play a role in carbon (C) cycling in the soil, as fungi and bacteria are major contributors to the decomposition of plant litter. In vineyards, the soil itself usually has a coarse texture, is limited in its development and has a minimal capacity to prevent soil organic matter to bind to minerals (Jakšić et al., 2020). This all leaves vineyard soils vulnerable to being broken down more than other soils (Jakšić et al., 2020). Tillage is such a practice that leads to soil organic material mineralization, loss of fertility, higher prevalence of erosion (Jakšić et al., 2020).

Phosphorus (P), an essential element for plant nutrition, but also immobile in most soils, becomes available to plants via numerous microorganisms. Within the soil it is mostly microorganisms which mineralize organic P (Richardson et al., 2009). For studied tropical forest soils, arbuscular mycorrhizal fungi transform unavailable P into more easily available organic P forms, and then acid phosphatase enzymes hydrolyze organic P into inorganic phosphate, which a

plant can uptake (Liu et al., 2021). In addition, there are free-living bacteria and fungi, within the plant rhizosphere which can mobilize orthophosphate from organic and inorganic forms of P (Kucey et al., 1989). Furthermore, within the rhizosphere, are Plant Growth Promoting Rhizobacteria (PGPR) which act as biofertilizers and are involved in enzymatically converting organic and inorganic P into phosphates (Richardson et al., 2009).

In summary, vineyard soils are hosts to plant mutualistic microorganisms due to their myriad of beneficial features. They improve grapevine performance, provide an enhanced nutrient uptake, give advanced protection against pathogens and pest attacks, as well as tolerance against abiotic stress factors, including those related to climatic shifts (Barea et al., 2005; Mendes et al., 2013; Richardson et al., 2009; Trouvelot et al., 2015). For this reason, it is necessary to ensure a good soil health through conscious soil management practices. The preservation of functional soil microbial communities, including those involved in nutrient cycling as well as plant symbiotic activity, will be crucial for sustaining an optimal grapevine growth, development, and productivity, especially in a context of climate change.

1.4.1 Arbuscular Mycorrhizal Fungi

Fungi within the diverse soil community that are especially worthy of mention, due to their relation to grapevines, are arbuscular mycorrhizal fungi (AMF). They are from phylum *Glomeromycota* and are largely present in vineyard soils and grapevine roots (Bouffaud et al., 2016; Trouvelot et al., 2015). Within plant roots of more than 80% of terrestrial plants, including those of the grapevine, these fungi operate as obligate symbionts, where these symbioses are usually mutualistic and biotrophic (Smith & Read, 2008). Such a relationship is proven functional due to the grapevine host providing a source of C to the AMF (a total of four to twenty percent of the grapevine's photosynthetically fixed C), while the AMF provide access to water and nutrients to its host (Smith & Read, 2008). Once the colonization of the roots has occurred, the grapevine is able to take up nutrients either through the roots, or through extraradical mycorrhizal fungal mycelium (Smith & Read, 2008). In the case of P uptake, when plants are colonized by AMF, the acquisition of this element happens almost exclusively through the symbiotic pathway (Smith et al., 2011)

Arbuscular mycorrhizal fungi have intraradical and extraradical structures. In the intraradical phase, there are arbuscules, intercellular hyphae, and vesicles (lipid storage organs), while in the extraradical phase there are ramified hyphae, spores and resistance propagules

(Porcel et al., 2012) (Figure 2). The extensive extraradical mycelium network formed by AMF is responsible for absorbing nutrients from the soil and delivering them into the roots of their host plants (Oehl et al., 2003). With every centimeter (cm) of plant root colonized by mycorrhizal fungi, there is 15 cm^3 more soil volume explored (Sieverding et al., 1991). This is an immense advantage to grapevines, as they can receive more water and nutrients through this larger functional root area (Vanden Heuvel et al., 2020).

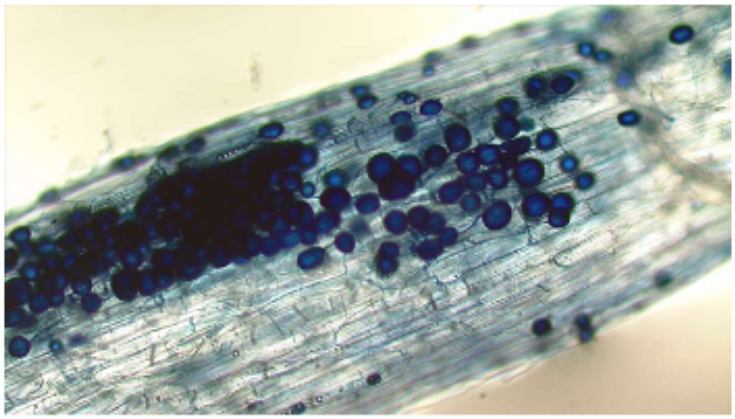

Figure 2. Intraradical spores in leek root (*Alium porrum* L.).

Studies show that arbuscular mycorrhizal fungi provide their host plant with a variety of assets; some include superior nutrient acquisition, higher yield, augmented soil stability and aggregation, protection from biotic and abiotic stress, acceleration of bud sprouting, acceleration of flowering, improved berry set, and alleviation of plant transplantation shock (Malhi et al., 2021; Trouvelot et al., 2015; Usha et al., 2005) (Figure 3). These symbionts also raise the hydraulic conductivity of the root system as well as the rate of photosynthesis of the canopy (Sánchez-Blanco et al., 2004). Furthermore, a rise in total phenols, sugar content, chlorophyll content, root colonization, and root length was found when grapevines were inoculated with AMF (Eftekhari et al., 2012).

A major issue facing vineyards on a global scale is the prevalence of pest and diseases. This fungal symbiosis can expand plant tolerance against pathogens. There are four mechanisms by which AMF can protect their hosts against root pathogens. The first mechanism is through direct competition or inhibition. It is commonly reported that the most effective competitive inhibition occurs when AMF are inoculated before pest infection. However, this is not universally true, as grapevine rootstocks inoculated with AMF had higher cases of *Ilyonectria* (Black Foot Disease) than non-mycorrhizal plants (Holland et al., 2019). The second is bettered or changed plant growth, morphology, or nutrition. In *Vitis vinifera* plants, inoculation with AMF contributes to a greater carotenoid concentration in the leaves up to 31% and the total phenolic concentration in the leaves up to 900% (Krishna et al., 2006). In addition, AMF enhanced the uptake of P, calcium (Ca), potassium (K), zinc (Zn), and copper (Cu) in grapevines (Biricolti et al., 1997;

Nicolás et al., 2014). These macro and micronutrients are vital to plant biological, chemical, and enzymatic processes. The third mechanism is induced resistance and plant defense mechanisms, which occur on a biochemical level (Vos et al., 2012). Other mechanisms involved in plant protection are the stimulation of the production of plant pathogen defense chemicals such as phenolic substances, phytoalexins, and chitinases (Barman et al., 2016). The last mechanism is the enhancement of the proliferation of pathogen-antagonistic microorganisms (Vos et al., 2012).

It has been shown that there is mycorrhizal protection against root pathogens (*Armillaria mellea*) in grapevines (Nogales et al., 2009). The spread of fungal, soil-borne pathogens *Fusarium oxysporum*, *Cylindrocarpon destructans,* as well as *Rhizoctonia solani*, was found to be controlled through the presence of certain AMF species of Glomerales (Whipps, 2004). *Glomus etunicatum* (*Claroideoglomus etunicatum*) was found to decrease the disease symptoms from *Verticillium dahlia*, a fungus causing wilt in grapevine (Matsubara et al., 1995). Furthermore, attacks of grapevine fungal diseases such as *Plasmopara viticola* and *Botrytis cinerea* on mycorrhizal grapevines have been observed to trigger enhanced defense reactions. This comprises the upregulation of genes involved in the stillbenoid biosynthesis pathway (Bruisson et al., 2016).

In the case of nematode infections, AMF showed local and/ or systemic induced bioprotection against the ectoparasitic nematode *Xiphinema index*, a vector of Grapevine fanleaf virus (Hao et al., 2012), and against *Meloidogyne incognita* (root knot nematode) (Li et al., 2006). In the first case, an upregulation and activation of plant defense genes including chitinase 1b, pathogenesis-related 10, glutathione, S-transferase, stilbene synthase 1, 5-enolpyruvyl shikimate-3-phosphate synthase, and a heat shock protein 70-interacting protein were found (Hao et al., 2012) in colonized roots. In the case of *M. incognita* infection, the inoculation of AMF induced a defense response in the grapevine against *Meloidogyne incognita* (root knot nematode) involving the transcriptional control of the VCH3 expression (Li et al., 2006).

In addition to relief from disease, mycorrhizal symbiosis can also elevate plant tolerance towards abiotic stress factors (e.g. high temperatures, salinity, nutrient deficiency, soil contamination) (Smith & Read, 2008). In maize plants experiencing high temperature stress, AMF can minimize membrane lipid peroxidation, reduce membrane permeability, elevate antioxidant activity, and allow more osmotic adjustment molecules to accumulate in the cell (Zhu et al., 2010). In another study involving maize plants under high temperature stress, AMF-colonized maize plants had a higher stomatal conductance, photosynthesis rate, and transpiration rate compared to non-mycorrhizal maize plants (Zhu et al., 2011). These symbiotic fungi also provide tolerance to heat stress to other plants, such as *Cyclamen persicum*, where the mycorrhizal plant received higher antioxidant activity, plant growth, and plant biomass (Maya &

Matsubara, 2013). In grapevines, it was also found that mycorrhiza-inoculated Touriga Nacional grapevines were more able to continue their grow following a heat stress than non-inoculated plants (Nogales et al., 2020).

Furthermore, AMF are able to raise the stomatal conductance of their host in situations of water stress (Augé et al., 2015). Augé, (2004) asserts that it is not only the colonization of AMF in plant roots that enhances plant stomatal conductance, but the colonization of AMF in the soil is of equal importance. This is due to the fungal mycelium stabilizing soil aggregates, and to a larger degree, impacting the soil structure, therefore affecting soil water retention (Augé, 2004). In the case of grapevines, Cabernet Sauvignon cultivars inoculated with *F. mosseae* and exposed to water stress reported higher shoot growth and leaf P, as well as superior predawn water potentials in comparison with non-mycorrhizal vines. A greater leaf transpiration and water uptake from the dry soil was found to be the main reasons for these findings (Nikolaou et al., 2003).

Soil salinity and soil contamination effects on grapevines can also be counteracted by AMF. Salinity affects the morphological development, physiological development, biochemical processes, and yield of many cultivated species. Grapevine rootstocks enduring various levels of salt stress and inoculated with *Rhizophagus intraradices* displayed a positive correlation between AMF inoculation and plant growth parameters, such as height, stem diameter, leaf area, and shoot and root biomass (Khalil, 2013). On the other hand, vesicles and spores serve as storage for salt ions and toxic substances (Barman et al., 2016; Ferrol et al., 2009). For instance, AMF can prevent toxic levels of Cu to enter the cytoplasm, perform intracellular complexation of Cu in the cytosol and compartmentalize the metal in the cell (Ferrol et al., 2009). Cu is displaced into subcellular compartments where its damage would be minimized; these compartments are most often vacuoles.

This symbiosis is also proven as an aid to soil stability and soil health. Mycorrhizae extend out of the grapevine roots to create a branched network of hyphae (up to 30 meters per gram of soil) which can provide support to soil particles (Wilson et al., 2009), and improve soil aggregation due to the secretion of Glomalin (Rillig, 2004) which in turn prevents erosion. This can be especially relevant in vertical planted and terraced vineyards, which are common in steep-slope and mountain viticulture regions, which are at high risk of soil erosion.

The symbiosis with AMF can be more complex than just interacting and providing benefits with the host plant. Arbuscular mycorrhizal fungi can form symbiosis with roots of plants that are also symbiotic with N fixing nodulating bacteria *Rhizobium* and *Frankia* and work synergistically with these bacteria (Fitter & Garbaye, 1994). As an example, in a low soil P scenario, AMF are able to improve P uptake, therefore profiting the bacteria, allowing its nitrogenase to work efficiently, and allowing it to fix more N (Fitter & Garbaye, 1994). The

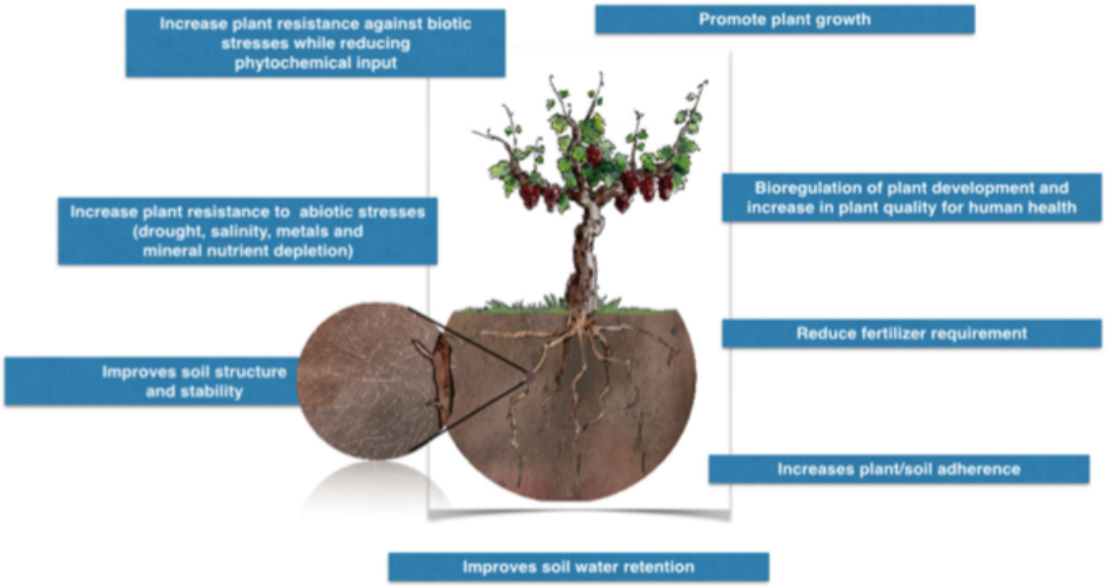

Figure 3. Benefits of arbuscular mycorrhizal fungi to grapevines (Trouvelot et al. 2015).

presence of rhizobia and leguminous plants in vineyards can indirectly benefit grapevines, due to the increased N incorporation into vineyard soils.

1.4.2 Mutualistic Soil Rhizobacteria

Soil mutualistic bacterial communities in vineyard soils are also of importance to grapevines, since this relationship ameliorates grapevine health, productivity, and response to climatic stressors (Vink et al., 2021). Research by Vink et al., (2021) illustrated that several grapevine factors (phenological stage and cultivar), and soil characteristics (soil management and soil chemical characteristics) impact soil bacterial communities, and these factors interact with each other.

These consortiums or single species of bacteria can ultimately alter their hosts' functional traits. A functional trait is a modification to morphology, physiology, or phenology of a plant which indirectly amplifies its fitness through its impact on growth, reproduction, or survival (Violle et al., 2007). Such examples could be: modified plant leaf area, nutrient uptake, and drought resistance (Friesen et al., 2011). For example, vine-associated soil bacteria undergo biogeochemical cycling, indirectly being advantageous to grapevines (Vink et al., 2021), as the cycling of nutrients, such as C, N, P, impacts plant available nutrients. In addition, microorganisms offer their hosts new biochemical pathways, can alter their hosts' pre-existing pathways, and create or change plant phytohormones (Friesen et al., 2011).

Rhizospheric bacteria that enhance plant growth are commonly referred to as plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 1980). It has been suggested that PGPR impact plant physiology the most out of all soil microorganisms (Saharan & Nehra, 2011). These bacteria produce auxins, gibberellins, cytokinins, which are plant growth promoting compounds-phytohormones (Sabir et al., 2012). For this reason it is no surprise that grapevine rootstocks inoculated with PGPRs experienced an increased vegetative growth (Sabir et al., 2012).

These bacteria can also enhance plant growth through other mechanisms, including phosphate solubilization, N fixation, production of siderophores, toxin production prevention, by the increase of abiotic plant stress tolerance through the production of antioxidant enzymes, phytohormones and other compounds and by the protection against pathogens through several mechanisms that include the induction of systemic resistance and the production antibiotic and lytic enzymes that degrade fungal pathogen cell walls (Backer et al., 2018).

Plant growth promoting rhizobacteria have been shown to be beneficial for grapevines. Work by Sabir et al., (2012) found that these PGPRs are useful to grapevine nutrient uptake; since all bacterial strains had a positive effect on nutrient uptake and plant photosynthesis. However, in this study, *A. brasilense* Sp 245 and *B. subtilis* OSU-142 were reported to be the most efficient of all the species and isolates used.

These mutualistic soil bacteria also deliver protection against pathogens. Strobel et al., (2005) elaborates that bacterial strain *Serratia marcescens* MSU-97 allows protection against *Plasmopara viticola,* a common pathogen in grapevines. Additionally, Khmel et al., (1998) found that *Pseudomonas aureofaciens* B-4117 protected plants against *Agrobacterium vitis*, causing crown gall. *Bacillus subtilis* AG1 protected against *Phaeomoniella chlamydospora*, causing Esca disease, a known disease in the viticulture sector (Alfonzo et al., 2009). *Botrytis cinerea*, a common disease of the grapevine, can be treated using bacterial biocontrol agents *Acinetobacter*, *Bacillus*, *Pantoea* and *Pseudomonas* spp. (Trotel-Aziz et al., 2008).

In addition to protection from biotic stress, selected PGPR bacterial isolates can protect plants from abiotic stress and allow them to better cope with drought conditions. Bacteria from genera *Pseudomonas*, *Enterobacter*, and *Achromobacter* produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which prevents plant growth in times of drought stress (Duan et al., 2021). In addition, two of these strains were efficient in solubilizing phosphate and N fixating (Duan et al., 2021). Arsenic poisoning, another abiotic stress, was studied in Malbec grapevines. Grapevines inoculated with PGPR bacterial species *Bacillus licheniformis*, *Micrococcus luteus*, *Pseudomonas fluorescens*, or a consortium of the three strains, experienced increased photosynthetic pigments and plant biomass, as well as reduced effects of arsenic poisoning of the grapevine (Funes Pinter et al., 2018).

Soil microbial communities, especially bacteria and fungi in this context, depend on certain factors to promote their proliferation, such as the soil resources and properties (e.g., soil organic matter, nutrition, pH) (Burns et al., 2016). Vineyard management practices impact these very properties that which soil microorganisms depend on.

1.5 Vineyard Management Practices

The goal of soil management practices is to develop the soil structure, manage soil fertility, plant growth and health in order to optimize plant productivity (Metay, 2020). Conventional viticultural practices include synthetic pesticides, fungicides, herbicides, tillage, and the use of mineral fertilizers. This use of agrochemicals to eliminate weeds, pests, diseases, and promote soil fertility, in excess, can lead to a loss of soil structure, decreased food quality, and a potential increase in plant diseases that may result from a decrease in soil microbial richness and diversity (Vogt, 2000).

Even though viticulture activity accounts for only three and a half percent of the agricultural area in the European Union Viticulture, it accounts for 15% of the pesticides used (Selim, 2020). Copper products and dithiocarbamate are used as protective agents against downy mildew (*Plasmopara viticola*) on grapevines, while powdery mildew (*Erysiphe necator*) is usually treated with different groups of anti-resistance sprays, or dusting, wetting, or pasting sulfur and sulfur compounds. Although Cu is commonly used as a fungicide, it can remain in the subsoil layers due to its immobility and may lead to soil contamination (Magalães et al., 1985).

In viticulture, the most commonly used herbicides contain active ingredients like glyphosate, glufosinate, and flazasulfuron (Bauer et al., 2017). Glyphosate is the most common herbicide in viticulture; it comprises 75% of herbicides used on the vineyard (Jacquet et al.,

2019). On the other hand, tillage is a conventional practice in viticulture that is used at many phases of a vineyard's establishment. Before vine plantation, deep tillage is used to break down the subsoil, remove rocks, and remove old plant material. Not only does tillage reduce nutrient content of the soil, it also destroys the extraradical mycelium of AMF (Brito et al., 2012). In terms of fertilization regime, it is important to keep nutrient inputs as low as possible to promote AMF proliferation within the soil (Van Geel et al., 2017).

Society's desire for a more sustainable agriculture and the demand for safer products for the environment has led to an increase in organic and biodynamic farming. The global area of organic cover of grapes is 437,153 ha, with 105,183 currently under conversion. Europe currently covers 387,061 ha of organic grape production, with 102,151 ha under conversion (FiBL, 2019). Portugal has 194 kha of vines (OIV, 2020), of which 3,997 ha are organic, and 961 ha are under conversion into organic (Figure 4) (FiBL, 2019).

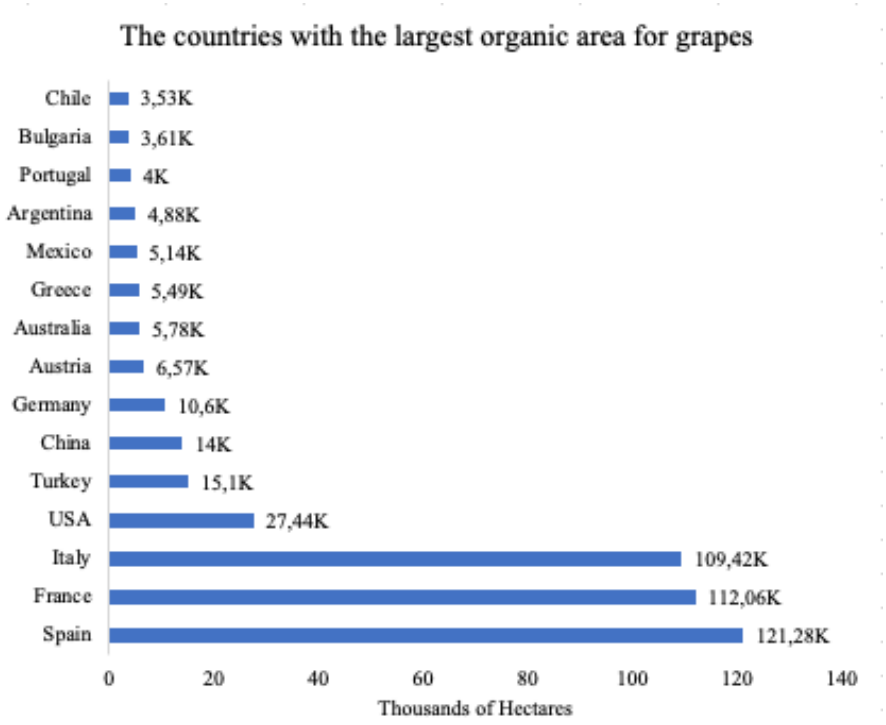

Figure 4. Area of organic grape production by country (FiBL, 2019).

Organic and biodynamic vineyard management initially started in the late 1960's (Danner, 1985). However, consumer demands for sustainable farming practices in recent decades has led to an expansion in organic food production (Yiridoe et al., 2005), and many practices used initially in organic viticulture are being increasingly integrated by conventional winegrowers. The

journal of the European Union asserts that organic production is as an overall system of farm management that prioritizes practices to protect and preserve the environment, climate, biodiversity, and natural resources, all while keeping its standards of production high (Radley-Gardner et al., 2016).

Organic and biodynamic viticulture follow similar principles and objectives, which are to encourage soil fertility, as well as its physical, chemical, and biological status. Manufactured synthetic fertilizers and synthetic chemical pesticides/fungicides are prohibited. The EU has set forth regulations for which products may or may not be used in organic farming, which is in Appendix II of Regulation (EC) No. 889/2008 (Döring et al., 2019). Biodynamic viticulture is guided by the principles of self-sufficiency, maintaining soil fertility, and environmental sustainability (Demeter, 2021). Like in organic viticulture, biodynamic viticulture does not use synthetic fertilizers, pesticides or genetically modified organisms (Lopes, 2020b). Few non-synthetic fertilizers are allowed and manual weed management (shallow tillage, cutting the grass) is used to sustain all facets of soil integrity (Coll et al., 2011). Application of organic fertilizers, especially to stimulate the N cycle, are recommended and used to improve soil fertility (Döring et al., 2019). This has led to a reduction in fertilizer use in organic exploitations by 34%-53%, while the pesticide input is decreased by 97% (Oehl et al., 2003). In addition biodynamic vineyards make "preparations" of horn manure to promote nutrient cycling and soil fertility (Demeter, 2021). However, organic and biodynamic exploitations result in lower yields compared to conventional vineyards (Döring et al., 2019). All in all, organic and biodynamic vineyard management have positive and negative aspects to their implementation.

1.5.1 The Impact of Vineyard Management Practices on Soil Microorganisms

Soil management has an influence on soil microbial communities, which subsequently impacts plant growth and nutrition. This is possibly due to these practices promoting the proliferation of microorganisms involved in nutrient cycling, or due to the proliferation of symbiotic microorganisms. However, the opposite is also possible, where vineyard management has a negative impact on the soil microbiome, which then will leave the plant more susceptible to environmental stressors.

There are consequences of certain vineyard management practices for soil microorganisms, soil macro-organisms, and plant health. For example, over 98% of pesticide sprays used in agriculture arrive somewhere other than to the target species, which can include

the air, water, and soil (Larramendy & Soloneski, 2019). This intrinsic error in pesticide spraying has the potential for severe implications on not only animal and human health, but also of the soil microbiota and plant health. Not to mention, even organic amendments were found to have changed the structure or the activity of soil microorganisms. This happens due to organic amendments introducing exogenous organisms to the soil with their own mechanisms of biodegradation and changing soil properties, thus causing disruptions to the native soil microbial activity (Carpio et al., 2020).

Concerning herbicides, the degree of damage done to soil microorganisms can vary depending on the type of herbicide used. For instance, in the study of Zaller et al., (2018), although the application of herbicides significantly affected the total number of colony-forming units (CFU) of soil microorganisms, the bacteria with the highest CFUs were the ones treated with glufosinate, while the lowest CFUs were found in the treatment of glyphosate (Zaller et al., 2018). Therefore, changes in the kind of herbicide applied can be a starting point for controlling the impact that herbicides have on soil functioning and plant performance.

1.5.2 The Impact of Vineyard Management Practices on Rhizosphere Bacteria

Studies have shown that land use and soil management practices impact soil bacterial communities. The diversity of the soil bacterial community in agricultural soils was smaller compared to natural soils (Ding et al., 2013). These arable soils were also found to contain less organic matter and phosphate concentration than natural soils, leading to the proliferation of only a small number of specific bacterial taxa that were able to occupy such a resource-limited niche (Ding et al., 2013). Furthermore, in a study where the impact of organo-mineral fertilization and conventional fertilization in soil bacterial communities was assessed, there was a difference in the community composition between treatments (Canfora et al., 2018).

On the other hand, tillage mobilizes the soil and is indeed linked to lower amounts of organic matter (Balesdent et al., 2000). Likar et al. (2017) showed that bacterial communities were very sensitive to vineyard soil management practices and that deep tillage resulted in the most profound impact in the bacterial community. This was in agreement with Burns et al., (2016), who found that low soil bacterial diversity was correlated to recent tillage. They explained these results as a consequence of the shifts in soil resources that happened after tillage, such as a reduction of particulate organic C, total N, and mineralizable N (Wander & Bollero 1999), which directly alters bacterial community structure.

However, some recent works demonstrate the improvement of the biodiversity and richness of soil bacteria through sustainable vineyard management practices. A study of the impact of vineyard practices on bacterial communities found that there was a significant difference in bacterial communities among conventionally and organically managed vineyards (Likar et al., 2017). Since there were no differences in plant-available soil Cu between conventional and ecological managed vineyards, this indicated that Cu levels were not responsible for this difference in bacterial communities (Likar et al., 2017). A work studying the impact of vineyard soil management on plant rhizosphere bacterial diversity did in fact find higher diversity and richness of bacterial communities in organically managed plots, although the organic and conventional vineyards did not have significant differences in chemical soil characteristics (Vega-Avila et al., 2015).

Nevertheless, additional study showed that bacterial species richness was similar in vineyard topsoil under different management regimes: integrated, organic, and biodynamic (Hendgen et al., 2018). However, the differences were observed in bacterial community composition, especially between soils under integrated management and organic and biodynamic managements (Hendgen et al., 2018).

1.5.3 The Impact of Vineyard Management Practices on Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungal diversity depends on several factors, including soil properties, soil depth, the host plant species, spatial variation within the plant community, as well as the vineyard management practices (Bouffaud et al., 2016; Wang et al., 2018). Among soil characteristics, the soil depth impacts the AMF community, which is due to changing soil conditions between the soil layers and different root presence in such layers, as well as the soil management practices, that can affect soil layers in different ways. For instance, fertilization may change mineral content in different soil layers, which in turn affects the presence of different AMF communities (Helgason et al., 1998; Johnson, 1993; Schreiner, 2020).

Conventional agricultural soil management practices have been shown to have a negative impact on AMF association, and agricultural soils tend to be poor in AMF species richness and abundance (Helgason et al., 1998; Menéndez et al., 2001). Overall, fertilization and herbicide treatments have shown negative effects on AMF colonization and diversity. The excess of P fertilization can lead to reduced AMF colonization and propagule density (Karagiannidis & Nikolaou, 1999). The excessive application these fertilizers leads to the disappearance of many AMF species as their role of nutrient acquisition is no longer needed (Van Geel et al., 2017). In

regards to herbicides, Zaller et al., (2018) found that all three within-row herbicide treatments used in that study, which included active ingredients flazasulfuron, glufosinate, and glyphosate, reduced grapevine root mycorrhization by more than 50% in comparison with mechanical weeding (Zaller et al., 2018).

Another soil management technique that impacts AMF communities is soil tillage. A reason for the reduced AMF diversity in vineyards is related to AMF extraradical mycelium disturbance due to soil tillage. This leads to a reduction in propagule density, i.e. extraradical infective hyphae, and consequently a lower capacity for root colonization (Dodd, 2000) (Boddington & Dodd, 2000), as it breaks up the soil and the extraradical mycelium within. In fact, conventional tillage can reduce AMF diversity by 40%, in comparison with no-till (Brito et al., 2012). This practice can promote the proliferation of the R-strategist type of AMF, such as some members of the *Glomeraceae* family. Many taxa of this AMF family thrive in disturbed habitats due to their high sporulation, low spore dormancy, and many primary and secondary infection points, while AMF of other families, can be k- strategists with a slower growing rate, and will appear long after a soil disturbance (IJdo et al., 2010). Soil tillage ultimately leads to a dominance of this first group and a consequent decrease in AMF species richness, as suggested by Bouffaud et al., (2016). This study conducted in Burgundy vineyards found that a majority of the AMF species in these soils were from the family *Glomeraceae*, therefore hinting at a low AMF diversity in these studied vineyards (Bouffaud et al., 2016). Additionally, Oehl et al., (2010) also mentions the tendency for vineyard soils to be environments for low-diversity niches of AMF.

In contrast to conventional vineyard practices such as soil tillage, organic soil management practices in general improve AMF spore abundance (Radić et al., 2014), as well as the topsoil AMF communities via reduced tillage (Säle et al., 2015).

1.5.4 The Impact of Vineyard Management Practices on Grapevine Performance

Vineyard management regime, in addition to influencing the soil characteristics and soil microbiological community, impacts vine performance as well. For example, the application of some agrochemicals can lead to soil contamination, which can induce phytotoxicity symptoms in plants. This has led to accumulation in vineyard soils at extremely high levels, especially in old vineyards (Mackie et al., 2012). This not only impacts plant and animal life, but also the yield and quality of grapes growing in this kind of soil (Ninkov et al., 2012).

The use of more sustainable vineyard management practices has been shown to have a

positive influence on plant performance through their indirect utility in the plant environment (Dal Ferro et al., 2017; Schrama et al., 2018). There have been studies showing the differences in yield among organic and conventional management. Among these, the study by Döring et al., (2015) found lower grapevine growth and yield of organic and biodynamic viticulture versus integrated vineyard management. However, the organic and biodynamic treatments had higher levels of N in the soil and leaves and there was no difference in grape quality between the different management systems. It is a matter of effectively eliminating diseases that will allow for organic and biodynamic viticulture to have the same yield and growth to that of conventional management (Döring et al., 2015).

1.6 Objective

The objective of this work was to evaluate the physical-chemical and biological characteristics of vineyard soils in comparison to non-agricultural soils from different Portuguese winegrowing regions, and to study the potential link between soil properties and the performance of Aragónez grapevines grafted onto two different rootstocks growing under controlled greenhouse conditions. The hypothesis of this project was that non-agricultural soils have a stronger soil fertility and microbiological presence than conventionally managed vineyards (due to their lack of soil tillage, mineral fertilization, and herbicide use) and this can lead to an enhanced grapevine growth and performance.

2. Materials and Methods

2.1 Soil Sampling

Eighteen soil samples were collected randomly from three locations in Portugal in spring 2021: Pegões, Bombarral, and Lisbon (Figure 1). Pegões is included in the Setúbal wine region, while Lisbon and Bombarral are part of the Lisbon wine region.

According to Köppen-Geiger climatic classification, Pegões and Lisbon had hot-summer Mediterranean climate (*Csa*), while Bombarral had a warm-summer Mediterranean climate (*Csb*). Table 1 shows the climatic normal for each location.

Table 1. Climatic Normals Collected from 1971-2000

Location	Average Minimum Temperature (˚C)	Average Maximum Temperature (˚C)	Average Daily Temperature (˚C)	Average Total Precipitation (mm)
Pegões *1*	10.1	22.3	16.2	673.6
Alcobaça *2* (47 km from Bombarral)	9.5	20.6	15.0	839.6
Lisbon *3*	11.9	20.9	16.4	680.4

1 (*Ficha Climatológica Pegões*, 2000); *2* (*Ficha Climatológica Alcobaça*, 2000); *3* (*Ficha Climatológica Lisboa*, 2000)

In each location, three soil samples were collected inside the vineyards, in the inter-row spaces, and another three in non-agricultural surrounding sites (Figure 1 and 2). The soil samples were collected until 10-20 cm of depth.

Figure 5. Localization of studied areas and sampling points in A) Pegões; B) Bombarral; C) Lisbon D) Map of the study areas and their geographical relation to one another.

All vineyards were conducted in a conventional management system and in the three cases, they were red variety vineyards planted in 2016, 2018 and 1996 (Pegões, Bombarral and Lisbon, respectively). The vineyards in Pegões and Bombarral had a conventional soil management, where weeds in the inter-row space were controlled by tillage and by herbicide application (glyphosate in Pegões, and glyphosate and flazasulfuron in Bombarral), leaving the inter-row space as bare soil. However, in Lisbon's vineyard, the vegetation in the inter-row was controlled by regular mowing, and therefore, there was a natural vegetation cover. This location was oriented on a slope, while the other two vineyards were flat.

In the case of the non-agricultural areas, in Pegões, it was characterized as having dispersed oak and pine trees and continuous natural herbaceous plants and grasses. In Bombarral, the non-agricultural area was characterized by a dense area of shrubs, dispersed pine trees and oak trees with and spontaneous herbaceous plants, and it was oriented on a slope. In Lisbon, pine

trees, oak trees, shrubs, and spontaneous herbaceous plants were present in the non-agricultural soil (Figure 2).

Figure 6. Images of the vineyard and surrounding non-agricultural soils where soil samples were collected. A) Pegões, vineyard soil B) Pegões, non-agricultural soil C) Bombarral, vineyard soil D) Bombarral, non-agricultural soil E) Lisbon, vineyard soil F) Lisbon, non-agricultural soil.

The soils from Pegões were developed on a complex of sedimentary rocks mostly composed of sands and minor concentrations of clays of Pliocene age, and are classified as

Arenosols (Nogales et al., 2019). The soils from Lisbon's sampling location were developed on post-Cretaceous rocks from the Volcanic Complex of Lisbon, which in the case of the vineyard location where the samples were collected consisted of tuffs and breccias originating a Tephric Eutric Regosol (Plano de Gestão Florestal Tapada da Ajuda, 2021). Non-agricultural soils from the surrounding area of the vineyard were developed on basalt rocks from the Volcanic Complex of Lisbon and were classified as Hipereutric Vertic Cambisols (Plano de Gestão Florestal Tapada da Ajuda, 2021). On the other hand, the soils collected in Bombarral region were developed on a complex of sedimentary rocks mainly composed of salt and gypsum marls and limestones of Jurassic age (Zbyszewski & Moitinho de Almeida, 1968) and were classified as Calcic Cambisols.

2.2 Soil Physical-Chemical and Biological Analysis

After soil sample collection, the eighteen samples (three per location and land use, i.e. vineyard or non-agricultural soil) were homogenized. A part of each soil sample was kept fresh (4 °C) for the analysis of biological parameters while the remaining subsample was air-dried and sieved at 2 mm to analyze physical-chemical properties. These analyses were of the pH in water (1:2.5 m:*V*) (Thomas, 1996), electrical conductivity in water (1:2.5 m:*V;* Rhoades, (1996)), organic C concentration by wet oxidation (Springer & Klee, 1954), total N (Kjeldahl method; Bremner, 1996), available P (Olsen, 1954). Also, concentration of macro- and micro-nutrients (K, Mg, Na, Ca, Cu, Fe, Mn and Zn) in the available fraction, and acid and non-acid exchange cations concentrations were measured. Macro- and micro-nutrients were extracted with a DTPA solution prior to their determination by atomic flame absorption spectrophotometry (Lindsay & Norvell, 1978). Acid exchange cations were analyzed by the exchangeable acidity method, and the non-acid cations were analyzed following the methodology designated by (Madeira et al., 2003) as 1 M NH_4OAc simplified method and quantified by atomic flame absorption spectrophotometry. The cation exchange capacities were calculated by the sum of acid and non-acid cations.

Furthermore, several soil biological parameters were also determined in fresh soil samples (< 2 mm). The following soil enzymes were determined: soil dehydrogenase activity (Tabatabai, 1994), ß-glucosidase activity (Eivazi & Tabatabai, 1988), acid phosphatase activity (Eivazi and Tabatabai, 1977), cellulase activity (Alef, 1995) and urease activity (Kandeler & Gerber, 1988).

In addition, the number of infective mycorrhizal propagules per gram of soil was determined in the soil samples using the most probable number (MPN) method (Porter, 1979; Powell, 1980) with leeks (*Alium porrum* L.) as trap plants. For this, leek seeds were previously surface disinfected and germinated in sterile sand under greenhouse conditions. Each fresh soil sample was diluted from 10^{-1} to 10^{-5} by mixing it with sterile sand (1 h at 121° C 1 atm pressure in the autoclave). Each diluted soil was placed in germination trays (5 pots per tray) and a three-weeks old leek plantlet was planted on each pot.

Four months later, leek root systems were collected from each pot and they were stained in Trypan blue using the procedure of Koske & Gemma, (1989); Phillips & Hayman, (1970). The root systems were observed under a binocular at 10X magnification and the number of mycorrhiza-colonized root systems per diluition factor and soil sample were annotated. Propagule density was then calculated using the excel program of (Jarvis et al., 2010).

2.3 Study of Grapevine Growth and Performance in Vineyard and Non-Agricultural Soils

2.3.1 Experimental Set-Up

Forty-eight non-rooted dormant vines of Aragónez *cv.* x 1103 Paulsen and 48 Aragónez *cv.* x 110 Richter were obtained from Viveiros Vitioeste, located in Bombarral, Portugal. 110 Richter is a very vigorous rootstock but often the response to rooting is poor. This rootstock has a resistance of up to 17% active limestone, but it is extremely sensitive to salinity and to the excess humidity. This rootstock has good adaptation to poor and dry soils (Infovini, 2021). The 1103 Paulsen rootstock is also very vigorous and has better rooting response than 110 Richter (Mottard et al., 1963).

Plants were washed with tap water before being planted into Styrofoam containers (65 x 35 x 16 centimeters) filled with sterile perlite (Figure 4A). Plants remained under greenhouse conditions for one month and were watered daily. Once per week, the vines were watered with a half-strength Hoagland and Arnon Solution (1950) while undergoing rooting (Hoagland & Arnon, 1950).

One month after plant growth in perlite (June 2021), when roots were already formed, plants were transplanted to forest pot containers (one liter) filled with the soil collected from each location/site (Figure 7B). Previously, the three subsamples collected from each soil had been

mixed at equal volumes. Plants were watered daily and every two weeks they were watered with a half-strength Hoagland and Arnon solution. Plants remained until the 15th of September 2021 under greenhouse conditions.

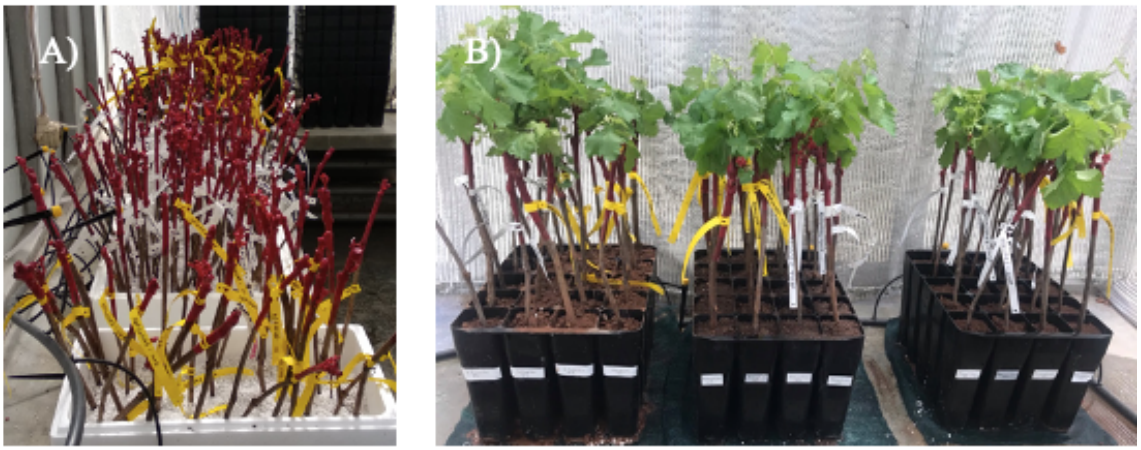

Figure 7. A) Grapevine rooting in perlite; B) Grapevine transplant into collected soils (Pegões, Bombarral, Lisbon).

2.3.2 Grapevine Growth and Physiology Monitoring

Periodically, Photochemical Reflectance Index (PRI) and Normalized Difference Vegetation Index (NDVI) were measured using PlantPen PRI 200 (PSI, Czech Republic) and PlantPen NDVI 300 (PSI, Czech Republic) portable devices, respectively (Figure 4A). Photochemical Reflectance Index is indicative of photosynthesis efficiency and plant stress levels (Garbulsky et al., 2011) while NDVI reflects plant vigor and, indirectly, chlorophyll status, P and N nutrition (Sembiring et al., 1998). These measurements were taken on two mature leaves per plant and per shoot, and the average value per plant was used for statistical analysis (Table 2).

Table 2. Measurement dates of grapevine performance parameters.

Date	Date after transplant	Measurement Taken
July 6th	One month	NDVI, PRI
July 20th	One month and a half	NDVI, PRI
August 4th	Two months	NDVI, PRI
August 24th	Two months and a half	NDVI, PRI
September 14th	Three months & one week	NDVI, PRI, shoot length, root biomass, root mycorrhizal colonization

After three and a half months of growth in the greenhouse, five plants per experimental treatment (i.e. three locations and two land uses per location, 60 plants in total) were harvested. Plant reflectance indices were measured, as well as shoot length and root biomass (Table 2). The roots were stained in Trypan blue using the procedure of Koske & Gemma, (1989) and Phillips & Hayman, (1970) and root mycorrhizal colonization was estimated by the gridline intersect method of Giovannetti & Mosse, (1980).

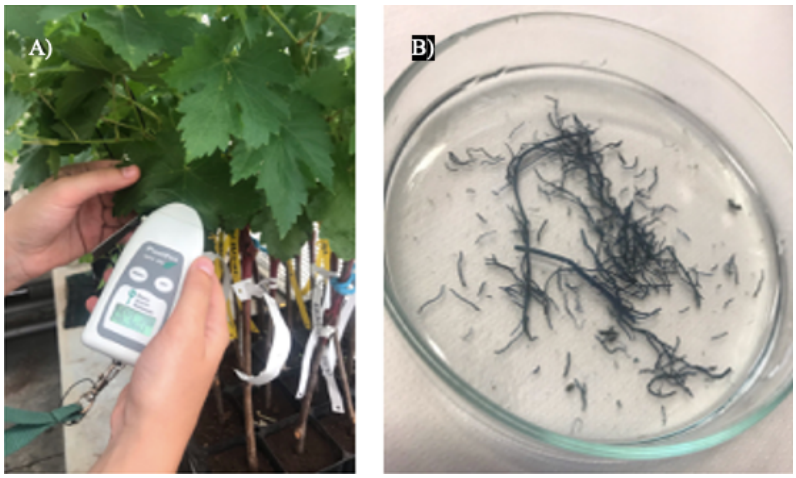

Figure 8. (A) Normalized Difference Vegetation Index measurement of plant leaves. (B) Grapevine root staining using Trypan blue solution.

2.4 Statistical Analysis

To analyze the effect of the location and land use as well as the interaction of the two factors on soil physical-chemical and biological properties, a two-way ANOVA was performed.

Previously normality of the residuals as well as variance homogeneity were studied. If the assumptions of normality and variance homogeneity were not met, data were inverse, or log transformed. If normality was still not achieved after transformation, a nonparametric Kruskall-Wallis test was executed. Then, a *post hoc* test (Duncan test) was conducted to determine significant differences among the experimental treatments.

In the case of the density of mycorrhizal infective propagules in the soils, a t-student test was conducted to detect potential differences between vineyard and non-agricultural soils. Furthermore, Pearson correlations analyses were performed on soil physical-chemical and biological parameters.

To analyze the effects of location, the land use and rootstock as well as of their interactions on NDVI and PRI, a 3-way ANOVA was conducted at each measuring time-point. Due to significant interactions among the factors, data were further analyzed at each location by a 2-way ANOVA where rootstock and land use were considered as main factors. A Duncan test was performed to find potential differences on the studied variables among the experimental treatments at each time point.

Shoot length, root biomass and mycorrhizal colonization rate were analyzed by a 3-way ANOVA considering location, land use, and rootstock as main factors. Due to significant interactions among the factors, data were further analyzed at each location by a 2-way ANOVA where rootstock and land use were considered as main factors. A Duncan test was performed to find potential differences on the studied variables. All statistical analysis were carried out with IBM SPSS program (Version 26).

3. Results

3.1 Soil Characteristics

3.1.1 Physical-Chemical Properties

The physical-chemical analysis of these soils was a preliminary inquiry into how these soils were similar and different, and their implications for grapevine growth and performance. When all soils collected from three winegrowing regions and different land uses were analyzed, a significant interaction between the location and the land use was observed for the pH of the soils (Table 3A). In Bombarral and Lisbon, the soil pH did not show significant differences related to the land use (vineyard or non-agricultural soil). In both locations the pH was between 7 and 8 (Table 3B). However, in Pegões, the vineyard soil had significantly higher pH than the non-agricultural soil. In fact, this soil presented the lowest pH of 5.51 ± 0.356 (Table 3B)

In addition, location had a significant impact on electrical conductivity (Table 3A); Pegões soils had significantly lower electrical conductivity compared to Bombarral and Lisbon (Table 3B).

Table 3. (A) Results of a two-way ANOVA with p-values representing the impact of location (Bombarral, Lisbon, or Pegões), land use (non-agricultural or vineyard), and their interaction on soil pH and electrical conductivity. Significant effect: p value ≤ 0.05. (B) Data represent average values (n=3) ± standard error. Different letters for same parameter indicate a significant difference based on Duncan test (p ≤ 0.05).

(A) Factor		**pH**	**Electrical Conductivity**
Location		<0.001	<0.001
Land use		0.482	0.657
Interaction		0.005	0.434
(B) Location	**Land use**	**pH**	**Electrical Conductivity (µs/cm)**
Pegões	Vineyard Soil	6.94 ± 0.210 b	36.34 ± 5.62 b
	Non-agricultural	5.51 ± 0.356 c	29.84 ± 3.32 b
Bombarral	Vineyard Soil	7.36 ± 0.229 ab	628.90 ± 219.56 a
	Non-agricultural	7.94 ± 0.141 a	252.03 ± 62.54 a
Lisbon	Vineyard Soil	7.58 ± 0.058 ab	113.37 ± 7.06 a
	Non-agricultural	7.95 ± 0.057 a	167.67 ± 2.812 a

Concerning soil CEC, a significant interaction was observed among the two factors: location and the land use (Table 4A). In Bombarral higher CEC was found in the non-agricultural soil compared to the vineyard soil, but Pegões and Lisbon's soils did not show this difference. Lisbon soils showed the highest CEC while Pegões had the lowest CEC.

When exchangeable cations were analyzed individually, it was observed that Ca was dominant in exchangeable complex, presenting the same trend as the overall CEC. However, for exchangeable Mg, Na, K and Al, the results were slightly different (Table 4A).

Looking at the results of exchangeable Mg and K, a significant interaction was seen among location and land use, but this was not seen in Na or in Al (Table 4A). Lisbon's soils had significantly higher exchangeable Mg values, than Bombarral and Pegões (Table 4B). Differences in land use were only seen in Lisbon, where vineyard soil had significantly higher Mg than non-agricultural soil (Table 4B). Exchangeable K concentrations were maximum also in Lisbon, but the minimum values were not the same, as Pegões had the lowest exchangeable K values. Regarding land use, as for Mg, only in Lisbon non-agricultural soils had higher K levels than vineyard soils.

On the other hand, the location influenced the concentration of exchangeable Na; Lisbon had the highest, while Pegões had the lowest one. Although land use had a significant effect on exchangeable Na cation ($p=0.025$), the Duncan post-hoc test did not reveal significant differences among vineyard and non-agricultural soils.

Regarding exchangeable Al, only land use had a significant effect on its concentration (Table 4A). Overall, non-agricultural soils had higher values than vineyard soils, especially in Bombarral (Table 4B).

Table 4. (A) Results of a two-way ANOVA with p-values representing the effect of location (Bombarral, Lisbon, or Pegões), land use (non-agricultural or vineyard), and their interaction on exchangeable cations (Ca, Mg, Na, K, Al) and overall cation exchange capacity. (B) Exchangeable cation concentration in vineyard and non-agricultural soils from different locations (Bombarral, Lisbon, or Pegões). Data represent average values (n=3) ± standard error. Different letters for the same parameter indicate a significant difference based on Duncan test or Dunn test ($p \leq 0.05$).

(A) Factor		**Ca**	**Mg**	**Na**	**K**	**Al**	**Cation Exchange Capacity**
Location		<0.001	<0.001	0.005	<0.001	0.269	<0.001
Land use		0.001	0.010	0.025	0.516	0.025	0.078
Interaction		0.007	0.036	0.705	0.031	0.241	0.007
(B) Location	**Land use**	**Exchangeable cation concentration in the available fraction ($cmol_c kg^{-1}$)**					
		Ca	**Mg**	**Na**	**K**	**Al**	**Cation Exchange Capacity**
Pegões	Vineyard Soil	2.42 ± 0.447 d	0.40 ± 0.015 c	0.29 ± 0.012 c	0.13 ± 0.013 d	0.13 ± 0.022 ab	3.24 ± 0.467 d
	Non-agricultural	1.86 ± 0.515 d	0.36 ± 0.028 c	0.37 ± 0.012 bc	0.10 ± 0.005 d	0.23 ± 0.063 ab	2.68 ± 0.549 d
Bombarral	Vineyard Soil	10.22 ± 1.348 c	2.16 ± 0.095 c	0.34 ± 0.015 bc	0.68 ± 0.167 c	0.11 ± 0.005 b	13.41 ± 1.354 c
	Non-agricultural	27.16 ± 1.326 b	2.18 ± 0.768 c	0.52 ± 0.010 ab	0.33 ± 0.065 cd	0.20 ± 0.007 a	30.19 ± 0.613 b
Lisbon	Vineyard Soil	41.05 ± 3.498 a	15.08 ± 0.985 a	0.51 ± 0.028 abc	1.31 ± 0.179 b	0.12 ± 0.016 ab	57.95 ± 2.368 a
	Non-agricultural	46.64 ± 0.234 a	5.98 ± 0.122 b	0.69 ± 0.120 a	1.96 ± 0.168 a	0.12 ± 0.005 ab	55.28 ± 0.422 a

The results of soil macronutrient concentrations are presented in Table 5. Significant interactions between the location and the land use were observed in concentrations of organic C, total N and in K and Ca concentrations in the available fraction. In Lisbon, organic C concentration was 36.7% lower in vineyard soil, in Bombarral, C concentration was 71.5% lower in vineyard soil, and in Pegões, C concentration was 57% lower in vineyard soil. For total N, in Lisbon, vineyard soil had 44.4% less total N than the non-agricultural soil, and in Bombarral, total N in vineyard soil was 52.6% lower than in non-agricultural soil. In Pegões, the vineyard soil had 42.3% less N than the non-agricultural soil.

In the case of available P, land use had a significant effect on its concentrations (Table 5A). Overall, vineyard soils had higher P concentration than non-agricultural soils (Table 5B). Lisbon had a 110% higher soil available P in vineyard soils, Bombarral had 860% higher soil P in vineyard soils, and Pegões had 217% higher soil available P in vineyard soil.

Differences in soil available K concentrations between land uses were only seen in Lisbon, where available K levels in vineyard soils were 56.1% lower than non-agricultural soils (Table 5B).

However, soil available Ca concentrations showed a different pattern. Its concentrations were highest in both Lisbon soils (in vineyard and non-agricultural soils) (approximately 14 g Ca/kg), and the lowest were in Pegões (approximately 10 g Ca/kg). Differences in land use were significant in Bombarral, where non-agricultural soils had the higher values (13.75 ± 39.257 g Ca/kg vs. 11.32 ± 21.537 g Ca/kg) but in Lisbon and Pegões, both land uses were not significantly different in Ca concentration (Table 5B).

In the case of soil Mg concentrations, the location and land use both had a significant effect (Table 5A). The highest Mg values were found in Lisbon's soils (876 mg Mg/Kg, on average), while lowest ones were in Pegões (29 mg Mg/kg, on average). Although a significant effect of land use factor was observed in Mg (p-value: 0.045) (Table 5A), Duncan post-hoc test did not show differences among vineyard and non-agricultural soils in any location (Table 5B).

Available Na concentrations were not significantly impacted by either factor ($p > 0.05$) (Table 5A).

Total N and Ca were significantly positively correlated with organic C (Appendix 3B).

Table 5. (A) Results of a two-way ANOVA with p-values representing the impact of location (Bombarral, Lisbon, or Pegões), land use (non-agricultural or vineyard), and their interaction on macronutrients (C, N, P, K, Na, Ca, Mg). (B) Macronutrient concentration in available fraction in vineyard and non-agricultural soils from different locations (Bombarral, Lisbon, or Pegões). Data represent average values (n=3) ± standard error. Different letters for the same parameter indicate a significant difference based on Duncan test ($p \leq 0.05$).

(A) Factor		**Organic C**	**Total N**	**Available P**	**Available K**	**Available Na**	**Available Ca**	**Available Mg**
Location		<0.001	<0.001	0.285	0.002	0.275	<0.001	<0.001
Land Use		<0.001	<0.001	0.026	0.282	0.092	0.002	0.045
Interaction		0.016	0.027	0.114	0.047	0.548	0.001	0.508
(B) Location	**Land Use**	**Organic C (g kg^{-1})**	**Total N (g kg^{-1})**	**Available P (mg kg^{-1})**	**Available K (mg kg^{-1})**	**Available Na (g kg^{-1})**	**Available Ca (g kg^{-1})**	**Available Mg (mg kg^{-1})**
Pegões	Vineyard Soil	4.72 ± 0.638 c	0.30 ± 0.087 d	14.23 ± 1.417 bc	50.68 ± 10.069 bc	3.03 ± 67.081 ab	10.39 ± 11.077 d	31.53 ± 1.811 b
	Non-agricultural	10.98 ± 1.701bc	0.52 ± 0.036 cd	4.48 ± 0.434 c	31.06 ± 2.876 c	3.05 ± 35.856 ab	9.98 ± 27.987 d	27.19 ± 0.859 b
Bombarral	Vineyard Soil	8.44 ± 0.656 c	0.92 ± 0.106 bc	57.96 ± 15.780 a	215.27 ± 67.630 ab	3.01 ± 84.792 ab	11.32 ± 21.537 c	229.22 ± 9.938 ab
	Non-agricultural	29.60 ± 1.721 a	1.94 ± 0.288 a	6.04 ± 0.410 c	166.61 ± 55.073 bc	3.17 ± 71.676 a	13.75 ± 39.257 b	211.16 ± 6.429 ab
Lisbon	Vineyard Soil	17.58 ± 2.842 b	1.34 ± 0.043 b	43.77 ± 9.685 ab	160.40 ± 24.871 bc	2.87 ± 59.942 b	14.22 ± 29.677 ab	1303.26 ± 114.345 a
	Non-agricultural	27.78 ± 1.492 a	2.41 ± 0.139 a	20.10 ± 1.939 bc	365.20 ± 29.905 a	3.04 ± 22.905 ab	14.78 ± 38.051 a	449.24 ± 22.517 a

Concerning micronutrients, as shown in Table 6, there were significant interactions between the main factors in Mn, Cu, and Fe soil concentrations. For Mn, Lisbon and Bombarral had the highest values, approximately 37 mg Mn/kg and 43 mg Mn/kg, respectively, while Pegões had the lowest concentration (approximately 2 mg Mn/kg). Differences in land use were only observed in Lisbon, where vineyard soil had significantly higher Mn concentrations (60% higher) than the non-agricultural soil. However, for Cu, the difference between land uses was only observed in Pegões, where the vineyard soil had 60350% higher concentrations (Table 6B).

Location impacted Zn values (Table 6A), and the highest Zn concentrations were observed in Lisbon (average of 9.8 mg Zn/kg), and the lowest in Bombarral and Pegões (averages of 2.56 mg Zn/kg and 3.08 mg Zn/kg, respectively) (Table 6B). Additionally, in Lisbon, Zn concentration in the vineyard soil was 69.1% lower than non-agricultural soil. For Fe, no differences were observed between vineyard soils and non-agricultural soils. Lisbon vineyard soil had the highest levels (123.36 mg Fe/kg), while Bombarral non-agricultural soil had the lowest (42.64 mg Fe/kg).

Table 6. (A) Results of a two-way ANOVA with p-values representing the impact of location (Bombarral, Lisbon, or Pegões), land use (non-agricultural or vineyard), and their interaction on micronutrients (Mn, Cu, Zn, Fe). (B) Micronutrient concentration in available fraction in vineyard and non-agricultural soils from different locations (Bombarral, Lisbon, or Pegões). Data represent average values (n=3) ± standard error. Different letters for the same parameter indicate a significant difference based on Duncan test ($p \leq 0.05$).

(A) Factor		**Available Mn**	**Available Cu**	**Available Zn**	**Available Fe**
Location		<0.001	0.785	0.002	0.330
Land use		0.055	0.109	0.084	0.500
Interaction		0.044	0.026	0.201	0.026
(B) Location	**Land use**	**Available Mn (mg kg^{-1})**	**Available Cu (mg kg^{-1})**	**Available Zn (mg kg^{-1})**	**Available Fe (mg kg^{-1})**
Pegões	Vineyard Soil	2.03 ± 0.254 c	12.09 ± 2.384 a	3.03 ± 0.186 b	53.49 ± 11.603 ab
	Non-agricultural	1.71 ± 0.440 c	0.02 ± 0.000 b	3.13 ± 0.690 b	114.16 ± 17.439 ab
Bombarral	Vineyard Soil	43.18 ± 3.199 a	5.55 ± 0.391 ab	2.04 ± 0.100 b	72.86 ± 13.515 ab
	Non-agricultural	42.71 ± 1.145 a	9.88 ± 4.263 a	3.08 ± 0.467 b	42.64 ± 3.614 b
Lisbon	Vineyard Soil	46.27 ± 3.979 a	7.71 ± 1.357 ab	4.64 ± 0.686 b	123.36 ± 29.417 a
	Non-agricultural	28.80 ± 3.707 b	4.70 ± 1.282 ab	15.01 ± 3.301 a	56.81 ± 7.904 ab

3.1.2 Soil Biological Properties

Land use had a significant effect on both acid phosphatase and ß-glucosidase activities (p-value= 0.008 and p< 0.001, respectively) (Table 7A). In both cases, non-agricultural soils tended to have the highest enzymatic activities. However, when comparing acid phosphatase activities in each location pairwise, these differences were not obvious, as no significant differences were found between vineyard and non-agricultural soils. In the case of ß-glucosidase activities, the same pattern was observed, but in that case, in Lisbon, the difference between both land uses was significant: non-agricultural soil had 76% higher activity than vineyard soil.

Location also had a significant effect on acid phosphatase and ß-glucosidase activities (p=0.008 and < 0.001, respectively) (Table 7A). In Pegões, acid phosphatase and ß-glucosidase activities were lower (on average, 0.34 and 0.19 µmole p-Nitrophenol g^{-1} dry soil matter h^{-1}, respectively) than in Bombarral (on average, 0.725 and 0.915 µmole p-Nitrophenol g^{-1} dry soil matter h^{-1}, respectively) and Lisbon (on average, 0.94 and 1.33 µmole p-Nitrophenol g^{-1} dry soil matter h^{-1}, respectively) (Table 7B).

Regarding cellulase activity, no interaction or significant influence of land use (p=0.643, Table 7) and location (p= 0.276, Table 7) was found; all values fell in the same range: 0.24-0.56 µmole glucose g^{-1} dry soil matter $16h^{-1}$ (Table 7A, 7B). However, interactions occurred between factors in both urease and dehydrogenase activities. In Bombarral and Lisbon, urease activity was significantly higher in the vineyard soils (971% and 262% higher, respectively), but in Pegões no significant differences were found despite the vineyard soil activity being 93% higher than in the non-agricultural soil. On the other hand, regardless of the significant interaction found in soil dehydrogenase activity, no differences were found between vineyard and non-agricultural soils in any location (Table 7B).

Soil pH was positively significantly correlated with ß-glucosidase activity (R^2= .650, p= .005) (Appendix 3D). Regarding soil chemical characteristics, organic C was significantly positively correlated with ß-glucosidase activity, while also significantly negatively correlated with acid phosphatase activity (R^2= .771, p= .000; R^2= -.718, p= .001, respectively) (Appendix 3D).

Table 7. (A) Results of a two-way ANOVA with p-values representing the impact of location (Bombarral, Lisbon, or Pegões), land use (non-agricultural or vineyard), and their interaction on soil enzyme activity. (B) Soil enzyme activity in vineyard and non-agricultural soils from different locations (Bombarral, Lisbon, or Pegões). Data represent average values (n=3) ± standard error. Different letters for the same parameter indicate a significant difference based on Duncan test ($p \leq 0.05$).

(A) Factor		**Acid Phosphatase**	**ß-glucosidase**	**Cellulase**	**Urease**	**Dehydrogenase**
Location		0.008	< 0.001	0.276	< 0.001	< 0.001
Land use		0.004	0.009	0.643	< 0.001	0.129
Interaction		0.122	0.108	0.301	0.012	0.048
(B) Location	**Land use**	**Acid Phosphatase[1]**	**ß-glucosidase[1]**	**Cellulase [2]**	**Urease[3]**	**Dehydrogenase[4]**
Pegões	Vineyard Soil	0.27 ± 0.033 b	0.18 ± 0.007 c	0.24 ± 0.031a	0.23 ± 0.049 c	67.65 ± 1.269 a
	Non-agricultural	0.41 ± 0.013 ab	0.20 ± 0.043 c	0.50 ± 0.081 a	0.12 ± 0.006 c	59.39 ± 4.282 ab
Bombarral	Vineyard Soil	0.35 ± 0.059 ab	0.68 ± 0.208 bc	0.56 ± 0.152 a	2.25 ± 0.387 b	33.46 ± 4.202 c
	Non-agricultural	1.10 ± 0.420 a	1.15 ± 0.172 b	0.55 ± 0.086 a	0.21 ± 0.071 c	46.80 ± 6.746 bc
Lisbon	Vineyard Soil	0.90 ± 0.186 ab	0.96 ± 0.155 b	0.42 ± 0.060 a	15.47 ± 3.006 a	6.74 ± 1.484 d
	Non-agricultural	0.97 ± 0.044 ab	1.69 ± 0.033 a	0.32 ± 0.102 a	4.27 ± 0.478 b	19.33 ± 2.311 d

1- µmole p-Nitrophenol g^{-1} dry soil matter h^{-1}; 2- µmole glucose g^{-1} dry soil matter $16h^{-1}$; 3- µmole $N\text{-}NH_4^+$ g^{-1} dry matter $2h^{-1}$; 4- µg TPF g dry matter^{-1} $16h^{-1}$

Table 8 outlines the estimated number of mycorrhizal infective propagules per gram of soil in the six treatment groups (three locations: Pegões, Bombarral, Lisbon; and two land uses: vineyard and non-agricultural). These data showed that overall, non-agricultural soils tended to have a higher mycorrhizal propagule number than vineyard soils (Table 8), although it was not statistically significant (p-value of the student's t-test: 0.069)

In Pegões, the overall number of mycorrhizal infective propagules in both land uses was low, with 3 and 1 propagule per gram of soil in non-agricultural and vineyard soils, respectively. The highest density of mycorrhizal propagules was found in Lisbon, with 170 infective mycorrhizal propagules in the non-agricultural soil sample (Table 8).

Table 8. Number of mycorrhizal infective propagules of each soil sample that were grown in soils from different locations (Bombarral, Lisbon, Pegões) and land use (vineyard, non-agricultural). Leeks (*Alium porrum* L.) were used as the trap plant. Each soil sample was diluted from 10^{-1} to 10^{-5}. Number of mycorrhizal infective propagules (n=5 per dilution) were estimated using the of Most Probable Number Method (MPN) (Jarvis et al., 2010).

Land use	Location	Number of mycorrhizal infective propagules g^{-1}	95% Confidence Limits	
			Lower	Upper
Non-agricultural soil	Pegões	3	1.1	7.9
	Bombarral	71	22	230
	Lisbon	170	49	570
Vineyard soil	Pegões	1.1	0.33	3.4
	Bombarral	3.3	1.1	9.9
	Lisbon	15	4.6	51

3.2 Plant Performance

3.2.1 Vegetative Indexes

Concerning the three-way ANOVA of NDVI values, at every measurement date, there were significant interactions between location and land use, and on August 4th and 24th, significant interactions between location and rootstock (Appendix 2A). For this reason, a two-way ANOVA was conducted for NDVI separately at each location.

In Bombarral, one month after transplant, land use had a significant effect on NDVI, especially in plants grafted onto 1103 Paulsen, who presented higher values in vineyard soils than

in non-agricultural soils. Two months after transplant, land use factor was significant again, and plants grown in vineyard soils also tended to have higher NDVI. Afterwards, this trend disappeared. The effect of the rootstock varied along the time: one month after grapevine transplant, NDVI was higher in vines grafted onto 1103 Paulsen, but two weeks later the trend changed, and plants grafted onto 110 Richter had the highest index values. Two months after transplant, 110 Richter still had the higher NDVI values, but at end of the experiment, no significant differences were found between the rootstocks.

Looking at Lisbon soils, land use had a significant effect one and two months after transplant. In those cases, plants grown in the non-agricultural soils had higher NDVI values than the ones growing in the vineyard soils. Two weeks later, the effect of land use was not significant. But, three months after transplant, the trend completely changed and plants growing in vineyard soils had higher NDVI values than the ones in the non-agricultural soil. The effect of the rootstock was significant on July 6th and July 20th. The 110 Richter rootstock had the highest values on July 20th. Two weeks later, there was no significant effect of the rootstock, but then two and a half months after transplant (August 24th), the 110 Richter rootstock again had the higher NDVI values.

In Pegões, one month and one month and half after transplant, there were significant interactions between rootstock and land use: while NDVI was higher in plants grafted onto 110 Richer and grown in non-agricultural soils than the ones grown in vineyard soils, in plants grafted onto 1103 Paulsen, this difference was not significant. Two months and a half after transplant, land use was a significant factor, and plants grown in the non-agricultural soils had higher NDVI values than the ones in the vineyard soils. Rootstock factor was significant two months after transplant (August 4th), and 1103 Paulsen had higher NDVI values than 110 Richter rootstock. Two weeks later, the trend changed, and 110 Richter had higher NDVI values. Finally, three months after transplant, there were no significant differences between either land use or rootstock.

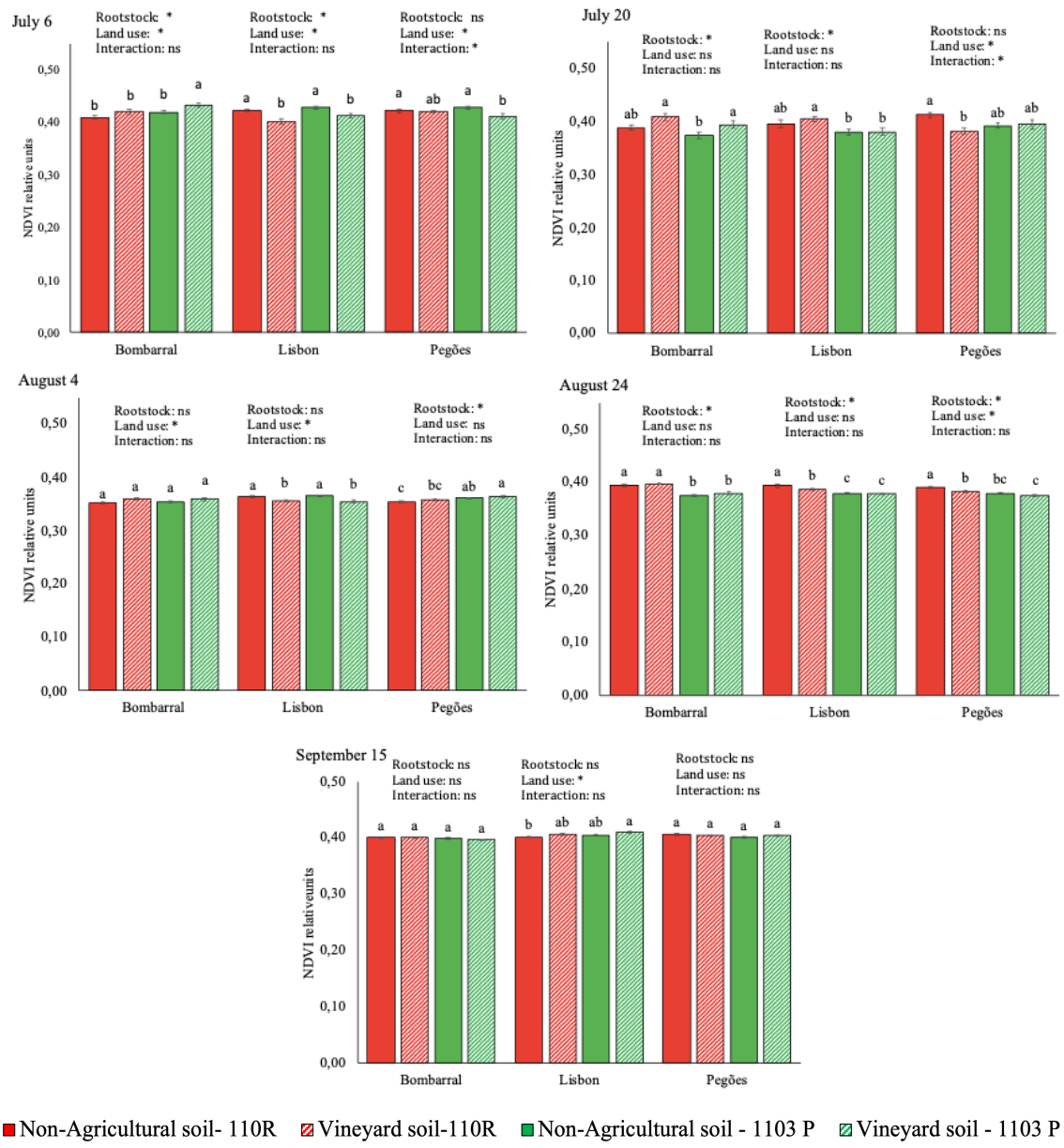

Figure 9. Normalized Difference Vegetation Index (NDVI) of grapevine Aragónez grafted onto rootstocks 110 Richter or 1103 Paulsen and grown in soils from different land use (vineyard, non-agricultural) and location (Bombarral, Lisbon, Pegões). Measurements were taken at five time points. Data represent average values (n=10) ± standard error. The significance of the effects of the two main factors (land use and rootstock) as well as of their interaction for each location is represented above the bars.
"*" indicates significant effect and "ns" indicates non-significant effect. Significant effect: p value ≤ 0.05.

One month after grapevine transplant to pots filled with vineyard and non-agricultural soils, vegetative indices were measured every 2 weeks. In the first measurement time, the location

had a significant effect on PRI values (p= 0.012) (see Appendix 2B). From this time onwards, PRI values showed significant interactions among the different factors (rootstock, location and land use) in several measuring times (see Appendix 2B), and for this reason, data were analyzed separately at each location.

In Bombarral, from July 20th to August 24th, grapevines grown in vineyard soils had significantly higher PRI values than the ones growing in non-agricultural soils (Figure 10). By the end of the experiment, in September, these differences were no longer significant. The rootstock did not have a significant effect on PRI values.

In Lisbon, land use had a significant effect from August 4th to 24th. However, the opposite trend was seen at both measuring times: non-agricultural soils had higher PRI on the 4th August, but grapevines grown in vineyard soils had higher PRI on the 24th. The rootstock had a significant effect on July 20th and August 4th, but again, the opposite trend was observed in both dates: while in the first one 110 Richter were the ones with the higher values, in the second one 1103 Paulsen were the ones with the highest values. In the following dates, the effect of the rootstock was no longer significant.

In Pegões, the effect of the land use was only significant on July 20th, and plants grown in the non-agricultural soil had higher PRI values than plants grown in the vineyard soil. This effect was especially obvious in plants grafted onto 1103 Paulsen rootstock. However, on August 4th, although the land use did not have a significant effect, PRI was higher in vines grafted onto 1103 Paulsen grown in the vineyard soil than in the non-agricultural soil. No differences were observed in plants grafted onto 110 Richter rootstock. From August 24th, differences related to the land use or to the rootstock were no longer significant.

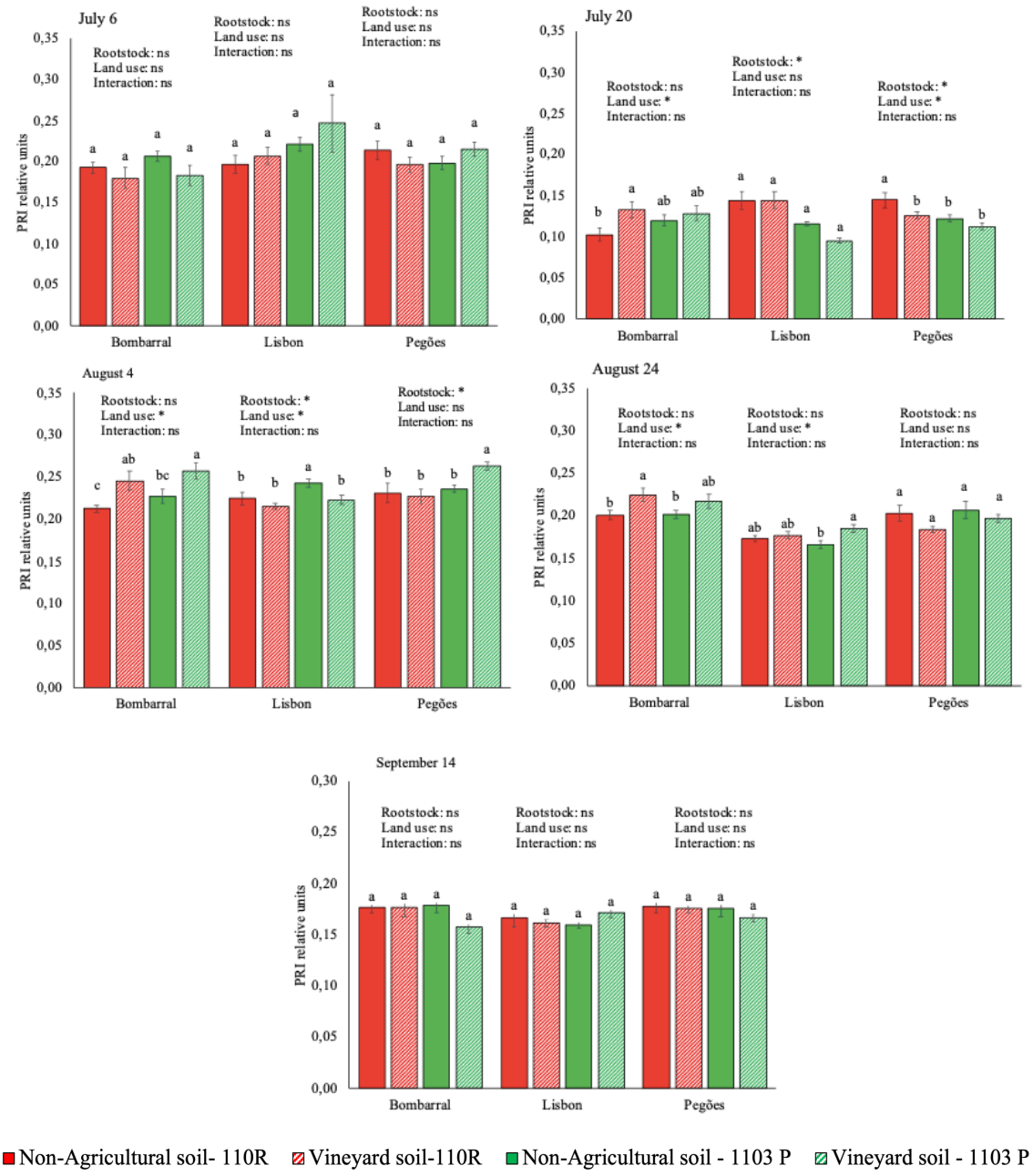

Figure 10. Photochemical Reflectance Index (PRI) of grapevine Aragónez grafted onto rootstocks 110 Richter or 1103 Paulsen and grown in soils from different land use (vineyard, non-agricultural) and location (Bombarral, Lisbon, Pegões). Measurements were taken at five time points. Data represent average values (n=10) ± standard error. The significance of the effects of the two main factors (land use and rootstock) as well as of their interaction for each location is represented above the bars.

"*" indicates significant effect and "ns" indicates non-significant effect. Significant effect: p value ≤ 0.05.

3.2.2 Shoot length, Root Biomass and Root Colonization

The shoot length of grapevines grown in Bombarral and Lisbon soils was significantly affected by the land use three months after transplant. Plants growing in the vineyard soils had higher shoot lengths (Figure 11). Besides, in plants grown in Lisbon soils, the rootstock had a significant effect. Grapevines grafted onto 110 Richter had higher shoot lengths than the ones on 1103 Paulsen (on average, 11.5 and 10.4, respectively) (Figure 11).

In Pegões soils, although non-agricultural soils tended to promote higher shoot lengths in grapevines than the vineyard soils, the effect of the land use was not significant.

Contrastingly, root biomass of grapevines grown in soils from the different locations was not affected by any of the factors (land use, rootstock, location) and no significant interactions were observed among them (Appendix 2D). Nevertheless, data were analyzed individually at each location. In grapevines grown in the soils collected from Bombarral, a significant effect of land use was observed on the root biomass. Grapevines grown in non-agricultural soil had a higher root biomass compared to those in vineyard (Rootstock 110 Richter: 9.83 ± 0.734 vs. 6.66 ±0.859; Rootstock 1103 Paulsen: 10.85 ± 1.015 vs. 8.59 ± 0.764, respectively). However, in Lisbon and Pegões, there were no significant effects for either factor.

Concerning grapevine root mycorrhizal colonization, the three-way ANOVA showed that this parameter was indeed affected by rootstock ($p \leq 0.001$). The rootstock 1103 Paulsen promoted the highest root colonization. However, due to the significant interactions between rootstock and location ($p \leq 0.050$) and between rootstock and land use ($p \leq 0.005$) (Appendix 3D), mycorrhizal colonization rate was also analyzed individually at each location.

In grapevines grown in Bombarral soils, there was an interaction between land use and rootstock. While no differences were detected for the 1103 Paulsen rootstock, plants grafted onto 110 Richter, had a higher root colonization in the non-agricultural soil.

In Lisbon soils, rootstock type had a significant effect on root colonization, where 1103 Paulsen rootstocks tended to have a higher colonization rate than 110 Richter rootstocks (Figure 11). In addition, although land use did not have a significant effect, in 110 Richter rootstock, significantly lower root colonization was found in plants grown in vineyard soils when compared to the ones in the non-agricultural soil.

In Pegões, no differences were found according to the land use. However, the rootstock factor also had a significant effect on mycorrhizal root colonization, as it happened in Lisbon's soils; grapevines grafted with 1103 Paulsen rootstocks had a higher root mycorrhizal colonization compared to 110 Richter.

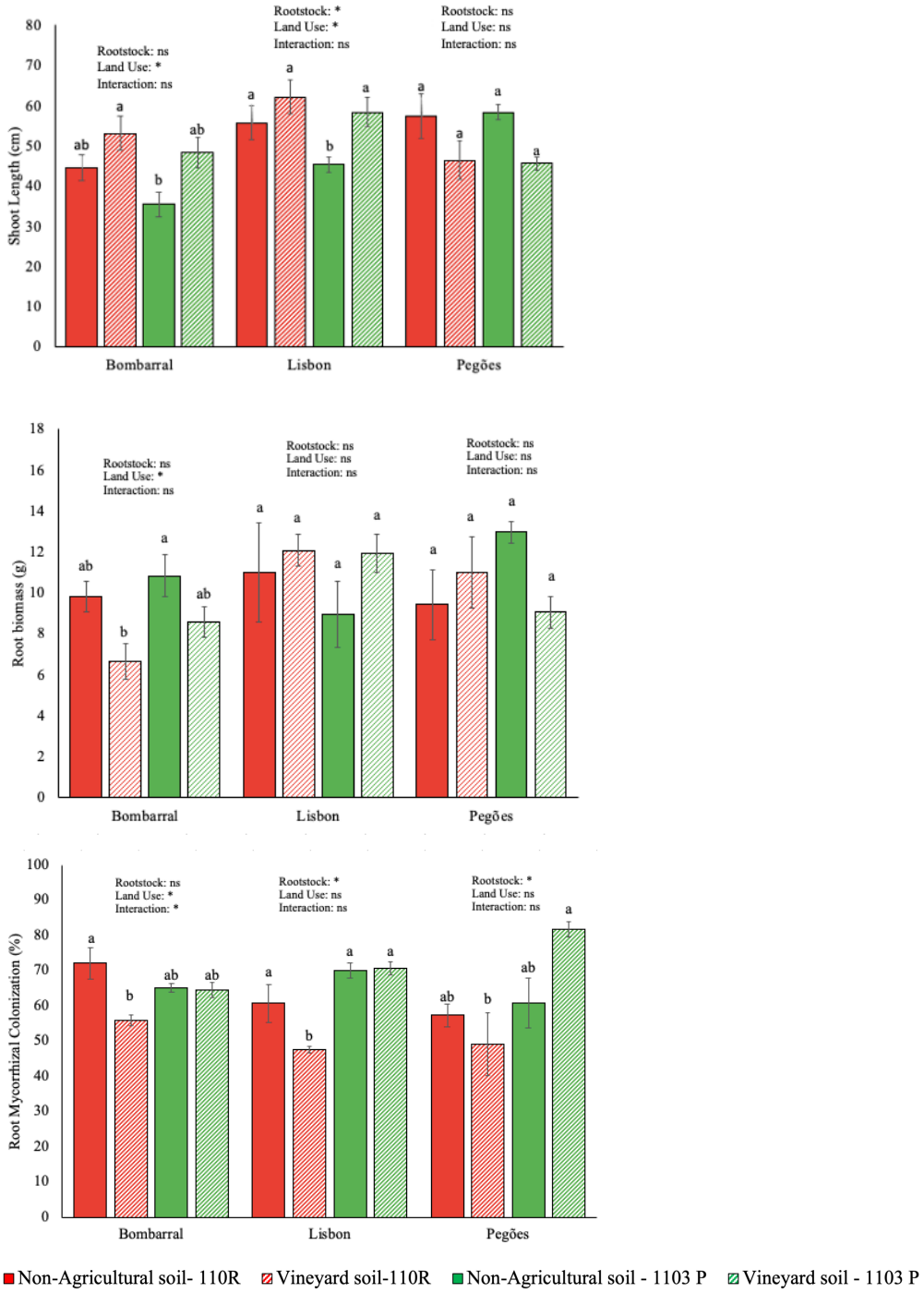

Figure 11. Shoot length, root biomass, and root mycorrhizal colonization rate of grapevine variety Aragónez grafted onto rootstocks 110 Richter or 1103 Paulsen and grown in soils from different land use (vineyard, non-agricultural) and location (Bombarral, Lisbon, Pegões). For shoot length, each bar represents the average value of 10 plants ± standard error. For root biomass, each bar represents the average value of 5 plants ± standard error. For mycorrhizal colonization, each bar represents the average value of 3

plants ± standard error. Different letters indicate significant differences between the average values at each location. The significance of the effects of the two main factors (land use and rootstock) as well as of their interaction for each location is represented above the bars.

"*" indicates significant effect and "ns" indicates non-significant effect. Significant effect: p value ≤ 0.05.

4. Discussion

4.1 Differences in Soil Properties Among Locations

Soils from the three studied locations (Lisbon, Pegões and Bombarral) differed in various physical-chemical and biological characteristics. This was most likely due to the different nature of the bedrocks of each location, on which the soils developed, as well as the vineyard management/land use over time. The soils from Pegões tended to have the lowest pH, electrical conductivity (Table 3), and cation exchange capacity (CEC) (Table 4). The low amount of organic matter (OM), assessed by the organic C concentration (Table 5) found in those soils, could have contributed to a lower CEC, which is in agreement with previous studies showing that OM and clay content are the most reliable factors to predict CEC (Manrique et al., 1991; Martel et al., 1978). Indeed, it is usually found that sandy soils, as the ones in Pegões (Nogales et al., 2019), have a smaller CEC in comparison to soils with high clay content (Binkley & Vitousek, 1989; Rashidi & Seilsepour, 2008), such as the ones found in Lisbon (Nogales et al., 2021). Future particle size analysis of fraction lower than 2 mm are needed in the collected soils to explain CEC differences in Bombarral. The soils from Pegões also had a low total N concentration, as well as low concentrations of certain nutrients (e.g. Mn, Ca and Mg) in the available fraction (Table 3B, 4B). In fact, sandy soils can to have low nutrient and organic matter content (Boul et al., 2003; Wambeke, 1992), which can often limit agricultural productivity (Yanai et al., 2005), and therefore require higher fertilization than other kinds of soils with higher nutrient levels.

4.2 Differences in Soil Properties Between Vineyard and Non-Agricultural Soils

Differences in soil properties due to the dissimilar land uses have been long studied (Brito et al., 2012; Vogt, 2000; Wander & Bollero, 1999). Varying fertilization regimes have an effect on the vineyard microbial communities (Canfora et al., 2018). The application of chemical fertilizers in the soil, in particular Nitrogen-Phosphorus-Potassium fertilizers, is commonly practiced in conventionally managed vineyards to replenish soils with the nutrients that are essential for grapevine growth (Arrobas et al., 2014). However, application of fertilizers is often targeted to meet the N needs of the plant, which may result in excessive P application to the soils, which builds up over time (Brady & Weil, 2002). This fact could explain the significantly higher

soil available P concentrations found in vineyard soils than in the non-agricultural soils (Table 5). In fact, grapevines generally have adequate soil available P levels (115-135 mg/kg), due to their narrow P requirements, and besides, the presence of AMF in their root system and phosphate solubilizing bacteria in their rhizosphere can additionally ensure that the plant gets the required P from the soil (Jackson, 2014; Khan et al., 2000; Schreiner & Osborne, 2018).

In this study it was found that the density of mycorrhizal infective propagules was low in vineyard soils (Table 8), which is in agreement with other works that showed that fertilizer inputs, especially in the case of soluble P, reduce the number of mycorrhizal propagules in vineyards (Karagiannidis & Nikolaou, 1999; Nikolaou et al., 2002).

Soil enzymatic analysis was used as indicator of soil microbial community functioning associated to nutrient cycling (Trasar-Cepeda et al., 2008). The acid phosphatase activity was also found to be lower in vineyard soils than in non-agricultural soils ($p=0.004$; Table 7). This was corroborated by Spiers & McGill, (1979), in whose work additions of P fertilizers resulted in a decrease in acid phosphatase activity. Acid phosphatases mineralize organic P and convert it into inorganic phosphate, which is further taken up by grapevine roots (Schmidt & Laskowski, 1961; Speir & Ross, 1978). Acid phosphatases are mainly derived from the microbial community of the soil, but can also be released by plant roots (Beever & Burns, 1981; Dick & Tabatabai, 1983; Estermann & McLaren, 1961). However, if P-containing fertilizers provide the plants with enough P, the activity of these enzymes is not required.

The lower organic C ($p< 0.001$, Table 5) and total N ($p< 0.001$, Table 5) found in vineyard soils in comparison to non-agricultural soils, especially in Bombarral and Lisbon, corroborates the results reported by Miguéns et al., (2007) and Richardson & King, (1995). These studies found lower total C, total N, and available P in the vineyard soils and tilled soils compared to natural soils and no-tillage soils. This may point to the degradation of the functionality of agricultural soils due to intense and prolonged agricultural activity, as discussed by Trivedi et al., (2016). In this global meta-analysis, temperate regions (including Portugal) had significantly lower total C (55 g/kg) and total N (4 g/kg) in agro-systems compared to natural soils (Trivedi et al., 2016).

Similarly, lower ß- glucosidase activity was found in vineyard soils when compared to non-agricultural soils ($p= 0.009$, Table 5), especially in Bombarral and Lisbon. In fact, a positive correlation was found between ß- glucosidase activity and organic C ($R^2 =0.771^{**}$, $p< 0.001$) (Appendix 3D). This result agrees with what is already known about this enzyme. ß- glucosidase activity derives from a glycosidase enzyme that catalyzes the hydrolysis of water-soluble di- and oligo-saccharides to release monosaccharides (Dick, 2020), and that therefore is involved in

organic matter decomposition. The simple sugars released by this enzymatic activity can be used as a source of carbon for soil microorganisms, and therefore it is often used as indicator of soil quality (Caldwell & Gri, 1999; Stege et al., 2010). It is sensitive to soil management, as observed by Caldwell & Gri, (1999) and Stege et al., (2010). Studies found lower ß- glucosidase activity levels in agricultural lands compared to natural soils with no agricultural activity (Caldwell & Gri, 1999; Miguéns et al., 2007). In particular, Miguéns et al., (2007) found that ß-glucosidase activity was smaller in the vineyard soil compared to soils with a natural, non-agricultural vegetation.

On the other hand, cellulase, an enzyme that converts disaccharides and soluble oligosaccharides into simple sugars, was found to not have any statistically significant differences in its activity among the factors land use ($p= 0.643$, Table 7) and location ($p= 0.276$, Table 7). In its simplest definition, this an enzyme which degrades cellulose, which could be lignified stems or mulch (Dick, 2020). Miguéns et al., (2007) found that cellulase activity in vineyard soils amounted to only 20% of the enzymatic activity found in natural soils, but this difference may depend on the vineyard practices (e.g. if lignified shoots are left in the soil after grapevine winter pruning or not), as well as on the vegetation type in the non-agricultural surrounding areas (e.g. dominated by woody plant species or by herbaceous plants).

In the case of urease activity in Lisbon and Bombarral, differences were observed regarding the land use, where vineyard soils had significantly higher values than the non-agricultural soils ($p< 0.001$, Table 7). This result does not agree with other results found in the literature (Giacinto et al., 2020; Miguéns et al., 2007), where conventionally managed vineyard soils had a lower urease activity in comparison with natural vineyards. Urease is produced by many microorganisms such as fungi and bacteria, and is naturally found in most soils (Zantua & Bremner, 1977). Urease catalyzes the hydrolysis of urea to carbon dioxide and ammonia, which is a form of N available for plants, and is used as an indicator of N mineralization and the release of ammonium carbonate into the soil (Dick, 2020). In non-agricultural lands, complex organic compounds from the dead plants and fauna are incorporated into the soil, which are further converted into inorganic N, which is an available form for plant uptake (Dick, 2020). Therefore, urease activity in non-agricultural soils is expected to be higher than in vineyards where there is applied available N and usually less vegetation. In the case of Lisbon, a potential reason could be the presence of interrow cover crops based on native plant species which could be contributing to decrease of N in soils and, consequently, promotion of microbial community associated to N cycle. However, currently, there is no literature studying the topic of interrow vegetation species and its influence on soil urease activity in vineyards. In the case of Bombarral, urease activity was

higher in the vineyard soil, which was maintained as a bare soil. This fact can contribute to the loss of applied N by leaching, and consequently, lead to more activity of microorganisms associated to N cycle.

Concerning micronutrients, the land use effect did not have an overall significant effect on any of the measured elements. However, in Lisbon's soils, Mn concentration was higher in the non-agricultural soil than in the vineyard soil, and Zn followed the opposite trend. Nutrient antagonism could be at play between Mn and Zn in this case, as it has been found in the study of Rietra et al., (2017), that showed 17 antagonistic interactions between nutrients in multiple crop species.

On the other hand, in the case of soil Cu in the available fraction, differences were only observed in Pegões (Table 6). At this location, the vineyard soils had higher concentrations than non-agricultural soils. There is no information in this study about the fungicides used in that location, but it is reasonable to hypothesize that Cu-based fungicides were being used on the vineyard to treat downy mildew as well as other diseases. These Cu-based fungicides have been used for centuries in the viticulture sector, which leads to an accumulation of this element in the soils over the time. Vineyard soils in Europe on average have 49.26 mg/kg of Cu (Ballabio et al., 2018), and in the Douro Valley of Portugal, total Cu varies between 17.8 and 211 mg Cu/kg (Patinha et al., 2013), while in central Portugal, total Cu varies between 58 and 130 mg Cu/kg (Magalhães et al., 1985). These values are higher than the values found in the three vineyards of this study: 12.09 ± 2.384 in Pegões, 5.55 ± 0.391 in Bombarral, and 7.71 ± 1.357 Cu/kg in Lisbon (Table 6), and therefore, Cu soil concentrations are below the average compared to other vineyards in Europe, and below the ones found in other wine growing regions in Portugal.

The density of mycorrhizal infective propagules tended to be higher in non-agricultural soils, although not statistically significant ($p= 0.06$). It is known that tillage impacts the mycelial network within the soil (Trouvelot et al., 2015). Brito et al., (2012) also highlights this fact, showing a 40% decrease in AMF diversity in a tillage ago-system compared to no-tillage. Besides, the use of some pesticides can be detrimental to AMF populations (Hage-Ahmed et al., 2019) which may explain the decrease in the number of propagules.

4.3 Grapevine Growth and Performance in Vineyard and Non-Agricultural Soils

In all three locations, mycorrhizal colonization in the plants grafted onto 110 Richter rootstock tended to be lower in vineyard soils than in the non-agricultural soils, especially in

Bombarral and Lisbon soils. Since soil P values were significantly higher in the vineyard soils of these two locations (Table 5) in comparison to their respective non-agricultural soil, and high soil phosphate concentrations are known to have a negative impact on mycorrhizal colonization (Breuillin et al., 2010; Mosse, 1973; Smith & Read, 2008), this could explain the lower root colonization found in 110 Richter rootstock.

Contrastingly, no differences in root colonization were observed in plants grafted onto 1103 Paulsen rootstocks. This could mean that this rootstock is less selective (had a lower discrimination for species of AMF) than 110 Richter, and therefore, allows higher root colonization by the AMF species of the vineyard soil, even if these can be less mutualistic AMF species, as often observed in agricultural soils (Werner & Kiers, 2015). Such a case was found where some C_4 grasses were unable to reduce colonization and formation of hyphal networks of non-mutualistic AMF and succumbed to parasitism, while some C_3 grasses successfully prevented AMF colonization by reducing their allocation of C to these species (Grman, 2012). Additionally it has been considered that some species of AMF have evolved from being dependent on their host for C to non-dependent (Estaún et al., 2010). Sequencing and identification of mycorrhizal communities could lead to more information regarding such relationships.

The rootstock also had significant impact on shoot length in grapevines grown in Lisbon and Bombarral soils. In both cases, grapevines grafted onto rootstocks 1103 Paulsen had a lower shoot development. An explanation of this result is that they are not well adapted to those soil conditions. It is considered that 1103 Paulsen is a vigorous rootstock that is more suitable for soils that present low moisture conditions (Pl@ntGrape, 2022), such is the case for sandy soils in Pegões, as they have a lower water holding capacity than clay soils. For this reason, rootstock 1103 Paulsen is recommended for use in Pegões soil. Richter 110 is a rootstock that is also adapted to be grown in drought conditions (Camprubí et al., 2008). There is no information regarding its optimal soil conditions for growing grapevine, however, it can be speculated that it is more vigorous when planted in soils with high clay content, such as Lisbon and Bombarral soil.

To determine whether soil characteristics and land uses influence grapevine vegetative development, Aragónez grapevine's physiological status and vigor was assessed by the measurement of vegetative indices. Normalized Difference Vegetation Index is a parameter of plant vigor and physiology as well as an indirect measurement of leaf chlorophyll status and P and N contents (Sembiring et al., 1998).

For grapevines grown in Lisbon's soils, the NDVI values one month and two months after transplant tended to be lower in plants grown in vineyard soils when compared to the values

obtained from the ones grown in the non-agricultural soil. The vineyard soils of that location had lower total N concentration than non-agricultural soils (44.4% less N), which could explain the observed NDVI values. Nitrogen is easily absorbed by plants in the form of NO_3 and NH_4 and is very mobile within the plant. It aids in plant root and shoot growth as well as in chlorophyll synthesis (Crouse, 2018; Hossain & Hamid, 2008). In fact, chlorophyll content in leaves is what it is indirectly assessed by NDVI measurements, and therefore, it is not surprising that plants grown in soils with high N content have higher chlorophyll content and yield higher NDVI values.

Grapevines grown in Pegões soils followed the same trend, with overall lower NDVI values in plants grown in vineyard soils compared to the non-agricultural soils. Total nitrogen content was also 42.3% lower in vineyard soils, which can explain the lower NDVI values when compared to plants grown in the non-agricultural soil.

Nevertheless, even if in Bombarral soil, N concentration was also 52.6% lower in the vineyard soil that in the non-agricultural soil, NDVI values presented the opposite trend as in the other two locations. From July 6th to July 20th (one month to one month and a half after grapevine transplant), grapevines that grew in vineyard soils had higher NDVI values. In that case, this could be explained by soil the P concentrations found in those soils, which were 9.6 times higher in the vineyard soil than in the non-agricultural soil (57.96 ± 15.780 versus 6.04 ± 0.410, respectively, Table 5). This could have contributed to a better P nutrition in plants grown the vineyard soil from Bombarral, and consequently a higher chlorophyll concentration, which may have reflected in higher NDVI values. Like N, P is also a necessary element to produce chlorophyll (Fredeen et al., 1990).

Photochemical Reflectance Index (PRI) measurements are based on the reflectance of chlorophylls and xanthophylls, which have the role of protecting against harmful, non-photochemical light (Filella et al., 2009). Photochemical Reflectance Index can be used as a useful indicator of abiotic stress (e.g. water stress) in plants (Ballester et al., 2018; Kohzuma et al., 2021; Thenot et al., 2002).

In Bombarral, during three consecutive measuring dates (July 20th, August 4th, August 24th), land use had a significant effect on PRI, and plants grown in vineyard soils tended to have higher values (Figure 6). As it occurred for NDVI, the big difference in soil available P concentrations between vineyard and non-agricultural soils found in that location (9.6 times higher concentration) may be also responsible for the higher PRI values observed in those grapevines (Table 5B). Phosphorus is known to play a role in plant root development (Sharma & Yadav, 1997), plant growth and reproduction (Brady & Weil, 2008), and for contributing to plants stress management for factors such as drought, high temperatures, and disease (Brady &

Weil, 2002). Therefore, it is reasonable to find higher PRI values in plants grown in soils with high P concentrations.

In the case of plants grown in Pegões and Lisbon's soils, no clear pattern was observed on PRI data. For example, the effect of land use in Lisbon was significant on August 4th and on August 24th, but the opposite trend was observed in both measuring dates: while in the first case PRI values were higher in grapevines grown in the non-agricultural soils, in the second case, PRI values were higher in plants grown in the vineyard soil (Figure 6). Factors such as fluctuating air temperature or soil humidity at measuring dates, which may all affect the reflectance of xanthophyll pigments, and therefore also PRI values, may have masked the effect of the experimental factors (rootstock and land use) (Ferrini et al., 1995; Xu & Zhou, 2011).

Concerning growth parameters, plants grown in vineyard soils from Bombarral and Lisbon had significantly higher shoot length than plants grown in the non-agricultural soil ($p= 0.047$ and $p= 0.014$, respectively). The higher shoot development of those plants in both locations may be also related to higher P concentration in the soil, and in the case of Bombarral, maybe also be due to a higher soil Mn content, which is an element necessary for photosynthesis (Brady & Weil, 2008), that could have also contributed to higher shoot development.

On the other hand, despite shoot length being higher, root biomass was lower in plants grown in the Bombarral vineyard soil than in the ones from the non-agricultural soil (Figure 7). In these soils, grapevines invested more in shoot growth than in root growth. The shoot to root ratio gives information on the soil nutrient and water conditions as well as above ground conditions, such as sunlight, humidity, and temperature (Ericsson, 1995; Mullins et al., 1992). Plants that invest more in the aerial development than on root development could have an adequate mycorrhizal colonization and do not require root development for water or nutrient acquisition (Trouvelot et al., 2015). Additionally, the scion and rootstock species can influence the extent of root development (Daulta & Chauhan, 1980).

5. Conclusion

All three winegrowing regions of this study showed differences in soil physical-chemical and biological properties. However, the key point of this study is the differences in soil physical-chemical and biological characteristics that were tied to the land use. In comparison with non-agricultural soils, conventionally managed vineyards tended to have lower levels of organic C, total N, acid phosphatase activity, ß- glucosidase activity, and mycorrhizal infective propagule density.

Accordingly, the performance of Aragónez grapevines varied among soil locations and land uses. The NDVI reflectance index showed to be a more reliable index to assess plant status, as PRI fluctuated more over the time. Grapevines grown in the vineyard soils from Lisbon and Pegões tended to have lower NDVI values than the ones grown in the non-agricultural soils which is in accordance with the working hypothesis. In contrast, the vineyard soil from Bombarral led to plants with better performance than those grown in non-agricultural soil, in terms of NDVI and shoot length. The high P content of this soil may explain the higher shoot development.

Based on the data in this study, it can be concluded that vineyard soils may result in lower performance in Aragónez *cv.* grapevines due to the poorer soil physical-chemical conditions, them being organic C and total N. Nevertheless, in some vineyard soils, the higher P content can compensate for the expectable growth and fitness depression. In addition, excessive concentrations of this element in the soil can be detrimental to AMF communities, and therefore, plants couldn't profit from the additional benefits that these symbionts may provide to their hosts in terms of biotic and abiotic stress tolerance.

A deeper study of the microbial communities in vineyard and surrounding non-agricultural soils and their relation to grapevine growth and nutrition could help identify which mutualistic microorganisms are crucial for grapevine performance. Furthermore, additional analysis of the taxa composing these communities would be useful as it could provide more information regarding species diversity and richness. In this manner, it would be possible in the future to determine which soil management practices should be conducted to preserve them and harness their benefits for grapevines and soil health.

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7. Appendix

Appendix 1. Coordinates of each soil collected at the three locations: Pegões, Bombarral, and Lisbon.

Location	**Coordinate Location 1**	**Coordinate Location 2**	**Coordinate Location 3**
Pegões Vineyard	38º64'66.9"N 8º 64' 33.7"W	38º64'76.0"N 8º 64' 48.1"W	38º.64'93.9" N -8º 64’ 39.2" W
Pegões Non-Agricultural	38º65'04.1"N 8º 64' 53.0"W	38º65'08.7" 8º 64'50.6" W	38º65'08.8" 8º 64’50.3" W
Bombarral Vineyard	39°18'38.0"N 9°12'37.0"W	39°18'38.9"N 9°12'36.4"W	39°18'39.7"N 9°12'37.0"W
Bombarral Non-Agricultural	39°18'34.0"N 9°12'34.3"W	39°18'33.1"N 9°12'37.6"W	39°18'35.1"N 9°12'35.7"W
Lisbon Vineyard	38°42'30.8"N 9°11'14.5"W	38°42'31.4"N 9°11'13.6"W	38°42'33.3"N 9°11'12.6"W
Lisbon Non-Agricultural	38°42'39.5"N 9°11'15.8"W	38°42'38.3"N 9°11'17.1"W	38°42'35.9"N 9°11'15.4"W

Appendix 2. Results of a three-way ANOVA with P-values representing the impact of rootstock, soil use, location, and their interaction on A) Normalized difference vegetation index (NDVI), B) Photochemical reflectance index (PRI), C) Shoot length and D) Root biomass E) Mycorrhizal colonization rate. Significant effect: p value ≤ 0.05.

A) Normalized difference vegetation index

Factor	**P values**				
	July 6	July 20	August 4	August 24	September 15
Rootstock	0.004	0.001	0.039	0.000	0.302
Location	0.171	0.541	0.073	0.006	0.091
Land use	0.019	0.356	0.768	0.066	0.169
Rootstock*Location	0.031	0.223	0.046	0.006	0.621
Rootstock*Land use	0.633	0.283	0.304	0.091	0.718
Location*Land use	0.000	0.002	0.000	0.001	0.205
Rootstock*Location*Land use	0.115	0.079	0.930	0.739	0.118

B) Photochemical reflectance index

Factor	P values				
	July 6	July 20	August 4	August 24	September 15
Rootstock	0.097	0.018	0.001	0.576	0.302
Location	0.012	0.124	0.055	0.000	0.091
Soil Use	0.257	0.773	0.034	0.137	0.169
Rootstock*Location	0.603	0.000	0.758	0.440	0.621
Rootstock*Soil Use	0.105	0.093	0.568	0.485	0.718
Location*Soil Use	0.105	0.071	0.000	0.001	0.205
Rootstock*Location*Soil Use	0.257	0.576	0.130	0.425	0.118

C) Shoot length

Factor	P values
	September 15
Rootstock	0.056
Location	0.014
Land use	0.152
Rootstock*Location	0.518
Rootstock*Land use	0.281
Location*Land use	0.004
Rootstock*Location*Use	0.719

D) Root biomass

Factor	P values
Rootstock	0.619
Location	0.117
Land use	0.434
Rootstock*Location	0.392
Rootstock*Land use	0.578
Location*Land use	0.056
Rootstock*Location*Use	0.107

E) Mycorrhizal colonization rate

Factor	P values
Rootstock	0.001
Location	0.798
Land use	0.360
Rootstock*Location	0.050
Rootstock*Land use	0.003
Location*Land use	0.113
Rootstock*Location*Use	0.506

Appendix 3. Pearson correlations of chosen factors A) plant parameters, B) soil macro and micronutrients, C) soil enzyme activity, D) CEC, pH, organic C, total N, and soil enzyme activity. R^2 values represent the strength of the correlation between the two factors (-1 to 1).
Significant effect, Sig ≤ 0.05.

A)

		Mycorrhizal Colonization	NDVI	PRI	Shoot length	Root biomass
Mycorrhizal Colonization	Pearson Correlation	1	-0.029	-0.211	0.110	0.009
	Sig. (2-tailed)		0.867	0.216	0.525	0.961
	N	36	36	36	36	36
NDVI	Pearson Correlation	-0.029	1	0.004	0.001	0.355**
	Sig. (2-tailed)	0.867		0.970	0.990	0.006
	N	36	116	115	114	58
PRI	Pearson Correlation	-0.211	0.004	1	0.065	-0.175
	Sig. (2-tailed)	0.216	0.970		0.493	0.188
	N	36	115	115	114	58
Shoot length	Pearson Correlation	0.110	0.001	0.065	1	-0.018
	Sig. (2-tailed)	0.525	0.990	0.493		0.892
	N	36	114	114	114	58
Root biomass	Pearson Correlation	0.009	0.355**	-0.175	-0.018	1
	Sig. (2-tailed)	0.961	0.006	0.188	0.892	
	N	36	58	58	58	58

**. Correlation is significant at the 0.01 level (2-tailed).

B)

		Organic C	Total N	P	Fe	Mn
Organic C	Pearson Correlation	1	0.899**	-0.100	-0.038	0.474
	Sig. (2-tailed)		0.000	0.703	0.886	0.054
	N	17	17	17	17	17
Total N	Pearson Correlation	0.899**	1	0.002	-0.179	0.568*
	Sig. (2-tailed)	0.000		0.994	0.492	0.017
	N	17	17	17	17	17
P	Pearson Correlation	-0.100	0.002	1	0.507*	0.499*
	Sig. (2-tailed)	0.703	0.994		0.038	0.041
	N	17	17	17	17	17
Fe	Pearson Correlation	-0.038	-0.179	0.507*	1	0.130
	Sig. (2-tailed)	0.886	0.492	0.038		0.618
	N	17	17	17	17	17
Mn	Pearson Correlation	0.474	0.568*	0.499*	0.130	1
	Sig. (2-tailed)	0.054	0.017	0.041	0.618	
	N	17	17	17	17	17
Zn	Pearson Correlation	0.549*	0.661**	-0.102	-0.019	0.148
	Sig. (2-tailed)	0.023	0.004	0.696	0.941	0.572
	N	17	17	17	17	17
Cu	Pearson Correlation	0.036	0.083	-0.035	-0.229	0.175
	Sig. (2-tailed)	0.892	0.750	0.895	0.377	0.502
	N	17	17	17	17	17
Ca	Pearson	0.778**	0.86	-0.047	-0.166	0.656*

	Correlation		1**			*
	Sig. (2-tailed)	0.000	0.000	0.858	0.524	0.004
	N	17	17	17	17	17
Mg	Pearson Correlation	0.332	0.346	0.380	0.477	0.640**
	Sig. (2-tailed)	0.193	0.174	0.133	0.053	0.006
	N	17	17	17	17	17
Na	Pearson Correlation	0.089	0.057	-0.166	-0.459	-0.201
	Sig. (2-tailed)	0.733	0.827	0.524	0.064	0.438
	N	17	17	17	17	17
K	Pearson Correlation	0.564*	0.781**	0.253	-0.134	0.572*
	Sig. (2-tailed)	0.018	0.000	0.327	0.609	0.016
	N	17	17	17	17	17

C)

		Urease	Dehydrogenase	ß- Glucosidase	Cellulase	Acid Phosphatase
Urease	Pearson Correlation	1	-0.889**	0.575*	-0.040	-0.317
	Sig. (2-tailed)		0.000	0.016	0.880	0.215
	N	17	17	17	17	17
Dehydrogenase	Pearson Correlation	-0.889**	1	-0.683**	-0.038	0.508*
	Sig. (2-tailed)	0.000		0.003	0.885	0.038
	N	17	17	17	17	17
ß- Glucosidase	Pearson Correlation	0.575*	-0.683**	1	0.126	-0.657**
	Sig. (2-tailed)	0.016	0.003		0.629	0.004
	N	17	17	17	17	17
Cellulase	Pearson Correlation	-0.040	-0.038	0.126	1	-0.399
	Sig. (2-tailed)	0.880	0.885	0.629		0.113
	N	17	17	17	17	17
Acid Phosphatase	Pearson Correlation	-0.317	0.508*	-0.657**	-0.399	1
	Sig. (2-tailed)	0.215	0.038	0.004	0.113	
	N	17	17	17	17	17

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

D)

		Urease	Dehydrogenase	ß-Glucosidase	Cellulase	Acid Phosphatase
Urease	Pearson Correlation	1	-.889**	.575*	-.040	-.317
	Sig. (2-tailed)		.000	.016	.880	.215
	N	17	17	17	17	17
Dehydrogenase	Pearson Correlation	-0.889**	1	-.683**	-.038	.508*
	Sig. (2-tailed)	0.000		.003	.885	.038
	N	17	17	17	17	17
ß-Glucosidase	Pearson Correlation	0.575*	-.683**	1	.126	-.657**
	Sig. (2-tailed)	0.016	.003		.629	.004
	N	17	17	17	17	17
Cellulase	Pearson Correlation	-0.040	-.038	.126	1	-.399
	Sig. (2-tailed)	0.880	.885	.629		.113
	N	17	17	17	17	17
Acid Phosphatase	Pearson Correlation	-0.317	.508*	-.657**	-.399	1
	Sig. (2-tailed)	0.215	.038	.004	.113	
	N	17	17	17	17	17
pH	Pearson Correlation	0.574*	-.525*	.650**	-.120	-.184
	Sig. (2-tailed)	0.016	.030	.005	.647	.479
	N	17	17	17	17	17
Total N	Pearson Correlation	0.506*	-.585*	.855**	-.021	-.629**

	Sig. (2-tailed)	0.038	.014	.000	.936	.007
	N	17	17	17	17	17
Organic C	Pearson Correlation	0.315	-.457	.771**	.085	-.718**
	Sig. (2-tailed)	.218	.065	.000	.745	.001
	N	17	17	17	17	17
CEC	Pearson Correlation	.792**	-.857**	.825**	.036	-.585*
	Sig. (2-tailed)	.000	.000	.000	.892	.014
	N	17	17	17	17	17