

Evaluation of rice straw and eucalyptus leaves mulching for weed management in vineyards

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Resumo alargado

A vinha (*Vitis vinifera* L.) é uma cultura característica de Portugal, da Europa mediterrânica e na Europa em geral, e com grande importância socioeconómica em todo o mundo. De acordo com dados de 2020 da European Commission Statistics, Portugal é o décimo maior produtor de vinho do mundo, e o quinto maior da Europa, com aproximadamente 0,19 milhões de hectares da Superfície Agrícola Utilizada dedicados a esta cultura.

Nas linhas e entrelinhas das vinhas, desenvolve-se vegetação espontânea mais ou menos diversa, consoante o historial de gestão do solo, as condições edafoclimáticas e a biogeografia. Em particular, a flora associada à vinha, quando afeta negativamente a cultura por competição ou alelopatia, quer seja na quantidade quer seja na qualidade do produto, e se esses prejuízos são superiores ao balanço dos custos do controlo e benefícios para os ecossistemas, é reconhecida como flora infestante ou ervas-daninhas. Para além de competir por água, espaço e nutrientes, as infestantes também podem afetar as vinhas indiretamente, podendo ser hospedeiras de doenças, especificamente causados por vírus e fungos, e pragas, tais como ácaros tetraníquidos e outras pragas polifágicas.

O controlo da vegetação espontânea das vinhas é uma atividade geralmente imprescindível na Europa Mediterrânica, e existem vários métodos para o fazer. Idealmente, a melhor forma de gestão do solo das vinhas será a que permite a presença da flora espontânea em condições que permitam aproveitar pelo menos uma parte dos seus aspetos úteis e evitar os seus aspetos desfavoráveis.

A gestão de infestantes nas vinhas da Europa mediterrânica nos últimos 40 anos, tem consistido principalmente na aplicação de herbicidas (incluindo glifosato) na linha, e/ou mobilização e mobilização ou corte da vegetação nas entrelinhas. A utilização generalizada de herbicidas deve-se ao facto de que a sua aplicação requerer geralmente menos custos e menos mão-de-obra humana. A utilização de herbicidas pode aumentar a produtividade, mas tem demonstrado ser prejudicial para a saúde e sustentabilidade dos agroecossistemas. Neste âmbito, o Plano Nacional de Acção para a Utilização Sustentável dos Produtos Fitofarmacêuticos (2018-2023) fomenta as boas práticas e promove a agricultura biológica e a produção integrada, de modo a minimizar os efeitos prejudiciais da utilização destes produtos. Existem também várias alternativas aos herbicidas para o controlo de infestantes da vinha, incluindo métodos físicos,

biológicos e culturais. No primeiro grupo (métodos físicos), a lavoura é dispendiosa e pode ter efeitos negativos na fertilidade do solo e na promoção da erosão; a monda térmica envolve elevados custos de equipamento e grande consumo em água, para além de não ser muito eficaz em espécies vivazes ou perenes. Nos métodos biológicos para controlo da vegetação em vinha têm sido utilizados animais, por exemplo rebanhos de ovelhas, mas geralmente necessitam de ser integrados com outros métodos.

O uso de coberturas do solo em vinhas tem sido equacionado, sobretudo para aplicação nas entrelinhas. A cobertura do solo da vinha pode ser morta ou inerte (*mulching*) ou por relvamento natural ou semeado (*cover crops*). Nas coberturas inertes inserem-se as telas de plástico ou de produtos biodegradáveis e coberturas orgânicas. As coberturas mortas orgânicas podem ser conseguidas à base de materiais como palha de gramíneas ou restos de culturas, lenha de poda destrozada, serradura, feno, composto, folhas e casca de árvores, entre outras. Os seus benefícios incluem protecção contra variações de temperatura, redução da evapotranspiração, melhoria da estrutura física do solo, e da saúde microbiana.

Face aos cenários previstos de alterações climáticas para a bacia Mediterrânica, com aumento de temperatura média e precipitação mais reduzida e irregular, eventos extremos mais frequentes, como ondas de calor, o uso de mulches poderá ser um método com o duplo objetivo de reduzir a temperatura na vinha e as perdas de água e controlar as infestantes. A utilização de folhas de eucalipto como material para *mulching* tem atraído a atenção de investigadores, devido à elevada presença de produtos químicos e óleos alelopáticos, o que as torna um possível bioherbicida. Outros materiais, como a palha de arroz, com baixo conteúdo em lenhina e celulose e uma boa capacidade de evitar a imobilização de nutrientes do solo e de resistir à degradação por apodrecimento têm sido estudados como mulches em várias culturas, incluindo a vinha. No entanto, em Portugal, desconhecem-se estudos do uso de mulches de palha de arroz ou de resíduos da exploração florestal de eucalipto.

O principal objectivo deste estudo é avaliar a eficácia de duas coberturas orgânicas (palha de arroz, RS, e folhas de eucalipto, EL) no controlo de infestantes na linha e entrelinha de vinhas em Portugal. O ensaio experimental foi estabelecido em março de 2022 em duas vinhas do *campus* do Instituto Superior de Agronomia (ISA), Lisboa e duas vinhas na Quinta do Pinto, Merceana-Torres Vedras (QDP), em parcelas onde estão instaladas castas 'Alvarinho' e 'Syrah', em ambos os locais. Foi colocada palha de arroz (0,25 cm de espessura) e folhas de eucalipto (0,15 cm de espessura) na linha e entrelinha de vinhas em 3 parcelas (correspondendo a 3 repetições) com ca. 4-5

metros x 2,5 metros. A modalidade de controlo corresponde ao tratamento usual para a totalidade da vinha. Os dados florísticos (abundância e fenologia) foram recolhidos nas várias parcelas na primeira semana de cada mês desde abril até setembro de 2022. Os dados (n=216 inventários florísticos) foram organizados em folhas de cálculo do software Microsoft Excel. Foi calculada a soma da cobertura das espécies, a cobertura média, frequência absoluta e relativa, e o Índice de Valor de Importância (IVI) para cada repetição, globalmente e por local e/ou casta ('Alvarinho', Syrah) e por tipo de tratamento. Espécies com IVI superior a 45 foram caracterizadas quanto à possibilidade de fornecer um determinado serviço ecossistémico. Foram realizadas análises de variância (ANOVA) e testes pos-hoc, no sentido de analisar se existem diferenças significativas nos vários parâmetros calculados entre modalidades, castas e locais. Foi realizada uma contabilização dos custos associados às modalidades em estudo.

Durante os seis meses de duração do ensaio foram inventariados 59 taxa, pertencentes a 24 famílias, das quais as Poaceae, Asteraceae e Geraniaceae são as mais representadas em número de taxa. As espécies dominantes (mais frequentes e abundantes) foram *Convolvulus arvensis*, *Avena sterilis*, *Hordeum murinum*, *Picris echioides*, *Calendula arvensis*, *Bromus diandrus*. Os resultados da ANOVA mostraram não haver diferença significativa entre a riqueza e abundância de espécies entre EL e C, com a modalidade RS a garantir uma riqueza e cobertura reduzida ao longo dos seis meses de observações e diferente das outras modalidades (Média da riqueza <2; média da Soma das coberturas <55). A modalidade RS manteve uma dominância elevada no ISA e QDP, com sucesso no controlo da maior parte das espécies à exceção da *C. arvensis*, que ocorre, mas com significativamente menor abundância que em C. EL e C revelaram uma maior diversidade Shannon-Wiener e equitabilidade, mas também maior riqueza e abundância com diferenças significativas entre meses, com maio a ter a maior diferença entre RS e EL/C. Há diferenças pontuais na composição florística entre ISA e QDP e entre as parcelas Syrah e Alvarinho, não obstante as conclusões sobre os tratamentos manterem RS como o tratamento com maior sucesso no controlo da vegetação infestante. No entanto, em relação à RS, a *Convolvulus arvensis* foi a única espécie com IVI > 45. Muitas das espécies presentes nas parcelas em ensaio contribuem para o bem estar humano, providenciando vários serviços dos ecossistemas e nas várias categorias. Por exemplo, *Convolvulus arvensis* tem propriedades medicinais e presta serviço de regulação e manutenção (polinização e hospedeira de fauna auxiliar).

Relativamente aos custos dos tratamentos, foi evidente que as modalidades de mulching são mais onerosas que a modalidade de controlo. Os custos associados à palha de arroz são superiores ao mulching de folhas de eucalipto, devido ao custo do material e à distância entre a disponibilidade de material e as vinhas em estudo. A análise realizada foi apenas indicativa, uma vez que o procedimento não foi otimizado, podendo os custos ser muito inferiores. Por outro lado, não está ainda estudada a longevidade dos mulchings à base de EL e RS e se será necessário adicionar material para garantir o controlo da vegetação. No entanto, foi evidente com este trabalho, que a modalidade EL será a mais sustentável para os ecossistemas e mais viável economicamente, necessitando provavelmente de uma maior espessura de material para melhorar o controlo de algumas infestantes, como *C. arvensis*, *Hordeum murinum* e *Avena sterilis*.

Na sequência deste trabalho, seria interessante estudar outros benefícios do *mulching*, como o efeito na redução da temperatura do solo e das perdas de água, bem como a melhoria das condições do solo em relação à vida microbiana e à fertilidade. A análise da composição dos mulching será também uma linha de investigação que poderá trazer conhecimento sobre o efeito das folhas de eucalipto e também da palha na quantidade e qualidade da uva. Finalmente, estudos de novos produtos como telas à base de fibras e óleos essenciais de eucalipto conjugados com palhas poderão resultar em soluções intermédias com boas potencialidades no controlo da vegetação e na regulação climática.

Palavras-chave: indicadores de diversidade, coberturas orgânicas, infestantes das vinhas, alternativas a herbicidas, gestão do solo.

Resumo

As vinhas (*Vitis vinifera* L.) são culturas características das paisagens rurais portuguesas, de grande relevância socioeconómica na Europa mediterrânica e no mundo. As linhas e entrelinhas das vinhas suportam, geralmente, um grande e diverso número de plantas que frequentemente necessitam de ser controladas, devido à interferência com a cultura, ou por serem refúgio de pragas e agentes fitopatogéneos. O principal objetivo deste estudo é a avaliação do potencial de dois tipos de coberturas orgânicas mortas (0,25 cm palha de arroz, RS; 0,15 cm folhas e ramos de eucaliptos, EL) para controlo de infestantes das vinhas. O ensaio experimental teve lugar em duas

vinhas do *campus* do Instituto Superior de Agronomia (ISA), Lisboa e em duas vinhas na Quinta do Pinto, Torres Vedras (QDP) das castas 'Alvarinho' e 'Syrah' em ambos os locais, com três réplicas. A gestão do solo usual no ISA/QDP foi usada como Controlo (C). Foram realizados inventários florísticos mensais (n=216) desde março a setembro de 2022 e calculados indicadores de abundância, frequência e diversidade. Efetuou-se a comparação por estatística descritiva e por ANOVA entre locais, observações mensais e tratamentos. Observaram-se 59 taxa florísticas pertencentes a 24 famílias. A espécie mais frequente e abundante foi *Convolvulus arvensis*. RS foi significativamente diferente de EL e C com reduzida riqueza específica, diversidade e indicadores de abundância. RS teve maior valor de dominância devido à prevalência de *C. arvensis*. Detetaram-se diferenças florísticas entre locais e estações do ano. Os maiores custos foram com RS, seguidos de EL e de C. No entanto, estes custos podem ser otimizados e devem ser incluídos os benefícios ecológicos das coberturas. Assim, EL foi considerada a opção mais sustentável para a gestão do solo. Estudos futuros deverão incluir a análise química das coberturas, análises biológicas e minerais do solo e testes de longevidade das coberturas no campo.

Palavras-chave: alternativas a herbicidas, coberturas orgânicas, indicadores de diversidade, infestantes das vinhas, gestão do solo.

Abstract

Vineyards (*Vitis vinifera* L.) are typical of Portuguese rural landscapes and a crop with high socioeconomic relevance across Mediterranean Europe and the world. Vineyard rows and inter-rows support usually large and diverse plant communities that frequently need to be controlled due to the interference with the vines, or indirectly as a refuge for pests and disease agents. The main objective of this study is to evaluate the potential of two organic mulches (0,25 cm rice straw, RS, 0,15 cm eucalyptus leaves, EL) to control vineyard weeds. The experimental trial took place at two vineyards of the ISA *campus*, Lisbon and two vineyards in Quinta do Pinto, Torres Vedras (QDP), in 'Alvarinho' and 'Syrah' varieties in both locations, with three replicates. The usual soil management of ISA/QDP was used as Control (C). Floristic surveys (n=216) were carried out from March to September 2022 and indicators of abundance, frequency, and diversity were calculated and compared by descriptive statistics and ANOVA between locations,

monthly observations, and treatments. We found 59 taxa from 24 families. The most frequent and abundant species was *Convolvulus arvensis* which prevail in all treatments, locations and monthly observations. RS was significantly different from EL and C with lower species richness, diversity and abundance indicators. RS had higher dominance values due to *C. arvensis* prevalence. There were some floristic differences between locations and seasons, but the difference between RS and EL/C was maintained for all indicators. RS followed by EL had much higher costs than C, however, costs were not optimized, and the beneficial effects of weeds must be considered. Therefore, EL was considered the most sustainable option for soil management. Future research should include mineral and biological soil analysis, tests for the longevity and the benefits of mulching in the field, and analysis of the mulch composition.

Keywords: diversity indicators, organic mulching, vineyard weeds, alternatives to herbicides, soil management.

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1. Introduction

Vineyards are characteristic of the Mediterranean Basin and Europe in general, accounting for 3.2 million hectares of vineyards in the European Union (European Commission Statistics, 2020). Approximately 0.19 million hectares of Portugal's Utilized Agricultural Area (SAU) are devoted to vineyards (INE, 2019). Portugal is the tenth-largest wine producer in the world, and the fifth-largest in Europe, having produced around 6.37 million hectoliters during the 2019/2020 harvest season (IVV, 2021a).

In vineyards, floral communities consist of a variety of plants that develop in the rows and between the rows and are influenced by soil management measures. Specifically, these plant communities are referred to as weeds when they negatively affect the quality or quantity of agricultural products or have other direct or indirect negative effects on agroecosystems (Portugal *et al.*, 2017). Most conventional vineyards' soil can be divided into two distinct zones: (i) the under vine or row, where weed management is essential because weeds can compete with the vines for space, water, and nutrients; and (ii) the inter-row, where herbaceous vegetation (natural or planted) can be maintained to provide physical protection to the soil, stabilizing and reducing soil erosion from atmospheric agents and wheel traffic, minimizing compaction, and increasing water infiltration.

Managing weeds is crucial to agriculture and will play a significant part in our ability to supply food for future generations (Westwood *et al.*, 2018). Controlling spontaneous vegetation is a standard activity in agriculture across Europe, and there are various methods for doing so. In the face of a spontaneous plant infestation, the best course of action is to let their presence be under conditions that allow them to take advantage of at least a portion of their helpful aspects and avoid their unfavorable aspects. Among the positive attributes, weeds reduce soil erosion (Gyssels *et al.*, 2005; Gómez *et al.*, 2009; López-Vicente *et al.*, 2016), enhance the portability of agricultural equipment (Reintam *et al.*, 2016) and the soil fertility and structure (Folorunso *et al.*, 1992; Gómez *et al.*, 2009, 2011). They operate as an auxiliary reservoir (Carlos *et al.*, 2004; Sabugosa-Madeira *et al.*, 2008; Goncalves *et al.*, 2012; Calha e Portugal, 2014; Furtado *et al.*, 2017), being at specified times and under specific conditions capable of enhancing the quality of the production (Lopes *et al.*, 2008; Portugal *et al.*, 2015). In addition, they can be a source of nutrition for livestock, and although in modest numbers, several taxa are utilized for medicinal, culinary, and aromatic uses (Sabugosa-Madeira *et al.*, 2008; Cunha *et al.*, 2011). Some species, such as the genus *Plantago*, benefit bees, which are crucial pollinators of orchards (Wood *et al.*, 2017).

The most notable losses are a drop in production as a result of competition for water and nutrients, and the declines in output quality (Afonso *et al.*, 2003; Lopes *et al.*, 2011; Portugal *et al.*, 2015). Weeds are also hosts of illnesses, specifically viruses and mycoses and pests, such as tetranichid mites and other polyphagous pests, particularly during the period of vegetative rest, allowing survival in some cases and life cycle continuation in others (Santos, 2011). They interfere in cultural operations, prolonging their execution (Colbach *et al.*, 2014).

Herbicides remain the primary technique of weed control due to their ease of application, great efficacy, and low cost (Portugal *et al.*, 2017; Sahin, 2019).

However, pesticide use poses other concerns, including the poisoning of groundwater, residues in food, and declines in animal and pollinator populations, among others (Rifai *et al.*, 2000).

There has been an increased need for alternatives to the use of plant protection agents in agriculture as a result of pressure from society and governments; in fact, numerous nations are introducing legislation to restrict the use of herbicides (Merfield *et al.*, 2017). As said in the Green Deal (https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal_en), in the context of combatting climate change and limiting its impacts, it is crucial to research options that are efficient, simple to implement, economically viable, and environmentally friendly.

According to Neri and Mia's (2020) study of sustainable alternatives to chemicals for weed control, "the goal is to implement an alternative weed management method (single or integrated) as opposed to relying solely on potentially hazardous chemicals". Modern equipment integrated with a shallow tillage approach can provide excellent weed control in tree rows, as well as enhanced tree performance and soil biodiversity. Living mulch lowers weeds and improves biodiversity, although the selection of less competitive and pest-attractive plants is essential. Plastic covers provide long-term weed control, but extra nutrient inputs are necessary to preserve the soil's natural fertility balance. Where the ingredients are readily available on or near the farm and where perennial weeds are few, wood chip mulch is recommended. For perennial crops, high-pressure water and robotic systems are still in their infancy, requiring additional research to demonstrate their effectiveness.

The organic mulching materials include agricultural wastes (straw, stalks), wood industrial wastes (sawdust), processing residues (rice hulls), and animal wastes (manure). The inorganic mulching materials include petroleum-based polyethylene

plastic sheets and synthetic polymers Kyrikou and Briassoulis, 2007; Gill, 2014). Other organic and inorganic mulching materials exist as well. Adhikari *et al.* (2016) and Yang *et al.* (2015) outlined various new varieties of biodegradable and photodegradable plastic films as environmentally friendly materials and offered sprayable and biodegradable polymer films for easy application and adaptability. Some readily available special materials, including sand and concrete, have also been used for mulching, but not frequently due to their inherent disadvantages. For example, sand mulching lowers soil nutrients (Gan *et al.*, 2008), and concrete mulching is highly expensive to construct (Lei *et al.*, 2004). Each sort of mulching material possesses a unique set of qualities. The selection of appropriate mulching material is contingent upon the local climate, cost-effectiveness (Wang *et al.*, 2015), and crop viability. Researchers are now investigating novel forms of mulching materials. To determine the efficacy and cost-effectiveness of the new mulching materials, it is essential to conduct extensive field studies with diverse mulching materials on an ongoing basis. The main purpose of this study is to evaluate the efficacy of two organic mulches (rice straw and eucalyptus leaves) for weed control in rows and inter-rows of vineyards. For this, floristic surveys were carried out along the vegetative cycle of the vineyards and the presence and abundance of weeds were compared with the control treatment, which is the method generally used for weed control. Besides evaluating the use of rice straws and eucalyptus leaves mulches, this study aims also to promote a circular economy mindset: testing paper and rice industries' waste material for weed management practices and arouse interest in further experimentation on this important topic.

The work consists of a literature review of the themes under investigation, as well as a description and characterization of all parts of the practical work and the methods employed. In addition, the results, their discussion, and recommendations for best practices for the examined cases are provided.

2. State of the art

2.1. Floristic communities of vineyards

Vineyards are characteristic of the Mediterranean Basin and Europe and are a crop with relevant socioeconomic importance. They include a wide diversity of flora, which might result in economic losses due to competition with the permanent crop, the grapevine (*Vitis vinifera* L.). Weeds are populations of certain plant species that, at specific levels and under particular ecological limits, are accountable for unacceptable "net" losses

(negative benefit-harm balance) in economic, ecological, or societal terms (Monteiro and Luz, 2017). According to Moreira *et al.* (2000), the most prevalent weeds in Portuguese vineyards and orchards come from over 40 plant families (pteridophytes and vascular plants) and include both native and foreign species.

Fall-winter vegetation, whose first germination occurs after the first autumn rains and consists primarily of annual species, can be said to exist in general. At the beginning of spring, typically beginning in March, there is a new period of germination, depending on the year and region, of spring/summer species, which are primarily annuals (Pacheco *et al.*, 2009) but also include perennials (e.g., the geophytes *Convolvulus arvensis* and *Cynodon dactylon*) and perennial hemicryptophytes (e.g., *Rumex* species; Monteiro and Luz, 2017).

2.2. Methods for weed control in vineyards

In the twenty-five years following World War II, various synthetic pesticides were registered and introduced on the market, which had a significant impact on the increase in agricultural output and the quality of food provided by farmers as a result of the vast improvement in crop protection (Oerke, 2006).

Weed management in the vineyards of Mediterranean Europe has consisted primarily of herbicide application (including glyphosate) in the row, as well as tillage in row and inter-row, for the past 40 years and still today. The generalized use of herbicides is due to the fact that their application generally requires fewer costs and less human labor (Guerra *et al.*, 2022).

2.2.1. Herbicides

Numerous molecules and their mixtures have been introduced in agriculture and they can be categorized according to their mode of action, selectivity, chemical family, and time of application against a specific phenological phase of the weeds (pre-planting, pre-emergence, post-emergence) (Scavo and Mauromicale, 2020).

Every three years from the 1950s to the 1980s of the 20th century, a new mode of action plant protection product was introduced (Duke, 2012). It was a moment of rapid invention of new pesticides, which encouraged carelessness in their application. Farmers utilized these goods, especially herbicides, without worry for decades, unaware of their negative

long-term impacts. The problem of herbicide-resistant weeds is currently a worldwide concern, especially since no new mode of action has been developed in the last thirty years (Frisvold *et al.*, 2017; Westwood *et al.*, 2018). However, Hashisu (2021) refer that is shortly expected the launch of an inhibitor of dihydroorotate dehydrogenase (DHOD), able to control resistant biotypes in the field, and that there is an investment in chemotypes to cope with non-target site resistance of weeds, which are related to metabolism, uptake and sequestration of the herbicidal active substance. The most prevalent chemical herbicides used in European vineyards now are listed in Table 1.

Table 1. Most prevalent chemical herbicides used in vineyards in the European Union (European Parliamentary Research Service, Scientific Foresight Unit (STOA) PE 690.043 – September 2021). S - systemic, R - residual, C - contact).

HRAC CODE	TARGET MECHANISM	CHEMICAL FAMILY	ACTIVE SUBSTANCE	ACTION
A	ACCase inhibitor	Aryloxyphenoxypropionates	Quizalofop-P-etile	S, R, C
//	(AcetylAcarboxylase)	//	Fluazifop-P-butile	S
//	//	Cyclohexanodione	Cicloxidime	S
B	ALS inhibitor	Sulfonylureas	Flazasulfuron	S, R
C2	Photosystem II inhibitor (D protein)	Ureas	Linuron	S, R, C
E	Potoporphyrinogen oxydase (PPO) inhibitor	Diphenylethers	Oxyfluorfen	C
F1	Carotenoids biosynthesis inhibitor (PDS)	Nicotinilide	Diflufenicion	R
F3	Carotenoids biosynthesis inhibitor (unknown)	Triazol	Amitrol (ammonium thiocyanate can be added as stabilizer)	S, R
G	EPSPS synthetase inhibitor	Glycines	Glyphosate	S
H	Glutamin synthetase inhibitor	Phosphinic acid	Glufosinate ammonium	C
K1	Cellular division inhibitor (mitosis)	Dinitroanilines	Pendimethaline	R, C
K3	Cellular division inhibitor	Benzamide	Propizide	S, R
L	Cell wall inhibitor (cellulose)	Benzamide	Isoxaben	R
F3+C2	Carotenoid & photosynthesis inhibitor	Triazol + Ureas	Amitrol + Linuron	S, R
//	Carotenoid & D protein inhibitor	Triazol + Triazines	Amitrol + Terbutylazine + Ammonium thiocyanate	S, R
F1+G	PDS & EPSPS inhibitor	Nicotinilide + Glycines	Diflufenicion + Glyphosate	S, R
D+G	Photosynthesis & EPSPS inhibitor	Bipyridyls + Glycines	Diquat + Glyphosate	S, C

G+C2	D protein & EPSPS inhibitor	Glycines + Ureas	Glyphosate + Linuron	S, R, C
G+C2+C1	EPSPS & D protein & Cellular division inhibitor	Glycines + Ureas + Triazines	Glyphosate + Linuron + Terbutylazine	S, R, C
G+E	EPSPS & PPO inhibitor	Glycines + Diphenylethers	Glyphosate +	S, R, C
G+C1	EPSPS & Photosystem II inhibitor	Glycines + Triazines	Glyphosate +	S, R, C

With the advent of glyphosate-tolerant crops in the 1990s, glyphosate has become the most frequently produced herbicide in the world (Cressey, 2015). The annual expense of treating weeds resistant to this pesticide currently surpasses one billion dollars in the United States alone (Frisvold *et al.*, 2017).

In order to minimize the negative environmental impact, farmers who use herbicides should strive to make the applications as efficient as possible and in the smallest number necessary, based on a thorough understanding of the phenological phases of the weeds infesting their fields and the use of sprayers of the most recent generation (Partel *et al.*, 2019).

The use of these chemical molecules may increase fruit yield, but they are detrimental to ecosystem health and sustainability (Shorette 2012; Meng *et al.*, 2016), promote soil acidification (Kibblewhite *et al.*, 2008), soil infertility, and contamination of natural underground water (Meng *et al.*, 2016), particularly if they are not used correctly or in excessive doses. It has also been noted that chemical herbicides can impair the microbial (Grossbard and Davies, 1976) and earthworm populations (Gaupp Berghausen *et al.*, 2015), as well as reduce the soil's nutrients and biodiversity (Gangatharan, Neri, 2012).

Due to all this scientific information, Europe's herbicide rules are pressuring farmers to minimize their pesticide use, as well as their impact on the environment and us all (Directive 2009/128/CE, 2009).

Point 2.5.2 of Portugal's National Action Plan for the Sustainable Use of Plant Protection Products (2018-2023) states: "It is intended to promote the reduction of plant protection product use to levels deemed acceptable from an economic and ecological standpoint, as well as the adoption of sustainable production modes, namely Organic Agriculture and Integrated Production" (DGAV, 2018).

In response, there are a number of alternatives to herbicides for vineyard weed control, including physical methods, but farmers are reluctant to adopt these options (Moss, 2019).

2.2.2. Mechanical practices

Hand weeding is the oldest and most traditional method of weed control, and it is still used in underdeveloped nations where human labor is less expensive and there are more employees available (Hammermeister, 2016).

Hoeing is the next step in the tillage alternatives, being simple and dependable, especially for the management of annual and biennial weeds with shallow roots. In agricultural regions where the geography does not permit mechanization, the hoe is also the primary implement for tilling (Bond and Grundy, 2001).

Mechanized tillage is widely used in vineyards due to its effectiveness in controlling weeds while requiring fewer labor hours. However, mechanized tillage is more expensive than unmechanized tillage due to the need for modern tillage equipment that runs on gasoline and must be operated by a licensed worker. Furthermore, if the machines are operated improperly, they can cause trunk damage to the vines (Hammermeister, 2016) and result in excessive compaction of the soil.

Mechanized tillage can have negative effects on soil quality, biological diversity, soil structure, and water-holding capacity (Merwin *et al.*, 1994), reducing the carbon and nitrogen available to microbes (Sanchez *et al.*, 2001, Hoagland *et al.*, 2008) and the soil cation exchange capacity (Hoagland *et al.*, 2008). (Granatstein and Sanchez 2009).

Integrated mowing is a method of automated weed treatment that involves the simultaneous use of a rotary brush weeder and a mower. Due to the necessity of being attached to a tractor, this method can still cause some soil-related issues. However, it can ensure precision weed management even in the sections closest to the trunk without causing harm (Neri *et al.*, 2020).

Finger weeders are the most advanced mechanical weeders. A finger weeder consists of two rotatory devices: one with a horizontal rubber weeder designed to kill weeds between plants, and another with vertical metallic fingers (of varying hardness) designed to break the soil's crust and collect it under the vines (www.bertima.it). The recommendation is to utilize these machines on generally looser soils and in the early

phases of weed growth, as weed maturity reduces the treatment's effectiveness (Pannacci *et al.*, 2017).

The ongoing evolution of technology is lately giving birth to new weed-control options, and some of them are overviewed below:

- **Thermal weed management**

This sort of weed management refers to a transference of heat by water, flame, or steam to the plants from an energy source causing an increase in local temperature plants. For instance, it can be carried out using a tractor-trailer fitted with suitably adapted propane burners that emit heat through one or two metal orifices at a temperature between 60°C and 70°C for optimal efficiency (Wei *et al.*, 2010). This technique has some drawbacks, such as fire hazards, fire damages, and gas consumption (Stefanelli *et al.*, 2009), while it is more expensive than unmechanized techniques due to the need for modern tillage equipment that must be operated by a licensed worker and, if operated improperly, can cause vine trunk damage (Hammermeister, 2016) and result in excessive compaction of the soil.

Another increasingly used technique is the hot foam application on plants, which differs from hot steam by the additional use of biodegradable foaming agents, that maintain for a longer time the temperature of the treated plants. Besides the high price of the equipment (around 30000 €), there are some drawbacks to the use of hot foam and steam, such as the high volume of water needed, and the time needed to refill the tank. Nevertheless, it controls most annual weeds and seedlings of vineyards and orchards, but the control of perennial species is more limited. Electrothermal weed control (using electroshocks) and UV-radiation are also included in thermal methods, as well as cryogeny, and have the same basis, which is the increasing or decreasing, respectively, of the temperature in the plant cells to induce damage (Antonopoulos, 2023). Equipment for electroshocks and UV radiation are also expensive.

- **High-pressure weed management (water jet blasting)**

This is one of the most revolutionary alternatives to chemical compounds for weed management. This innovative weed control method consists of a tractor-trailer that

employs a high-pressure water blast (up to 1150 bar) to kill the roots and leaves of weed plants and bury them up to a few centimeters into the soil (Fig. 1).

This approach uses around 2000L per hectare in vineyards with 2.5m wide vine rows and only two sprays per year to control weeds (www.caffini.com). The biggest worry is the expensive prices for these trailers, which start from 30. 000 euros.

Due to the requirement for contemporary tillage equipment that runs on gasoline and requires a licensed operator to operate it, this kind of weed control is more expensive than most of other physical control procedures. Furthermore, if the machinery is operated improperly, it can cause trunk damage to the vines (Hammermeister, 2016) and result in excessive compaction of the soil.



Figure 1. Caffini GrassKiller in action (Caffini Italy, 2022).

- **Precise weed management (robotic systems)**

In recent years, automation has been at the core of a revolutionary rise; robotic systems for precise weed management may be the finest alternative to pesticides in terms of modern technology (Bajwa *et al.*, 2015). These robots are developed with four primary

considerations in mind: (i) guidance, (ii) weed recognition and distinguishing from crops, (iii) great precision during in-row operations, and (iv) mapping (Slaughter *et al.*, 2008).

According to Bakker *et al.* (2010), the greatest challenge for this technology is to become the key that will enable sustainable crop production in the world's agriculture, as Intelligent camera-based systems capable of guiding mechanical weeding devices are effective but too costly to be adopted by small farms, which still favor low-tech and low-cost solutions (Peruzzi *et al.* 2017).

In conclusion, mechanical weeding techniques have been developed for three main applications: full-field weeders, inter-row weeders, and intra-row weeders (removal of weeds in the row, between the plants). Full-field weeding can be used before the vineyard establishment, it takes more time but does not make use of plant protection products (PPPs), net resulting in either decreased or increased weed control costs, depending on the conditions and the specific techniques applied.

Another option is intra-row weeding, which is extremely precise work in order to avoid damage to vines. Therefore, an RTK-GPS system is required besides the investment in the weeder itself. An RTK-GPS system cost roughly €15.000 (Inagro, 2017). The investment in standard GPS-equipment takes €4,000 but this will be a less accurate version. Some precise applications even require GPS-equipment on both the tractor and the weeding device. However, such an investment gives a difference between 2 cm and 5 cm accuracy and allows for a higher driving speed, which increases the capacity (in the area treated per hour).

The cost of mechanical weeding greatly depends on the cost of labor in the different regions, since mechanical weeding costs more time per ha than spraying. Therefore, the viability of such techniques also depends on the availability and cost of labor (G. Roccuzzo, pers. comm., May 2021).

2.2.3. Biological weed management

Biological management of weeds relies on the use of natural enemies or a complex of natural agents involving parasitism, herbivory, or other natural mechanisms that diminish weed development or contribute to its suppression from the area of interest. These agents include phytophagous arthropods (insects and mites), plant diseases (fungi, bacteria, and viruses), birds, and other animals (ex. sheep, goats, ducks).

By grazing in the vineyards, sheep can be employed as a biological management strategy (Nóbrega, Pedreira, and Goncalves, 2017). However, careful consideration must be given to the time of year that livestock enters the vineyard: sheep can nibble on buds and young branches, and they can also remove bark; goats often prefer woody browse to most herbaceous plants.

SheepIT is an experimental project that aims to exploit the sheep grazing behavior for vineyard weeding in a controlled manner: a system of electric collars and beacons locates each sheep in the flock and shocks them with a small electric charge if they get too close to the crops, ensuring the safety of the young buds (Nóbrega, Pedreira, and Goncalves, 2017).



Figure 2. Sheep grazing in the vineyards of Quinta do Pinto, Portugal (2022).

2.3. Vineyard weed management using mulches

Mulching, also known as stuffing, is the practice of covering the soil with weed-preventative materials such as wood chips, bark, gravel, straw, sawdust, and canvas (Amaro, 2003). This technique is regarded as one of the most effective alternatives to chemical techniques for reducing weed problems in farmed fields. This method's primary purpose is to preserve soil structure and suppress weed development (Mia *et al.*, 2020).

Other intriguing benefits include protection from temperature variations, enhancement of vineyard biodiversity, and enhancement of the soil's physical structure, chemical composition, and microbial health (Polvergiani *et al.*, 2013a, 2013b).

Mulching can come in many forms, such as living mulches (including cover crops), organic materials such as straw, sawdust, hay, compost, tree leaves and bark chips, weeds, or other plant residues. Plastic films of various colors and biodegradable synthetic materials can be also used such as spun and woven cloth, and plain and oiled paper. Stacking with plant remains inhibits the growth of weeds, lowers direct soil moisture evaporation, and provides an abundance of organic matter. However, it has drawbacks such as being expensive, making fertilizing difficult, and providing habitat for rodents and other pests. Moreover, spreading dry straw in orchards throughout the summer can significantly increase the risk of fire (Cerqueira, 1994).

Utilizing mesh-based mulches has the primary advantages of boosting soil temperature, preserving soil moisture, and minimizing weed emergence, which results in increased crop yields. However, the use of plastic-based screens is a pollutant, leading to the development of numerous organic and biodegradable alternatives (Kasirajan and Ngouajio, 2012).

2.3.1. Types of mulching

Living mulch

This strategy entails retaining specific plant strains in the vineyard, chosen for their favorable qualities and influence on the environment of the vineyard. Keeping vineyard plants under control is highly effective against soil erosion (Teddars, 1983), improves soil structure by constructing an organic matter layer (Liang and Huang, 1994), creates a habitat for beneficial insects (Lacey *et al.*, 2006), and stimulates the soil biota that feeds on the weeds' root exudates (Wardle *et al.*, 2001). These live mulches may consist of either spontaneous ground vegetation from the existing floristic community or intentionally planted (cover crops) plant strains to suppress weed communities. Cover crops can also be reintegrated into the soil via a technique known as green manure, to increase the soil's ready assimilable nitrogen. Cover crops should be viewed as a valuable strategy for vineyard soil management when the objective is to increase or preserve soil organic matter.

The primary disadvantage of this method is the potential conflict between the mulch and the vines for water and nutrients (Tahir *et al.*, 2015).

In vineyards, more than 50 plant species are often used as cover crops (McGourty 1994; Bugg *et al.* 1996, Ingels *et al.*, 1998,). According to Ingels *et al.* (1998), there are three primary plant categories: grasses, legumes, and forbs.

Grasses

These plants are Gramineae (or Poaceae) family members and monocotyledons (the grass family). They are all capable of creating substantial volumes of carbon-containing chemicals above and below the soil's surface. They are useful for aggregating soils and delivering considerable amounts of carbon to the root zone via their fibrous roots. The annual production of aboveground biomass per hectare may range from 2 to 10 tons of dry matter, depending on the species and management. Grass typically has a Carbon-Nitrogen (C/N) ratio of more than 20:1, especially if allowed to grow (Hirschfelt, 1998). They can compete with vines for water and nutrients as they grow (especially N). The residue will take a long time to decay if left on the earth's surface, but it will function as mulch and aid prevent soil erosion if left there. If integrated into the soil, cellulose-rich plant biomass will deplete available N, drastically lowering the quantity of N available for vines. Grasses are beneficial as cover crops if their growth is controlled mechanically to reduce their competition with crops. However, grasses should not be used if soil reintegration (green manure) is planned due to their high C/N ratio, which could lead to soil N depletion, and their slow decomposition rates.

Legumes

These plants belong to the Leguminosae (or Fabaceae) family (pea and bean family). When infected with symbiotic strains of Rhizobia bacteria, most of them are capable of fixing nitrogen. Depending on species and management, the total quantity of fixed nitrogen might range from 30 kg to more than 200 kg per hectare. Legumes can easily fix enough nitrogen for the annual needs of grapevines. Typically, these plants have C/N ratios below 20:1, and when introduced into damp soil, they breakdown rapidly and N is released promptly. The majority of plant C is respired by soil microorganisms, and little soluble organic matter accumulates. Left as mulch on the soil's surface, some materials are consumed by soil-dwelling organisms, while others decompose. The root morphology of these plants ranges from fibrous to tap, and they are not as effective as grasses at improving soil structure (Ingels *et al.*, 1998). *Trifolium repens* is an example of a leguminous plant that is beneficial and suited for use as a cover crop due to its ability

to add nitrogen and root exudates to the soil (Nielsen and Hogue, 2000; Granatstein and Mullinix, 2008), which are beneficial to the planted crop.

Briefly, we can say that legumes are very important for their ability to fix nitrogen and that they can be planted alone in the rows as green manure or as permanent cover crops, particularly when combined with other herbaceous strains that improve soil structure.

Forbs

There are countless other herbaceous and broad-leaved plants besides legumes and grasses. Forbs, such as mustards, composites, and buckwheats, serve a variety of purposes, such as growing carbon (*Brassica* spp. and *Rhaphanus* spp.), assimilating nitrogen to prevent it from leaching during winter rains (*Phacelia* sp.), creating insectaries for beneficial insects (various Umbelliferae or Apiaceae and Labiatae or Lamiaceae), suppressing nematodes and soil-born diseases. For this reason, cover crop species such as mustard are utilized; if mowed during late flowering and then disked into the soil, they will provide readily assimilable nitrogen for the crops.

In the experiment conducted by Sexton and Plant (2007), mustard (*Sinapis alba*) was seeded with *Brassica juncea* at a seed rate of 10 lb/acre, mowed at late blooming, and then disked into the soil. The following season, tuber yield at each site was assessed and compared to that of potatoes grown in barley (*Hordeum vulgare*) control plots. Averaged across all sites, overall tuber output was 8% better when mustard green manure was applied as opposed to barley.

Many cover crops improve soil structure, but plants with taproots may help create huge soil macropores as they disintegrate, so enhancing water infiltration and root penetration for subsequent plant growth (McGourty, 1994).

Numerous grape growers use cover crop species to concurrently create habitat for beneficial insects, enhance soil structure, inhibit nematodes and illnesses, and produce nitrogen. For soil organisms to breakdown cellulose and allow carbon to build, N sources must also be present. This is a further argument for growing a mixture of legumes, grasses, and forbs as a cover crop (Bugg *et al.*, 1996).

Another valid option could be adopting a "sandwich system" similar to the one Weibel *et al.* (2007) implemented in Switzerland, which consisted of a 50cm band of living mulch maintained between the tree rows, while in-row competition was regulated by tillage on both sides of the lines.

Plastic mulch

As stated in Abouziena and Haggag (2016) review, historically the introduction of synthetic mulches into agriculture, the mechanization of their application to meet the needs of large-scale and small-scale agriculture at relatively low costs, and their favorable effects on yield and earliness and weed control favored the use of synthetic mulches over organic mulches (Abdul-Baki and Teasdale, 1993). The use of 15 mm thick cellulose sheets (kraft paper sandwiched between a biodegradable biopolymer layer of polylactic acid, one clear and the other colored with black carbon) for weed and pest control in small orchards produced acceptable weed control (Benoit *et al.*, 2006). Black synthetic fabric is provided for mulching. It is sold commercially in 6-foot-wide rolls and is machine-applied when trees are planted. It is simple to apply and inhibits the germination of most annual weed seeds (Zimdahl, 2013).

This method of mulching consists of layers of opaque plastic film that are discarded over bare soil, impeding the growth of light-sensitive seeds by shading them from sunlight and physically preventing the appearance of weed seedlings (Schonbeck, 2012). Plastic mulch has an excellent cost-to-benefit ratio since it may provide us with excellent and long-lasting outcomes at a very low cost (Hammermeister, 2016). The most crucial aspect of this mulching technique is the accurate positioning of the plastic layer, as the improper placement will allow weeds to grow through it (Grieshop *et al.*, 2012).

In the past, the color of the plastic layer and its opacity were the primary drawbacks of this technique: for instance, black plastic has a high light absorption, which makes it very effective at preventing weed growth but can promote very high soil temperatures during the summer, whereas white plastic keeps the soil fresher and reflects the sunlight on the canopy but is less effective than black plastic at preventing weed growth (Lamont 2005).

As previously said, black, opaque screens are the most efficient in weed management because they restrict solar radiation from reaching the soil and, consequently, the weeds. Additionally, they have a warming impact on the soil, resulting in some solarization. This greatly restricts the germination, growth, and development of weeds (Chang *et al.*, 2016).

Another advantage of using screens is that, if they are reflective, they can improve the quality of the fruit produced by increasing light reflection onto the crops. As a result, Leo *et al.* (2018) evaluated the usage of these screens to increase the red color of apples and discovered that the reddest apples were found on trees whose inter-row had the more reflecting white screen.

Mia *et al.* (2020) suggests that in the future, plastic screens should be constructed with white plastic on top and dark plastic below so that the black plastic filters the sun and suppresses weeds, while the white plastic reflects light into the trees and prevents the soil from scorching. The described benefits, along with the weed control provided by the screens, make them particularly appealing as control approaches for orchards and vineyards.

Nowadays the market is expressing the need for the usage of biodegradable plastics as a result of the contaminating potential of plastic mulching layers, especially if they are not disposed of correctly (Malinconico, 2017).

Even though the scenario is extremely optimistic, this revolutionary change to bioplastics for mulching is still in its infancy; there are still issues to resolve, such as the short durability of these materials in the field (Alins *et al.*, 2012; Touchaleaume *et al.*, 2016).

Fully biodegradable composites must have comparable qualities to commercial materials in order to be a viable option. As a result, Delgado-Aguilar *et al.* (2018) sought to produce PLA-based composites reinforced with a commercial bleached kraft eucalyptus pulp. The results of this study support the ability of wood fiber-reinforced PLA composites to replace other glass fiber-reinforced polypropylene composites in terms of tensile properties. In addition, the micromechanics research revealed that it is possible to obtain strong interphases between the PLA and the reinforcement without the need for any coupling agent.

Adopting biodegradable plastics instead of PE mulching materials is more expensive from an economic standpoint, but considering the entire process, including waste management and recycling expenses, biodegradable materials are more convenient (Mar *et al.*, 2019).

Organic mulch

This mulching technique consists of covering the soil with a layer of any type of organic material that must be at least 15 to 20 centimeters thick (Lisek, 2014) and can last from a few months to three years, depending on the nature of the mulch, while conserving 20% of irrigation water (Granatstein and Sanchez 2009).

Abouziena and Haggag (2016) refer that mulching the soil with plant wastes or synthetic mulches is one of the management strategies for lowering soil evaporation; it promotes water retention, hence improving crop fields' water usage efficiency and weed control

(Hegazi, 2000; Awodoyin *et al.*, 2007). This also provides a more uniform distribution of moisture throughout the soil profile, which improves water use even further. Indirectly, organic mulches also improve water use efficiency. As the mulch decomposes, humus is added to the soil, increasing its capacity to retain water (Unger *et al.*, 1997). A mulch layer hinders the growth of weed seedlings by preventing light from penetrating the soil surface. Reduced weed prevalence substantially increases water use efficiency (Ossom *et al.*, 2001). Many examples can be given. For instance, Xu *et al.* (2009) observed that straw mulching (wheat straw after ear harvest) dramatically reduced weeds, increased soil microbial quantity and activity, prevented powdery mildew, and increased pumpkin fruit production.

Organic mulches are a viable alternative to chemical herbicides for weed control (Ingels *et al.*, 2013); however, the main problem is the cost of a large amount of material required to mulch the entire vineyard (Tahir *et al.*, 2015); therefore, the best source could be any organic waste with no commercial value (yet) that is available in large quantities near the vineyard.

The primary disadvantage of organic mulches occurs when high lignin-based mulches, such as wood chips or wood barks, are employed: the decomposition of carbon polymers results in a high C/N ratio in the soil, which causes nutrients immobilization (Larsson 1997, Treder *et al.*, 2004).

2.3.2. Effects of mulching on vineyards

Mulching application is a cost-effective practice with many positive outcomes, both on the vine and the soil, available to both large and small companies; as stated in Fraga and Santos' studies (2018), organic mulches, in addition to being effective in weed management, can be an effective adaptive measure to combat yield losses due to climate change, improving and/or preserving soil characteristics (biome vitality, vineyard biodiversity, soil structure) and saving water frost damage. According to Abouzienna and Haggag (2016) mulch protects and even enhances soil humus. It also increases the cation exchange capacity, or the capacity of the soil to hold nutrients, after that mulch boosts the activity of soil organisms and protects and enhances organic materials, while frequently increasing the availability of phosphorus, potassium, and magnesium).

To sum up, and according to Müller-Samann and Kotschi (1994), Farooq (2011), and Abouzienna (2015) and Abouzienna and Haggag (2016), the benefits of mulching soil are

manifold: i) weed management, ii) minimize erosion, iii) maintain soil structure, iv) water economy, v) improve crop root development, vi) improve the chemical qualities of the soil, vii) preserve soil life viii) improve agricultural production.

There are, however, several negative aspects and constraints to mulching usage. First, the costs and availability frequently restrict the usage of mulches. Then, for some perennial weeds, mulches are not successful. For instance, weeds like field bindweed (*Convolvulus arvensis*) and nutsedge (*Cyperus esculentus*) typically have adequate root reserves to penetrate thick mulches (>15 cm). Even plastic mulches do not control these creeping perennials and as these weeds can puncture the plastic, the light can induce the germination of other weeds. In warm areas, mulches quickly break down and require frequent refilling. When agricultural wastes are employed as mulch, the crop's seeds may germinate and cause issues.

2.3.3. Climate change

Premium wine grapes are much more responsive to slight climatic fluctuations than other crops (Furer, 2006; Hannah et al., 2013). The shift in global temperature trends may displace premium grape-growing regions from their current locations and also trigger a shift in grape variety cultivation.

There is greater warmth over land, with greater warming at the higher latitudes, particularly in the Northern Hemisphere (IPPC, 2022), which has a significant impact on agriculture. Changing weather patterns may also increase the demand for freshwater supplies as certain regions continue to dry out (Hannah et al., 2013). In the next half century, the National Academy of Sciences predicts a major shake-up in the regional distribution of wine production due to the general movement of warmer temperatures poleward (Hannah et al., 2013). The economic and practical implications would be massive. The premium wine-producing regions would relocate to the poles.

The impact of global warming on wine-growing regions in Europe would be substantial. The absence of the Gulf Stream would chill Bordeaux and parts of Spain, necessitating the replanting of grapes that thrive in cooler climates (Furer, 2006). Other locations, however, would become warmer. Alsace, for instance, has experienced a shrinking growing season and a shift of harvest from October to September over the past three decades, while Burgundy may soon come to “resemble” Bordeaux. In the Chianti region of Tuscany, grapes are ripening far too early, necessitating a shift in grape varieties

(Furer, 2006). Alentejo region in Portugal and central Spain may "find it difficult to survive" due to the effects of rising temperatures and water shortages (Furer, 2006). It has been demonstrated that the Alentejo winemaking industry will require appropriate adaptation measures to combat the negative effects of climate change. Fraga et al. (2018), for example, suggested irrigation as a suitable adaptation measure (when water is available), although it may not be sufficient to maintain present yields. Consequently, a combination of adaptation methods may be required, with mulching as a strong possibility. Grapevine cultivators can employ mulching as a short-term method that is reasonably economical. Other adaptation measures include the adoption of training systems that support shorter trunks (e.g. Gobelet), the selection of more drought-tolerant varieties/rootstocks/clones (Bota et al., 2016), and the modification of soil management practices, which should also be considered (Bahar and Yasasin, 2010).

By 2050, vast swaths of Europe's Mediterranean coast, including Italy, Portugal, Spain, and France, may become hostile to grape growing, while Southern England is going to resemble Champagne with its produced notable vintages (Furer, 2006).

In order to minimize climate change's effects, should be good to consider:

- 1) Introducing winter cover crops in regions capable of supporting such crops during the winter to minimize soil erosion and optimize nutrient and water storage as a result of changes in precipitation patterns (Schultz, 2000).
- 2) "Reuse, treatment, and recycling of water to minimize waste" as well as minimizing the costs of water usage and removal to counteract dwindling water supplies and combat global warming (E-VitiClimate).
- 3) Using deficit irrigation strategies, such as partial root drying, sustained deficit irrigation, and regulated deficit irrigation, to compensate for reduced water supply that increase water use efficiency and promote optimal grape maturity and wine quality (Fraga et al., 2012).
- 4) Counteracting a rise in vineyard temperature, consider enhancing cooling strategies such as strategic vine orientation/trellising treatments and water-efficient micro-misters (Hannah *et al.*, 2013).

Water loss and soil erosion are major agricultural challenges, particularly in the Mediterranean region. In the experiment done by Prosdocimi *et al.* (2016 a), a barley straw cover of around 59% (median value) applied at a rate of 750 kg/ha resulted in

delayed times to ponding, runoff, and runoff in outflow during low frequency-high magnitude rainfall events in Mediterranean vineyards. In addition, it reduced i) surface runoff rates from 52.59 to 39.27 percent (bare to straw), ii) sediment content in runoff from 9.8 to 3.0 g/L, and iii) soil loss rates from 2.81 to 0.63 Mg/ha*h. This reduction of soil and water losses was attained immediately after the straw application. Barley did not grow in the same area where the plots were located, rather it was grown in the surrounding fields, mulched and then transferred. Straw mulch was confirmed to be a very inexpensive and effective soil conservation strategy that can be applied by winegrowers to lower the high erosion rates in semiarid areas, based on the results obtained.

In 2018 Fraga *et al.* simulated a future situation of mulch used as climate change adapting measures for vineyards in Alentejo, Portugal. While both non-mulching and mulching simulations indicated a progressive yield decline in the future, mulching mitigated 10 to 25% of the negative climate change consequences, depending on the subregion and time period. The results indicated that mulching could lower the expected yield decline (from 0.75% per year to 0.65% per year), hence aiding in the maintenance of sustainable yearly yield levels. In fact, mulching is anticipated to increase yields during the whole duration of the simulation, although the advantages should be more pronounced with time (2061–2080). However, it should be emphasized that the climate model predicted a stabilization of the hydric indices over this period. On a sub-regional scale, most interior regions, i.e. Granja Amareleja, Portalegre, Reguengos, and Redondo are anticipated to suffer the greatest yield losses, therefore mulching may have a significant adaptation potential in these areas.

Under a harsh future scenario, the yields of Alentejo grapevines as well as other regions are anticipated to decrease dramatically if no mitigation measures are taken. Given these future estimates, the viticulture industry must identify acceptable adaptation methods. Mulching is a short-term, cost-effective adaptation method that can be easily adopted by growers (whether they are small farmers or major corporations). However, this measure may not be sufficient to fully mitigate the projected yield losses, and other complementary strategies should be implemented to ensure a thriving and competitive winemaking industry in all of Europe.

2.4. Available materials for mulches in the Mediterranean area

According to Prosdocimi *et al.* (2016 b), the most popular methods of mulching used in Mediterranean vineyards are organic mulches, including materials such as straw, wood chips, needles or mulches made from vine winter pruning, and plastic mulches. But hybrid approaches such as combinations of mulching and grass cover, mulching and tillage, or mixed mulching are also prevalent.

2.4.1. Use of Eucalyptus leaves

In recent years, the use of Eucalyptus leaves, particularly *Eucalyptus globulus* leaves, for mulching has attracted attention because of the high presence of allelopathic chemicals and oils, which makes them a possible bioherbicide (Puig *et al.*, 2018). In fact, according to Puig *et al.* studies (2018), aqueous extract from blue-gum leaves has phytotoxic effects on lettuce plants. Although certain phytotoxic effects were seen on the physiology and morphology of mature lettuce plants, leading to the abandonment of the use of eucalyptus aqueous extract as a post-emergence bioherbicide, it shows promise as a pre-emergence bioherbicide and for perennial crops. It is determined that *E. globulus* produces a cocktail of phytotoxic chemicals that, when released from leaf remains into the soil, inhibit the germination and growth of other species.

In addition, due to its high carbon content, *E. globulus* is one of the most widely distributed wood species in the world, making it highly profitable for the paper-making process (Puig *et al.*, 2019). The leaves, which are not used to create paper, can be a fantastic source of organic mulching material at cheap prices and, as far as we can tell, also have good results in weed management on certain annual crops such as *Stevia rebaudiana* (Taak *et al.*, 2021) and *Cicer arietinum* (Khan *et al.*, 2019). (Fig. 3).



Figure 3. Application of eucalyptus leaves mulch in Quinta do Pinto vineyard (March 2022).

In Canhoto *et al.* studies (1996), the leaf decomposition of *Eucalyptus globulus* and three native deciduous tree species, *Alnus glutinosa* (alder), *Castanea sativa* (chestnut), and *Quercus faginea* (oak), were studied. Negative exponential curves match the weight loss of leaves over time for all leaf species. Alder ($K = 0.0161$) > chestnut ($K = 0.0079$) > eucalyptus ($K = 0.0068$) > oak ($K = 0.0037$) had the highest rate of mass loss, confirming low decomposition rate for oak and eucalyptus leaves which could translate in more durable mulches.

One other reason that could promote eucalyptus leaves' use for mulching purposes is that, as leaves aren't used in the paper production process, they are left in the field, which combined with the hot Portuguese summer temperature and low decomposition rates, makes them a possible fire starter.

2.4.3 Use of Rice straw

Straws are often useful for mulching due to their widespread availability and the ability to compress them into bales and reduce their transportable area (Duan et al., 2015).

The low quantities of lignin and cellulose in rice straws (Japan Institute of Energy, 2002; Barmina et al., 2013) decrease the likelihood of nutrient immobilization, making rice straws one of the best types of straw for usage as mulch (Figure 4). Furthermore, rice straws are less susceptible to mold due to the aquatic nature and properties of rice (*Oryza sativa*) (Gummert et al., 2020).

According to Oliveira et al. (2014), the inhibitory effect of organic mulch on weeds may be due to both the physical (the reduced passage of solar radiation and temperature range on soil superficial layer) effect of emergence suppression and the possible chemical effects arising from allelochemicals released by straw that may have contributed to emergence reduction. In addition, allelopathic interaction and chemical/biological effects of mulching include alterations in soil pH and nutrient dynamics.

Abouziena and El-Saeid (2014) tested at 45 days after transplanting an onion field, the rice straw mulch treatments, which considerably decreased the total dry weight of weeds. Mulching treatments were more effective against broad-leaved weeds than against grassy weeds. In addition, the application of rice straw mulch increased bulb yield by 118%. In another study under mandarin trees, two layers of rice straw mulch reduced weeds by 85 to 98 percent (Abouziena et al., 2008).

There are several studies on the use of rice straw in vineyards. Zhang et al. (2014) studied the effect of rice straw mulching in China with diverse types of irrigation of grapevines and the results showed that the higher yield, longer shoot length, and larger berry with surface irrigation and rice-straw mulch. Other authors confirmed the increased water use efficiency when rice straw mulch is applied, and reported 30-50% savings in irrigation water (Chaudhry *et al.*, 2004, Laila and Ali 2011). Recently, Abo-Ogiala and Khalafallah (2019), in an experiment on King Ruby grape vineyard in the Delta Nile, Egypt using diverse restrictions to water irrigation with and without rice straw mulching, highlighted the role of mulching in keeping soil moisture, especially under severe drought stress.



Figure 4. Rice straw mulch in Quinta do Pinto vineyards, Portugal (March 2022).

3. Materials and methods

3.1 Characterization of the case studies

Four vineyards located in the Região Vitivinícola de Lisboa, Portugal were used for the evaluation of rice straw (*Oryza sativa*) and eucalyptus leaves (*Eucalyptus globulus*) mulching. Two of them are part of the vineyards of the campus of the Instituto Superior de Agronomia da Universidade de Lisboa (38.422461N, -9.110553W), ISA hereafter, and the other two belong to private landowners of the Quinta do Pinto winery (39.0878454N, -9.1262779W), QDP hereafter.

The places of study are located in the district of Lisboa, ISA inside Lisboa municipality, and Merceana at Torres Vedras municipality. The climate of the region is Mediterranean with hot summers and mild winters (Csa, Köppen-Geiger classification). Climate normal from ISA's meteorological station indicates that the annual average air temperature is 16.4°C with July and August as the warmest months (mean maximum of 22°C and 23°C, respectively). The annual average rainfall is 591 mm, concentrated mostly in November,

December and January (Figure 5). Climate normal from Torres Vedras indicates that the annual average air temperature is 15.8°C with July and August as the warmest months (mean maximum of 20°C and 20.5°C, respectively). The annual average rainfall is 638 mm, concentrated mostly in November, December and January (Figure 5).



Figure 5. Graphics representations of the average temperatures and precipitations in Lisboa (left graph) and Torres Vedras (right graph) municipality areas (www.climate-data.org; consulted on September 20th 2022).

In the selection of the vineyards under study, the climatic conditions were considered, as well as the characteristics of the vineyard (conduction system, cultivars, age of the vineyard) and other local variables. These precautions allowed us to limit the effects of the characteristics of the vineyards and environmental factors on the results and possible conclusions regarding the effects of rice straw and eucalyptus leaves mulch on floristic communities.

3.1.1 Vineyards of ISA campus

The experimental trial ISA is located at the Instituto Superior de Agronomia *campus* in Lisboa. Vines are trained in a vertical shoot positioning trellis system with two pairs of movable wires. The vineyard plots consist of the spur-pruned white cultivar ‘Alvarinho’ and the spur-pruned red cultivar ‘Syrah’ of *Vitis vinifera*, both grafted onto 1103 Paulsen rootstock, planted respectively in 2006 and 1998, spaced 1.0 m within and 2.5 m between rows.

The soil of the vineyard was classified as clay soil with a pH ranging from 6.3 to 6.6, and with an average organic matter content (approximately 1.6- 3%). It contains a very high

concentration of K, Mg, Fe, Mn, Cu, a high concentration of Ca, and a medium-high concentration of P, according to a recent analyses report (Ervedeiro, 2021).

Respectively, the Syrah plot (Figure 6A; 38.7092806 N, -9.1867691 W) has an exposition towards South-South-East, while the Alvarinho plot (Figure 6B; 38.7071370 N, -9.1844298 W) has an exposition towards South.



Figure 6. Satellite views of the vineyards located at the campus of Instituto Superior de Agronomia (A- 'Meia encosta', B – 'Almotivo') and Quinta do Pinto (C, D). The red line indicates the location of the experimental plot used in the trial: A and C – cv. 'Syrah', B and D- cv. 'Alvarinho' (Source: Google Earth, 2022).

3.1.2 Vineyards of Quinta do Pinto

The experimental site, QDP, is located in Aldeia Galega de Merciana in the Região Vitivinícola de Lisboa. Vines are trained in a vertical shoot positioning trellis system with two pairs of movable wires. The vineyard plots consist of spur pruned white cultivar, cv. 'Alvarinho', and spur pruned red cultivar, cv. 'Syrah' of *Vitis vinifera*, both grafted onto 1103 Paulsen rootstock, planted respectively in 2014 and 2004, spaced 1.0 m within and 2.5 m between rows. The plots' soil is characterized by being highly alkaline with approximately 1.01% organic matter and a pH of 8.4. Respectively, the Syrah plot (Figure. 6C; 39.0900787 N, -9.1271680 W) has an exposition towards South-South-East, while the Alvarinho plot (Figure 6D; 39.0864172 N, -9.12286749 W) has an exposition towards North-West.

3.2 Weed management of vineyards

In all studied seasons, the sites were subject to similar standard cultural practices during the growing cycle, including water shoot removal, shoot trimming at 1.2 m above the cordon, shoot positioning and fertilization.

The weed management method used for both the Alvarinho and the Syrah vineyards at ISA *campus*, consisted of a combination of inter-row mowing and under-row glyphosate spraying, which were conducted four times along the vegetative part of the grapevine life cycle (April-May-June-July). Soil management and pest control were conducted by the Núcleo de Espaços Verdes (NEV) of ISA and were the same as in the rest of the vineyard.

The common weed management method used for both the Alvarinho and the Syrah QDP vineyards consisted of a combination of alternated inter-row tillage (one row tilled, one row no tillage), which was carried out twice during the vegetative season (May-July), and biological weed control using sheep flocks in April.

3.3 Experimental design and treatments

Each one of the four plots (ISA Alvarinho, ISA Syrah (Figure 9), QDP Alvarinho (Figure 8), QDP Syrah) that characterized our experiment was extended over 4 rows, 3 inter-rows about 2,5 meters each making this side around 8,5 meters (2,5 m x 3 + under-

rows), while the long side was between 12 and 15 meters along the row, depending from the plot; each row portion was divided into 3 sections of 4 meters each (Figure 7), which were treated in the following way:

- 4-5 meters of roughly 0,25 meters thick rice straw mulch (RS, hereafter), for which around 500 kg per plot (2000 kg total) were used, making it 104 tonnes/hectare treatment;
- 4-5 meters of roughly 0,15 meters thick eucalyptus leaves mulch (EL, hereafter), for which around 625 kg per plot (2500 kg total) were used, making it 130 tonnes/hectare treatment;
- 4-5 meters of control (C, hereafter), which was treated in the same way the rest of the vineyard where each plot was found was usually treated (3.2.1.; 3.2.2.).

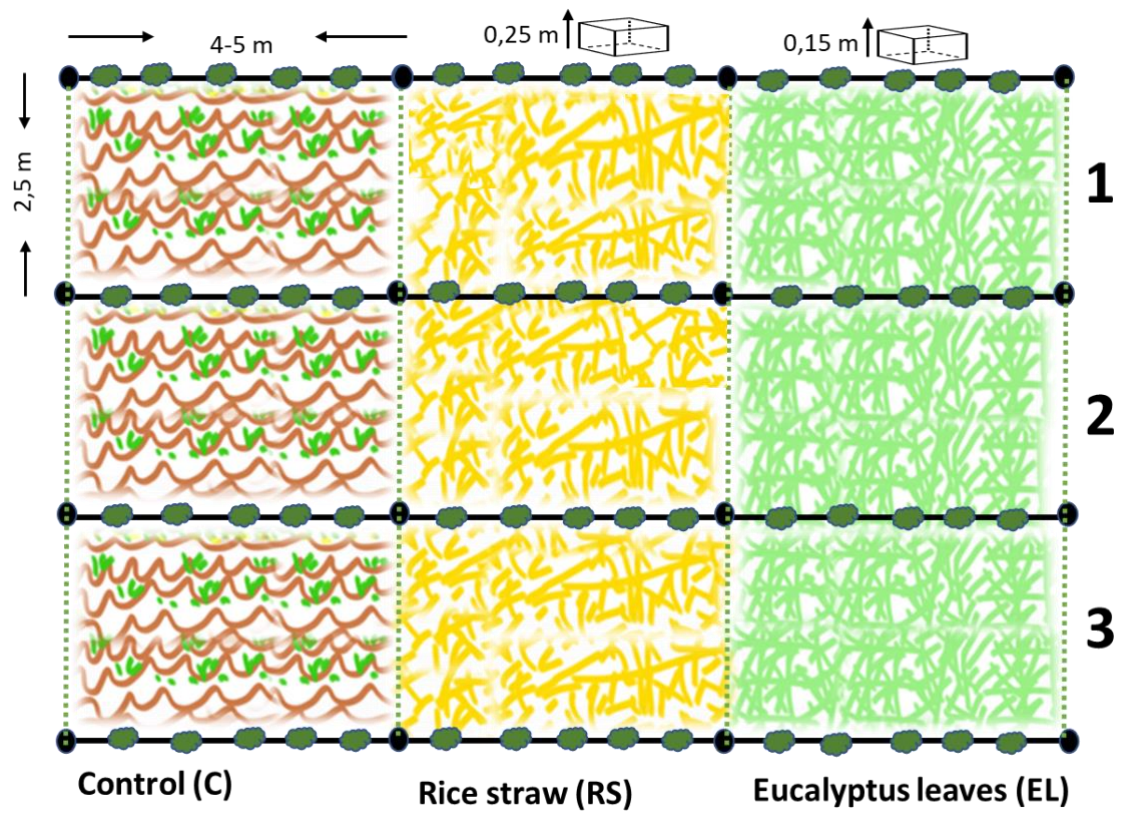


Figure 7. Schematic representation of the disposition of the different sections of eucalyptus leaves mulch, rice straw mulch and control inside all of the different plots in ISA and QDP.



Figure 8. Side view from the QDP Alvarinho plot, moments after both the eucalyptus leaves and the rice straw mulches were established in the selected plot of Quinta do Pinto's vineyards.



Figure 9. Side view from the ISA Syrah plot, moments after both the eucalyptus leaves and the rice straw mulches were established in the selected plot of Instituto Superior de Agronomia's vineyards.

The *Eucalyptus globulus* leaves and the *Oryza sativa* straws were analyzed with the courtesy of Researcher Solange Araújo from ISA, which results of the chemical analysis can be found in Annex 1.

3.4 Floristic surveys details

Floristic surveys occurred once a month in each plot, from April to September (6 surveys in 4 vineyards), totalizing 216 surveys. Each one of the surveys was conducted personally by me and, when available, along with the thesis supervisor Francisca Aguiar. The identification was supported by technical literature, such as the book “Ervas Daninhas das Vinhas e Pomares” (Moreira et al., 2000), and national floras (e.g. Franco, 1971; 1984). Some specimens were identified at Herbário João de Carvalho e Vasconcellos (LISI), Lisbon.

The abundance of the weeds (percentage of coverage of each *taxon*) was visually estimated for each plot, using an adaptation of the Domin scale (Table 2). It was also noted the phenological state of the species inventoried according to 5 classes (1-plantling; 2-roset; 3-adult plant; 4-flowering; 5-fructification).

Table 2. Abundance scale based on Domin scale for the visual estimation of the percentage covered by each *taxon*.

Class	Cover scale
1	> 0-2,5%
2	> 2,5-5,0%
3	> 5,0-10,0%
4	> 10,0-15,0%
5	> 15,0-25,0%
6	> 25,0-50,0%
7	> 50,0-67,5%
8	> 67,5-100%

3.5 Complementary data

In the experimental essay at ISA, Alvarinho plot was used for the investigation of the effect of the eucalyptus leaves and rice straw mulches and the control on soil temperature. This work was carried out by the Master student Guglielmo Piazzoli and

supervised by Professor Miguel Costa. The work had also the aim of calibrating the Flir A35 thermal camera (Annex 2). This work was conducted during the 2022 summer months (June, July and August).

In order to have a wider range of information and to give more context to future research on the topic, a sampling collection of the eucalyptus leaves and the rice straw took place on February 15th of 2022 at ISA and February 20th at QDP. Samples were analyzed by Solange Araújo, a Senior Researcher on the line ForTec of the Forest Research Centre of the University of Lisbon (CEF/ISA), which some preliminary results of the chemical analysis can be found in Annex 1.

3.6 Data treatment

The data that was collected monthly from each plot was organized in an Excel spreadsheet, using the acronyms to identify each section (Figure 10): for example, the acronym EL_SyISA2_07 identifies the survey of July at the section located in the second interrow (2nd repetition), 'Syrah' vineyard of the ISA campus, treated with eucalyptus leaves mulch (Annex 5). Thus, each plot is divided into 9 sections which are identified by weed management type, row, variety and location, with the taxa identified in rows and the sections in columns, counting the record of the abundance of each species and their phenological state.

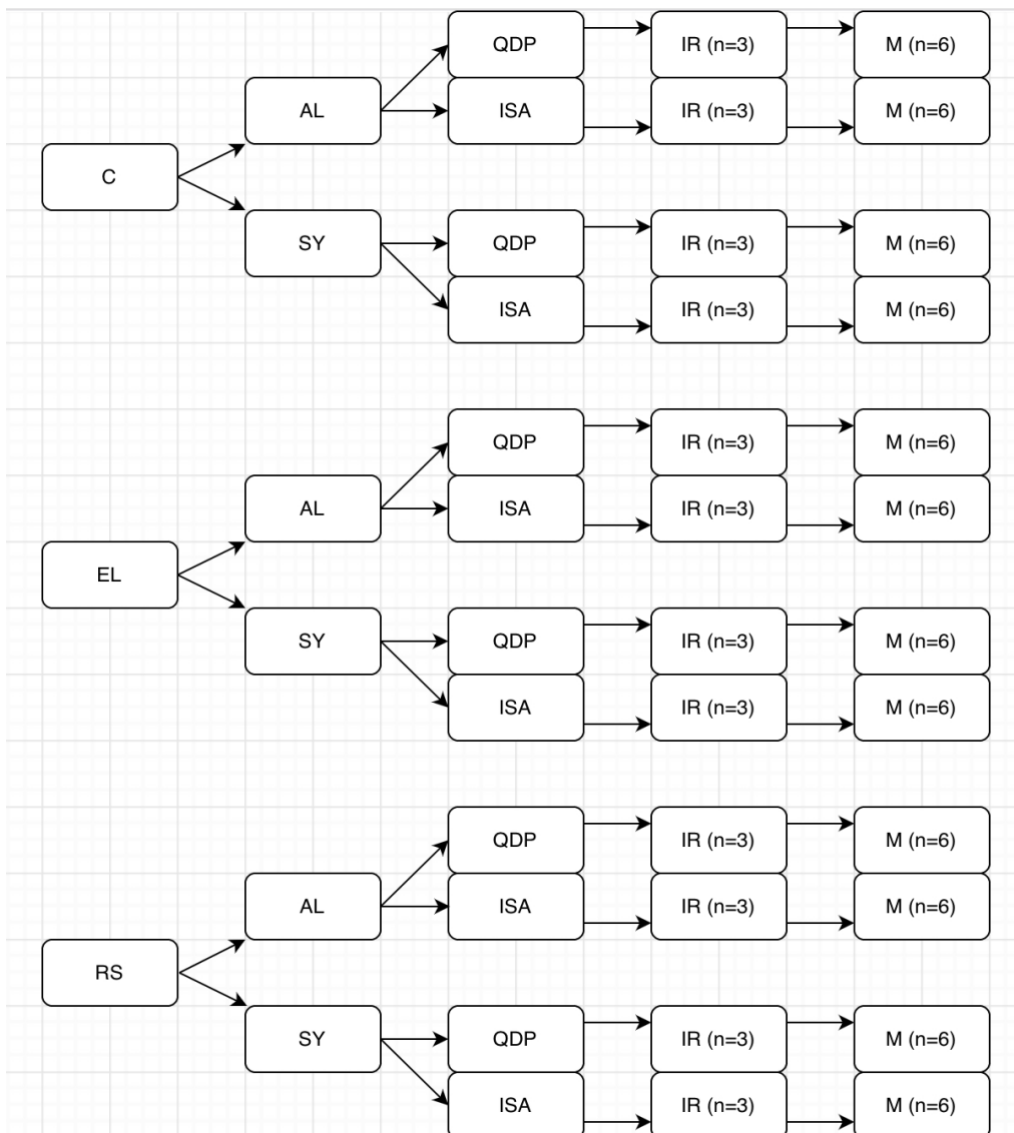


Figure 10. Scheme of the experimental delineation. C=Control, EL=Eucalyptus Leaves, RS=Rice Straw, AL=Alvarinho, SY=Syrah, QDP=Quinta do Pinto, ISA=Instituto Superior de Agronomia, IR=Interrow, M=Month.

The sum cover and median cover were calculated for each section, globally and by each plot. In addition to that four diversity indices were calculated: specific richness (S = number of species in each quadrat), Shannon-Wiener diversity index [$H' = \sum p \times \ln(p_i)$], Simpson's dominance [$1-D$; in which $D = \sum p_i^2$], Equitability (J), where $J = H' / \ln(S)$. The calculation of these indexes was performed with the RStudio and R software (The R Foundation for Statistical Computing, 2019), PAST4.03 (Oyvind Hammer for Windows, 2020) and Microsoft Excel software.

The comparison by weed management techniques is presented visually through graphs. The existence of significant differences between averages of the richness of the various treatments was carried out through analysis of variance (ANOVA). The analysis of variance tests the hypothesis of the equality of averages of two or more populations, checking if there is a significant difference between the averages. The assumptions of the ANOVAs are: the samples are random and independent; the populations have normal distribution and the population variances are the same. Tests were carried out to prove these assumptions and the subsequent option for parametric or non-parametric tests. The hypotheses tested are: H0 Null hypothesis, the population averages are equal; H1 Alternative hypothesis, the population averages are different, that is, there is at least one different average from the others (Annex 3).

In the case of rejection of the null hypothesis, 'post-hoc' tests were carried out that allow the modalities to be separated from each other. In the case of parametric tests, the Tukey test was performed and in the case of non-parametric tests, Dunn's and the Ryan, Einot, Gabriel, Welsch (REGW) test was performed, with $\alpha=0.05$ (Annex 3).

The median abundance (AbM), absolute frequency (FrA) and relative frequency (FrR) were calculated for each taxon, globally and by plot.

For all the taxa found was then calculated the Importance Value Index (IVI) by each type of weed management. This index was calculated by the sum of AbM and FrR and it's very useful because allows us to investigate the existence of species of weeds with greater importance in the vineyards tested.

Species with IVI greater than 45 in the rows of each weed management technique were characterized as to the possibility of providing a certain ecosystem service. Thus, ecosystem services with interesting characteristics for the culture in question were selected, based on scientific articles, databases and bibliography found. The characterization was made according to the categories of Provision, SProv (animal food, human food, medicinal use, use of materials and structures, use of essences) and Regulation and Maintenance, SReg (pollination service, regulation as auxiliary hosts, erosion control and soil quality improvement, dispersion of seeds by ants, dispersion of seeds by birds) (Almeida, 2013, Pires, 2022).

Under SProv there are the following categories: i) animal feed services, plants used as forage or for siding; ii) human food, all edible plants are considered, either as a vegetable or for the preparation of sweets or drinks; iii) medicinal use, describes the species used in the confection of pharmaceutical compounds; iiiii) essences, encompasses the plants

that are used for extraction of compounds or that have industrial potential (Almeida, 2013; Haines-Young and Potschin, 2018).

Under SReg there are the following categories: i) plants that contribute to pollination; ii) plants that have aptitude as hosts of auxiliary fauna; iii) plants that provide food for birds and ants, which in turn can contribute to the dispersion of seeds, endozooecoria and mirmecoria, respectively (Haines-Young and Potschin, 2018); iv) all plants that contribute to the maintenance of the soil structure and consecutively to the control of erosion.

4. Results e discussion

4.1 Cost analysis

In order to have a better understanding of the economic aspects of our experiment, Table 3 shows the costs we had to face to provide both of the raw materials, including the costs that ISA and QDP had to face for weed management in their vineyard (control plots). While looking at this table we have to consider some important facts that influenced the prices: i) the rice straw's price per kilogram was higher than usual at the moment we bought it, due to a period of severe drought which raised the price as a consequence of a lack of cattle feed; ii) the price of the eucalyptus leaves transfer could have been cheaper if we had the opportunity to organize it with the eucalyptus farm's trucks (as we did for the rice straws), which we didn't have, so we had to organize it with Torrestir, a Portuguese package delivery company; iii) the other costs consisted in the price for 20 carpentry bigbags that were used to gather and transport the leaves, plus 7 sickles and 7 pairs of working gloves that were given to the gathering crew; iv) even though the rice straws transport cost is higher than the eucalyptus leaves one, we have to consider that the first ones were brought from Alcácer do Sal to ISA and to QDP in two separated trips (208 km total), while the second ones needed only the trip to be brought to ISA (59 km) since they were gathered in a site next to QDP; v) the total costs per hectare per year of the two control methods strongly depend from the incidence of these treatments along this period, in fact tillage in combination with glyphosate spraying can be conducted up to 4/5 times a year due to the usage limits of chemical herbicides, while simple tillage doesn't have limitations and can be effectuated even more than 15 times a year, especially in the case of alternated row tillage; vi) in order to completely evaluate the

case of the QDP control we have to consider that biological control through flocks of sheep grazing into the vineyard was provided by the company's animals, which, if not, could have raised the final price vii) last but not least, the obvious comment that comes up looking at the eucalyptus leaves mulch's and rice straw mulch's total costs per hectare and total costs per hectare per year (total cost per hectare divided by 4 because this treatments should last 4 years) in Table 3 is that both are totally unacceptable from an economic point of view: it's important to keep in mind that under (better) commercial circumstances between professional activities, such as an hypothetic contract between the wine/grape producer, the eucalyptus farm/paper production company and a transport organization as a third party, the prices of both of the organic mulches could be lower by far; furthermore the ongoing problems concerning pollution by herbicides on a global scale, or fire hazard in eucalyptus plantations on a national scale (Portugal) could arouse the interest from European/National governments in calling a fund or calling for concessions to promote the use of eucalyptus leaves mulch and help to lower the price.

Table 3. Costs and costs per hectare of eucalyptus leaves and rice straw mulching application, compared to the cost per hectare of the weed management methods adopted by ISA and QDP. Subheadings i) to vii) are explained in the section 4.1.

Mulches	Absolute cost (€)				Total cost (€/ha)	Total cost (€/ha*year)		
	Material	Transport	Other	Total				
Eucalyptus leaves (2500 kg)	0	330 (ii)	129 (iii)	459	27.000 (vii)	6.750 (vii)		
Rice straw (2000 kg)	500 (i)	400 (iv)	0	900	52.000 (vi)	13.000 (vi)		
Control	Cost/area (€/ha)						Total (1 treatment)	Total (1 year)
	Herbicide	Equipment depreciation	Labor	Diesel	Water			
ISA	72,35	0,75	1,5	1,5	0,73	76,83	300 - 500 (v)	
QDP	-	0,75	1,5	1,5	-	3,75	30 – 60 (v-vi)	

4.2 Species richness and abundance

The frequency of occurrence and average abundance of plants in vineyards are simple indicators of the problems with weed communities and the domination of a single or few species. Figure 11 to 13 shows RGB images of the vineyards and mulching effects on weeds after almost three months of the installation of the experiment.



Figure 11. Rice straw mulch weed controlling performance during the floristic survey conducted in May (almost 3 months after being laid in the field) in the QDP Syrah plot.



Figure 12. Eucalyptus leaves mulch weed controlling performance during the floristic survey conducted in May (almost 3 months after being laid in the field) in the QDP Syrah plot.



Figure 13. Control treatment weed suppressing performance during the floristic survey conducted in May (almost 3 months after being laid in the field) in the QDP Syrah plot.

The first test conducted was a One-Way ANOVA concerning all of the global Richness (nr.) that was detected in each one of the four plots and under each treatment (control, eucalyptus leaves and rice straw mulch); the tests of the Normality of Residuals (es: Skewness) rejected the hypothesis of Normality, then the tests of Equality of Group Variances (es: Levene) rejected the hypothesis of having equal variances. Then the Kruskal-Wallis One-Way ANOVA on Ranks was effectuated with H0: All medians are equal and H1: At least two medians are different. The test rejected the H0, in consequence, a Dunn's Test was conducted and the results showed that there were no significant differences between the control treatment and the eucalyptus leaves mulch effects, but both of them were significantly different from the rice straw mulch, which performed better. Details are in Annex 3.

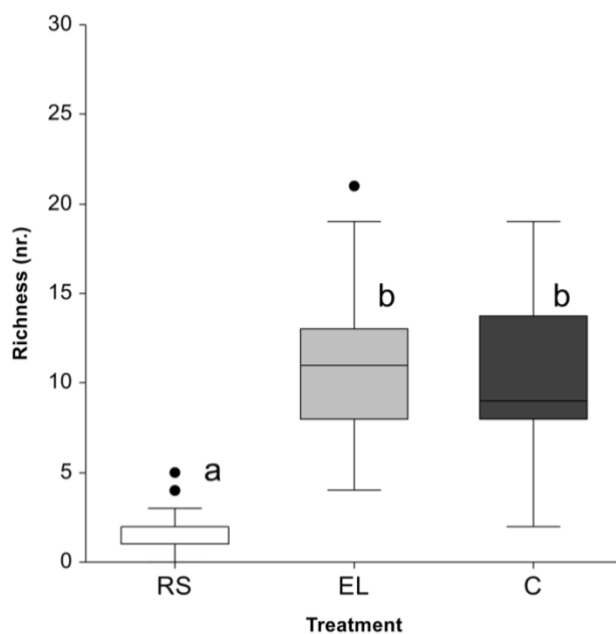


Figure 14. Box-plots of the species richness for the the rice straw (RS) and Eucalyptus leaves (EL) mulches and Control (C). Different letters show significant differences between treatments (Kruskall-Wallis on ranks and Dunn's test; $p < 0,05$). Outliers – black circles.

Results showed that there are significant differences between the average number of species on rice straw mulch and both eucalyptus leaves mulch and control (Figure 14).

The second test conducted was a One-Way ANOVA concerning all of the global the Sum of Species Cover (%); the tests of the Normality of Residuals (es: Skewness) rejected the hypothesis of Normality, then the tests of Equality of Group Variances (es: Levene) rejected the hypothesis of having equal variances. Then the Kruskal-Wallis One-Way

ANOVA on Ranks was effectuated with H0: All medians are equal and H1: At least two medians are different.

The test rejected the H0, in consequence a Dunn's Test was conducted and the results shown that there were no significant differences between the control treatment and the eucalyptus leaves mulch effects, but both of them were significantly different from the rice straw mulch, which performed better.

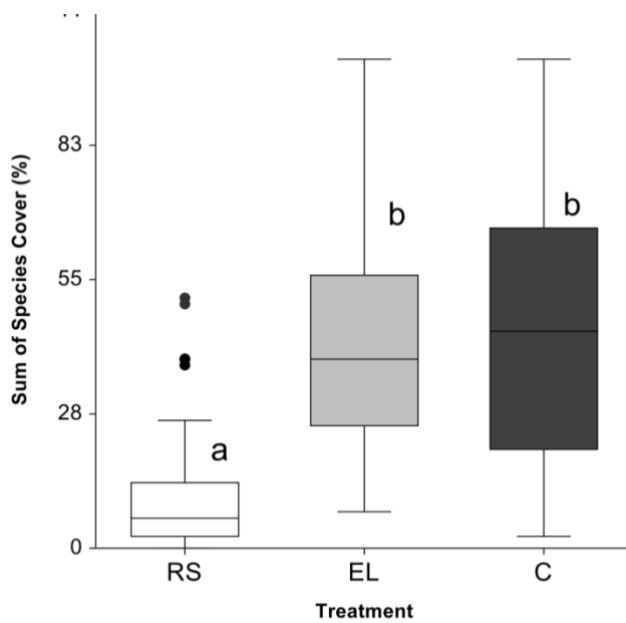


Figure 15. Box-plots of the Sum of Species Cover (%) for the rice straw (RS) and Eucalyptus leaves (EL) mulches and Control (C). Different letters show significant differences between treatments (Kruskall-Wallis on ranks and Dunn's test; $p < 0,05$). Outliers – black circles.

Results of Kruskal-Wallis and Dunn's test showed significant differences between the rice straw mulch and both eucalyptus leaves mulch and control, which weren't significantly different one to the other.

To perform a further certification, other ANOVAs were conducted.

The first one was a Two-Ways ANOVA concerning the analysis of variance of the global Richness that were detected in each one of the four plots and under each treatment (control, eucalyptus leaves mulch, rice straw mulch), in relationship with the effects of the combinations of Treatment and Location (ISA and QDP), and Treatment x Location; the results showed a slightly significant difference between the ISA and QDP locations, while eucalyptus leaves mulch and control were confirmed similar to each other and both *different from rice straw* (Figure 16).

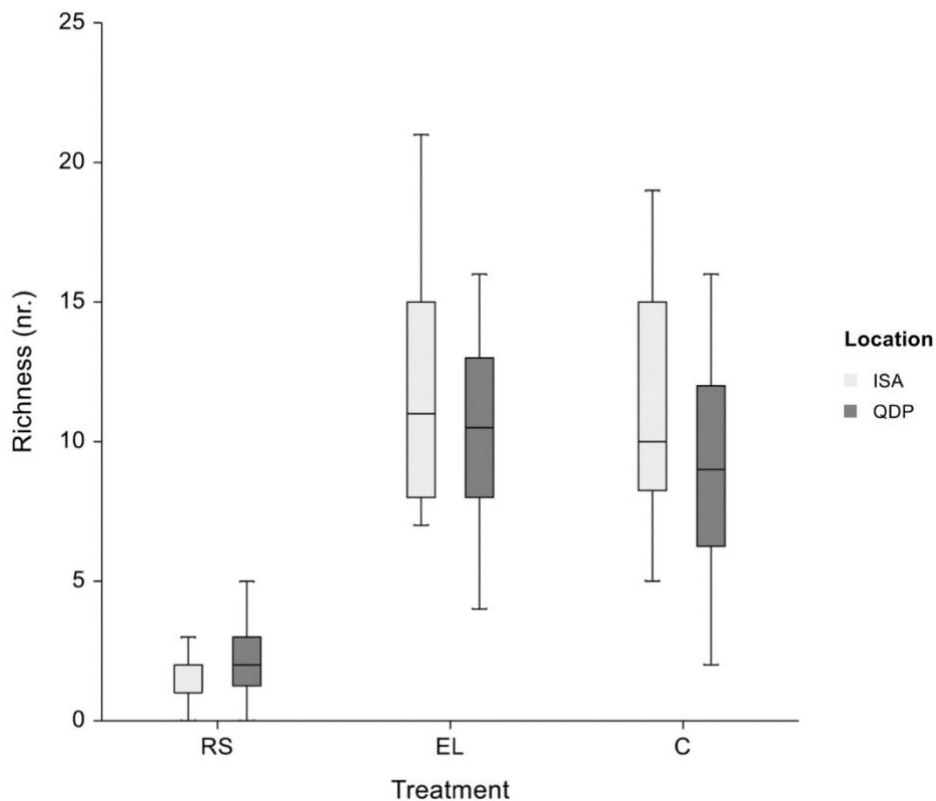


Figure 16. Box-plots of the Richness for the rice straw (RS), Eucalyptus leaves (EL) mulches and Control (C) by each location (ISA and QDP).

Two-Ways ANOVA concerning all the global Richness (nr.) found during all the floristic surveys in all of the plots, in relationship with the effects of the combinations of Treatment and Location (ISA and QDP), and Treatment x Location; the results show differences between the rice straw (RS) mulch detected Richness and both eucalyptus leaves (EL) mulch and control (C), which weren't significantly different one to the other, and slightly significant difference between the ISA and QDP locations.

The second one was a Two-Ways ANOVA concerning the analysis of variance of the global Richness that were detected in each one of the four plots and under each treatment (control, eucalyptus leaves mulch, rice straw mulch), in relationship with the

effects of the combinations of Treatment and Month (period April-September in which the floristic surveys were conducted monthly, n=6), and Treatment x Month; the results showed a slightly significant differences between the monthly results of the floristic surveys, with May registered as having the most different results from the other months, especially from the point of view of the control Richness results being significantly lower (probably due to the mechanical weed management practices conducted in all the fields in this month, see 3.2.1. and 3.2.2.) and the eucalyptus leaves mulch Richness results being significantly higher (probably because of May being the 5th month in a row with average precipitations >45mm, see Figure 5).

From the point of view of the Treatment, eucalyptus leaves mulch and control were confirmed similar to each other in all monthly floristic surveys and both of them were confirmed different from rice straw (Figure 17).

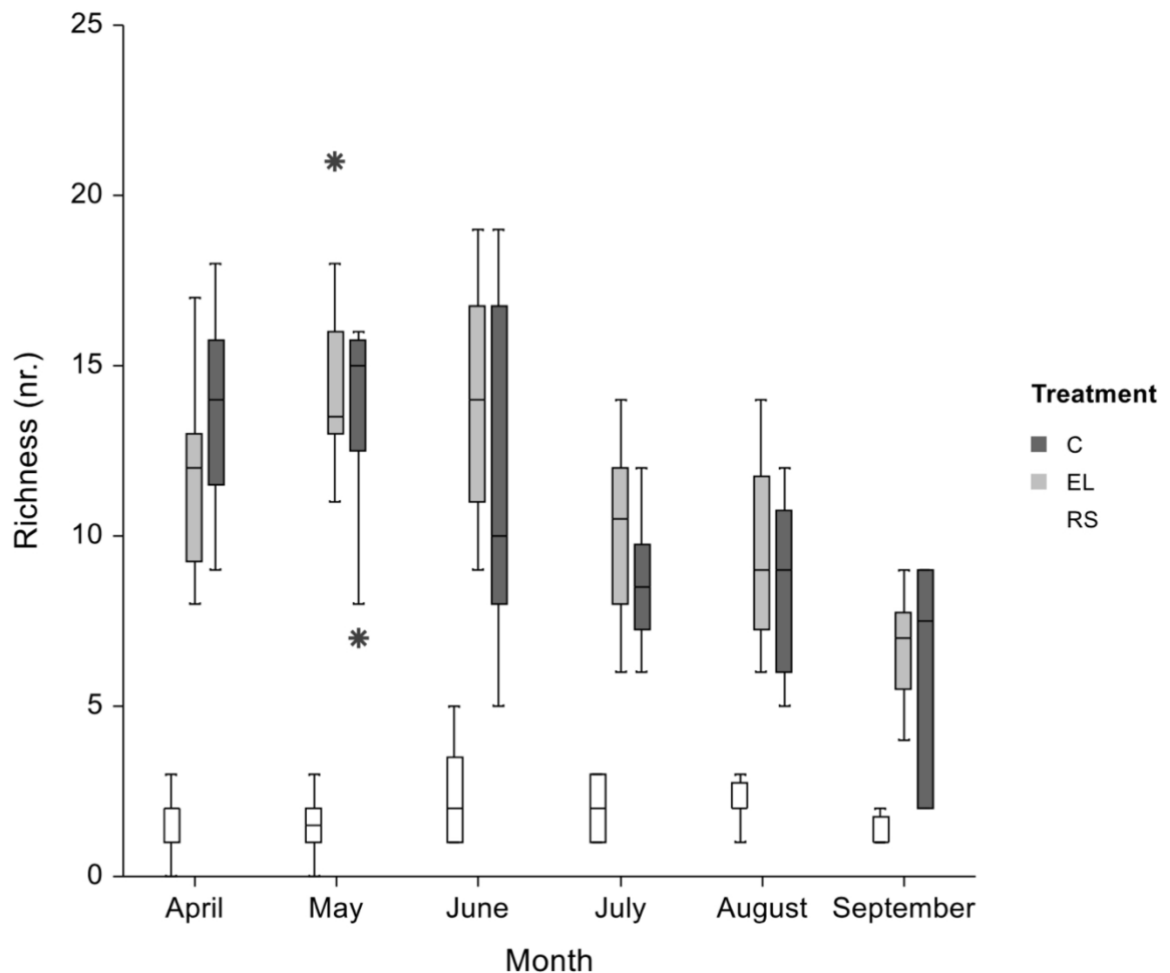


Figure 17. Box-plots of Richness along the six observations (April to September) and for the rice straw (RS), Eucalyptus leaves (EL) mulches and Control (C).

The Two-Ways ANOVA concerning the Richness (nr.) found during all the floristic surveys in all plots, in relation to the effects of the combinations of Treatment and Month (n=6), and Treatment x Month showed differences between RS and the treatments EL and C, which weren't significantly different. Slightly significant differences between the monthly results of the floristic surveys are shown, with May registered as having the most different results from the other months, especially from the point of view of the control and the eucalyptus leaves mulch results.

The last one was a complex Two-Ways ANOVA with the Block, concerning the analysis of variance of the global Richness that were detected in each one of the four plots and under each treatment (control, eucalyptus leaves mulch, rice straw mulch), in relationship with the effects of the combinations of Treatment and Location (ISA and QDP) and Variety (Syrah and Alvarinho), with the Month as a Block (considered as a factor and not as a number, n=6); the analysis of the variance table of the global Richness showed a highly significant influence on the detected results given by both the Treatment and the Month, while a lower, but still significant, the influence was given also by the Location in which the Richness was registered (Table 4).

*Table 4. Table of the ANOVA of the global Richness showing a highly significant influence (***) on the detected results given by both the Treatment and the Month, while a lower, but still significant, influence (**) was given also by the Location in which the Richness was registered.*

Response: Richness					
Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Treatment	2 3805.8	1902.88	264.7321	< 2.2e-16 ***	
Variety	1 17.2	17.23	2.3966	0.123131	
Location	1 67.8	67.78	9.4300	0.002422 **	
Month	5 721.2	144.23	20.0657	2.755e-16 ***	

In conclusion, also the Two-Ways ANOVA with the Block showed no significant differences between the control treatment and the eucalyptus leaves mulch effects, but both of them were significantly different from the rice straw mulch, which performed better.

In conclusion, all the tests performed showed that rice straw mulch was the best-performing treatment, having lower values in both Richness and Sum Cover in all of the plots, all of the locations and during all the months in which the experiment was

conducted.

This is really interesting because eucalyptus leaves mulch performed as good as two of the most common and spread weed management methods throughout the world's vineyards and arouses even more interest if we consider that glyphosate spraying (used in ISA) is seriously risking becoming illegal from December 15th 2022 in all Europe (https://food.ec.europa.eu/plants/pesticides/approval-active-substances/renewal-approval/glyphosate_en) and has anyway seriously detrimental effects on the agroecosystem scientifically proven since many years (2.2.1.), while tractor passing between the rows and tillage (used in both ISA and QDP) can result in soil compaction and also promote soil erosion (2.2.2).

4.3 Characterization of the flora

We found in total 59 different *taxa*, belonging to 24 different families (Table 5). Overall, the families with more *taxa* were: Asteraceae (15 *taxa*), Poaceae (10 *taxa*), Geraniaceae (4 *taxa*) and Polygonaceae (4 *taxa*). There were some differences between the flora of ISA and QDP, with the latter having more species (n=50) than ISA (n= 38), but both sharing Poaceae, Asteraceae and Geraniaceae as the most represented families. More than one-quarter of the species found were the sole elements of a given family (Figure 18). For instance, QDP had 17 species from different families, such as *Chenopodium album* (Amaranthaceae), *Ranunculus repens* (Ranunculaceae), *Anagallis arvensis* (Primulaceae), *Galium aparine* (Rubiaceae), *Rubus ulmifolius* (Rosaceae).

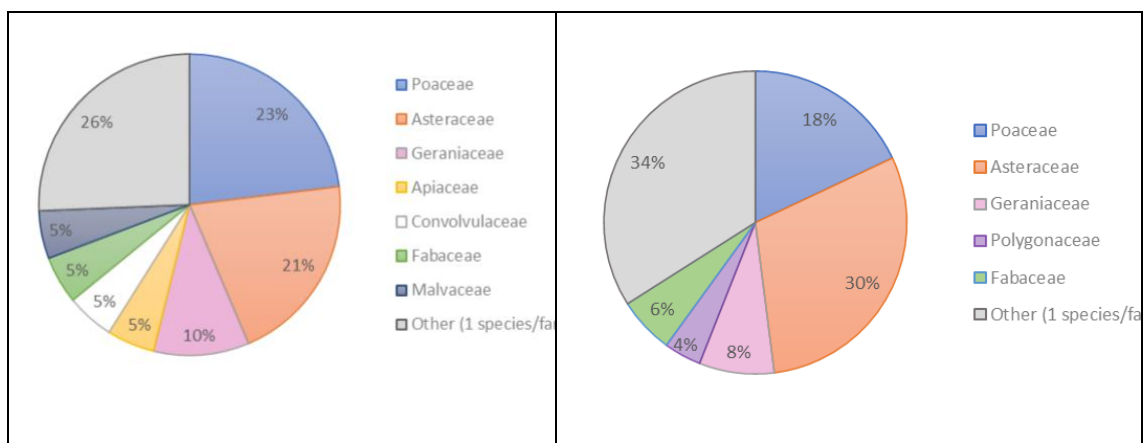


Figure 18. Representation of all the different taxonomic families of the various taxa encountered during the floristic surveys in the experimental plots: ISA (left graph) and QDP (right graph).

The Asteraceae and Poaceae families predominate in crops due to the easy spread of the Asteraceae and a generally good adaptation to the cultural cycles of the Poaceae (Caiado, 1992). The identified taxa are described in Table 5.

Table 5. Taxonomic characterization of the 59 total taxa found during all the floristic surveys in all of the tested plots. Eudicot – Eudicotyledon (Magnoliopsida); Monocot – Monocotyledon (Liliopsida). Life span and physiognomic type.

Taxon	Family	Phisionomy	Life span	Class
<i>Ammi majus</i>	Apiaceae	Terophyte	Annual/bienal	Eudicot
<i>Anagallis arvensis</i>	Primulaceae	Terophyte	Annual	Eudicot
<i>Andryala integrifolia</i>	Asteraceae	Hemicryptophyte	Perennial	Eudicot
<i>Avena sterilis</i>	Poaceae	Terophyte	Annual	Monocot
<i>Bromus diandrus</i>	Poaceae	Terophyte	Annual	Monocot
<i>Calendula arvensis</i>	Asteraceae	Terophyte	Annual	Eudicot
<i>Celtis australis</i>	Cannabaceae	Macrophanerophyte	Perennial	Eudicot
<i>Chamaemelum fuscatum</i>	Asteraceae	Terophyte	Annual	Eudicot
<i>Chenopodium album</i>	Amaranthaceae	Terophyte	Annual	Eudicot
<i>Chrysanthemum segetum</i>	Asteraceae	Terophyte	Annual	Eudicot
<i>Cichorium intybus</i>	Asteraceae	Hemicryptophyte	Perennial	Eudicot
<i>Cirsium arvense</i>	Asteraceae	Geophyte	Annual	Eudicot
<i>Coleostephus myconis</i>	Asteraceae	Terophyte	Annual	Eudicot
<i>Convolvulus arvensis</i>	Convolvulaceae	Proto-hemicryptophyte	Perennial	Eudicot
<i>Convolvulus tricolor</i>	Convolvulaceae	Proto-hemicryptophyte	Perennial	Eudicot
<i>Cynodon dactylon</i>	Poaceae	Proto-hemicryptophyte	Perennial	Monocot
<i>Echinochloa crus-galli</i>	Poaceae	Terophyte	Annual	Monocot
<i>Epilobium tetragonum</i>	Onagraceae	Proto-Hemicryptophyte	Perennial	Eudicot
<i>Equisetum telmateia</i>	Equisetaceae	Geophyte	Perennial	Eudicot
<i>Erodium malacoides</i>	Geraniaceae	Terophyte	Annual	Eudicot
<i>Erodium moschatum</i>	Geraniaceae	Terophyte	Annual	Eudicot
<i>Euphorbia helioscopia</i>	Euphorbiaceae	Terophyte	Annual	Eudicot
<i>Galactites tomentosa</i>	Asteraceae	Terophyte	Annual	Eudicot
<i>Galium aparine</i>	Rubiaceae	Terophyte	Annual	Eudicot
<i>Geranium dissectum</i>	Geraniaceae	Terophyte	Annual	Eudicot
<i>Geranium molle</i>	Geraniaceae	Terophyte	Annual	Eudicot
<i>Hedera helix</i>	Araliaceae	Microphanerophyte	Perennial	Eudicot
<i>Holcus lanatus</i>	Poaceae	Proto-hemicryptophyte	Perennial	Monocot
<i>Hordeum murinum</i>	Poaceae	Terophyte	Annual	Monocot
<i>Hypochaeris radicata</i>	Asteraceae	Hemicryptophyte	Perennial	Eudicot

<i>Lactuca serriola</i>	Asteraceae	Proto-hemicryptophyte	Perennial	Eudicot
<i>Lavatera cretica</i>	Malvaceae	Terophyte	Annual	Eudicot
<i>Lavatera trimestris</i>	Malvaceae	Terophyte	Annual	Eudicot
<i>Leontodum taraxacoides</i>	Asteraceae	Hemicryptophyte	Perennial	Eudicot
<i>Lolium perenne</i>	Poaceae	Terophyte	Annual	Monocot
<i>Lythrium junceum</i>	Lythraceae	Hemicryptophyte	Perennial	Eudicot
<i>Medicago polymorpha</i>	Fabaceae	Terophyte	Annual	Eudicot
<i>Melilotus segetalis</i>	Fabaceae	Terophyte	Annual	Eudicot
<i>Mentha suaveolens</i>	Lamiaceae	Proto-hemicryptophyte	Perennial	Eudicot
<i>Oxalis pes-caprae</i>	Oxalidaceae	Geophyte	Annual	Eudicot
<i>Panicum repens</i>	Poaceae	Proto-hemicryptophyte	Perennial	Monocot
<i>Phalaris minor</i>	Poaceae	Terophyte	Annual	Monocot
<i>Picris echioides</i>	Asteraceae	Proto-hemicryptophyte	Perennial	Eudicot
<i>Plantago lanceolata</i>	Plantaginaceae	Hemicryptophyte	Annual	Eudicot
<i>Poa annua</i>	Poaceae	Terophyte	Annual	Monocot
<i>Polygonum aviculare</i>	Polygonaceae	Terophyte	Annual	Eudicot
<i>Ranunculus repens</i>	Ranunculaceae	Hemicryptophyte	Perennial	Eudicot
<i>Raphanus raphanistrum</i>	Brassicaceae	Terophyte	Annual	Eudicot
<i>Rubus ulmifolius</i>	Rosaceae	Magnoliophyte	Perennial	Eudicot
<i>Rumex crispus</i>	Polygonaceae	Proto-hemicryptophyte	Perennial	Eudicot
<i>Senecio vulgaris</i>	Asteraceae	Terophyte	Annual	Eudicot
<i>Solanum nigrum</i>	Solanaceae	Proto-hemicryptophyte	Perennial	Eudicot
<i>Sonchus asper</i>	Asteraceae	Terophyte	Annual	Eudicot
<i>Sonchus oleraceus</i>	Asteraceae	Terophyte	Annual	Eudicot
<i>Stachys arvensis</i>	Lamiaceae	Terophyte	Annual	Eudicot
<i>Torilis arvensis</i>	Apiaceae	Terophyte	Annual/biennial	Eudicot
<i>Trifolium repens</i>	Fabaceae	Proto-hemicryptophyte	Perennial	Eudicot
<i>Veronica persica</i>	Plantaginaceae	Terophyte	Annual	Eudicot
<i>Vicia sativa</i> subsp. <i>nigra</i>	Fabaceae	Terophyte	Annual	Eudicot

The highest species richness was registered in the QDP Alvarinho plot, with 37 different *taxa* registered in the section treated with eucalyptus leaves mulch, followed by QDP Alvarinho control (n=32), ISA Alvarinho eucalyptus leaves mulch (n=31), ISA Syrah control (n=28), ISA Alvarinho control and ISA Syrah eucalyptus leaves mulch (both n=27), QDP Syrah eucalyptus leaves mulch (n=25), QDP Syrah control (n=23).

An interesting fact can be seen if looking at the sum of the relative frequencies registered per each *taxon* found in Figure 19, in fact, despite the Convolvulaceae family being represented by only two *taxa*, *Convolvulus arvensis* is the most frequent *taxon* throughout all the floristic surveys, being the only *taxon* that was registered in all the different plots and treatments in every month. Not all the bad comes to hurt, in fact *Convolvulus arvensis* is known for his white flowers, which attract pollinating fauna and

auxiliary fauna (Prokop *et al.*, 2014), while also having interesting chemical constituent and pharmacological effects (Al-Snafi, 2016).

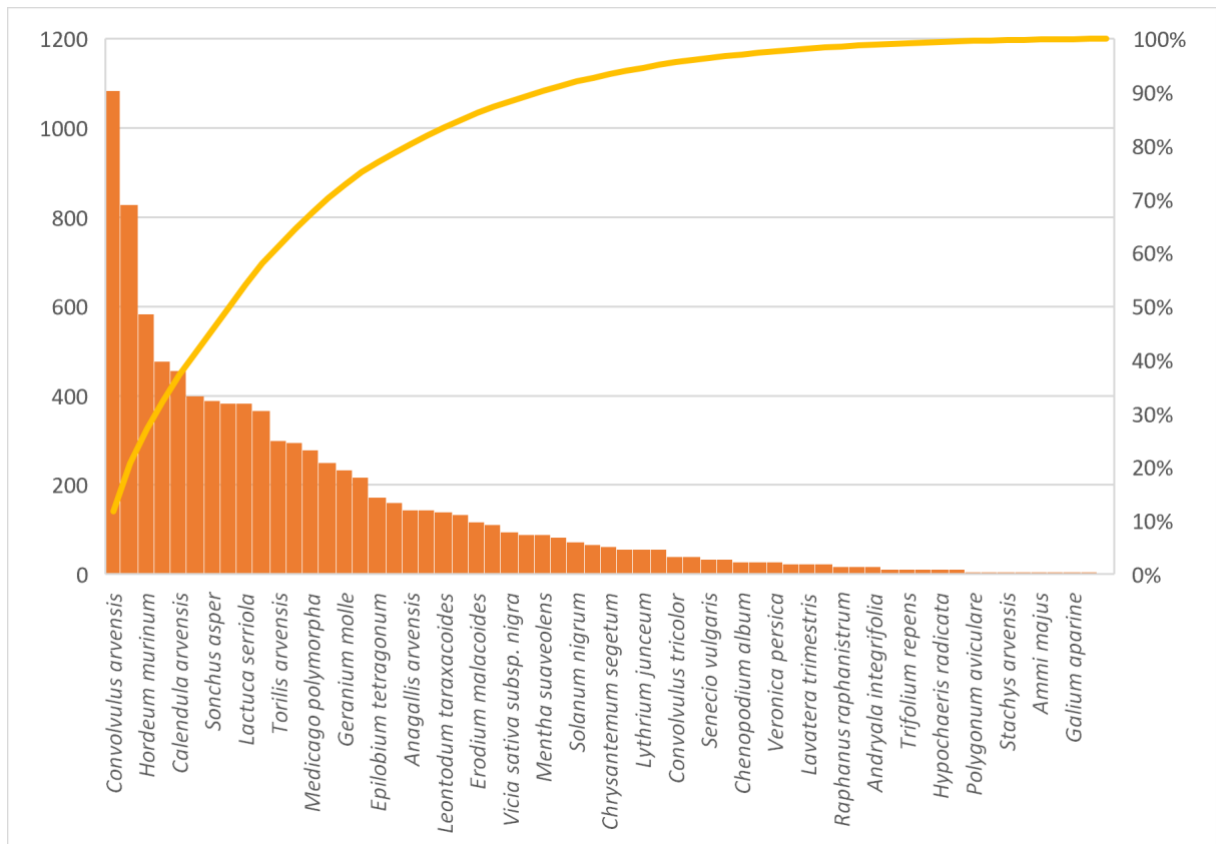


Figure 19. Graphical representation of the sum of all the relative frequencies (orange) registered in all of the different plots tested during the 6 months of testing (April-September) per each taxon found.

4.4 Diversity indexes

To have a better understanding of the ecology of the plots in which our experiment was conducted, taking inspiration from many scientific papers on habitat ecology and from the master thesis “Influence of vineyard soil management on floristic communities: contribution to the Alentejo Wine Sustainability Plan” (Pires, 2022), the most important habitat ecology analyzing indexes were calculated: starting from the specific Richness of each section of each treatment tested in each plot, Shannon’s Index, Simpson’s Index, Equitability and Dominance, as well as the average Richness of each weed management technique.

As shown by the results in Table 6, all the different indexes found eucalyptus leaves mulch and control comparable to each other, and both different from rice straw mulch.

Table 6. Results of the diversity indexes: Shannon index, Simpson index and Equitability were calculated per treatment in each one of the four plots tested.

		Shannon-Wiener	Simpson	Equitability	Dominance
ISA	SY				
	RS	0,178 b	1,241 b	0,373 b	0,785 b
	EL	1,344 a	19,62 a	0,939 a	0,123 a
	C	1,335 a	18,28 a	0,922 a	0,157 a
	AL				
	RS	0,494 b	2,202 b	0,635	0,862 b
	EL	1,358 a	19,53 a	0,910	0,141 a
	C	1,316 a	17,75 a	0,919	0,172 a
QDP	SY				
	RS	0,622 b	3,303 b	0,799	0,645 b
	EL	1,294 a	16,62 a	0,925	0,203 a
	C	1,256 a	15,07 a	0,922	0,223 a
	AL				
	RS	0,835 b	5,348 b	0,835	0,353
	EL	1,438 a	22,66 a	0,917	0,116
	C	1,341 a	17,55 a	0,891	0,147

The Shannon index was calculated by the negative sum of the results of the specific frequencies divided by the total of them, multiplied by their natural logarithm. This index shows how similar the abundance of different weed species is in one community; in Figure 20 we can see graphically the differences between the average abundances of *taxa* per weed management method in both ISA and QDP tested plots.

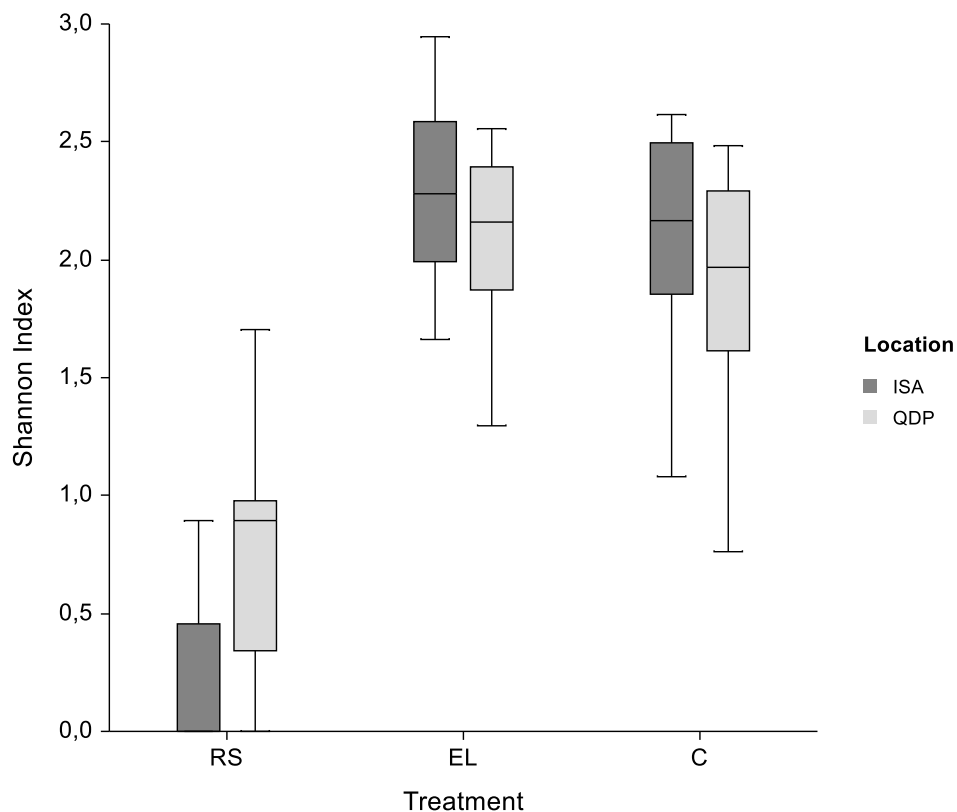


Figure 20. Graphical results of the comparing of average abundance levels (Shannon index) throughout the different weed management (eucalyptus leaves – EL; rice straw – RS; control – C) methods tested in ISA and QDP plots, showing significant differences between EL and RS, and between C and RS.

The Simpson index was calculated by dividing one by the sum of the squares of each one of the specific frequencies divided by the total of them. This index shows how high the diversity of different weed species is in one community; in Figure 21 we can see graphically the differences between the differences of taxa per weed management method in both ISA and QDP tested plots.

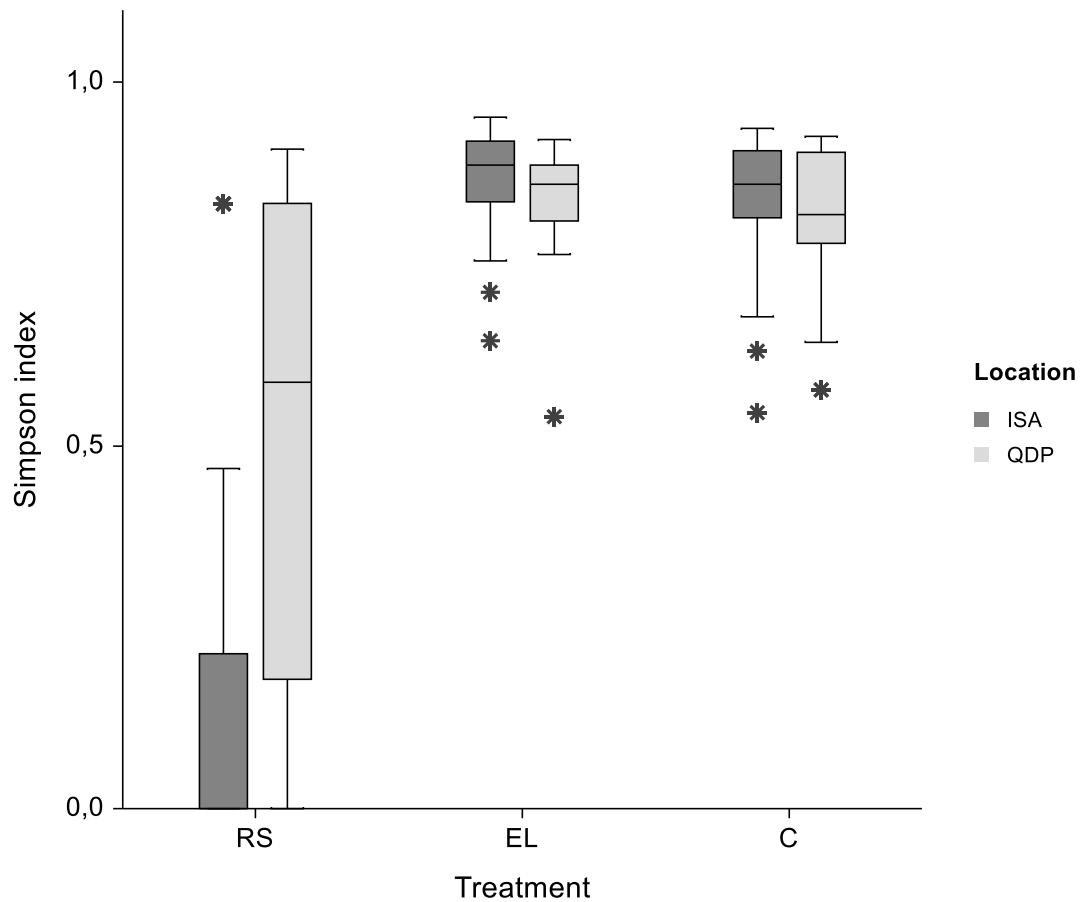


Figure 21. Graphical results of the comparing of diversity levels (Simpson index) throughout the different weed management (eucalyptus leaves – EL; rice straw – RS; control – C) methods tested in ISA and QDP plots, showing significant differences between EL and RS, and between C and RS.

The Equitability index was calculated by dividing the Shannon index by the maximum possible diversity found in each weed management method. This index shows the extent of the representation by equal numbers of individuals of different species of a given community; in Figure 22 we can see graphically the differences between weed management methods in both ISA and QDP tested plots.

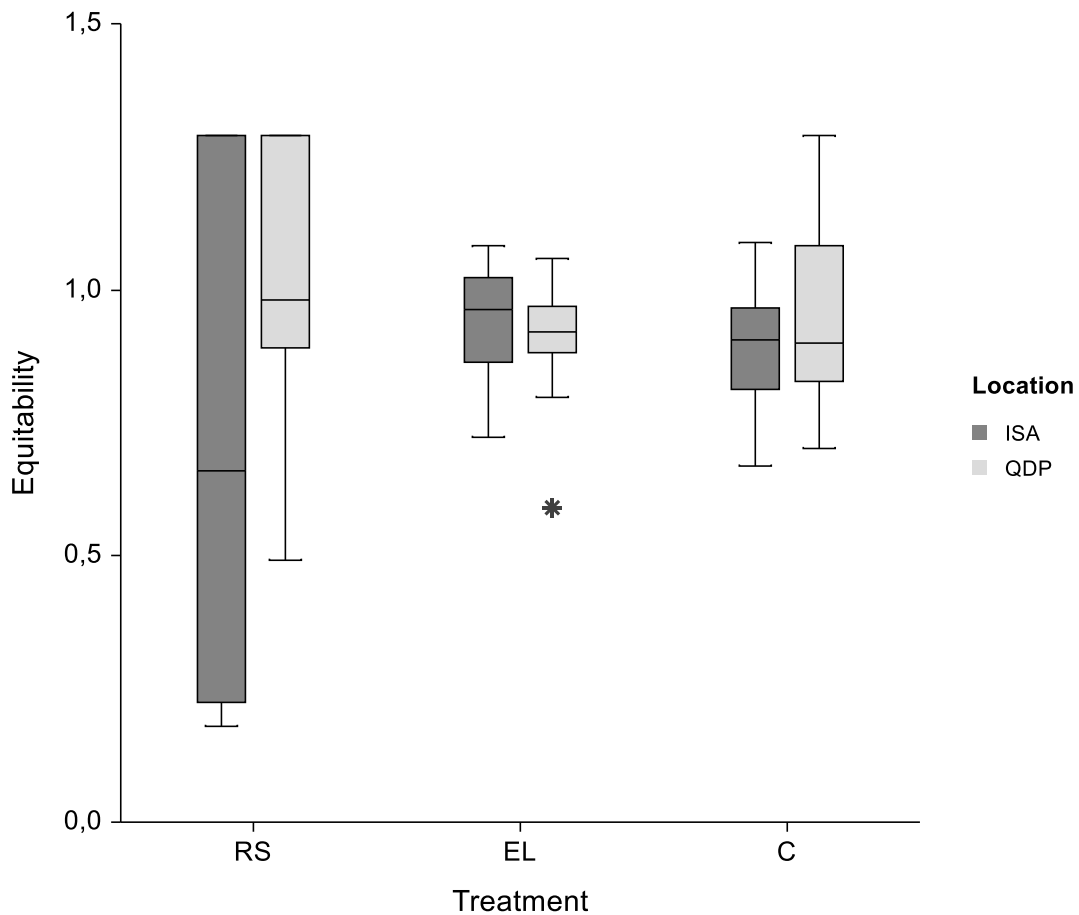


Figure 22. Graphical results of the extent of the representation by equal numbers of individuals of different species of a given community (Equitability) throughout the different weed management (eucalyptus leaves – EL; rice straw – RS; control – C) methods tested in ISA and QDP plots, showing significant differences between EL and RS, and between C and RS.

In Figure 23 we can see the graphical results of the comparison of average Dominance levels throughout the different weed management methods tested in ISA and QDP plots. This graph shows us the dominance registered under each weed management method both in ISA and QDP, confirming the situation encountered during the floristic surveys of the rice straw mulch having few and very dominant *taxa* surviving it (es. *Convolvulus arvensis*), while in both EL and C it was registered many more *taxa* with obviously less dominance for each one.

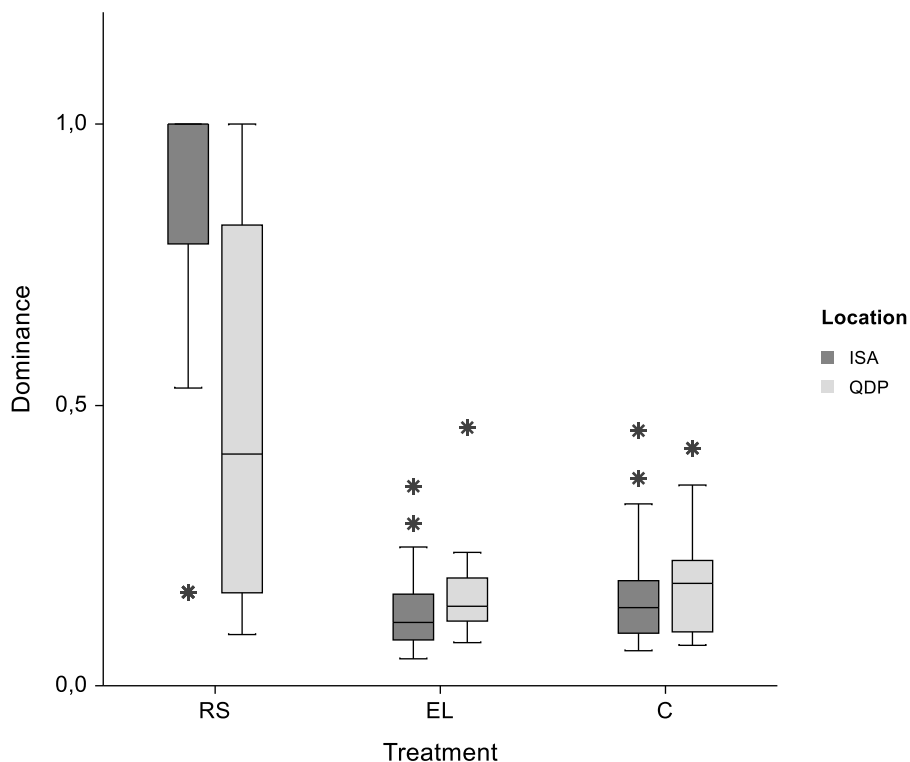


Figure 23. Box-plots of the Dominance index throughout the different weed management (eucalyptus leaves – EL; rice straw – RS; control – C) methods tested in ISA and QDP plots, showing significant differences between EL and RS, and between C and RS.

Overall, all the different indexes are similar between the EL and C, with both showing a higher diversity. On the other hand, both of them are often significantly different from the results given by the RS, which were the lowest from all the diversity indices, except for the dominance, which resulted from the resistance of *Convolvulus arvensis* to shadowing and the potential high recuperation from radicular buds.

4.5 Value of Importance Index

In order to select the most relevant *taxa* for the case study, the ones with more influence inside the vineyard agroecosystem, the Value of Importance Index (IVI) was calculated for each one of the species in each weed management method (Annex 4), then the species with $IVI > 45$ were pointed out in Table 7. The sections with the higher number of *taxon* with $IVI > 45$ were the ones characterized by the control treatments and the ones treated with the eucalyptus leaves mulch; were respectively found 7 *taxa* in the QDP Syrah section, 9 *taxa* in the QDP Alvarinho section, 10 *taxa* in the ISA Syrah section

and 10 *taxa* in the ISA Alvarinho section for the control treated sections of the four plots, while for the sections treated with eucalyptus leaves mulch, the numbers of *taxa* were respectively 5 in the QDP Syrah section, 9 in the QDP Alvarinho section, 9 in the ISA Syrah section and 12 in the ISA Alvarinho section.

Overall the rice straw mulch is the treatment that had a lower incidence of *taxa* and the only *taxon* found with high frequency enough to have IVI > 45 was *Convolvulus arvensis* in all four plots, both ISA's and both QDP's (*C. arvensis* IVI was even 2 or almost 3 times greater than 45).

Table 7. *Taxa identified at ISA and QDP at rice straw, eucalyptus leaves and control from Syrah (SY) and Alvarinho (AL) plots with Index of Importance Value (IVI) higher than 45. The common species from all treatments t each of the four locations is highlighted in bold.*

	Rice straw	IVI	Eucalyptus leaves	IVI	Control	IVI
ISA	SY					
	Convolvulus arvensis	93	<i>Avena sterilis</i>	113	<i>Avena sterilis</i>	114
			<i>Hordeum murinum</i>	93	Convolvulus arvensis	104
			Convolvulus arvensis	86	<i>Hordeum murinum</i>	76
			<i>Sonchus asper</i>	57	<i>Sonchus asper</i>	68
			<i>Erodium moschatum</i>	55	<i>Anagallis arvensis</i>	57
			<i>Lolium perenne</i>	54	<i>Lavatera cretica</i>	51
					<i>Lolium perenne</i>	48
	AI					
	Convolvulus arvensis	80	Convolvulus arvensis	87	Convolvulus arvensis	102
			<i>Hordeum murinum</i>	86	<i>Avena sterilis</i>	101
			<i>Picris echioides</i>	75	<i>Picris echioides</i>	82
			<i>Torilis arvensis</i>	69	<i>Torilis arvensis</i>	75
			<i>Epilobium tetragonum</i>	69	<i>Hordeum murinum</i>	75
			<i>Rubus ulmifolius</i>	57	<i>Epilobium tetragonum</i>	68
			<i>Lolium perenne</i>	52	<i>Mentha suaveolens</i>	53
			<i>Calendula arvensis</i>	51	<i>Lolium perenne</i>	52
			<i>Geranium dissectum</i>	46	<i>Bromus diandrus</i>	51
QDP	Sy					
	Convolvulus arvensis	98	<i>Lactuca serriola</i>	95	<i>Avena sterilis</i>	113
			<i>Hordeum murinum</i>	86	<i>Hordeum murinum</i>	105
			Convolvulus arvensis	74	<i>Calendula arvensis</i>	100
			<i>Picris echioides</i>	69	Convolvulus arvensis	96
		<i>Sonchus asper</i>	65	<i>Lavatera cretica</i>	80	

		<i>Bromus diandrus</i>	62	<i>Lactuca serriola</i>	76
		<i>Lavatera cretica</i>	57	<i>Torilis arvensis</i>	64
		<i>Geranium molle</i>	52	<i>Picris echioides</i>	64
		<i>Lolium perenne</i>	51	<i>Bromus diandrus</i>	57
				<i>Geranium molle</i>	46
AI					
<i>Convolvulus arvensis</i>	124	<i>Convolvulus arvensis</i>	99	<i>Convolvulus arvensis</i>	113
		<i>Picris echioides</i>	91	<i>Avena sterilis</i>	103
		<i>Bromus diandrus</i>	91	<i>Lactuca serriola</i>	88
		<i>Lavatera cretica</i>	87	<i>Lavatera cretica</i>	81
		<i>Sonchus asper</i>	82	<i>Calendula arvensis</i>	72
		<i>Avena sterilis</i>	81	<i>Lolium perenne</i>	68
		<i>Calendula arvensis</i>	74	<i>Bromus diandrus</i>	62
		<i>Lactuca serriola</i>	62	<i>Picris echioides</i>	58
		<i>Medicago polymorpha</i>	60	<i>Solanum nigrum</i>	52
		<i>Erodium moschatum</i>	52	<i>Sonchus asper</i>	51
		<i>Lolium perenne</i>	51		
		<i>Hordeum murinum</i>	46		

4.6. Ecosystem services of weedy flora

After identifying the most dominant taxa inside the vineyard agroecosystem, a literature search was conducted on their actual roles and possible uses inside and outside the vineyard. Overall, the total herbaceous taxa with IVI > 45 were 18, with a range between 45 and over 123 (Table 8). *Rubus ulmifolius* and *Celtis australis*, a liana and a phanerophyte species were not considered in this analysis.

Table 8. Uses and ecosystem services of taxa with IVI >45. Data collected from: CABI - Invasive Species Compendium (<https://www.cabi.org/isc/>); PFAF nature.uevora.pt/Especies-e-<https://pfaf.org/user/Default.aspx>) habitats/Plantas); CONECT-e (<https://www.conecte.es/index.php/es/plantas/buscar>); Savage et al. (1969); Waddington (1976); Almeida (1996); Wolff and Debussche (1999); Jain et al. (2011), Sharrif and Hamed (2012); Williams et al. (2020); Ben-Nasr et al. (2015); Minkey e Spafford (2016); Lovas-Kiss et al. (2019); Eyal et al. (2019); Rajasab A.H. (2022); Saleem et al. (2020); Carapeto et al. (2021), Pires (2022).

Taxon	Cattle feed	Human food	Medicinal use	Essences	Pollination	Auxiliary fauna	Seed dispersion	Soil structure
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<i>Anagallis arvensis</i>		X	X		X			
<i>Avena sterilis</i>	X	X	X				X	X
<i>Bromus diandrus</i>	X						X	X
<i>Calendula arvensis</i>		X	X	X	X			
<i>Convolvulus arvensis</i>			X		X	X		
<i>Epilobium tetragonum</i>			X	X	X			
<i>Erodium moschatum</i>		X	X	X	X			X
<i>Geranium molle</i>		X	X	X				X
<i>Hordeum murinum</i>	X	X					X	X
<i>Lactuca serriola</i>		X	X		X			
<i>Lavatera cretica</i>		X	X	X	X			
<i>Lolium perenne</i>	X				X		X	X
<i>Medicago polymorpha</i>	X	X				X		X
<i>Mentha suaveolens</i>	X	X	X	X	X			
<i>Picris echioides</i>					X	X		
<i>Solanum nigrum</i>			X					
<i>Sonchus asper</i>	X	X	X	X	X	X		X
<i>Torilis arvensis</i>				X				

All this work was done to expand our knowledge about the possible benefits brought to the vineyard ecosystem by the weed strains that were more present in our experiment and in order to have a better understanding of whether we should improve the efficiency of our weed management techniques or if we could let them coexist with our treatment since, while being genuinely controlled in order not to compromise yield and crop quality, they can bring various beneficial effects to the vines and the vineyard agroecosystem.

5. Conclusions and future perspectives

This study allowed us to evaluate the effects of two mulching methods which aren't usually used in vineyards, while also comparing them to two very common practices in worldwide vineyard weed management such as tillage and herbicides usage. Furthermore, this study allowed the inventory of the flora of these vineyards of the Lisboa region and to notice that some species are becoming tolerant to various control management methods, namely *Convolvulus arvensis*. The characterization of the floristic

community allowed us to know the services provided to the ecosystems of the vineyard. In this sense, the calculation of various indicators of diversity and the exploitation of ecosystem services taking into account the abundance of species and the services they can provide made it possible to recognize their importance for the biodiversity of the vineyards of this region.

All the different tests that were conducted, from the points of view of the cover, the richness, of the diversity, gave very similar results: the eucalyptus mulch effects were comparable to the effects of both control methods from the two different sites where the tested vineyards were located, while the rice straw mulch effects were significantly different and way more efficient than the other weed management methods.

In conclusion both tests shown that rice straw mulch is significantly different from eucalyptus leaves mulch and control, both for global Richness (number of species observed) and global Sum of Cover (%) of all species observed (Figure 15).

Rice straw mulch was the most effective treatment to control weeds in all the plots tested, having an average sum cover and an average specific richness around 80% lower than eucalyptus leaves mulch and control ones. But being this highly effective could also become detrimental, especially from the point of view of the biodiversity of the vineyard agroecosystem: for this reason, rice straw mulch could be used in vineyards with ongoing weed infestations, more in particular weeds that have developed resistances to the previously used weed management techniques and are now out of control, affecting the yield and the quality of the grapes.

Eucalyptus leaves mulch, instead, was less effective than rice straw mulch, but its results were very similar to the control weed management techniques in both ISA and QDP plots, which are treated the same way as the rest of the vineyards in which the plots are located.

If eucalyptus leaves mulch was tested even further in vineyards, it could conquer the attention of wine producers, making it possible to organize a supply chain with the collaboration of eucalyptus farmers, paper production industries and transport organizations, with the final goal of lowering the price of distribution of this organic waste material and making it become a serious answer to the need of keeping the vineyard agroecosystem under control while respecting it. However, some caution has to be made, as the composition of Eucalyptus leaves was not studied in relation to the effects on the vines and their production, as well as on the fauna auxiliary communities, and soil microbiota and mineral composition.

Starting from here, future studies could be conducted on organic mulches such as eucalyptus leaves, rice straw (and maybe new others) using different quantities, testing these mulches for longer periods in the field, focusing on their effects on the soil and its microbiome, studying more deeply their total effects on the vineyards agroecosystem.

Further studies could even include new mulching techniques such as alginate disposal on the nude soil (Immirzi *et al.*, 2009) mixed with eucalyptus and/or rice straw fibers and eucalyptus essential oils.

Another possibility could even be represented by the development and testing of a new mulching product starting from the results of the research conducted by Delgado-Aguilar and collaborators in 2018 on the technical reinforcement of corn starch-derived bioplastic with eucalyptus fibers.

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Annexes

Annex 1. Results for the chemical analysis of the mulching materials.

Chemical Analysis	<i>Eucalyptus leaves</i>	<i>Rice straws</i>
Ash (% of o.d)	5,2	10,5
Total extractives (% of o.d)	43,8	23,7
CH ₂ Cl ₂	19,9	3,6
EtOH	17,1	14,8
H ₂ O	6,8	5,3
Total lignin (% of o.d)	19,5	17,7
Klason lignin	16,8	15,6
Soluble lignin	2,7	2,1
Sugars (% of o.d)	28,5	45,9
ramnose	0,7	0,3
arabinose	7,0	3,8
galactose	3,5	1,5
glucose	21,5	34,6
xylose	8,0	16,8
mannose	-	-
galacturonic acid	6,9	0,6
glucuronic acid	0,1	0,2
acetic acid	2,8	2,3

Annex 2. Results from the thermal camera analysis of the ISA Alvarinho tested plots.

June	Flir A35					
	9h			15h		
	Control	Eucalyptus Leaves	Rice Straw	Control	Eucalyptus Leaves	Rice Straw
	27,849	25,952	24,789	31,245	29,84	25,415
	26,743	23,196	23,841	28,091	29,105	23,461
	27,643	25,968	25,035	30,807	32,422	24,061
	25,79	23,29	23,707	28,32	29,296	23,556
	29,248	25,148	25,152	29,464	31,953	26,545
	26,819	23,754	23,853	28,568	30,351	23,487
	29,047	25,392	26,182	31,093	33,69	25,278
	26,814	24,303	22,97	30,459	29,809	22,997
	28,567	26,255	25,053	32,648	33,053	25,89
	24,684	22,15	21,331	28,069	28,337	23,464
	21,024	21,967	24,145	31,408	17,753	29,647
	18,381	21,601	21,777	28,514	14,699	29,86
	22,049	22,982	25,645	30,672	17,41	32,204
	17,948	21,237	21,056	29,08	16,519	30,193
	21,02	25,125	26,4	32,58	18,048	30,878
	18,856	20,936	21,97	28,944	16,251	30,916
	19,26	22,688	24,361	31,455	19,067	32,729
	17,615	20,149	21,599	28,491	15,554	31,153
	22,154	22,02	23,438	29,708	16,44	33,894
	17,64	20,978	21,213	28,658	14,826	31,096
Average	23,45755	23,25455	23,67585	29,9137	23,72115	27,8362
July	Flir A35					
	9h			15h		
	Control	Eucalyptus Leaves	Rice Straw	Control	Eucalyptus Leaves	Rice Straw
	26,799	24,115	22,631	29,776	22,295	24,132
	24,784	22,678	20,851	28,313	20,416	23,477
	25,35	26,009	23,224	28,617	23,317	27,583
	23,591	23,226	21,172	27,411	21,169	24,461
	26,201	25,544	22,557	28,566	23,524	26,712
	25,54	23,391	23,039	28,128	22,246	24,641
	26,882	25,49	24,254	29,565	23,953	25,5
	26,329	23,061	22,441	27,384	22,729	24,517
	26,735	25,885	24,007	29,956	24,058	27,034
	24,396	25,096	19,544	26,791	22,523	23,734
	28,259	20,215	22,24	26,449	29,963	27,681
	26,994	18,781	20,198	24,955	27,657	25,414
	29,81	21,699	24,868	26,032	29,146	26,993
	26,036	18,844	21,604	25,538	28,121	25,379

	28,446	22,541	25,913	26,723	30,844	29,08
	26,672	20,441	22,119	26,232	30,146	26,367
	29,176	20,386	22,55	27,109	31,716	27,178
	27,597	17,867	21,94	24,189	29,739	25,922
	30,072	19,741	22,858	25,142	31,921	27,982
	26,925	19,155	20,312	26,611	29,039	25,137
Average	26,8297	22,20825	22,4161	27,17435	26,2261	25,9462
August	Flir A35					
	9h			15h		
	Control	Eucalyptus Leaves	Rice Straw	Control	Eucalyptus Leaves	Rice Straw
	26,5	25,93	14,45	27,28	28,05	27,77
	23,83	24,97	12,63	25,43	26,29	26,02
	26,07	24,45	15,31	26,01	27,36	27,23
	23,94	25,24	13,78	25,79	27,11	26,52
	25,69	24,86	14,96	26,65	29,15	28,25
	23,49	24,08	16,72	24,69	26,71	26,87
	26,39	25,01	17,15	26,67	29,85	27,78
	22,06	25,66	12,27	25,55	28,42	27,84
	28,32	26,01	14,82	26,71	28,59	27,8
	22,26	26,61	10,81	24,79	27,67	27,27
	25,64	27,46	26,91	27,03	26,55	24,78
	24,21	28,01	24,76	25,75	25,36	23,9
	26,21	29,76	27,85	26,73	27,89	27,19
	24,09	27,91	24,86	25,7	26,69	25,11
	26,99	28,39	26,36	28,27	28,34	26,44
	24,84	28,18	24,41	25,65	27,23	25,07
	28,17	29,1	25,79	27,87	29,6	27,19
	26,47	29,32	24,67	25,26	25,59	24,44
	26,96	28,72	25,28	27,42	28,37	27,89
	25,58	28,33	24,63	26,69	26,95	25,98
Average	25,39	26,9	19,92	26,3	27,59	26,57

Annex 3. ANOVAs

RICHNESS

One-Way Analysis of Variance Report

Dataset ISA/QDP global
Response Richness

Tests of the Normality of Residuals Assumption

Test	Value	Level	ProbReject Normality? ($\alpha=0,20$)
Normality Attributes			
Skewness	1,4784	0,13929	Yes
Kurtosis	1,8660	0,06204	Yes
Skewness and Kurtosis (Omnibus)	5,6677	0,05879	Yes

Tests of the Equality of Group Variances Assumption

Test Name	Value	Level	ProbReject Equal Variances? ($\alpha=0,20$)
Brown-Forsythe (Data - Medians)	34,4044	0,00000	Yes
Levene (Data - Means)	43,2863	0,00000	Yes
Conover (Ranks of Deviations)	78,3799	0,00000	Yes
Bartlett (Likelihood Ratio)	127,0607	0,00000	Yes

Assumptions rejected -> Kruskal-Wallis

Kruskal-Wallis One-Way ANOVA on Ranks

Hypotheses

H0: All medians are equal.

H1: At least two medians are different.

Test Results

Chi-Squared Method	Prob DF	Reject H0? (H)	Level	Prob ($\alpha=0,05$)
Not Corrected for Ties	2	137,2647	0,00000	Yes

Group Detail

Sum of Group	Mean Count	Ranks	Rank	Z-Value	Median
C	72	10027,50	139,27	5,1166	9
EL	72	10656,50	148,01	6,5693	11
RS	72	2752,00	38,22	-11,6859	2

Kruskal-Wallis Multiple-Comparison Z-Value Test (Dunn's Test)

Richness	C	EL	RS
----------	---	----	----

C	0,0000	0,8421	9,7402
EL	0,8421	0,0000	10,5823
RS	9,7402	10,5823	0,0000

Regular Test: Medians significantly different if z-value > 1,9600

Bonferroni Test: Medians significantly different if z-value > 2,3940

RS≠ C ; RS≠ EL; EL=C

SUM COVER

Dataset ISA/QDP global

Tests of the Normality of Residuals Assumption

Test	ProbReject Normality?		
	Value	Level	($\alpha=0,20$)
Normality Attributes			
Skewness	4,2210	0,00002	Yes
Kurtosis	0,7803	0,43520	No
Skewness and Kurtosis (Omnibus)	18,4253	0,00010	Yes

Tests of the Equality of Group Variances Assumption

Test	ProbReject Equal Variances?			Kruskal-Wallis	One-Way	ANOVA	on	Ranks
	Value	Level	($\alpha=0,20$)					
Test Name								
Brown-Forsythe (Data - Medians)	26,5362	0,00000	Yes					
Levene (Data - Means)	28,3824	0,00000	Yes					
Conover (Ranks of Deviations)	50,8023	0,00000	Yes					
Bartlett (Likelihood Ratio)	55,4485	0,00000	Yes					

Hypotheses

H0: All medians are equal.

H1: At least two medians are different.

Test Results

Chi-Squared Method	Prob DF	Reject H0? (H)	Level	($\alpha=0,05$)
Not Corrected for Ties	2	94,6704	0,00000	Yes
Corrected for Ties	2	94,7847	0,00000	Yes

Number Sets of Ties 44

Multiplicity Factor 12156

Group Detail

Sum of Group	Mean Count	Ranks	Rank	Z-Value	Median
C	72	9930,50	137,92	4,8926	44,375
EL	72	9906,50	137,59	4,8372	38,75
RS	72	3599,00	49,99	-9,7298	6,25

Kruskal-Wallis Multiple-Comparison Z-Value Test (Dunn's Test)

Sum_Cover	C	EL	RS
C	0,0000	0,0320	8,4474
EL	0,0320	0,0000	8,4153
RS	8,4474	8,4153	0,0000

Regular Test: Medians significantly different if z-value > 1,9600
 Bonferroni Test: Medians significantly different if z-value > 2,3940

RS ≠ C ; RS ≠ EL; EL = C

Conclusions: Rice straw mulch is significantly different from Eucalyptus leaves mulch and control, both for richness (number of species observed) and sum of cover of all species observed.

RICHNESS vs TREATMENT vs LOCATION

Response	Richness	Variance	Table	for	Richness	
Analysis of Variance Table for Richness						
Source	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0,05)
A: Treatment	2	3805,75	1902,875	188,71	0,000000*	1,000000
B: Location	1	67,78241	67,78241	6,72	0,010192*	0,732660
AB	2	101,5648	50,78241	5,04	0,007305*	0,812520
S	210	2117,528	10,08347			
Total (Adjusted)	215	6092,625				
Total	216					

* Term significant at alpha = 0,05

Effect of Treatment and Location, and Treatment x Location

Means	and	Standard	Errors	of	Richness
Standard					
Term	Count	Mean	Error		
All	216	7,708333			
A: Treatment					
C	72	10,25	0,3742301		
EL	72	11,08333	0,3742301		
RS	72	1,791667	0,3742301		
B: Location					
ISA	108	8,268518	0,3055575		
QDP	108	7,148148	0,3055575		
AB: Treatment,Location					
C,ISA	36	11,5	0,5292412		
C,QDP	36	9	0,5292412		
EL,ISA	36	11,88889	0,5292412		
EL,QDP	36	10,27778	0,5292412		
RS,ISA	36	1,416667	0,5292412		
RS,QDP	36	2,166667	0,5292412		

Month

Analysis of Variance Table for Richness

Source	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0,05)
A: Treatment	2	3805,75	1902,875	323,29	0,000000*	1,000000
B: Month	5	721,1528	144,2306	24,50	0,000000*	1,000000
AB	10	400,3055	40,03056	6,80	0,000000*	0,999997
S	198	1165,417	5,885943			
Total (Adjusted)	215	6092,625				
Total	216					

* Term significant at alpha = 0,05

Analysis of Variance Table for Richness

Source	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level
A: Treatment	2	3805,75	1902,875	323,29	0,000000*
B: Month	5	721,1528	144,2306	24,50	0,000000*
AB	10	400,3055	40,03056	6,80	0,000000*
S	198	1165,417	5,885943		
Total (Adjusted)	215	6092,625			
Total	216				

* Term significant at alpha = 0,05

Means and Standard Errors of Richness

Standard Term	Count	Mean	Error
All	216	7,708333	
A: Treatment			
C	72	10,25	0,2859182
EL	72	11,08333	0,2859182
RS	72	1,791667	0,2859182
B: Month			
April	36	8,972222	0,4043494
August	36	6,666667	0,4043494
July	36	6,888889	0,4043494
June	36	9,194445	0,4043494
May	36	9,916667	0,4043494
September	36	4,611111	0,4043494
AB: Treatment,Month			
C, April	12	13,58333	0,7003536
C, August	12	8,416667	0,7003536
C, July	12	8,583333	0,7003536
C, June	12	11,41667	0,7003536
C, May	12	13,58333	0,7003536
C, September	12	5,916667	0,7003536
EL, April	12	11,66667	0,7003536

EL, August	12	9,5	0,7003536
EL, July	12	10,08333	0,7003536
EL, June	12	13,91667	0,7003536
EL, May	12	14,66667	0,7003536
EL, September	12	6,666667	0,7003536
RS, April	12	1,666667	0,7003536
RS, August	12	2,083333	0,7003536
RS, July	12	2	0,7003536
RS, June	12	2,25	0,7003536
RS, May	12	1,5	0,7003536
RS, September	12	1,25	0,7003536

RICHNESSvsTREATMENT+VARIETY+LOCATION with block(SEASON)

```

> MODELLO<-lm(RICHNESS~Treatment+Variety+Location+Season,
data=DATA_NCSS)
> anova(MODELLO)

```

Analysis of Variance Table					
Response: RICHNESS					
Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Treatment	2 3805.8	1902.88	264.7321	< 2.2e-16	***
Casta	1 17.2	17.23	2.3966	0.123131	
Location	1 67.8	67.78	9.4300	0.002422	**
Season	5 721.2	144.23	20.0657	2.755e-16	***
Residuals	206 1480.7	7.19			

```

---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> shapiro.test(MODELLO$residuals)

```

Shapiro-Wilk normality test		
data: MODELLO\$residuals		
W	= 0.98914	p-value = 0.1021

```

> ols_test_normality(MODELLO$residuals)

```

Test	Statistic	pvalue
Shapiro-Wilk	0.9891	0.1021
Kolmogorov-Smirnov	0.043	0.8198
Cramer-von Mises	15.178	0.0000
Anderson-Darling	0.4922	0.2160

> MODELLOLevene<-lm(res_abs~Treatment+Variety+Location+Season, data = DATA_NCSS)			
> anova(MODELLOLevene)			
Analysis of Variance Table			
Response: res_abs			
Df	Sum Sq	Mean Sq	F value Pr(>F)
Treatment	2	17.00	8.4986 4.1962 0.016359 *
Variety	1	16.75	16.7542 8.2725 0.004448 **
Location	1	1.47	1.4739 0.7277 0.394608
Season	5	33.88	6.7765 3.3459 0.006293 **
Residuals	206	417.21	2.0253

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1			
Study: MODELLO ~ c("Treatment")			
HSD Test for RICHNESS			
Mean Square Error: 7.187927			
Treatment, means			
RICHNESS	std	r	Min Max
C	10.250000	4.191709	72 2 19
EL	11.083333	3.714266	72 4 21
RS	1.791667	0.918319	72 0 5
Alpha: 0.05 ; DF Error: 206			
Critical Value of Studentized Range: 3.338647			
Minimun Significant Difference: 1.054887			
Treatments with the same letter are not significantly different.			
RICHNESS groups			
EL	11.083333	a	
C	10.250000	a	
RS	1.791667	b	

Annex 4. IVIs calculated for each *taxa* found in all tested plots.

CONTROL QDP SY		EUCALYPTUS QDP SY		RICE STRAW QDP SY	
TAXON	IVI	TAXON	IVI	TAXON	IVI
Raphanus raphanistrum	11,4	Calendula arvensis	39,6	Convolvulus arvensis	93,4
Calendula arvensis	28,9	Convolvulus arvensis	86	Avena sterilis	45
Convolvulus arvensis	104	Erodium moschatum	54,9	Panicum repens	11,3
Erodium moschatum	36,4	Geranium dissectum	34,3	Vicia sativa subsp. nigra	5,63
Geranium dissectum	28,3	Geranium molle	33,8	Rumex crispus	16,9
Geranium molle	34,2	Sonchus asper	56,8	Poaceae	22,8
Sonchus asper	67,6	Picris echioides	39,4		
Picris echioides	16,9	Avena sterilis	113		
Avena sterilis	114	Lactuca serriola	28,1		
Lactuca serriola	28,1	Torilis arvensis	22,5		
Torilis arvensis	5,63	Medicago polymorpha	28,8		
Medicago polymorpha	35,9	Oxalis pes-caprae	5,63		
Oxalis pes-caprae	5,63	Hordeum murinum	93,3		
Hordeum murinum	76	Sonchus oleraceus	11,3		
Sonchus oleraceus	17	Leontodum taraxacoides	29		
Leontodum taraxacoides	28,8	Lavatera cretica	51,3		
Lavatera cretica	51	Bromus diandrus	16,9		
Bromus diandrus	11,3	Anagallis arvensis	22,6		
Anagallis arvensis	56,9	Veronica persica	5,63		
Lolium perenne	48,3	Lolium perenne	54,1		
Vicia sativa subsp. nigra	34,1	Vicia sativa subsp. nigra	34,4		
Rumex crispus	11,3	Cichorium intybus	5,63		
Senecio vulgaris	22,5	Rumex crispus	33,9		
		Senecio vulgaris	11,3		
		Hypochaeris radicata	11,3		

CONTROL QDP AL		EUCALYPTUS QDP AL		RICE STRAW QDP AL	
TAXON	IVI	TAXON	IVI	TAXON	IVI
Raphanus raphanistrum	5,63	Calendula arvensis	50,8	Calendula arvensis	11,3
Calendula arvensis	5,76	Convolvulus arvensis	86,5	Convolvulus arvensis	79,7
Convolvulus arvensis	102	Erodium moschatum	17	Avena sterilis	16,9
Erodium moschatum	16,9	Erodium malacoides	5,63	Rubus ulmifolius	52,7
Erodium malacoides	11,3	Geranium dissectum	45,7	Torilis arvensis	5,63
Geranium dissectum	40,6	Geranium molle	28,3	Bromus diandrus	11,3
Geranium molle	22,5	Sonchus asper	17	Epilobium tetragonum	39,4
Sonchus asper	11,3	Picris echioides	74,7	Chrysanthemum segetum	5,63
Picris echioides	81,6	Avena sterilis	108	Lythrium junceum	5,63
Avena sterilis	101	Euphorbia helioscopia	22,5	Poaceae	23,8
Euphorbia helioscopia	16,9	Rubus ulmifolius	57,3		
Rubus ulmifolius	39,9	Lactuca serriola	16,9		
Lactuca serriola	5,63	Torilis arvensis	69		
Torilis arvensis	75,1	Medicago polymorpha	34,7		
Medicago polymorpha	36,5	Poa annua	5,63		
Hordeum murinum	74,9	Hordeum murinum	86,3		
Bromus diandrus	50,8	Sonchus oleraceus	5,76		
Veronica persica	11,3	Lavatera cretica	5,63		
Lolium perenne	51,6	Bromus diandrus	11,3		
Mentha suaveolens	52,8	Anagallis arvensis	34		
Vicia sativa subsp. nigra	22,9	Veronica persica	11,3		

Ranunculus repens	11,3	Lolium perenne	52,3		
Trifolium repens	11,6	Mentha suaveolens	41,1		
Chenopodium album	5,63	Galium aparine	5,63		
Epilobium tetragonum	68,3	Chamaemelum fuscum	5,63		
Rumex crispus	17	Equisetum telmateia	35,5		
Phalaris minor	11,5	Galactites tomentosa	5,63		
Chrysanthemum segetum	11,4	Epilobium tetragonum	68,8		
Polygonum aviculare	5,97	Rumex crispus	22,5		
Hedera helix	5,63	Phalaris minor	42,8		
Cirsium arvense	5,76	Chrysanthemum segetum	11,3		
Lythrium junceum	22,9	Coleostephus myconis	16,9		
		Andryala integrifolia	17,2		
		Holcus lanatus	11,9		
		Cirsium arvense	34,3		
		Lythrium junceum	28,4		
		Poaceae	22,8		

CONTROL ISA SY		EUCALYPTUS ISA SY		RICE STRAW ISA SY	
TAXON	IVI	TAXON	IVI	TAXON	IVI
Calendula arvensis	99,51	Calendula arvensis	96,3	Convolvulus arvensis	98,4
Convolvulus arvensis	96,2	Convolvulus arvensis	74,4	Sonchus asper	5,63
Erodium moschatum	17	Erodium moschatum	34,5	Avena sterilis	5,63
Erodium malacoides	34,2	Solanum nigrum	5,63		
Geranium dissectum	16,9	Erodium malacoides	22,8		
Geranium molle	46,2	Geranium dissectum	33,8		
Sonchus asper	41,9	Geranium molle	51,9		
Picris echioides	63,8	Sonchus asper	64,7		
Avena sterilis	113	Picris echioides	69,4		
Euphorbia helioscopia	16,9	Avena sterilis	102		
Lactuca serriola	75,9	Euphorbia helioscopia	33,9		
Torilis arvensis	64,4	Lactuca serriola	95,3		
Medicago polymorpha	36,9	Torilis arvensis	34,2		
Poa annua	16,9	Medicago polymorpha	36		
Hordeum murinum	105	Poa annua	17,3		
Sonchus oleraceus	34,6	Hordeum murinum	86,1		
Leontodum taraxacoides	29,7	Sonchus oleraceus	34,3		
Lavatera cretica	80,3	Leontodum taraxacoides	34,5		
Bromus diandrus	56,7	Lavatera cretica	57,1		
Panicum repens	17,3	Bromus diandrus	61,9		
Anagallis arvensis	5,63	Panicum repens	17,3		
Lolium perenne	22,8	Anagallis arvensis	28,6		
Chenopodium album	17	Lolium perenne	51,3		
Galactites tomentosa	11,3	Galactites tomentosa	5,63		
Celtis australis	104	Celtis australis	91,7		
Rumex crispus	11,3	Melilotus sagetalis	5,63		
Melilotus sagetalis	16,9	Chrysanthemum segetum	16,9		
Chrysanthemum segetum	16,9				

CONTROL ISA AL		EUCALYPTUS ISA AL		RICE STRAW ISA AL	
TAXON	IVI	TAXON	IVI	TAXON	IVI
Calendula arvensis	71,6	Calendula arvensis	73,5	Convolvulus arvensis	124

Convolvulus arvensis	113	Convolvulus arvensis	99,4	Sonchus asper	5,63
Solanum nigrum	51,5	Erodium moschatum	52,4	Sonchus oleraceus	11,3
Erodium malacoides	11,4	Solanum nigrum	16,9	Chenopodium album	5,63
Geranium dissectum	17,3	Erodium malacoides	34,9	Celtis australis	28,3
Geranium molle	5,63	Geranium dissectum	39,5	Rumex crispus	5,63
Sonchus asper	51,2	Geranium molle	16,9		
Picris echioides	57,6	Sonchus asper	81,7		
Avena sterilis	103	Picris echioides	91,1		
Lactuca serriola	87,5	Avena sterilis	80,7		
Torilis arvensis	16,9	Lactuca serriola	62,2		
Medicago polymorpha	41,1	Torilis arvensis	17		
Poa annua	39,4	Medicago polymorpha	59,7		
Oxalis pes-caprae	11,4	Poa annua	33,9		
Hordeum murinum	45	Oxalis pes-caprae	5,63		
Sonchus oleraceus	17,3	Hordeum murinum	45,8		
Leontodum taraxacoides	18,4	Sonchus oleraceus	34,2		
Lavatera cretica	81	Leontodum taraxacoides	5,63		
Bromus diandrus	62,2	Lavatera cretica	87,4		
Panicum repens	5,63	Bromus diandrus	90,8		
Lolium perenne	68,1	Panicum repens	34,4		
Convolvulus tricolor	17,2	Lolium perenne	50,9		
Cynodon dactylon	34,8	Convolvulus tricolor	22,8		
Celtis australis	57,3	Cynodon dactylon	22,5		
Rumex crispus	16,9	Celtis australis	22,7		
Lavatera trimestris	17,6	Stachys arvensis	5,63		
Plantago lanceolata	5,69	Echinochloa crus-galli	5,63		
		Ammi majus	5,63		
		Lavatera trimestris	5,69		
		Plantago lanceolata	5,63		
		Phalaris minor	5,63		

Annex 5. Floristic survey matrix on Excel (APRIL Floristic survey).

FLORISTIC SURVEY**Date: 01/04/2022****Vineyard: ISA SYRAH****Mulching type/treatment: Rice straw (RS) Eucalyptus leaves (EL) Control (C)**

	RS_SyISA1_0 4	RS_SyISA2_0 4	RS_SyISA3_0 4	EL_SyISA1_0 4
Taxon	Cover	Cover	Cover	Cover
Raphanus raphanistrum				
Calendula arvensis				1,25
Convolvulus arvensis	1,25	1,25		1,25
Erodium moschatum				3,75
Solanum nigrum				
Erodium malacoides				
Geranium dissectum				1,25
Geranium molle				1,25
Sonchus asper				1,25
Picris echioides				1,25
Avena sterilis	1,25			1,25
Euphorbia helioscopia				1,25
Rubus ulmifolius				
Lactuca serriola				
Torilis arvensis				
Medicago polymorpha				3,75
Poa annua				
Oxalis pes-caprae				
Hordeum murinum				
Sonchus oleraceus				1,25
Leontodum taraxacoides				3,75
Lavatera cretica				1,25
Bromus diandrus				
Panicum repens				
Anagallis arvensis				1,25
Veronica persica				
Lolium perenne				3,75
Mentha suaveolens				
Vicia sativa subsp. nigra				
Galium aparine				
Chamaemelum fuscum				
Cichorium intybus				
Ranunculus repens				84
Equisetum telmateia				

