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Evaluación del interés del uso de una cubierta vegetal bajo la línea como estrategia de gestión del suelo en viñedos cultivados en condiciones de clima mediterráneo

Evaluation of an under-vine cover crop as soil management strategy for Mediterranean climate vineyards

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F. JAVIER ABAD ZAMORA
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Evaluación del interés del uso de una cubierta vegetal bajo la línea como estrategia de gestión del suelo en viñedos cultivados en condiciones de clima mediterráneo

Evaluation of an under-vine cover crop as soil management strategy for Mediterranean climate vineyards

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Resumen

El manejo del suelo de los viñedos en condiciones de clima mediterráneo siempre ha primado reducir la competencia que pudieran generar otras plantas por los recursos nutricionales y, en especial, por los recursos hídricos. Sin embargo, los cambios de sistemas de producción, con desplazamiento de los viñedos a terrenos más fértiles, muchas veces dotadas de riego; la necesidad de buscar alternativas a los herbicidas como elementos de control de la vegetación adventicia, en especial en la zona ubicada bajo las cepas; plantean la posibilidad, o incluso la necesidad, de trabajar el suelo de manera diferente.

En los últimos años, se ha avanzado de manera relevante en el estudio de las implicaciones del introducir las cubiertas vegetales como alternativa al uso de herbicidas y al laboreo, habiendo quedado de manifiesto el interés agronómico y ambiental de este cambio de manejo, que, como cualquier otro, debe acometerse considerando todas sus implicaciones en función de las particularidades de cada viñedo y objetivo agronómico-empresarial. Sin embargo, la práctica totalidad de las actuaciones encaminadas a introducir el uso de cubiertas en viñedos de zonas mediterráneas implican un cambio de gestión del suelo las calles, pero no del suelo bajo la línea de cultivo. En este contexto, la posibilidad de emplear una cubierta vegetal bajo la línea de cultivo que permitiera competir con otras adventicias pero no lo hiciera de manera relevante con el viñedo podría ser una alternativa de mucho interés.

En esta Tesis Doctoral se ha estudiado el comportamiento de una cubierta de *T. fragiferum* (UV) frente a un manejo con laboreo intercepas (T). Dicho ensayo se realizó durante tres campañas consecutivas (2018, 2019 y 2020) en un viñedo de Merlot ubicado en terrenos de Bodegas Ochoa en Traibuenas (Navarra-España), sobre un suelo Typic Calcixerepts y con un clima húmedo-templado mediterráneo según la clasificación de Papadakis.

El empleo de la cubierta consiguió una cubrición del suelo de un 80% en el primer año, llegando al 100% en los dos siguientes. En 2018, *T. fragiferum* representaba el 26% de la superficie del suelo, en 2019 el 67,5% y en 2020 el 85%. La presencia de la cubierta afectó al estado hídrico del viñedo, siendo este efecto puntual en las dos primeras campañas y más constante en la última, si bien las diferencias no eran muy marcadas. La presencia de la cubierta pareció aumentar, de manera muy ligera, el riesgo de daños por heladas primaverales. El crecimiento vegetativo, cuantificado por el peso de madera de poda, no se vio afectado; aunque en el año 2019 se produjo una disminución del número de pámpanos allí donde se empleó la cubierta. De manera similar, el rendimiento no se vio afectado por la presencia de la cubierta, ni tampoco el tamaño de los racimos ni de las bayas; aunque si se apreció una menor tasa de cuajado en las cepas con cubierta en 2019. La composición de la uva tampoco se vio apenas afectada, lo que coincide con las pocas diferencias observadas entre los vinos obtenidos tanto en la evaluación fisicoquímica como organoléptica. Las cepas de levaduras autóctonas de *Saccharomyces cerevisiae* presentaron una menor diversidad cuando había presencia de cubierta.

En lo referente al efecto sobre las características del suelo, la implantación de la cubierta provocó un aumento en los niveles de carbono orgánico del suelo (COS) y el carbono particulado (POC) al año de su implantación. La abundancia de agregados estables en agua (WSA) y el tamaño medio de los agregados (MWD) también se incrementaron de manera relevante en el mismo periodo. Los parámetros de actividad microbiana (respiración basal) y

la biomasa microbiana del suelo fueron incrementándose progresivamente a lo largo de las tres campañas.

Finalmente, según el estudio económico realizado, el empleo de esta cubierta vegetal en condiciones de clima semi-árido requiere asegurar la supervivencia de la misma por un periodo mínimo de tres años para poder competir económicamente con otras técnicas como la aplicación de herbicidas o la realización de labores intercepas.

El conjunto de los resultados obtenidos permite concluir que el empleo de una cubierta de *T. fragiferum* bajo la línea de cultivo del viñedo en condiciones de clima mediterráneo constituye una alternativa realista al uso de herbicidas o laboreos intercepas. En las condiciones de ensayo, su uso ha redundado en una mejoría notable de los parámetros de calidad del suelo, sin apenas alterar el comportamiento agronómico y enológico de las cepas, resultando económicamente viable cuando se consigue una supervivencia de la cubierta mínima de tres años.

Abstract

Soil management in vineyards under Mediterranean climate conditions has always focused on reducing competition from other plants for nutritional resources and, in particular, for water resources. However, changes in production systems, with vineyards moving to more fertile land, often with irrigation, and the need to find alternatives to herbicides to control adventitious vegetation, especially in the area under the vines, have raised the possibility, or even the need, to work the soil in a different way.

In recent years, significant progress has been made in the study of the implications of introducing plant cover as an alternative to the use of herbicides and tillage, and the agronomic and environmental interest of this change in management has become clear, which, like any other, must be undertaken considering all its implications according to the particularities of each vineyard and agronomic-business objective. However, practically all the actions aimed at introducing the use of canopies in vineyards in Mediterranean areas involve a change in the management of the soil in the lanes, but not of the soil under-vines. In this context, the possibility of using a cover crop under-vines that could compete with other adventitious plants but would not compete significantly with the vineyard could be a very interesting alternative.

In this Doctoral Thesis, the behaviour of a *T. fragiferum* (UV) cover crop was studied against a management with inter-vines (T) tillage. This trial was carried out during three consecutive seasons (2018, 2019 and 2020) in a Merlot vineyard located on land belonging to Bodegas Ochoa in Traibuenas (Navarra-Spain), on a Typic Calcixerepts soil and with a humid-temperate Mediterranean climate according to the Papadakis classification.

The use of the cover achieved a ground cover of 80% in the first year, reaching 100% in the following two years. In 2018, *T. fragiferum* represented 26% of the soil surface, in 2019 67.5% and in 2020 85%. The presence of the canopy affected the water status of the vineyard, with a one-off effect in the first two seasons and a more constant effect in the last one, although the differences were not very marked. The presence of the canopy seemed to increase, very slightly, the risk of spring frost damage. Vegetative growth, as measured by the weight of pruned wood, was not affected, although in 2019 there was a decrease in the number of shoots where the canopy was used. Similarly, yield was not affected by the presence of the canopy, nor was bunch or berry size, although there was a lower fruit set rate on canopied vines in 2019. Grape composition was hardly affected either, which coincides with the few differences observed between the wines obtained in both the physicochemical and organoleptic evaluations. The indigenous *Saccharomyces cerevisiae* yeast strains showed less diversity when cover was present.

Regarding the effect on soil characteristics, mulching caused an increase in soil organic carbon (COS) and particulate carbon (POC) levels one year after mulching. The abundance of water stable aggregates (WSA) and mean aggregate size (MWD) also increased significantly over the same period. Microbial activity parameters (basal respiration) and soil microbial biomass increased progressively over the three seasons.

Finally, according to the economic study carried out, the use of this plant cover in semi-arid climate conditions requires ensuring its survival for a minimum period of three years in order

to be able to compete economically with other techniques such as the application of herbicides or inter-vine tillage.

All the results obtained allow us to conclude that the use of a *T. fragiferum* cover crop under-vine under Mediterranean climate conditions is a realistic alternative to the use of herbicides or inter-vine tillage. In the trial conditions, its use has resulted in a significant improvement in soil quality parameters, with hardly any alteration in the agronomic and oenological behaviour of the vines, and is economically viable when a minimum of three years of canopy survival is achieved.

“A los mayores les gustan mucho las cifras”
El Principito. Antoine de Saint-Exupéry

Confío haber madurado durante estos años de tesis y…
quien sabe, igual hasta me haya hecho mayor.

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0. INTRODUCCIÓN Y ESTRUCTURA DE LA TESIS

El cultivo de la vid ha estado vinculado al clima mediterráneo desde tiempos remotos, si bien el manejo de los viñedos ha ido evolucionando de manera continua a lo largo del tiempo. En este sentido, existen algunos eventos que marcaron particularmente la manera de cultivar, e incluso las regiones en las que se cultiva la vid, siendo los más importantes aquellos ocurridos a lo largo del siglo XIX, con la aparición de dos nuevas enfermedades, el oídio (*Uncinula necator*) (Piqueras Haba, 2010), y el mildiu (*Plasmopara viticola*) (Barrios Sanroma y Reyes Aybar, 2004) y la llegada de la plaga de la filoxera (*Daktulosphaira vitifoliae*), que obligó a emplear portainjertos tolerantes (Piqueras Haba, 2005).

El final del siglo XX y el inicio del XXI se corresponden también con un periodo de grandes cambios en la viticultura, no causados por la irrupción de ningún patógeno alóctono, sino por la "modernización" del cultivo. Aunque pueda parecer un cambio menor, es necesario subrayar que en 30-40 años se ha transformado el sistema de conducción, pasando de configuraciones y sistemas de poda tradicionales al uso masivo de elementos de apoyo y conducciones en espaldera, se ha trasladado el viñedo de zonas marginales a suelos mucho más fértiles, se han introducido el riego e incluso la fertirrigación, el material vegetal ha pasado a ser clonal y libre de los virus principales, y se han mecanizado buena parte de las operaciones de cultivo. Algunos de estos cambios han producido desequilibrios entre el desarrollo vegetativo y producción, pero también han permitido incrementar las producciones o la calidad de la uva, permitiendo que la planta se mantenga fotosintéticamente activa hasta el final del ciclo.

Con cierto retraso sobre los cambios anteriores, se está produciendo también un cambio importante, al menos en lo conceptual, en el manejo del suelo. Tradicionalmente, y motivado por la ubicación de los viñedos en terrenos pobres de secano, se intentaba mantener el suelo absolutamente libre de vegetación adventicia, para evitar así la competencia entre el cultivo y dicha vegetación por los recursos hídricos y nutricionales. La eliminación de la vegetación se realizaba inicialmente mediante labores mecánicas del suelo, si bien desde la aparición de los herbicidas, el control químico ha ido cobrando un protagonismo creciente, manteniéndose un uso combinado de laboreo mecánico y químico como estrategia de gestión más habitual. Sin embargo, tal y como se ha adelantado, el planteamiento que hacía absolutamente prioritario mantener el suelo libre de cubierta está cambiando por las siguientes razones:

- Menor necesidad de "competencia cero": el traslado de los viñedos a zonas con suelos más fértiles, el uso del riego y las limitaciones al rendimiento establecidas por voluntad propia o por mandato de los consejos reguladores hacen que no sea necesario eliminar de manera completa la vegetación, lo que hace que incluso en los viñedos en los que se pretende mantener el suelo desnudo, el manejo pueda ser relativamente laxo con la eliminación de la vegetación adventicia.
- Lucha contra la perdida de suelo: la capa superficial de suelo es la más fértil y a su vez la más afectada por los procesos de erosión, tanto hídricos como eólicos. Toda desprotección de la superficie del suelo incrementa el riesgo de pérdida del mismo. Además, prácticas como el laboreo aceleran la oxidación de la materia orgánica, uno de los principales estructurantes del suelo, a parte de la ruptura física de la estructura que genera *per se*, lo que incrementa el riesgo de pérdida del suelo.

- Aparición de una conciencia social contraria al uso de herbicidas: la sociedad civil apuesta de manera decidida por la reducción del uso de productos fitosanitarios, que afecta de manera muy directa al caso de los herbicidas. De hecho, en algunos casos, esta oposición ha terminado con la prohibición de algunos de ellos. Estas restricciones han implicado e implicarán en un futuro cercano la retirada de numerosas materias activas de los registros de productos por parte de las entidades competentes.
- Puesta en valor de la biodiversidad del viñedo: desde la Unión Europea, a través de su "Estrategia sobre la biodiversidad de aquí a 2030" se percibe la necesidad de integrar la biodiversidad dentro de los ecosistemas agrícolas, buscando armonizar la producción agrícola con los ecosistemas naturales.

A la vista de todo lo anterior, queda en evidencia que existe un interés creciente en entender las implicaciones que el empleo de cubiertas vegetales tiene sobre los viñedos, habiéndose realizado un número muy importante de estudios a nivel global en esta temática. Sin embargo, resulta complicado tener una visión de conjunto, ya que la cubierta empleada y las condiciones de cultivo varían mucho entre ensayos. Al objeto de arrojar luz sobre este tema, y como herramienta para ayudar en la toma de decisiones en el viñedo, en los **Apartados 1.1.** y **1.2.** se presenta una revisión bibliográfica sistemática que recoge los resultados obtenidos en ensayos publicados en los últimos 20 años en los que se evaluaban cubiertas vegetales. Ambos apartados han sido publicados en la revista OenoOne, abordando el primero las implicaciones de las cubiertas sobre el suelo y la biodiversidad del viñedo, y el segundo las implicaciones agronómicas.

Esta revisión sistemática, además de permitir conocer las implicaciones que el empleo de las cubiertas vegetales tiene en el viñedo, puso en evidencia que el empleo de cubiertas se circunscribe, de manera abrumadoramente mayoritaria, a las calles del viñedo, manteniéndose el terreno bajo las cepas libre de vegetación. Existe muy poca investigación que haya valorado, a pesar de su interés agronómico y ambiental, el uso de cubiertas en la línea del cultivo, y dichos trabajos se han realizado mayoritariamente en condiciones de cultivo de climatologías frías y/o elevadas pluviometrías, donde el desarrollo vegetativo elevado resulta ser uno de los principales problemas. Únicamente los trabajos realizados en Australia, con una climatología de tipo mediterránea, tenían por objetivo emplear cubiertas vegetales como alternativas económicamente viables al empleo de herbicidas bajo las cepas. En el **Apartado 1.3.** se presenta una breve recopilación de todos estos trabajos.

Todo lo anterior sirve de contexto a esta Tesis, que surge como resultado de unos trabajos iniciales que la Estación de Viticultura y Enología de Navarra (EVENA), perteneciente al Gobierno de Navarra, inició en 2016 para estudiar alternativas al manejo del suelo bajo la línea de cultivo. Para ello, se sembraron 6 cubiertas de especies que cumplieran los siguientes criterios: capacidad de adecuada cubrición del suelo para poder competir correctamente con las plantas adventicias; buena persistencia en el tiempo, bien por su carácter perenne o bien por su alta capacidad de semillar y autosembrarse; porte bajo que no requiera de labores de siega, y potencial interés de fijación de nitrógeno en el caso de las leguminosas. Estos trabajos preliminares permitieron valorar la adecuación de dichas especies, y plantearon el interés de establecer un ensayo en el que se evaluar el potencial de una de ellas (*Trifolium fragiferum*) para evaluar de manera más exhaustiva las implicaciones que tendría su empleo, tanto sobre el viñedo, como sobre la calidad de la uva y vinos, y sobre la calidad del suelo. Esta tesis es el resultado final de dicha evaluación. En esta memoria, se recoge dicha evaluación agrupada en los siguientes capítulos:

- **Capítulo 3**: Aunque la estructura de los siguientes capítulos, dada su configuración de artículo científico, ya presenta sus propios apartados de material y método, se ha querido incluir una descripción más detallada del ensayo y presentar de manera agrupada las metodologías empleadas a lo largo de todo el trabajo de manera que el lector pueda obtener una visión de conjunto de manera sencilla.
- **Capítulo 4**: En este capítulo se hace referencia a los aspectos agronómicos del empleo de esta cubierta, desde la instalación de la propia cubierta y su competencia con otras hierbas adventicias, pasando por su implicación en el estado hídrico del viñedo, producción, crecimiento vegetativo y parámetros de composición de las bayas.
- **Capítulo 5**: Este capítulo presenta los resultados de la elaboración de los vinos y su valoración. En el **Apartado 5.3.** se hace mención a los parámetros químicos de los vinos, mientras que la valoración organoléptica se presenta en el **Apartado 5.4.**
- **Capítulo 6**: En este capítulo se muestran las implicaciones que el empleo de la cubierta tuvo sobre el suelo. Para ello se han agrupado los resultados en aspectos físico-químicos (**Subapartado 6.3.1.**) y aspectos biológicos (**Subapartado 6.3.2.**)
- **Capítulo 7**: Aunque alguno de los aspectos presentados en este capítulo podrían tener cabida dentro de los anteriores, estos se presentan de manera independiente ya que únicamente se dispone de información de una campaña, Así pues, aunque consideramos la información relevante para el lector, los resultados obtenidos deben tomarse con cautela. En el **Apartado 7.2.** se hacer referencia a las implicaciones de la cubierta sobre el riesgo de heladas de primavera; y en el **Apartado 7.3.** se hace referencia al estudio de la población de levaduras de los vinos cuando han sido elaborados con las levaduras autóctonas que presentaban las bayas. Por último, este capítulo se completa con el **Apartado 7.4.** donde se presenta un estudio económico para entender en qué condiciones esta alternativa al manejo habitual del suelo resulta interesante en términos económicos.
- **Capítulo 8**: En este último capítulo se presentan de manera agrupada las conclusiones a las que se ha llegado en los diferentes aspectos tras evaluar la cubierta de *T. fragiferum* bajo la línea de viñedo como alternativa al empleo de herbicidas o laboreos.

1. ANTECEDENTES

1.1. Cover crops in viticulture. A systematic review (1): Implications on soil characteristics and biodiversity in vineyard

Javier. Abad, Iranzu Hermoso de Mendoza, Diana Marín, Luis Orcaray and Luis Gonzaga Santesteban

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1.1.1. Introduction

The planting schemes commonly used in viticulture, especially trellised systems, leave a large portion of the soil surface uncultivated. The management of this part of the soil has important effects on vegetative growth, yield, plant nutrition and water status, and grape and wine quality, and also on soil characteristics (nutrition, organic carbon, structure or erosion) and environmental factors (soil and vineyard biodiversity, gas emissions).

Although the management of vineyard soils through cover crops shows a growing trend worldwide, even in areas where their use was limited in the past due to lack of rainfall, it is very convenient to set the balance between the pros and cons. This is particularly relevant since there is a great diversity in what can be considered a "cover cropping" in a vineyard. According to their origin, cover crops can be sown or spontaneous. When sown, mixtures of species, but also monocultures, are broadly used, being those in *Fabaceae* (legume) and *Poaceae* (grasses) the most widespread ones. Related to this, cover crops can be also classified as annual or permanent, according to the cover crop duration. Variation also occurs with cover crop management, sometimes including harvesting or destruction with tilling or herbicide application. There is also a certain degree of variation in the fraction of the vineyard covered with the crop, which is usually established in the alleys, sometimes covering all the vineyard, and more exceptionally established just under the vines.

Considering all the diversity mentioned above, and taking into account that some additional factors certainly affect their impact in the vineyard, such as variation in climate, soil type, rootstock and irrigation use, it is very relevant to examine in detail the balance between their potential advantages and disadvantages in every situation. Although there are some comprehensive reviews in this issue (Garcia *et al.*, 2018; Steenwerth and Guerra, 2012) that provide interesting compiled information on the effect of cover crops in viticulture, none of them approaches to all the effect of cover crops or, if it does, it is not performed following a systematic process to identify and select research on this topic.

This work aims to compile and analyse, in a systematic way, the information available in recent literature on the effect of cover crops between the rows. Given the extension of the study, this information is presented as two companion papers, this one dealing with the aspects related to soil characteristics and environment-related issues, while a second paper (Abad *et al.*, 2021b) analyses the direct impact of cover crops on vineyard performance.

1.1.2. Published data sourcing and selection

Although a standard or consensus definition of a systematic review does not exist (Krnic Martinic *et al.*, 2019), a systematic review is a review that reports or includes: (1) a research question, (2) sources that were searched, with a reproducible search strategy (naming of databases, naming of search platforms/engines, search date and complete search strategy) (3) inclusion and exclusion criteria, (4) selection (screening) methods.

In our case, we used the Scopus database as the source for extracting publications. The following search query was constructed and applied: TITLE-ABS-KEY ("cover crop" OR "green cover" OR "ground cover" OR "tillage") AND TITLE-ABS-KEY ("wine" OR "vitis" OR "vineyard" OR "grapevine" OR "grape"), between the years 1999 and 2018. A total number of 584 published papers were obtained (search day: January 20th, 2019).

To these papers, the following exclusion criteria were applied, analysing their titles and abstracts:

- Books, conferences or papers that are not based on a specific experiment.
- Publications about crops different from vines.
- When there is no mention (not even indirect) of cover crops ("nor till").
- Publications that refer to cover crops only as examples of organically managed vineyards, but not as the main objective of the study.
- Papers presenting results of modelling exercises, without experimental ground-truthing.
- Publications about table grapes.

The selection was independently completed by two people. Those articles excluded by both selectors were directly discarded, but those excluded just by one of the selectors were re-revised. After this process, there were 272 papers remaining. These articles were categorised according to their theme, and the following metadata were extracted:

- Location
- Vineyard: scion variety and rootstock, planting pattern, age and vine formation
- Experiment duration
- Cover crop characteristics (sown or spontaneous, monoculture or crop mixture, species, cover crop and row management)
- Climate: an illustrative classification was performed; cold (average T below 12 °C), mild (average T between 12 and 15 °C) and warm climate (average T above 15 °C)
- Cultural practices: irrigation (yes/no) and fertilisation (yes/no)
- Soil: texture, organic matter percentage (%OM) and studied horizons

Additionally, all the information regarding the effect cover crops had had on any characteristic relevant from an agro-ecological point of view was extracted and considered for global analysis. In the following sections, the information related to soil characteristics and environmental aspects is presented, whereas a companion compiled and discussed the impact of cover crops on vineyard performance.

1.1.3. Soil mineral composition

Grapevine is not very demanding in terms of fertilisation, among other reasons, because the main objective frequently lies in the achievement of high-quality levels more than maximising grape production. However, nitrogen (N) is considered as an important element in grape growing, due to its relevance on vegetative growth and its leaching potential in nitric form in the soil. Other nutrients, such as potassium (K), influence fermenting grape juice. In general, it is perceived that cover crops can compete with vines for soil nutrients (Celette *et al.*, 2009; Steenwerth and Belina, 2008).

1.1.3.1. Nitrogen

The impact of cover crops on soil N content is analysed in 14 of the articles selected (Table 1-1), covering 40 different management strategies. In general terms, the use of legumes as a cover crop provides an increased amount of total N (Ntot = organic + mineral N), as well as of mineral N (Nmin) in the soil due to their role in fixing air nitrogen when legume roots have Rhizobium (Peoples *et al.*, 2009). On the contrary, grasses act as major soil N scavengers from the soil, reducing the Ntot content to a greater extent than other families.

As mentioned above, the use of grasses as cover crops leads to decreased N levels in the soil as a general rule, this diminution being, as an average, around 25 % both Ntot and Nmin. This effect was reported in Varga *et al.* (2012), analysing the role of a spontaneous cover in Hungary, where an impact on vineyard yield was also shown. Commonly used grass cover crops in France (Celette *et al.*, 2009; Gontier *et al.*, 2014), barley cover crops in Turkey (Judit *et al.*, 2011) and La Rioja, Spain (Pérez-Álvarez *et al.*, 2013), and spontaneous or *Festuca arundinacea* cover crops in Italy (Mattii *et al.*, 2005) showed a reduction in soil N content. Occasionally, the observed N reduction could affect grape juice yeast assimilable nitrogen (YAN) (Pérez-Álvarez *et al.*, 2015b). Accordingly, Rodriguez-Lovelle *et al.* (2000b) identified a soil N reduction with the use of a 2-year *F. arundinacea* cover crop in Montpellier, France, linked to a 30-50 % reduction in leaf N content. It was also observed that other factors than N competition could explain the N reduction, such as reduced soil moisture.

Table 1-1. Total nitrogen (Ntot) and mineral nitrogen (Nmin) average content of the soil, grouped according to cover crop type/soil management. Prepared from the 14 mentioned articles and Table 1-2.

Soil management	**Ntot ($g·kg^{-1}$)**	**N data**	**Nmin ($mg·kg^{-1}$)**	**N data**
Grass	0.873	4	5.475	4
Grass + Legumes	1.716	7	-	
Legumes	1.555	2	17.458	5
Spontaneous vegetation	1.062	5	7.430	1
Tillage control	**1.193**	4	**7.515**	2
Herbicide control	**1.053**	3	**1.900**	1
Herbicide + tillage control	**0.935**	2	-	

Celett *et al.* (2009) demonstrated that *F. arundinacea* and a 3-year-barley cover crop in Montpellier, France, resulted in an N decrease due to a reduced soil N mineralisation caused by low soil moisture levels. The reduced soil mineralisation was more pronounced in the driest years. The same authors observed that a Festuca cover crop cutting at the beginning of May caused a reduction in the cover crop N uptake. However, an increase in N mineralisation potential was detected with the use of a *F. longifolia* and an 8-year spontaneous cover crop in La Rioja (Peregrina *et al.*, 2010). For their part, Klodd *et al.* (2016) did not observe N competition in the petiole due to the presence of a grass cover crop in cooler and more humid weather conditions (Virginia, USA). However, a 63 % reduction in the length of absorptive roots at 80 cm depth was shown, as well as a thin hair-root reduction of 49 % in the first 20 cm of soil. Mycorrhizal colonisation of the grapevines was unaffected (15-40 % of the radicular length) by the presence of the cover crop.

Concerning legume-based cover crops, they increased soil N content, this increase being, on average, around 30 % for Ntot and nearly 100 % for Nmin. (Fourie *et al.*, 2007c; Messiga *et al.*, 2015; Ovalle *et al.*, 2007; Pérez-Álvarez *et al.*, 2015a; Sulas *et al.*, 2017). However, such N increase sometimes may not modify vine behaviour directly, as observed by Sulas *et al.* (2017), who estimated that only 10 % of the total 125 kg ha^{-1} $year^{-1}$N fixed by a *Medicago polymorpha* cover was used by the vines, and hypothesised that this limited absorption could be due to a combination of physical, chemical and microbiological processes.

1.1.3.2. Other elements

With respect to phosphorus (P), no significant differences have been generally detected on its availability when comparing the use of cover crops and tillage practices in adult vineyards (Biddoccu *et al.*, 2016; DeVetter *et al.*, 2015; Ferreira *et al.*, 2018; Mattii *et al.*, 2005; Pérez-Álvarez *et al.*, 2015a; Ruiz-Colmenero *et al.*, 2011a). Grapevine nutritional status remained unaltered concerning P content when cover cropping, according to petiole analysis (Mattii *et al.*, 2005; Pérez-Álvarez *et al.*, 2015a). However, Klodd *et al.* (2016) observed a reduced P content on cover cropped vines. This reduction could be explained by a redistribution of the vine root system towards deeper less fertile soil layers, due to the competition between cover crop and vine roots.

Regarding soil potassium (K), the general trend observed is that cover crops did not affect content (Fourie *et al.*, 2007; Mattii *et al.*, 2005; Pérez-Álvarez *et al.*, 2015a; Pou *et al.*, 2011). Vineyard soil mulching, specifically straw mulch, tended to increase P and K content in soil samples (DeVetter *et al.*, 2015). In some cases, supplemental fertiliser applications to ensure cover crop growth could account for the increased soil P and K content (Ovalle *et al.*, 2007). However, cover crops could increase P losses compared to soil tillage, in case of sloppy vineyards when the fertiliser is not covered with the soil (Napoli *et al.*, 2017). On the other hand, P, K and magnesium (Mg) losses in sloped vineyards were 70-95 % higher under tillage than in the presence of cover crops, due to sediment transportation by surface runoff of water (Ruiz-Colmenero *et al.*, 2011; Vrsic *et al.*, 2011).

There is much less evidence on the effect of grass cover crops on recently established vineyards, though under some conditions it can promote vine growth at the beginning of the season, probably due to the presence of organic compounds in the cover crop rhizosphere. Nevertheless, at the end of the cycle, the effect becomes negative because of the high degree of competition for nutrients and water (Brunetto *et al.*, 2017).

1.1.4. Soil organic matter

Cover crops increased soil organic carbon (SOC) accumulation in 13 out of the 19 articles selected (see Table 1-2). The increase observed in SOC was variable, depending on the cover crop type and its presence, both during the season and over the years.

A total of 16 grass cover crops increased SOC by an average of 68.5 % compared to the initial conditions. When legumes were grown as a cover crop, an average increase of 39.2 % was observed (9 cases in total). Finally, the data from 8 spontaneous cover crops, mainly composed of grass species, showed the most favourable results, with a 119.5 % increment in SOC content. In contrast, one of the analysed spontaneous cover crops caused a decline of 0.5 % in the SOC levels (Mattii *et al.*, 2005).

The temporal evolution of SOC in tilled vineyards is quite variable since in the 6 articles found measuring this evolution, in 2 of them it was observed to decrease (Peregrina, 2016; Pou *et al.*, 2011), in another 2 it was not affected (Belmonte *et al.*, 2016; Mattii *et al.*, 2005), while the remaining 2 (García-Díaz *et al.*, 2017; Peregrina *et al.*, 2010) showed an increased SOC content. As an average, in tilled vineyards, an average increase of 4.05 % per year was observed. Similarly, the evolution of SOC in soils weeded chemically was variable, since in one of the studies it was observed to decrease (Belmonte *et al.*, 2018), whereas, in the other one, it increased (Celette *et al.*, 2009), being the average change of -0.8 % per year.

The dynamics of SOC increase in cover cropped vineyards are slow, and, for instance, García-Díaz *et al.* (2016) reported that it took 5 years for the annual incorporation of a *Vicia faba* cover crop to increase SOC. Pou *et al.* (2011) observed a decrease in the SOC content with a spontaneous cover crop under deficit irrigation for 3 years in Mallorca, Spain. According to Belmonte *et al.* (2016), the increase in SOC content is not observed until the third year when a spontaneous grass cover crop was analysed in Italy. In other cases, the use of a *Brachypodium distachylon* cover crop led to a SOC increase during the first year (Marques *et al.*, 2010), while a rye cover crop did not show the same effect. A higher increase was shown when the cover crop included grasses (Messiga *et al.*, 2015), more than when they were only composed of legume (Table 1-2).

Table 1-2. Initial and final soil organic carbon (SOC), final nitrogen (N) content in the soil and aggregate stability from analysed publications.

N	Location	Duration	Soil management	Cover type	Initial SOC (g·kg^{-1})	Final SOC (g·kg^{-1})	ΔSOC (%)	Final soil N Ntot (g·kg^{-1}) Nmin (mg·kg^{-1})	Depth (cm)	Aggregate stability
1	Kreinbacher, Turkey	3	Tillage	CT	9.28			8.72 Nmin	0-30	
			Spontaneous vegetation	SV				7.43 Nmin		
2	La Caple, France	4	Herbicide + tillage	CTH	6.2	6.03	-2.74	0.60 Ntot	0-15	
			Permanent cover *Festuca rubra, Lolium perenne*	G		8.55	37.9	0.71 Ntot		
3	Agugliano, Italy	7	Tillage (depth 5–8 cm)	CT		7.52		1.64 Ntot	0-50	
			Spontaneous vegetation	SV		8.32		0.93 Ntot		
4	Tokaj, Hungary	3	Tillage (4/seasons)	CT				6.31 Nmin	0-30	
			Annual cover *Hordeum vulgare*	G				3.54 Nmin		
5	Montpellier, France	5	Herbicide	CH	7.5-8.7	8.58	5.93	0.78 Ntot	0-30	
			Permanent cover *Festuca arundinacea*	G		8.41	3.83	0.82 Ntot		
			Annual cover *Hordeum vulgare*	G		8.35	3.09	0.76 Ntot		
6	Santana do Livramento, Brazil	2	Herbicide	CH	10.7			0.52 Ntot	0-10	
			Spontaneous vegetation *Paspalum notatun, L. multiflorum, Bromus auleticu, Desmodium* spp., *Vicia sativa*	SV				0.50 Ntot		
7	Western Cape, South Africa	10	Herbicide	CH	1.3	1.28	-1.54	7.28 Nmin	0-15	
			Annual cover *Secale cereale*	G		2.5	92.31	6.88 Nmin		
			Annual cover *Avena sativa*	G		1.69	30.0	6.23 Nmin		
			Annual cover *A. strigosa*	G		1.92	47.69	5.25 Nmin		
			Annual cover *Medicago truncatula*	L		1.92	47.69	19.45 Nmin		
			Annual cover *Ornithopus sativus*	L		2.1	61.54	13.31 Nmin		
			Annual cover *V. dasycarpa*	L		2.39	83.85	18.53 Nmin		
8	Mallorca, Spain	3	Tillage	T	13.3	12.8	-3.76	1.8 Ntot		
			Permanent cover *Medicago* sp., *A. sterilis, Lotus ornithopodioides, Trifolium scabrum, Chrysanthemum coronarium*	GL		11.0	-17.29	1.7 Ntot		
			Annual cover *T. resupinatum, M. truncatula, T. subterraneum, Dactylis glomerata*	GL		13.3	0	1.9 Ntot		
9	California, U.S.A.	5	Tillage	CT		0.01			0-15	
			Annual cover *Triticale* x *Triosecale*	G		0.01				
			Annual cover *S. cereale*	G		0.01				
10	Región Maule, Chile	2	Herbicide	CH		8.99		1.90 Nmin	0-20	
			Permanent cover *T. subterraneum, M. polymorpha*	L		10.8		21.9 Nmin		
			Permanent cover *T. subterraneum, T. michelianum*	L		9.92		14.1 Nmin		
11	California, U.S.A.	3	Herbicide	CH	21.7	19.28	-11,15	1.86 Ntot	0-5	+
			Permanent cover *Vulpia myuros, B. hordeaceus, T. hirtum, T. pratenses*	GL		26.64	22.76	2.45 Ntot		
			Annual cover *Vicia faba, Pisum sativum, Triticum aestivum* or *S. cereale*	GL		17.75	-18.2	1.70 Ntot		
12	Ligurian Apennines, Italy	3	Tillage	CT	5.0	5.0	0		0-5	+
			Spontaneous vegetation	SV		11.8	136.0			
13	Brunello di Montalcino, Italy	5	Tillage (3/season, depth 20 cm)	CT	9,45	9.45	0	1.1 Ntot	0-15	
			Spontaneous vegetation	SV		9.40	-0,53	1.1 Ntot		
			Annual cover *T. subterraneum*	L		9.74	3.07	1.5 Ntot		
			Permanent cover *F. arundinacea*	G		12.76	35.03	1.2 Ntot		
14	Burgundy, France	10	Herbicide	CH		13.9			0-5	+
			Permanent cover clover	L		25.6				
			Permanent cover *Festuca* sp.	G		32.4				

15	Badajoz, Spain	1	Tillage (3/season, depth 10–15 cm)	CT		1.68		0.23 Ntot	0-10	+
			Spontaneous vegetation *Elytrichia repens, F. arundinacea, Portulaca oleracea*	SV		13.70		0.64 Ntot		
16	Madrid, Spain	4	Tillage (2–3/season, depth 15 cm)	CT	5.20–8.10	≈11.0	≈65.4		0-5	+
			Permanent cover *Brachypodium distachyon*	G		≈13.0	≈95.5			
			Spontaneous vegetation	SV		≈14.5	≈118			
17	La Rioja, Spain	4	Tillage (3–4/season, depth 15 cm)	CT	5.39	≈6.5	≈20.6		0-5	+
			Spontaneous vegetation *B. mollis, H. marinum, Diplotaxis erucoides, Sonchus asper, Sonchus oleraceus, Veronica latifolia, Coniza canadensis, Papaver hibridum*	SV		≈17.5	≈224			
			Permanent cover *Festuca glauca*	G		≈20.0	≈271			
18	Madrid, Spain	2	Tillage	CT		9.8			0-10	
			Annual cover *S. cereale*	G		10.4				
			Permanent cover *Brachypodium distachyon*	G		10.5				
19	Traisen Valley, Austria	10	Annual legumes cover with tillage (5/season, depth 5–10 cm)	L		27.3		1.61 Ntot	0-10	
			Spontaneous vegetation	SV		35.2		2.14 Ntot		
20	Nueva Escocia, Canada	2	Tillage + herbicide (depth 10 cm)	CTH		15.57		1.27 Ntot	0-15	
			Annual cover *A. sativa, Pisum sativum, V. villosa*	GL		15.57		1.42 Ntot		
			Annual cover *A. sativa, T. pratense*	GL		17.21		1.42 Ntot		
			Permanent cover *Pheum pratense* (70 %), *T. hibridum* (15 %), *T. pratense* (15 %)	GL		17.21		1.42 Ntot		
21	La Rioja, Spain	10	Tillage (3–4/season, depth 15 cm)	CT	<11.6	≈6.0			0-2.5	
			Spontaneous cover	SV		≈22.0				
			Permanent cover *F. longiflora*—4 years, *B. catharticus*—6 years	G		≈15.0				
22	Navarra, Spain	1-5	Tillage	CT		9.15			0-5	+
			Permanent cover *F. arundinacea, L. multiflorum*—1 year	G		15.7				
			Permanent cover *F. arundinacea, L. multiflorum*—5 years	G		12.5				
23	Iowa, U.S.A.	7	Tillage	CT					0-7.6	+
			Herbicide	CH						
			Straw mulch	M						
			Cover Festuca rubra	G						
24	Madrid, Spain	3	Tillage	CT						+
			Annual cover *S. cereale*	G						
			Permanent cover *Brachypodium distachyon*	G						

1: Varga *et al.* (2012); 2: Gontier *et al.* (2014); 3: Agnelli *et al.* (2014); 4: Judit *et al.* (2011); 5: Celette *et al.* (2009); 6: Brunetto *et al.* (2017); 7: Fourie *et al.* (2007); 8: Pou *et al.* (2011); 9: Steenwerth and Belina (2008); 10: Ovalle *et al.* (2007); 11: Belmonte *et al.* (2018); 12: Belmonte *et al.* (2016); 13: Mattii *et al.* (2005); 14: Bartoli and Dousset (2011); 15: López-Piñeiro *et al.* (2013); 16: García-Díaz *et al.* (2018); 17: Peregrina *et al.* (2010); 18: Marques *et al.* (2010); 19: Zehetner *et al.* (2015); 20: Messiga *et al.* (2015); 21: Peregrina (2016); 22: Virto *et al.* (2012); 23: DeVetter *et al.* (2015); 24: Ruiz-Colmenero *et al.* (2013).

N: number-author reference; Duration: years since cover crop establishment; Initial SOC: soil organic carbon at the beginning of the experiment; Final SOC: soil organic carbon at the end of the experiment; ΔSOC: variation between final SOC and initial SOC; Depth: sampling depth; Cover type: CT, tillage control; CH, herbicide control; CTH, tillage+herbicide control; G, grass; GL, grass+legume; L, legume; SV, spontaneous vegetation; M, mulch; Ntot: total N (organicN + mineral Nitrogen).

1.1.5. Soil structure

Altogether with the increase in SOC reported above, soil aggregate stability was also improved by the presence of cover crops when compared to tilled soils (Table 1-2). Such improvement required a relatively long period to appear. Thus, Belmonte *et al.* (2016) did not observe changes in aggregate stability until the third year of a spontaneous grass cover crop establishment, whereas Ruiz-Colmenero *et al.* (2013) detected an increase in SOC and aggregate stability from the second year onwards (30 % increase in aggregate stability compared to tillage). Under some circumstances, a direct interaction between soil microbial population and the development of more stable soil structure has been observed (Virto *et al.*, 2012). Cover crops have also been reported to increase meso- and macro-porosity (Ruiz-Colmenero *et al.*, 2013), or to enhanced pore connectivity and infiltration rates, even if pore size or volume remain unaltered (García-Díaz *et al.*, 2018). Pore connectivity and infiltration rates improved through better aggregate stability, although pore size or volume remained unaltered (García-Díaz *et al.*, 2018).

1.1.6. Soil erosion

The use of cover crops is directly associated with a considerable reduction in soil erosion. A number of 12 articles, comprising 29 different soil management practices, were selected for the systematic review (Table 1-3). As average figures, greater soil losses were detected in herbicide-treated (12 Mg ha^{-1} year^{-1}) and tilled control plots (11.4 Mg ha^{-1} year^{-1}). Grass cover crops (1.1 Mg ha^{-1} year^{-1}), mixtures of grass and legume (2.3 Mg ha^{-1} year^{-1}), spontaneous cover crops (2.4 Mg ha^{-1} year^{-1}) and legume cover crops (3.4 Mg ha^{-1} year^{-1}) were the treatments which showed higher erosion-reducing effects. Erosion rates did not directly match with runoff coefficients, that were higher in tilled control plots (21.8 %) compared to the use of grass cover crops (11.8 %) on average values. These data are biased by the results obtained in Gontier *et al.* (2014), where both the herbicide-treated control plot and grass cover crop vineyard showed a runoff coefficient of 34 %. Leaving this outlying data aside, the average runoff coefficient for grass cover crops would be 8.2 %, more in line with the observed erosion rates. This coefficient was 1.8 % (one single data) in grass or grass-legume mixtures, and around 8.7 % in case of spontaneous cover crops. There were no available data for legume cover crops.

Most of the studies evaluating the impact of cover cropping in erosion were performed in Mediterranean climate conditions (Table 1-3), where strong storms occur more frequently in the summer, showing that cover crops can play an important role on soil protection during this season (Bagagiolo *et al.*, 2018; Biddoccu *et al.*, 2015; Vrsic *et al.*, 2011). Nevertheless, attention should also be paid to erosion events occurring in autumn and winter. For instance, a study performed in Portugal, in an area where 1200 mm of annual rainfall concentrates in winter, showed the highest losses to occur at this time of year (Ferreira *et al.*, 2018). Similarly, in Sicily (Italy), Novara *et al.* (2013) observed that the autumn-winter rains resulted in greater runoff and the most severe erosion of the year; and also in Carpeneto, Italy, maximum runoff occurred in winter months, while most rainfall takes place in autumn (Biddoccu *et al.*, 2016). Factors that could explain this behaviour were the reduced cover crop density when the vegetative growth stops and the increased soil compaction due to the employed mechanical tillage practices. Rainfall erosivity was also related to the topography of the vineyard. In Italy, Bagagiolo *et al.* (2018) observed that high-intensity rains (>16 mm hour^{-1}) in 15 to 35 % sloped vineyards resulted in higher soil losses when vines were planted along contour lines.

On the contrary, in vineyards disposed following the maximum slope line, long-duration rainfall events (>50 hours) caused the highest soil loss.

The effectiveness of cover crops in the control of soil losses also depends on plant cover duration. The continuous presence of perennial cover crops showed reduced erosion rates compared to temporal plant covers that are mowed in spring (Ruiz-Colmenero *et al.*, 2011; Usón *et al.*, 1998).

Legume-based cover crops showed different effectiveness in controlling soil erodibility. A smaller erosion-reducing effect was associated with a *Vicia faba* annual cover crop in Sicily compared to a mixture of legume species or grass-legume mixtures, probably due to its lower biomass production (Novara *et al.*, 2011). Soil aggregate stability was found to be an important parameter affecting soil erosion (Ruiz-Colmenero *et al.*, 2013), but soil type could also account for reduced soil losses. In this sense, (García-Díaz *et al.*, 2016) observed that silty soils were more prone to erosion.

The effect erosion had on soil loss was accompanied by carbon and nutrient losses, dragged through soil particles. This fact was reported for K, P and N (both as ammonium and nitrates) in Biddoccu *et al.* (2016) and Ferreira *et al.* (2018), whereas García-Díaz *et al.* (2017) observed N losses occurring as nitrates while ammonium remained unaltered.

Table 1-3. Summary of runoff coefficients, soil erosion losses and vineyard description from analysed articles.

N	Location	AP (mm)	Slope (%)	Duration	Soil management	Cover type	C (%)	Annual erosion (Mg·ha^{-1}·year^{-1})
1	Toscana, Italy	695	4-30	8	Tillage (1/season)	CT	9.4	8.59
					Spontaneous vegetation	SV	8.3	7.78
2	La Caple, France	583	10	4	Herbicide	CH	34.0	≈ 12.0
					Permanent cover *Festuca rubra, Lolium perenne*	G	34.0	≈ 0.7
3	Champagne, France	757	5-7	7	Tillage	CT	80.0	
					Permanent cover *Lolium* sp.	G	0.4-77 (18.8 years average)	
4	Maribor, Slovenia	1045	34	5	Tillage alternately	CT		1.89
					Spontaneous vegetation	SV		0.09
5	Piemonte, Italy	905	15	14	Tillage (Depth 25 cm)	CT	27.1	12.3
					Permanent cover grass	G	9.6	2.2
6	Piemonte, Italy	965	15	12	Tillage (Depth 25 cm)	CT	17.4	10.4
					Tillage (Depth 15 cm)	CT	15.3	24.8
					Spontaneous vegetation	SV	10.3	2.3
7	Abruzzo, Italy		21	3	Tillage (2–3/season)	CT	5.6	
					Annual cover *Hordeum vulgare* (60 %), *Vicia faba* (40 %)	GL	1.8	
8	Piemonte, Italy	849	15	14	Tillage (Depth 25 cm)	CT	18.0	7
					Tillage (Depth 15cm)	CT	16.0	20.7
					Spontaneous vegetation	SV	10.0	1.8
9	Sicilia, Italy	589 ± 175	15.9	10	Tillage (3–4/season, depth 15 cm)	CT		8
					Annual cover *V. faba*	L		4.8
					Annual cover *V. faba, V. sativa*	L		2
					Permanent cover *Trifolium subterraneum, F. rubra, L. perenne*	GL		2.7
					Permanent cover *T. subterraneum, F. rubra, F. ovina*	GL		1.9
					Annual cover *Triticum durum*	G		3.5
					Annual cover *T. durum, V. sativa*	GL		2.4
10	Madrid, Spain	400	7-13.5	2	Tillage (3/season, depth 20 cm)	CT	28.0	
					Permanent cover *Brachypodium distachyon*	G	15.8	
					Spontaneous vegetation	SV	9.2	
11	Piemonte, Italy	850	15	10	Tillage (Depth 25 cm)	CT	21.0	11.15
					Tillage (Depth 15 cm)	CT	19.0	20.70
					Spontaneous vegetation	SV	14.0	2.60
12	Madrid, Spain	386	8-14	2	Tillage (3–4/season)	CT	4.6	0.008
					Permanent cover *Brachypodium distachyon*	G	0.9	0.001
					Annual cover *Secale cereale*	G	1.1	0.002
					Annual cover *H. vulgare*	G	2.7	0.003
					Spontaneous vegetation	SV	0.3	0.002

1: Napoli *et al.* (2017); 2: Gontier *et al.* (2014); 3: Morvan *et al.* (2014); 4: Vrsic *et al.* (2011); 5: Bagagiolo *et al.* (2018); 6: Biddoccu *et al.* (2015); 7: Ramazzotti *et al.* (2008); 8: Biddoccu *et al.* (2016); 9: Novara *et al.* (2011); 10: García-Díaz *et al.* (2017); 11: Biddoccu *et al.* (2014); 12: Ruiz-Colmenero *et al.* (2011).

N: number-author reference; AP: average annual precipitation; Duration: in years since the beginning of the experiment; C: runoff coefficient.

1.1.7. Soil biodiversity

The use of cover crops results as a general rule in a remarkable increase in soil microbial diversity (Table 1-4). The enhanced soil microbial biomass and activity associated to cover crops is majorly concentrated in soil top layers (0-5 cm), mainly as a consequence of the increase in SOC content mentioned above. In particular, it is the particulate organic matter C which most relevantly increases nutrient availability to microorganisms (Agnelli *et al.*, 2014; Belmonte *et al.*, 2018; García-Díaz *et al.*, 2018; Peregrina *et al.*, 2010; Peregrina *et al.*, 2014).

In this regard, López-Piñeiro *et al.* (2013) observed an improvement in soil microbial amount and biodiversity after a 6-year natural vegetation management regime in Spain. Soil microbial activity, measured separately for different groups of yeasts and bacteria, was positively affected by the presence of a spontaneous cover crop during 5 years (Peregrina *et al.*, 2014). Zehetner *et al.* (2015) observed an increased SOC content with a dense grass cover compared to tillage, which showed a positive influence on soil microorganisms.

The response of fungi and bacteria to the changes in soil management strategies is not the same, Likar *et al.* (2017) observing that bacteria were more sensitive to them. In a long term experiment (22 seasons), where the incorporation of the cover crop into the soil was done either every year or just mown, microbial activity was observed to be favoured by mowing (Belmonte *et al.*, 2018). Even distance between rows seemed to influence soil microorganisms. A distance of 70 cm of the grapevine row showed higher microbial activity than a 120 cm distance, regardless of the cover crop species (grasses, legumes or brassicas) (Mackie *et al.*, 2014).

Regarding earthworm populations, a three-fold increase in the number of individuals has been associated to cover crop inclusion in the management system and, conversely, a decrease is observed linked to herbicide applications (Vrsic *et al.*, 2011). Last, in a study comparing the influence of soil management on springtail species, tillage was observed not to affect their diversity, although there was a relevant decrease in the densities of the biggest species (Buchholz *et al.*, 2017).

1.1.8. Biodiversity in vineyard

The implication of cover crops on arthropods, small mammals and bird populations were analysed in 24 of the articles selected. In 72 % of the cases considered, cover crops increased the presence of species acting as natural enemies for vineyard pests. In particular, the *Hymenoptera* population increased in 86 % of cases, minute pirate bugs (*Anthocoridae*) in 80 %, spiders in 40 % and mites, as well as thrips (*Aeolothripidae*), in 100 % of cases. The diversity and density of pollinator insects, birds and small mammals also increased in all cases (Table 1-4).

Table 1-4. Collection of data from articles that study the influence of cover crops in vineyards on arthropods and vertebrates. *Control treatment.

N	Location	Soil management	Cover type	Climate	Irrigation	Duration	Arthropods
1	Douro Region-Portugal	Spontaneus vegetation	SV	M		1	
2	Córdoba-Spain	_Avena sativa_ (70%), _Vicia sativa_ (30%)	GL	W		1	
3	Francia	Bared soil* /Spontaneus vegetation	C/SV	M		2	
4	Douro Region-Portugal	Spontaneus vegetation	SV	M		1	
5	Barrosa-Australia	_A. sativa_* /_Austrodanthonia richardsonii_ /_Chloris truncata_ /_Atriplex sp._	CG/G/G/O	M	Y	2	+
6	Zadar-Croatia	Tillage* /Spontaneus vegetation	CT/SV	M		1	+
7	Modena-Italy	Grass* /_Lobularia maritima_ /_Phacelia tanacetifolia_ /_Fagopyrum esculentum_ /_V. faba_ /_A. sativa_	CG/O/O/O /L/G	M		3	+
8	Geneva Canto - Switzerland	Herbicide* /Spontaneus vegetation /_Festuca rubra_,_Trifolium repens_ /_T. repens_, _Lotus corniculatus_	CH/SV/GL/ GL	C		1	
9	New York County-U.S.A.	_Dactylis glomerata_* /_F. esculentum_ /_T. repens_	CG/O/L	C		2	
10	Nîmes-France	Tillage* /Herbicide* /Spontaneus vegetation	CT/CH/CV	M		2	
11	Zagreb-Croatia	_Agrostis alba_, _D. glomerata_, _F. rubra_, _Poa pratensis_, _L. corniculatus_ , _T. repens_	GL	C		2	+
12	California-U.S.A.	Tillage* /_F. esculentum_	CT/O	W	Y	1	
13	Valais-Switzerland			C		4	
14	California-U.S.A.	_Helianthus annuus_, _F. esculentum_	O	W		2	
15	Marche-Italy	Tillage* /Tillage, herbicide* /Spontaneus vegetation	CT/CTH/SV	M		2	
16	Malaga-Spain	Tillage* /Spontaneus vegetation	CT/SV	W	Y	1	
17	California-U.S.A.	Tillage* /_P. tanacetifolia_, _Ammi majus_, _Daucus carota_/Spontaneus vegetation	CT/O/SV	W	Y	1	
18	Califronia-EUA	Tillage* /_F. esculentum_	CT/O	W	Y	1	+
19	New South Wales-Australia	Tillage* /Spontaneus vegetation /_Brassica juncea_, _Borago officinalis_, _Coriandrum sativum_, _F. esculentum_, _L. maritima_	CT/SV/O	W		1	
20	California-U.S.A.	Untreated* / Tillage /Herbicide/ _Bromus carinatus_	CU/T/H/G	W	Y	2	-/=
21	California-U.S.A.	Tillage, spontaneus vegetation alternately* /_P. tanacetifolia_, _A. majus_, _D. carota_	T,SV/O	W	Y	2	+
22	California-U.S.A.	Tillage* /_H. annuus_, _F. esculentum_	T/O	W		2	
23	Melbourne-Australia	Adjecent vegetation	O	M		1	
24	Auckland-New Zeland	_T. subterraneum_ /_T. repens_ /_T. incarnatum_ /_T. fragiferum_	L	GH			

1: Gonçalves *et al.* (2017); 2: Barrio *et al.* (2012); 3: Vogelweith and Thiéry (2017); 4: Gonçalves *et al.* (2018); 5: Danne *et al.* (2010); 6: Franin *et al.* (2016); 7: Burgio *et al.* (2016); 8: Pétremand *et al.* (2017); 9: English-Loeb *et al.* (2003); 10: Renaud *et al.* (2004); 11: Barić *et al.* (2008); 12: Irvin *et al.* (2018); 13: Buehler *et al.* (2017); 14: Nicholls *et al.* (2000); 15: Minuz *et al.* (2013); 16: Duarte *et al.* (2014); 17: Wilson *et al.* (2018); 18: Irvin *et al.* (2016); 19: Begum *et al.* (2006); 20: Sanguankeo and Le (2011); 21: Wilson *et al.* (2017); 22: Nicholls *et al.* (2008); 23: Smith *et al.* (2015); 24: Sandanayaka *et al.* (2018).

N	Hymenoptera	Anthochorini	Cicadelidae	Spiders	Mites	Thrips	Others
1				= NE(D)			+/= Ants
2							+ P Rabbits
3			- P		-/+ PL, NE(D)		= NE *Phalangium opilio*
4							+ Predators
5						+ NE (D)	+ NE Dermaptera, tiphiid
6		+		=			- Coleoptera
7	+ NE (PP)				+ NE(D)		
8							+ Syrphid
9	+ NE (PP)						
10							+ Collembola
11							
12		+ NE		+ NE		+ NE (D)	
13							+ Woodlark
14	+ NE (PP)	+ NE (D)	- P			- P	+ NE (D) Coccinelids, *Chrysoperla*
15							= P Disease vectors
16							+ Passerine birds
17							+ Bees
18						+ NE (D)	
19	+ NE (PP)						+/= P *Epiphyas postvittana*
20							
21	=	+ NE	= P	+ NE			
22	+ NE (PP)	= NE	- P				= NE Coccinelids, syrphid
23	+ NE (PP)						
24							NE *Pseudococcus calceolariae*, *P. longispinus*

N: number-author reference; Duration: in years since the beginning of the experiment; C: cold climate (average T > 12 °C); M: mild climate (average T 12-15 °C); W: warm climate (average T < 15 °C); *:Control management; GH: green house; Cover type: CT: tillage control; CH: herbicide control; CTH: tillage+herbicide control; CU: Untreated control; T: Tillage; G: grass; GL: grass+legume; L: legume; SV: spontaneous vegetation; O: other cover crop group; PE: pest; NE: pest natural enemy; (D): predatory of pests; (PP): parasitic of pests.

Increasing plant biodiversity through cover crops was observed to cause a positive effect on the bee population. A study performed in California evaluated bee response to the use of different summer flowering cover crops (*Phacelia tanacetifolia*, *Ammi majus*, *Daucus carota*) compared to tilled soils and natural vegetation (Wilson *et al.*, 2018). The study revealed that diversity and abundance of wild bees were increased with the cover crops composed of flowering species.

The presence of cover crops favoured vertebrate abundance in comparison with that in bare soils. Buehler *et al.* (2017) observed that woodlarks prefer nesting in cover cropped plots, particularly in fields with taller and denser ground covers. Besides, nest predation risk was lower in the presence of cover crops. The abundance and diversity of passerine birds were higher in vineyards with herbaceous cover crops than in those under conventional management (bare soil and soil tillage) (Duarte *et al.*, 2014). An increase in the rabbit population in vineyards due to the presence of cover crops has also been reported (Barrio *et al.*, 2012).

In general, the number of natural enemies increased with the introduction of cover crops, though this variation in the natural enemies did not have a direct effect on pests in all studies (Danne *et al.*, 2010; Irvin *et al.*, 2016). One of the best studies of natural enemy groups is *Hymenoptera*, for which there are different examples of parasitoids, which increase when cover crops are used. The presence of *Anagrus*, egg parasitoids of *Cicadellidae*, increased with cover cropping (Begum *et al.*, 2006; Centinari *et al.*, 2016; English-Loeb *et al.*, 2003; Nicholls *et al.*, 2008, 2000; Smith *et al.*, 2015) and *Erythroneura* (*Cicadellidae*) population, in turn, decreased. The only exception found to this behaviour is an experiment performed in California, where the presence of cover crops did not affect Anagrus (Wilson *et al.*, 2017). The parasitism rate of *Epiphyas postvittana* (pest) and *Trichogramma carverae* (parasite) was also increased. In this study, the presence of a sown cover crop had the strongest effect on parasitism rate, in comparison to a spontaneous cover crop or tilled soils. However, some sown cover crops, such as *Lobularia marítima*, provided higher longevity for the pest than spontaneous cover crops or tillage, and there were no differences in *Borago officinalis* and *Fagopyrum esculentum* covers compared to control plots (Begum *et al.*, 2006).

The difference between sown cover crops and spontaneous vegetation was the presence of flowers. Some authors pointed to a link between parasitism rate increase and higher availability of floral nectar as a source of nutrition. English-Loeb *et al.* (2003) compared *Anagrus* longevity and parasitism rate of *Erythroneura* spp. in a laboratory study. Both parameters were greater when adults had access to flowering *Fagopyrum esculentum* rather than plants without flowers. Moreover, the longevity of *Anagrus* was increased when provided with honey or sugar water compared to water only. In field experiments, it was also observed that the rate of parasitism increased (Daane *et al.*, 2018; Nicholls *et al.*, 2008) or remained unaltered (Nicholls *et al.*, 2000) when cover crops were established.

Regarding arachnid populations, the effect of cover crops was highly variable. In some cases, the presence of cover crops led to a decrease in spider populations (Daane *et al.*, 2018), maintained them unaltered (Franin *et al.*, 2016; Gonçalves *et al.*, 2017), or caused an increase (Irvin *et al.*, 2018; Wilson *et al.*, 2017) in spiders known to be predators of pest insects. Cover crop management enhanced predatory mite densities (Burgio *et al.*, 2016). In France, an increase in the number of individuals of the predatory mite *Typlodromus pyri* was observed, while the number of the mycophagus mite *Orthotydeus lambi* and the pest mite *Panonychus ulmi* decreased in cover cropped vineyards (Vogelweith and Thiéry 2017).

A positive response of predatory thrips (spiders, *Nabis* sp., *Orius* sp., *Geocoris* sp., *Coccinellidae* and *Chrysperla* sp.) to cover cropping has also been observed, while reduced densities of western flower thrips pest (*Frankliniella occidentalis*) have been reported in cover-cropped plots (Nicholls *et al.*, 2000).

The presence of natural enemies in the cover crop does not mean that they will be present in the vines themselves. Gonçalves *et al.* (2018) showed that, although predators could colonise the vineyard, it is more probable that they feed primarily on vineyard pests that spend part of their life cycle on the ground or use plants from ground cover as alternative hosts. With this regard, the abundance of grape pests has been reported to be higher on grape leaves compared to their presence on the cover crop itself, while the presence of beneficial insects was higher on cover crop (Irvin *et al.*, 2016). Concerning the impact of the presence of cover crops, it has been observed that the increase in the populations of natural enemies of a cover cropped with respect to a tilled one is greater on the ground than on the grapevine canopy (Wilson *et al.*, 2017).

Cover crop mowing could be an effective tool to increase the abundance of natural enemies on vine canopy. In California, Nicholls *et al.* (2008) showed that numbers of leafhoppers declined in vines when the cover crop was mown, while the cutting of the cover crop vegetation increased *Anagrus* densities on the vines, especially one week after mowing.

The presence of natural enemies is also influenced by cover crop types. When three native cover crops were compared to a sown *Avena sativa* control in Australia, the abundance of arthropods, predators and parasitoids as well as potential pests, was observed to be higher in all native cover crops (Danne *et al.*, 2010). The comparison of sown and spontaneous cover crops generated varied results. Regarding *Anagrus* parasitism rate, in an experiment in Italy, it was higher in sown cover crops (Muscas *et al.*, 2017) than in spontaneous covers. However, under these conditions, an increase in the abundance and biodiversity of syrphids was observed in spontaneous vegetation compared to sown cover crops (Pétremand *et al.*, 2017).

1.1.9. Soil gas emissions

Like most economic sectors, agriculture contributes to greenhouse gas (GHG) emissions. However, agriculture can also participate as a sink for gas emission storage by means of carbon sequestration in the soil. As the use of cover crops increases SOC, the installation of a cover crop can contribute to mitigating CO_2 emissions.

In a comparative study of spontaneous and *Hordeum vulgare* cover crops, the emissions were higher after tillage than in mown treatments where plant biomass was incorporated to the soil (Steenwerth *et al.*, 2010). In the same way, Bogunovic *et al.* (2017) observed that higher CO_2 emissions were found in annual tillage treatment compared to tilling the soil every two years. Lower emissions were observed under continuous no-tillage treatment, indicating that the cover crop management has more influence on CO_2 emissions than the cover crop itself. When the cover crop was annually mown and tilled, a C loss was observed, while a barley cover crop under minimum tillage (superficial tillage every two years) accumulated 1.12 Mg CO_2 ha^{-1} $year^{-1}$ (Steenwerth *et al.*, 2010). However, higher N_2O emissions were detected when leguminous cover crops were mowed and left on the soil surface, under the row or between lines, compared to the incorporation of residues into the soil via conventional tillage (Garland *et al.*, 2011), although these increased emissions relate to the nitrogen that had been previously fixed by the cover crop from the atmosphere.

The dynamics of C sequestration and emission change along the season in cover-cropped vineyards, and at some points, due to their higher biological activity, increased emissions when the soil is moist (Peregrina, 2016; Steenwerth *et al.*, 2010). Nevertheless, on a yearly or long-term basis, the presence of cover crops contributes very actively to C sequestration through the increase in SOC.

1.1.10. Conclusions

The systematic review performed has allowed a complete synthesis of the knowledge generated in the last two decades regarding the influence of cover crops on soil characteristics and biodiversity in vineyards. This first part is focused on soil characteristics and environment-related issues, whereas their effect on agronomic performance will be presented in the second part of this work.

As part of this wide-scope analysis, it can be concluded that using cover crops has a positive effect on increasing SOC and thus in reducing greenhouse gases in the atmosphere. Covers crops do not, in general, constitute a major competition for nutrients to the vines except for nitrogen when grass covers are used. On the contrary, legume cover crops generally increase N in the soil, although the availability of this for plants is not immediate.

Cover crops improve aggregate stability and reduce erosion. Likewise, there is an increase in biodiversity, both in soil biodiversity and activity, as well as in populations of arthropods, birds and small mammals.

Both the SOC increases in the long run and the erosion reduction are greater when the cover is formed by grasses, the results being more variable when the used cover is formed by legumes.

There are other aspects where the implication of establishing a cover crop is more variable, generally affected by soil and climate characteristics. This review constitutes a tool that can help to have a preliminary idea on what could happen under certain growing conditions, as peer-reviewed scientific literature has been revised, and some characteristics of the vineyards studied in each article are provided.

1.2. Cover crops in viticulture. A systematic review (2): Implications on vineyard agronomic performance

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1.2.1. Introduction

Cover crops are one of the most appealing options for soil management in vineyards, because- as was shown in our companion paper (Abad *et al.*, 2021a) - they increase soil organic carbon, improve water infiltration and aggregate stability, reduce soil erosion and greenhouse gas emissions, and increase biodiversity in the vineyard. Nevertheless, as vines and cover crops coexist in the same space, they compete for nutrients and water at certain moments in the season, which can directly affect vineyard performance. Such competition can result in changes to shoot growth and leaf activity, which in turn can seriously affect shoot fertility, fruit set, berry development, susceptibility to pests and diseases, yield, and grape composition (Ibañez Pascual, 2013).

The intensity and implications of the aforementioned effects depend highly on many factors, such as cover crop features, soil type, climate and other vineyard characteristics. We therefore carried out a systematic review of research results obtained in recent decades to determine the main agronomic effects of cover crops in vineyards and the factors that modulate them. In this article, the second part of the review results is presented; only nutrition was included in the first part, as it was considered to be highly linked to other soil processes described therein.

1.2.2. Published data sourcing and selection

The methodology applied for this systematic review is detailed in the article, "Cover crops in viticulture. A systematic review (1): implications on soil characteristics and vineyard biodiversity" (Abad *et al.*, 2021a). In short, a systematic review can be defined as including (1) a research question, (2) sources that were searched with a reproducible search strategy (naming of databases, naming of search platforms/engines, search date and complete search strategy), (3) inclusion and exclusion criteria, and (4) selection (screening) methods (Krnic Martinic *et al.*, 2019). As such, the main features of the systematic review we performed on the implication of cover crops in vineyards are summarised below.

The Scopus database was used, with search query TITLE-ABS-KEY ("cover crop" OR "green cover" OR "ground cover" OR "tillage") AND TITLE-ABS-KEY ("wine" OR "vitis" OR "vineyard" OR "grapevine" OR "grape"). A total number of 584 published papers were obtained (search day: 20 November 2018). Two people worked independently from each other on the selection process in several steps with a final number of 272 papers being selected. The following data were extracted from the selected papers:

- Location
- Vineyard: scion variety and rootstock, planting frame, age and vine training
- Experiment duration
- Cover crop characteristics (sown or spontaneous, monoculture or crop mixture, species, cover crop and row management)
- Climate: an illustrative classification was performed; cold (annual average T^a below 12 ºC), mild (annual average T^a between 12 and 15 ºC) and warm climate (annual average T^a above 15 ºC)

- Cultural practices: irrigation (yes/no) and fertilisation (yes/no)
- Soil: texture, organic matter percentage (% OM) and studied horizons

The following sections provide information related to vineyard agronomic performance, whereas the aforementioned companion paper outlined soil characteristics and environmental aspects. Both papers together are a compilation of most of the factors that should condition the choice of soil management in a vineyard. It should be noted that other factors, such as spring frost risk, the necessity of soil amendments once the vineyard is established, or risk of excessive competition with young vines, need to be considered before choosing cover crop as the best solution; however, they were not considered in the systematic review as information on them was not available in the selected papers.

1.2.3. Vegetative development

Ensuring optimal vegetative development is one of the key issues for successful grape growing, with a balanced number and disposition of leaves being required. Although minimum leaf development is required to guarantee carbohydrate supply to all plant organs, excessive growth can be detrimental, as it may cause reduced fruit set (Dardeniz *et al.*, 2008; Parker *et al.*, 2016), increased susceptibility to fungal diseases (Valdés-Gómez *et al.*, 2011) and delayed ripening (Smart et *al.*, 2017). Therefore, it is of great interest to determine the ways in which cover crops can impact vine growth.

The effect of cover crops on vine vegetative growth - mostly evaluated by pruning weight measurement - was analysed in 51 of the selected articles. None of these articles reported that an increase in vegetative development was associated with the introduction of a cover crop, and only 3 studies (6 % of the cases) showed no changes in pruning weight due to its presence. Thus, the use of cover crops mostly caused a reduction in growth (Table 1-5). However, in 23 articles (45 %) the reduction in pruning weight was relatively small (by < 20 %), while in the remaining 25 articles (49 %) this reduction was > 20 %. When the potential impact of climate conditions was analysed (Figure 1-1), it was observed that vineyards in warmer regions showed a more pronounced decrease in growth than those in cooler areas.

The most drastic effect was observed in four studies, in which pruning weight reduction was shown to exceed 60 % (Coletta *et al.*, 2013; Gontier *et al.*, 2014; Olmstead *et al.*, 2012; Rodriguez-Lovelle *et al.*, 2000a). The most extreme growth diminution was observed in Olmstead *et al.*, (2012): the cover crop had been established at the time of vineyard planting and the reduction was between 70 and 90 %.

Nine experiments showed reductions in pruning weight of over 40 %, and, quite remarkably, in 5 of them the rootstock was SO4 (Coletta *et al.*, 2013; Coniberti *et al.*, 2018a; Coniberti *et al.*, 2017; Toci *et al.*, 2012; Wheeler *et al.*, 2005). The predominant cover crop species in these experiments was perennial *Festuca rubra* (Coletta *et al.*, 2013; Gontier *et al.*, 2014; Toci *et al.*, 2012), *Festuca arundinacea* (Coniberti *et al.*, 2018a; Coniberti *et al.*, 2017; Hatch *et al.*, 2011; Olmstead *et al.*, 2012; Rodriguez-Lovelle *et al.*, 2000a) and *Festuca ovina* (Coletta *et al.*, 2013; Toci *et al.*, 2012). The average age of these vineyards was 5 years, but it never exceeded 8 years of age, suggesting that under some circumstances the presence of cover crops during the initial years of vineyard's life can be too limiting for proper vineyard development.

Lastly, when the influence of the cover crop on growth was found to be milder (< 20 %) or even not observed at all, most of the experiments relied on irrigation (Gill Giese *et al.*, 2016; Jordan *et al.*, 2016; Klodd *et al.*, 2016; Mercenaro *et al.*, 2014; Monteiro *et al.*, 2008; Monteiro and Lopes, 2007; Steenwerth *et al.*, 2013; Steenwerth *et al.*, 2016; Tourte *et al.*, 2008); only in the minority of cases was irrigation not used (Pérez-Álvarez *et al.*, 2015b; Ripoche *et al.*, 2011; Vrsic *et al.*, 2011). In these cases, cover crops were mainly composed of cereals (*Triticum aestivum*, *Secale cereale* and *Avena sativa*), *Lolium* and mixtures of grass and legume. The average age of vineyards was around 12 years, which highlights that vineyard age is a key factor in the modulation of vineyard growth response.

Table 1-5. Impact of cover crop on vine vegetative growth (pruning weight) compared to tilled or to herbicide applied in the row.

				No trend				
1	Costello (2010b)	=	**2**	Jordan *et al.* (2016)	=	**3**	Wilson *et al.* (2017)	=
				Slightly negative				
4	DeVetter *et al.* (2015)	+(T)/-	**12**	Karl *et al.* (2016b)**	=/--	**20**	Ingels *et al.* (2005)*	-(T)/--
5	Krohn and Ferree (2005)	+(T)/--	**13**	Smith *et al.* (2008)	=/-	**21**	Reynolds *et al.* (2006)**	-(T)/--
6	Sweet and Schreiner (2010)*	+(T)/--	**14**	Klodd *et al.* (2016)	- (T)	**22**	Ripoche *et al.* (2011)*	- (T)/--
7	Tourte *et al.* (2008)	=/-(T)	**15**	Steenwerth *et al.* (2016)	- (T)	**23**	Giese *et al.* (2016)	-
8	Lopes *et al.* (2008)	=/--	**16**	Coniberti *et al.* (2018a)	- (T)/-	**24**	Steenwerth *et al.* (2013)	-
9	Mercenaro (2014)	=/-	**17**	Monteiro and Lopes (2007)	- (T)/-	**25**	Vrsic *et al.* (2011)	-
10	Pérez-Álvarez *et al.* (2015b)	=/-	**18**	Muscas *et al.* (2017)*	- (T)/-	**26**	Pérez *et al.* (2018)	-/--
11	Trigo-Córdoba *et al.* (2015)	=/-	**19**	Tomaz *et al.* (2015)	- (T)/-			
				Negative				
27	Rodriguez-Lovelle *et al.* (2000b)	+(T)/---	**36**	Palliotti *et al.* (2007)	--	**45**	Coletta *et al.* (2013)	---
28	Delpuech & Metay (2018)*	=/--	**37**	Pou *et al.* (2011)*	--	**46**	Coniberti *et al.* (2017)	---
29	Reeve *et al.* (2016)	-/---	**38**	Valdés-Gómez *et al.* (2011)	--	**47**	Gontier *et al.* (2014)	---
30	Coniberti *et al.* (2018b)	--	**39**	Caspari *et al.* (1997)	--/---	**48**	Hatch *et al.* (2011)	---
31	DePascali *et al.* (2014)	--	**40**	Guilàrt *et al.* (2017)	--/---	**49**	Olmstead *et al.* (2012)	---
32	Giese *et al.* (2015)	--	**41**	Matti *et al.* (2005)	--/---	**50**	Toci *et al.* (2012)	---
33	Hickey *et al.* (2016)	--	**42**	Muganu *et al.* (2013)	--/---	**51**	Wheeler *et al.* (2005)	---
34	Linares Torres *et al.* (2018)	--	**43**	Rodriguez-Lovelle *et al.* (2000a)**	--/---			
35	Lopes *et al.* (2011)	--	**44**	Silvestre *et al.* (2012)	--/---			

= denotes does not affect, no clear trend; -(T)/+(T) denotes reduction trend/general increase; -/+ denotes difference in reduction/increase lower than 20 %; --/++ denotes difference in reduction/increase between 20 and 40 %; ---/+++ denotes difference in reduction/increase higher than 40 %; * denotes differences among treatments in one or more years; ** denotes differences among controls in one or more years.

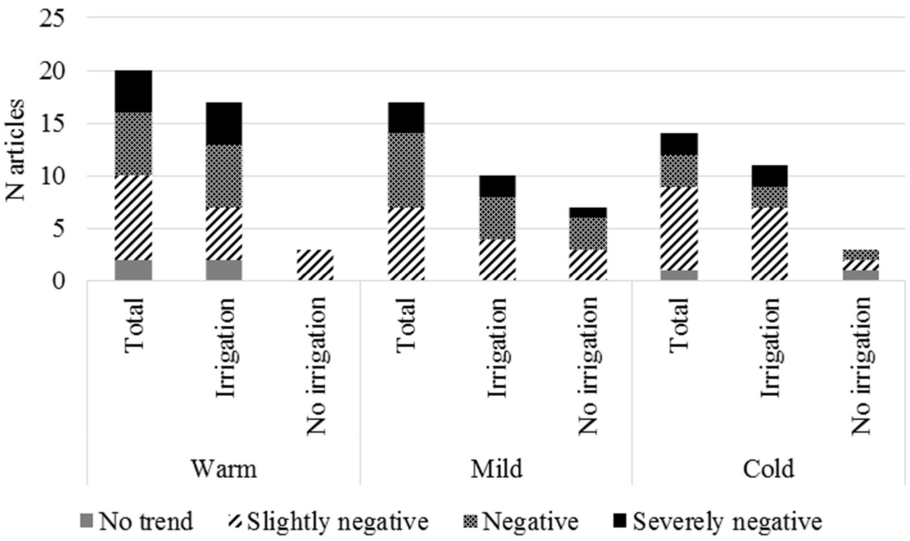

Figure 1-1. Effect of cover crop use on vine vegetative growth (pruning weight) according to climate and irrigation management.

1.2.4. Plant water status

Cover crop competition for soil water is a major constraint which needs to be considered when deciding whether to establish a cover crop in areas where a certain amount of water deficit can be expected in summer. In this review, 130 of the selected papers described at least one parameter related to water status: 40 articles measured leaf (25) or stem (15) water potential and, according to the criteria established by Carbonneau and Ojeda (2013), severe water stress was experienced in 5 % of the cases, while moderate to severe levels occurred in 45 % of the cases. Meanwhile, 40 % of the vineyards studied in the reviewed articles experienced mild to moderate water stress, and only 10 % of the vineyards experienced no water stress at all (Table 1-6).

In most cases, the presence of a cover crop implied a certain increase in water deficit, reaching its maximum around veraison, decreasing again as grape harvest approached, and fading away at the end of the grapevine cycle (Daane *et al.*, 2018; Pou *et al.*, 2011). However, it should be noted that in other cases this point of maximum stress is not so clear (Giese *et al.*, 2015; Hatch *et al.*, 2011; Jordan *et al.*, 2016). Cover-cropped treatments sometimes showed lower leaf water potential at the beginning of the cycle, while the control plots showed the most negative potential values during grape veraison (Steenwerth *et al.*, 2016) or after the start of irrigation (Toci *et al.*, 2012); this may be because water needs in tilled vineyards are greater at the end of the season due to their increased vigour and yield. Rainfall distribution during the grape growing seasons was found to have an extreme impact on plant stress responses; for instance, when rainfall was scarce in spring in one of the three growing seasons compared in Delpuech and Metay (2018) a 60 % cover crop soil coverage led to more negative water potential values than bare soil. Similarly, Pou *et al.* (2011) only observed significant differences between soil management treatments in the driest years.

Table 1-6. Minimum seasonal values for leaf and stem water potential depending on the cover used – information extracted from the different articles studied.

N	Place	Variety	Soil management	Irrigation	Measure	Year 1				Year 2				Year 3			
						Rain (mm)	Ψmax (Mpa)		Sig	Rain (mm)	Ψmax (Mpa)		Sig	Rain (mm)	Ψmax (Mpa)		Sig
1	Montpellier-France	Shiraz	Tillage *Medicago* mix cover Resilent cover	No	Ψ_{pd}	-	-0,57 -0,64 -0,58	End Aug.	*								
2	Nimes-France	Shiraz	Herbicide *Festuca rubra*	No	Ψ_{pd}	493 vc	-0,41 -0,58	End Aug.	*	321 vc	-0,8 -0,89	Mid Aug.	ns	301 vc	-0,51 -0,29	End Aug.	*
3	Gallargues-France	Sauvignon blanc	Herbicide *Festuca arundinacea*	-	Ψ_{pd}	650 ay	≈-0,17 ≈-0,16	Mid Jul.	ns	650 ay	≈-0,15 ≈-0,18	Mid Aug.	ns				
4	Alentejo-Portugal	Tempranillo	Tillage Resilent cover	Yes	Ψ_{pd}	-	≈-0,6 ≈-0,7	Mid Aug.	*	-	≈-0,4 ≈-0,5	Mid Aug.	*				
5	Tokaj-Hungary	Hárslevelü	Tillage *Hordeum vulgare* Straw	-	Ψ_{pd}	-	≈-0,55 ≈-0,5 ≈-0,4	-	*								
6	Alenquer-Portugal	Cabernet Sauvignon	Tillage Resilent cover 60% *Lolium-Festuca,* 40% *Trifolium*	No	Ψ_{pd}	942 y	≈-0,26 ≈-0,38 ≈-0,36	Mid Aug.	*	564 y	≈-0,18 ≈-0,24 ≈-0,25	Mid Aug.	*				
7	Madrid-Spain	Shiraz	Herbicide *Bromus hordeaceus* *Secale cereale*	Yes	Ψ_{md}	142 y	-1,5 -1,25 -1,4	Mid Aug.	*								
8	Sacramento County-USA	Merlot	Tillage *Hordeum vulgare*, *Avena sativa* Green manure Anual clover mix California native perennial grass	Yes	Ψ_{md}	-	-1,11 -1,08 -1,16 -1,22 -1,14	Jul.	*								
9	Fresno County-USA	Barbera	Tillage *Nassella cernua*	Yes	-	-	≈-0,92 ≈-0,92	Aug.	ns	-	≈-1,94 ≈-1,72	Aug.	ns				
10	Califronia-USA	Cabernet Sauvignon	Tillage 50% *Bromus carinatus*, 50% *Elymus glaucus*	No	Ψ_{md}	-	≈-1,0 ≈-0,9	Mid Aug.	ns	-	≈-1,1 ≈-1,0	Mid Aug.	ns				
11	Bairrada-Portugal	Fernão Pires	Tillage Resilent cover	No	Ψ_{pd}	137 vc	≈-0,38 ≈-0,38	Mid Jul.	ns	108-vc	≈-0,55 ≈-0,55	Aug.	ns				
12	Tekigdar-Turkey	Cabernet Sauvignon	Tillage Resilent cover	-	Ψ_{pd}	175 vc	≈-0,40 ≈-0,52	Aug.	ns								

N	Place	Variety	Soil management	Irrigation	Measure	Year 1				Year 2				Year 3			
						Rain (mm)	Ψmax (Mpa)		Sig	Rain (mm)	Ψmax (Mpa)		Sig	Rain (mm)	Ψmax (Mpa)		Sig
13	Canelones-Uruguay	Tannat	*F. arundinacea* -(Herbicide) *F. arundinacea* -(*F. arundinacea*)	Yes	Ψ_{md}	668 cv	≈-1.0 ≈-1.2	Mid May	-	1019 cv	≈-0.6 ≈-0,9	Mid May	-	1323 cv	≈-0.8 ≈-0.8	Mid May	-
14	Virginia County-USA	Cabernet Sauvignon	*F. arundinacea* , *Dactylis glomerata* - (Herbicide) Row *F. arundinacea, D. glomerata* -(*F. rubra*)	Yes	Ψ_{md}	721 cv	≈-0.65 ≈-0.75	Mid Aug.	ns								
15	Salento-Italy	Negroamaro	Tillage *F. rubra* + *F. ovina* + *Trifolium subterraneum*	Yes	Ψ_{md}	-	-1,54 -1,35	Early Aug.	*								
16	New York County-USA	Cabernet Franc	*F. arundinacea* -(Tillage) *F. arundinacea* -(Herbicide) *F. arundinacea* -(Native vegetation) *F. arundinacea* -(*T. repens*)	No	Ψ_{md}	597	-0,85 -1,05 -0,87 -0,91	End Aug.	*	654	-0,29 -0,26 -0,28 -0,28	Mid Aug.	*				
17	Ourense-Spain	Mencía	Tillage Native vegetation *Lolium perenne* *T. subterraneum*	No	Ψ_{md}	313 cv	≈-0.6 ≈-1.0 ≈-0.9 ≈-0.9	Early Sept.	*	163 cv	≈-1.0 ≈-1.15 ≈-0.95 ≈-0.8	Early Sept.	*	185 cv	≈-1.0 ≈-1.0 ≈-0.7 ≈-0.6	Early Sept.	*
18	Montevideo-Uruguay	Tannat	*F. arundinacea* -(Herbicide) *F. arundinacea* -(*F. arundinacea*)	Yes	Ψ_{md}	650 ay	≈-1 ≈-1.2	End Jan.	-	650 ay	≈-0.6 ≈-0.9	Mid Jan.	-				
19	New York County-USA	Cabernet Franc	Resident vegetation-(Tillage) Resident vegetation-(*Fagopyrum esculentum*) Resident vegetation-(*Lolium multiflorum*) Resident vegetation-(*Brassica rapa* var. *Rapa*)	No	Ψ_{md}	526 cv	≈-0.5 ≈-0.7 ≈-0.7 ≈-0.6	Mid Aug.	ns	450 cv	≈-0.6 ≈-0.8 ≈-1.0 ≈-0.9	Aug.	ns	350 cv	≈-1.1 ≈-1.2 ≈-1.25 ≈-1.2	Early Sept.	ns
20	Oregon County-USA	Pinot Noir	Tillage *F. rubra*	No	Ψ_{md}	356 cv	-0,89 -0,77	Mid Sept.	-								

1: Kazakou *et al*. (2016); 2: Delpuech and Metay (2018); 3: Celette *et al*. (2005); 4: Lopes *et al*. (2011); 5: Judit *et al*. (2011); 6: Lopes *et al*. (2008); 7: Linares Torres *et al*. (2018); 8: Ingels *et al*. (2005); 9: Costello (2010b); 10: Daane *et al*. (2018); 11: Cruz *et al*. (2012); 12: Bahar and Semih Yaşain (2010); 13: Coniberti *et al*. (2018a); 14: Hatch *et al*. (2011); 15: Toci *et al*.(2012); 16: Karl *et al*. (2016); 17: Trigo-Córdoba *et al*. (2015); 18: Coniberti *et al*. (2017); 19: Centinari *et al*. (2016); 20: Reeve *et al*. (2016)

() denotes under-vine soil management; Ψpd denotes predawn; Ψmd denotes middawn; * denotes significant differences; ns = no significant differences; vc = rainfall during the vine cycle (April-October); y = rainfall in a year; ay = average rainfall in a year

Apart from the above-described changes in plant water status, which reduce water availability for vines (due to cover crop transpiration), the installation of cover crops can affect water status through other processes that also need to be considered, particularly increased water infiltration or reduced evaporation losses. Regarding the former, Celette and Gary (2013) showed that cover cropping successfully increases the infiltration of water into soil in Montpellier (France), whereas in terms of the latter, some authors have also reported a decrease in soil evaporation at the end of the growing cycle (Steenwerth *et al.*, 2016). Nevertheless, the potential increase in water availability that these two factors cause does not usually compensate for cover crop transpiration. In Celette and Gary, (2013), although the presence of the cover crop was shown to improve winter soil water refilling, cover crop transpiration in spring led to similar water availability of grapevine compared to the control plot in the years with moderate water stress, whereas in the drier years it caused higher deficit from budbreak to flowering. As regards the reduction in transpiration, Klodd *et al.* (2016) observed that, if continuously mowed, a cover crop of F. arundinacea resulted in similar soil water content values than tilled soil, whereas when not mown, soil evapotranspiration increased by about 35-40 %, in both a temperate region (Virginia-USA) and a humid region (Bologna-Italy) (Centinari *et al.*, 2013).
Lastly, cover crop and vine competition for soil water can to a certain extent be compensated for by the different rooting depths of cover crops and vines (Hatch *et al.*, 2011); the compensatory growth of the grapevine root system occurs when a cover crop is established, forcing the vine roots to explore deeper soil horizons (Celette *et al.*, 2008).

1.2.5. Pest and disease incidence

The increase in the biodiversity of flora in the vineyard that can result from the introduction of a cover crop can increase the diversity of insects and indirectly improve the balance between insects and vineyards. Likewise, cover crop usually has the effect of reducing vine vegetative growth, and this can contribute to improved aeration in the vineyard and with it a lower incidence of fungal diseases. In general terms, pest populations in vineyards did not increase in the presence of cover crops in 95 % of the cases considered (whereas 45 % = no changes, and 50 % = decrease; Table 1-7). Only occasionally, at some specific moments, did *Epiphyas postvittana* and some homopters show an increase in population when cover crops were used.

The positive impact of cover crops on decreasing pest population is especially clear in the case of *Cicadellidae*. The pest reduction effect is mainly due to an increased presence of parasitoids of genus *Anagrus* (Daane *et al.*, 2018; Nicholls *et al.*, 2008). This increase in the population of *Anagrus* population was not observed in Nicholls *et al.* (2000) and, as a consequence, *Erythroneura* populations remained unaltered.

The influence of cover crop on grapevine diseases has been mainly studied for powdery mildew (*Erysiphe necator*), botrytis (*Botrytis cinerea*), downy mildew (*Uncinula necator*), black foot disease (*Ilyonectria liriodendri*) and grape black rot (*Guignardia bidwellii*). In 12 out of 18 evaluated situations (67 %), the presence of cover crops reduced disease incidence to a certain extent (Table 1-8).

The establishment of cover crops was found to reduce the incidence of powdery mildew in 2 out of 3 reviewed articles; no increase has ever been observed. In detail, Valdés-Gómez *et al.* (2011) compared the incidence of powdery mildew on two cover-cropped vineyards (perennial *vs.* annual) and two herbicide-treated control plots (fertilised and irrigated *vs.* not fertilised and not irrigated) in Montpellier. They observed that the powdery mildew incidence was higher in the fertilised and irrigated bare soils, but was slightly reduced in bare soils without fertilisation and irrigation practices. Both cover crop treatments showed a relevant decrease in disease incidence, being more significant in the case of a perennial cover crop in its second year of application. The differences among treatments were due to higher vegetative growth (greater number of leaves per shoot) when the cover crop competition was absent or the fertilisation rate increased. Conversely, Vogelweith and Thiéry (2017) did not observe any differences in Bordeaux (France) for powdery mildew incidence when vineyards with a spontaneous cover crop or bare soils were compared.

Table 1-7. Main characteristics and results of the impact of cover crops on vineyard pests.

N	Place	Variety	Soil management	Cover type	Climate
1	Villenave d' Ornon-France	Merlot	Bared soil*/Spontaneus vegetation	SV	M
2	Virginia-USA	Several	Tillage*/Spontaneus vegetation	TC/SV	M
3	Cerdeña-Italy	Carignano	Tillage*/*Medicago polymorpha, Trifolium yanninicum* /*Dactylis glomerata, Lolium rigidum*	TC/L/GL	W
4	Northern Dalmatia-Croatia	-	Tillage*/Spontaneus vegetation	TC/SV	M
5	Modena-Italy	Salamino	Tillage*/*Lobularia maritima* /*Phacelia tanacetifolia* /*Fagopyrum esculentum* /*Vicia faba* /*Vicia villosa, Avena sativa*	TC/O/O/O/L/GL	M
6	California-USA	Cabernet sauvignon	Tillage*/*Elymus glaucus, Hordeum brachyantherum, Bromus carinatus*	TC/G	W
7	California-USA	Chardonnay	Tillage*/*Helianthus annuus, Fagopyrum esculentum*	TC/O	W
8	Marche-Italy	Several	Tillage*/Spontaneus vegetation	TC/SV	M
9	New South Wales-Australia	Chardonnay	Tillage, Spontaneus vegetation*/*Brassica juncea* /*Borago officinalis* /*Coriandrum sativum* /*F. esculentum* /*L.maritima*	TC, SV/ O/O/O/O/O	W
10	California-USA	Zinfandel	Tillage,Spontaneus vegetation*/*B. carinatus*	TC, SV/G	W
11	California-USA	Cabernet sauvignon	*P. tanacetifolia, Ammi majus, Daucus carota*		W
12	Marlorough-New Zealand	-	*Spontaneus vegetation** /*V. faba*	SVC/L	M
13	California-USA		*H.annuus, F. esculentum* /Flower island	O/O	W
14	California-USA	Zinfandel	Tillage*/*Medicago sativa, A. sativa*	TC/GL	W

1: Vogelweith and Thiéry (2017); 2: Rijal *et al.* (2014); 3: Muscas *et al.* (2017); 4: Franin *et al.* (2016); 5: Burgio *et al.* (2016); 6: Daane *et al.* (2018); 7: Nicholls *et al.* (2000); 8: Minuz *et al.* (2013); 9: Begum *et al.* (2006); 10: Sanguankeo and León (2011); 11: Wilson *et al.* (2017); 12: Nboyine *et al.* (2018); 13: Nicholls *et al.* (2008); 14: Karban *et al.* (1997).

N	Duration	Cicadelidae	Spiders	Mites	Thrips	Others
1	2	- PE		- PE, NE		= NE *Phalangium opilio*
2	5					= PE *Vitacea polistiformis*
3	3					-/= PE *Planoccocus ficus*
4	1		=			- Coleoptera
5	3			+ NE(D)		+/= PE Homoptera
6	3	- PE	-			
7	2	- PE			- PE	+ NE(D) Coccinelids, *Chrysoperla*
8	2					= PE Disease vectors
9	1					+/= PE *Epiphyas postvittana*
10	2					
11	2	= PE	+ NE			
12	2					- PE *Hemiandrus* sp
13	2	- PE				= NE Coccinelids, syrphid
14	2			= PE		

N: number-author reference; Duration: in years from the beginning of the experiment; C: cold climate (average T > 12 ºC); M: mild climate (average T 12-15 ºC); W: warm climate (average T < 15 ºC); *Control management; Cover type: CT: tillage control; G: grass; GL: grass+legume; L: legume; SV: spontaneous vegetation; O: other cover crop group; PE: pest; NE: pest natural enemy; (D): predator of pests. Symbols: = denotes does not affect; - denotes negative effect compared to the control; + denotes positive effect compared to the control.

Table 1-8. Main characteristics and results of the impact of cover crops on grapevine diseases.

N	Place	Variety	Soil management
1	Villenave d' Ornon-France	Merlot	Bared soil*/Spontaneus vegetation
2	Sourthen-Uruguay	Tannat	Row *Festuca arundinacea**/Full cover of *F. arundinacea*
3	Navarra-Spain	Malvasia /Tempranillo[1]	Bared soil* /Sinapis alba
4	Blenheim-New Zealand	Chardonnay	Control*/*Phacelia tanacetifolia* /*Lolium perenne*
5	Montpellier-France	Aranel	Herbicide* /*F. arundinacea*, *L. perenne*
6	Madrid-Spain	Shiraz	Tillage /Herbicide/ Spontaneus vegetation
7	Sourthen-Uruguay	Tannat	Row *F. arundinacea**/Full cover of *F. arundinacea*
8	Montpellier-France	Aranel	Herbicide* /F. *arundinacea, Perennial ryegrass* /*Hordeum vulgare*
9	Bairrada-Portugal	Fernão Pires	Spontaneus vegetation* / Tillage
10	Sourthen-Uruguay	Tannat	Row *F. rubra**/Full cover of *F. rubra*
11	Sourthen-Uruguay	Tannat	Row *F. arundinacea**/Full cover of *F. arundinacea*
12	Montpellier-France	Shiraz	Tillage* /*Medicago truncatula, M. rigidula, M. polymorpha*
13	British Columbia-Canada		Exotic grass: *F. trachyphylla*, *Agropyron cristatum*, *F. rubra*, *L. perenne* /Exotic grass, *Lotus corniculatus*, *M. lupulina*, *Trifolium repens* /Native grass: *Bouteloua dactyloides*, *F. idahoensis*, *Pseudoroegneria spicata*, *Boteloua gracilis* /Native grass, *Nepeta racemosa*, *Origanum vulgare*, *Artemisia frigida*, *Achillea millefolium*, *Heterotheca villosa*, *Erigonium neveum*, *Erigeron filifolius*
14	Tokaj-Hungary	Hárslevelü	Tillage*/*Hordeum vulgare*
15	Ohio-USA	Seyval blanc	Bared soil*/ *Festuca arundinacea* /*Mazus japonicus albus* /*Mentha pulegium* /*Thymus serpyllum minus* /*T. fragiferum* /*Veronica prostratum* /*L. perenne* (75%), *F. rubra* (25%)

[1] Nursery planting material

1: Vogelweith and Thiéry (2017); 2: Coniberti *et al.* (2018a); 3: Berlanas *et al.* (2018); 4: Jacometti et al. (2007); 5: Valdés-Gómez *et al.* (2008); 6: Cordero-Bueso *et al.* (2011b); 7: Coniberti *et al.* (2017); 8: Valdés-Gómez *et al.* (2011); 9: Cruz *et al.* (2012); 10: Coniberti et al. (2018b); 11: Coniberti *et al.* (2018c); 12: Guilpart *et al.* (2017); 13: Vukicevich *et al.* (2018); 14: Judit *et al.* (2011); 15: Krohn and Ferree (2004).

N	**Cover type**	**Climate**	**Duration**	**Powdery mildew**	**Downy mildew**	**Botrytis**	**Black rot**	**Black foot**	***Ilyonectria liriodendri***
1	C/SV	M	2	=	=		=		
2	GC/G	M	3			-			
3	C/ O	M	2					-	
4	C/O/G	M	1			-			
5	CH/G	M	4			-			
6	CT/CH/SV	M	3						
7	GC/G	W	2			-			
8	CH/GR	M	5	-/=					
9	CSV/T	M	2			-/=			
10	GC/G	W	3			-			
11	GC/G	W	3			-			
12	TC/L	M	3	-/=		-/=			
13	G/GL/G/O	GH	1						-
14	CT/G	C	4			+			
15	C/G/O/O/ O/L/O/G	GH	1			=			

N: number-author reference; Duration: in years from the beginning of the experiment; C: cold climate (average T > 12 ºC); M: mild climate (average T 12-15 ºC); W: warm climate (average T < 15 ºC); Cover type: GH: green house; C: control bared soil; CT: control tillage; CH: control herbicide; SV: spontaneous vegetation; G: grass; GL: grass+legume; L: legume; O: other crop group. Symbols: = denotes does not affect; - donates negative effect compared to the control; + donates positive effect compared to the control.

The evaluation of botrytis incidence on cover-cropped vineyards resulted in no change or in a reduction of this disease in 80 % of the studied cases. In a single experiment in the Tokaj wine region, where *Botrytis cinerea* is used for the production of its famous sweet wines (and thus known as noble rot), the noble-rotted berries in the bunches from plots with a barley cover crop were reported to have increased by 18 % (Judit *et al.*, 2011). In France, vines with cover crop showed a reduced shoot growth, and thus a decrease in botrytis incidence (Valdés-Gómez *et al.*, 2008). The establishment of under-trellis grass cover crops in vineyards in a humid region in Uruguay also resulted in a reduction in both the incidence and the severity of this disease (Coniberti *et al.*, 2018a). The same authors observed that the extend of disease reduction depends more on the presence/absence of the cover crop than on the planting density (0.8 m x 2.8 m *vs.* 1.5 m x 2.8 m) (Coniberti *et al.*, 2018c). Likewise, Jacometti *et al.* (2007) in New Zealand confirmed that the incidence of botrytis was higher in bare soils compared to mulched plots with mowed or tilled cover crops. This is due to the increase in soil moisture and a higher rate of soil biological activity, increased vine debris degradation, reduced *B. cinerea* primary inoculum on the debris and decreased *B. cinerea* severity at flowering and harvest. Between the two studied cover crops, the presence of *B. cinerea* in *Phacelia tanacetifolia* cover cropped vineyards tended to be higher than in *Lolium perenne*, likely due to the reduced competition of soil biota with the fungus. As already mentioned, in some experiments no differences in botrytis incidence associated with the presence of cover crops were found. For instance, in Portugal, no changes were observed between spontaneous cover and till treatments (Cruz *et al.*, 2012). Lastly, in an experiment performed in a greenhouse to compare different cover crops, no differences were found in fungus incidence on the cover crop species in most cases (Krohn and Ferree, 2004).

A study conducted in the South of France by Guilpart *et al.* (2017) concluded that reduced plant growth had a direct effect on reducing grapevine susceptibility to powdery mildew and botrytis, and that it was directly linked to the reduced plant growth by water stress at flowering in the same year. However, grapevine yield (berry number per bunch and bud fertility) was closely linked to water potential at flowering in the previous year. Thus, appropriate management of cover crops could have a positive impact by reducing fungal diseases based on the climatic variability of the growing season.

The impact of cover crops on downy mildew (*P. viticola*) incidence on vines has been reported in a single study, in which no differences were detected between treatments (Vogelweith and Thiéry, 2017). The same study revealed that the presence of cover crops did not affect the incidence of black rot (*G. bidwellii*) either. Moreover, some cover crops have been found to control soil-borne fungal diseases; for instance, *Sinapis alba* biomass residues incorporated into the soil have shown potential for improving control of black foot disease in nursery planting material (Berlanas *et al.*, 2018). Under greenhouse experimental conditions on soils from different types of groundcover management, a reduction in *Ilyonectria liriodendra* was observed with cover crop. It seems that the presence of cover crops alters the root-associated fungal communities of soil biota, thus increasing the amount of plant-protective mycoparasites, which could explain the observed reduction in black foot disease incidence (Vukicevich *et al.* 2018).

1.2.6. Yield

Another aspect that needs to be examined when considering the appropriateness of installing cover crops is their impact on yield. As a general rule, it is assumed that cover crops compete with vines for soil resources (water and nutrients; Gómez, 2017), resulting in a decrease in yield. The analysis of the published papers is mostly in line with this general assumption, but there are some exceptions.

Sixty-eight articles analysed the effect of cover crops on vineyard yield (Table 1-9). In 16 % of these articles, the presence of cover crops was linked to a 20 to 40 % increase in yield compared to control plots; however, this percentage was outnumbered by articles with results showing that cover crops caused no change (28 %) or a decrease in yield (56 %). Among the latter, 26 articles (38 % of total cases) reported a moderate (< 20 %) reduction in yield, whereas in the remaining 12 papers (17 %) yield loss was > 20 % when cover crops were established.

In the studies in which yield increased when using a cover crop, the species used were annual, such as *A. sativa* (Fourie *et al.*, 2007b; Messiga *et al.*, 2016; Steenwerth *et al.*, 2013, 2016; Steinmaus *et al.*, 2008), or legumes like *Trifolium* sp. (Messiga *et al.*, 2016; Ovalle *et al.*, 2010; Susaj *et al.*, 2013) and *Vicia* sp. (Fourie and Freitag, 2010; Messiga *et al.*, 2016; Nboyine *et al.*, 2018; Steenwerth *et al.*, 2013, 2016; Steinmaus *et al.*, 2008). Conversely, permanent cover crops of *F. rubra* (De Pascali *et al.*, 2014; Gontier *et al.*, 2014; Toci *et al.*, 2012) and *F. arundinacea* (Celette, Wery, Chantelot, Celette, & Gary, 2005; Hatch et al., 2011; Mattii et al., 2005; Palliotti et al., 2007) led to a decrease in grape yields. In other cases in which grass- and legume-based cover crops were compared, no differences were observed (Ingels *et al.* 2005; Steinmaus *et al.* 2008; Trigo-Córdoba *et al.* 2015), although there were exceptions (E. Muscas et al., 2017).

The results obtained when comparing spontaneous versus sown cover crops were inconsistent. In some cases, spontaneous cover crops led to a higher grape yield compared to that of sown cover crops (Mercenaroddu *et al.*, 2014; Tomaz *et al.*, 2017; Trigo-Córdoba *et al.*, 2015), while in other cases the result was the opposite (Pérez *et al.*, 2018; Susaj *et al.*, 2013).

Finally, when comparing yields of vines with cover crops on every inter-row or every second inter-row, a greater decrease in yield was observed when the plant cover took up the whole inter-row soil surface (Reeve *et al.*, 2016; Rodriguez-Lovelle *et al.*, 2000a; Rodriguez-Lovelle *et al.*, 2000b). The application of vineyard soil mulch (straw and sawdust, etc), was generally found to lead to increased yields compared to living cover crops or bare soils (Fourie, 2011; Susaj *et al.*, 2013; Varga *et al.*, 2012), but not in all studied cases (Wheeler *et al.*, 2005).

The observed grapevine yield increases took place in areas of warm (average T^{a} above 15 ºC) and mild (average T^{a} between 12 and 15 ºC) climate. Only one experiment was performed in a cold climate (average T^{a} below 12 ºC), in which an increase in yield was found (Hatch *et al.*, 2011). The experiments showing increased yields were located in areas like California (USA) (Steenwerth *et al.*, 2013; Steenwerth *et al.*, 2016; Steinmaus *et al.*, 2008), South Africa (Fourie, 2011; Fourie, Agenbag, et al., 2007b), New Zealand (Nboyine *et al.*, 2018) or Chile (Ovalle *et al.*, 2010). However, the vineyards that suffered a loss of yield were located in the Mediterranean climate area (France, Italy and Spain), with the exception of a single experiment in Virginia (USA) (Hatch *et al.*, 2011). Although it is difficult to determine the reasons for these geographical differences, they may be related to a combination of plant water deficit and temperature: the higher these two variables, the greater the reduction in yield (Figure 1-2).

When climate was analysed alongside irrigation practices, it was shown that, in areas of warm climate, almost all the experiments were under irrigation and positive results were only observed when vineyards were irrigated. In the few experiments performed with irrigation in mild climate areas there was no reduction in grape yield (Figure 1-2).

Table 1-9. Cover crop impact on grape yield compared to tilled and inter-row herbicide-treated control plots.

				Higher, slightly higher				
1	Messiga *et al.*, 2016	++	**5**	Fourie, 2011	=/++	**9**	Marques *et al.*, 2018	-(T)/+++
2	Fourie *et al.*, 2007b*	++	**6**	Steenwerth *et al.*, 2013* **	=/+	**10**	Ovalle *et al.*, 2010*	-/+
3	Nboyine *et al.*, 2018	+	**7**	Steenwerth *et al.*, 2016*	=/+	**11**	Ripoche *et al.*, 2011*	-/+
4	Susaj *et al.*, 2013* **	+	**8**	Steinmaus *et al.*, 2008	=/+			
				No trend				
12	Bettoni *et al.*, 2016	=	**19**	Ingels *et al.*, 2005	=	**26**	Rodriguez-Lovelle *et al.*, 2000b*	=
13	Coniberti *et al.*, 2018a	=	**20**	Lopes *et al.*, 2008	=	**27**	Smith *et al.*, 2008	=
14	Costello, 2010a*	=	**21**	Mercenaro, 2014	=	**28**	Sweet & Schreiner, 2010	=
15	DeVetter *et al.*, 2015	=	**22**	Monteiro & Lopes, 2007	=	**29**	Tourte *et al.*, 2008	=
16	Donkó *et al.*, 2017	=	**23**	Pérez-Álvarez *et al.*, 2013	=	**30**	Wolff *et al.*, 2018	=
17	Giese *et al.*, 2015	=	**24**	Pérez-Álvarez *et al.*, 2015a	=			
18	Giese *et al.*, 2016	=	**25**	Pérez-Álvarez *et al.*, 2015b	=			
				Slightly lower				
31	Jordan *et al.*, 2016	=/-(T)	**40**	Tomaz *et al.*, 2015	-(T)/-	**49**	Klodd *et al.*, 2016	-
32	Ruiz-Colmenero *et al.*, 2011	=/-	**41**	Coniberti *et al.*, 2017	-(T)/- -	**50**	Linares Torres *et al.*, 2018*	-
33	Muscas *et al*, 2017*	=/- -	**42**	Coniberti *et al.*, 2018c	-(T)/- -	**51**	Lopes *et al.*, 2011	-
34	Reeve *et al.*, 2016	=/- -	**43**	Karl *et al.*, 2016a**	-(T)/- -	**52**	Pérez-Bermúdez *et al.*, 2016	-
35	Bahar *et al.*, 2010	-(T)	**44**	Pérez *et al.*, 2018*	-(T)/- -	**53**	Rodriguez-Lovelle *et al.*, 2000a*	-
36	Marques *et al.*, 2010	-(T)	**45**	Pou *et al.*, 2011	-(T)/- -	**54**	Vrsic *et al.*, 2011	-
37	Trigo-Córdoba *et al.*, 2015*	-(T)	**46**	Varga *et al.*, 2012**	-/=	**55**	Nicolosi *et al.*, 2016	-/- -
38	Wheeler *et al.*, 2005	-(T)	**47**	Hickey *et al.*, 2016	-	**56**	Coletta *et al.*, 2013	- -/ -
39	Reynolds *et al.*, 2006	-(T)/-	**48**	Judit *et al.*, 2011	-			
				Lower				
57	Cruz *et al.*, 2012	=/- - -	**61**	Delpuech & Metay, 2018*	- -	**65**	Palliotti *et al.*, 2007	- -/- - -
58	Guilpart *et al.*, 2017	-(T)/- - -	**62**	Kazakou *et al.*, 2016*	- -	**66**	Silvestre *et al.*, 2012	- -/- - -
59	Celette *et al.*,2005	- -	**63**	Mattii *et al.*, 2005*	- -	**67**	Gontier *et al.*, 2014	- - -
60	De Pascali *et al.*, 2014	- -	**64**	Toci *et al.*, 2012	- -	**68**	Hatch *et al.*, 2011	- - -

= denotes does not affect, no clear trend; -(T)/+(T) denotes reduction trend/general increase ; -/+ denotes difference in reduction/increase lower than 20 % ; --/++ denotes difference in reduction/increase between 20 and 40 %; ---/+++ denotes difference in reduction/increase higher than 40 % ; * denotes differences among treatments in one or more years; ** denotes differences among controls in one or more years.

Unfertilised vineyards never showed increased yields. However, vineyard fertilisation management showed higher or similar grapevine yields in cover cropped vineyards (Table 1-10).

Table 1-10. Comparison of cover crop impact on grape yield when the vineyard was fertilised or not.

	Fertilised	**Not fertilised**
Positive, slightly positive	31% (6)	0%
No trend	42% (8)	36%(5)
Slightly negative	21% (4)	36%(5)
Negative	5% (1)	29%(4)

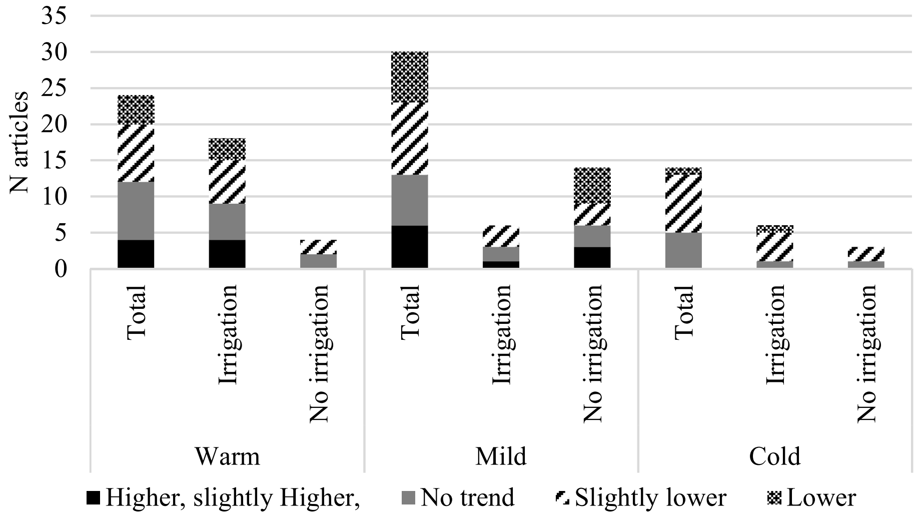

Figure 1-2. Impact of cover crops on grapevine yield according to climate conditions and irrigation practices.

Some yield trends were also observed regarding rootstock. For instance, the use of SO4 mainly resulted in a relevant yield decrease (Celette *et al.*, 2005; Cruz *et al.*, 2012; De Pascali *et al.*, 2014; Delpuech and Metay, 2018; Palliotti *et al.*, 2007; Toci *et al.*, 2012), while increases occurred in some of the vineyards with 110R and 99R (Fourie, 2011; Marques *et al.*, 2018; Steenwerth *et al.*, 2013, 2016). When we grouped data according to rootstock tolerance to drought (Figure 1-3), it was possible to confirm that in the presence of cover crops yield only increased with drought tolerant rootstocks (Fercal, 110R, 140Ru, 99R and 779P). With the remaining rootstocks, (of medium resistance, such as 3309C, SO4, 1103P, 41B; or drought-sensitive, such as 101-14, 420A, 5BB, Teleki 5C), yield was observed to decrease in the presence of cover crops (Figure 1-3).

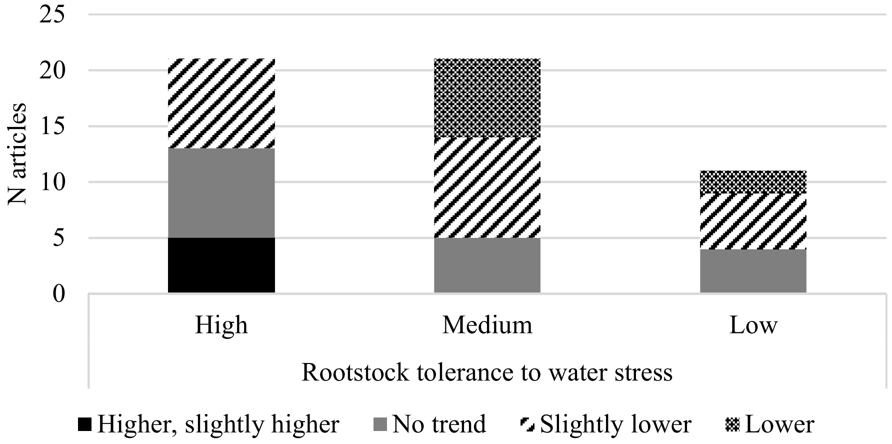

Figure 1-3. Number of reviewed papers on the impact of cover crops on grapevine yield grouped according to the rootstock resistance to water stress.

Classification of drought tolerance was performed according to the characteristics of the vine rootstocks published in Vivai Cooperativa Rauscedo (2013), grouped as high (Fercal, 110R, 140Ru, 99R, 779P), medium (3309C, SO4, 1103P, 41B) and low tolerance (101-14, 420A, 5BB, Teleki 5C).

1.2.7. Grape composition

When examining the potential impact of cover crops on grape composition, it is important to take into account that it is intrinsically related to vigour, yield and canopy photosynthetic activity, which are all modified by cover crops. In addition, the presence of cover crops can affect berry size (reduced in 35 % of articles), which may also be associated with changes in berry composition.

Details of grape composition are provided in the following sub-sections. In general terms, total soluble solids (TSS) remained unaffected in 68 % of cases (30 out of 44 papers), whereas in 8 cases they showed an increase, and only 4 reported a decrease in TSS content associated with the introduction of a cover crop (Table 1-11). When an increase in TSS was observed (6 cases), it was mainly associated with a decrease in yield and berry size (4 cases), although in two of the studies no changes in yield or berry size were reported. Conversely, when a decrease in TSS was observed, it was associated with a reduction in yield, indicating that cover crops were the cause of strong weakening of vineyard. Concerning acidity, 90 % of studied articles (29) reported no change in the pH, whereas in 4 of them a decrease was observed, and only one showed a pH increase associated with cover crop installation. Similarly, cover crops did not alter titratable acidity (TA) in the majority of the studies (23 out of 32, 72 %), a certain decrease was observed in 8, and only 3 reported increased TA.

As regards phenolics, anthocyanins were analysed in 8 studies, out of which 3 showed an increase in anthocyanin content and 5 showed no effect. Meanwhile, for total phenolics the effects were more limited: in 4 out of 7 studies the content remained unchanged, while it increased in 2 and decreased in 1 (Table 1-11). Lastly, in terms of competition for nutrients, yeast assimilable nitrogen (YAN) was one of the grape composition parameters to be mostly affected by the presence of a cover crop, as it only remained unchanged in 39 % of the experiments and decreased in half of them; only 2 out of 18 studies considered it to have increased. The general implications of cover crops for nitrogen nutrition were reviewed in our companion article (Abad *et al.* 2021a).

Table 1-11. Grape juice quality components.

N	Variety	Climate	Irrigation	Fertilization	Duration	Yield	Berry weight	TSS (ºBrix)	pH	TA	YAN	Total anthocyanins	Total polyphenols
1	Cabernet Sauvignon	W	Yes	Yes	2		=/-	=	=	=		=/+	
2	Tempranillo	W	Yes	No	2	-	-	=/+	=	-		=	=
3	Sauvignon blanc	W	Yes	Yes	10	+/=	=	-/=			+/=		
4	Cabernet Sauvignon	W	No	Yes	3	=		=	=	-		+	+
5	Manto Negro	W	Yes		3	-		=	=			=	=
6	Pedro Ximénez	W	No		3	-	=	+	=	=	=		
7	Canaiolo nero/Trebbiano giallo	W			2			=	=	=			=/+
8	Merlot	W	Yes	Yes/No	2/3	=		=	=	=			
9	Sangiovese	W		No	2	-	-	=	+	+			
10	Carignano	W	Yes		3	-	-	=/+				+/-	+/-
11	Negroamaro	W	Yes		1	-	=	=	=	=			
12	Cabernet Sauvignon	W	Yes		1			-/=	-/=	+/=			-
13	Tempranillo	W	Yes		2	=/-						=(w)	
14	Negroamaro	W	Yes		1	-	=	=	=	=		+(w)	=(w)
15	Cabernet Sauvignon	W	Yes	Yes	2						=		
16	Mazuelo	W	Yes	Yes	5	=	=	=	=	=		=/+	
17	Chardonnay	W	Yes		5	=	=	=	=	=/-			
18	Chardonnay	W	Yes	Yes	12	=/+	-/=	=	=	=	=/-/+		
19	Pinnot noir	C	No		2	=		=	=	=	=		
20	Pinnot noir	C			4			=					
21	Furmint/Hárslevelü	C			2	=		=		=			
22	Cabernet Sauvignon	C		Yes	7	=	=	=	=	=	-		
23	Mencía	C			2							+/=(w)	=(w)
24	Gewurztraminer	C	Yes		6	-	=	-	=/-	=/+			
25	Maréchal Foch	C	Yes	Yes	7	=	=	=	=	+			
26	Pinot noir	C	No	Yes	3	=/-		=	=	-	-		
27	Shiraz	M	No	No	2						-		

N	Variety	Climate	Irrigation	Fertilization	Duration	Yield	Berry weight	TSS (ºBrix)	pH	TA	YAN	Total anthocyanins	Total polyphenols
28	Furmint	M			3	=		=		=			
29	Aranel	M	No	No	5/7	-	=	-/+			-		
30	Sauvignon blanc	M	Yes		4	=	=	-		-			
31	Tempranillo	M	Yes	No	3	=	=	=	=	=			=
32	Sangiovese	M			3		-	+	=	-			
33	Kallmet	M	No	Yes/No	3	-	=	=		=			
34	Merlot	M	Yes	Yes	3	=	=	=	=	=			
35	Leon Millot	M		Yes	2	+		=/+					
36	Merlot	M			2	-	-	+	=	-			
37	Mencía	M	No		3	=/-	=/-	=	=	=		=(w)	
38	Grechetto	M	No		6						=/-		
39	Merlot, Cabernet franc, Sauvignon branc	M		No	2	-					-		
40	Tempranillo	M	No		1	=	=	=	=	=			
41	Fernão Pires	M	No		2	-	-	-	=	=			
42	Tempranillo	M	No	No	4	=		=	=	=			
43	Cabernet Sauvignon	M			1	=		=		=		=	
44	Cabermet Sauvignon	M			2	=	=	+	=	-	-		
45	Tempranillo	M		No	1	=						+	
46	Tempranillo	M	No	No	3	=	=				=/-		

1: Lee and Steenwerth (2013); 2: Lopes *et al*. (2011); 3: Fourie, *et al*. (2007b); 4: Lopes *et al*. (2008); 5: Pou *et al*. (2011); 6: Pérez *et al*. (2018); 7: Muganu *et al*. (2013); 8: Ingels *et al*. (2005); 9: Mattii *et al*. (2005); 10: Muscas *et al*. (2017); 11: Toci *et al*. (2012); 12: Nazrala (2008); 13: Silvestre *et al*. (2012); 14: Coletta *et al*. (2013); 15: Lee and Steenwerth, (2011); 16: Mercenaro *et al*. (2014);17: Smith *et al*. (2008); 18: Fourie (2011); 19: Sweet and Schreiner (2010); 20: Gouthu *et al*. (2012); 21: Donkó *et al*. (2017); 22: Giese *et al*. (2015); 23: Bouzas-Cid *et al*. (2016); 24: Reynolds *et al*. (2006); 25: DeVettere *et al.*, (2015); 26: Reeve *et al*. (2016); 27: Kazakou *et al*. (2016); 28: Varga *et al*. (2012); 29: Ripoche *et al*. (2011); 30: Caspari *et al*. (1997); 31: Pérez-Bermúdez *et al*. (2016); 32:Ferrini *et al.*, (1996); 33: Susaj *et al*. (2013); 34: Steenwerth *et al*. (2016); 35: Messiga *et al*. (2016); 36: Rodriguez-Lovelle *et al*. (2000a); 37: Trigo-Córdoba *et al*. (2015); 38: Palliotti *et al*. (2007); 39: Rodriguez-Lovelle *et al*. (2000b); 40: Marques *et al.*, (2010); 41: Cruz *et al*. (2012); 42: Pérez-Álvarez *et al*. (2015a); 43: Bahar and Semih Yaşain (2010); 44: Wheeler *et al*. (2005); 45: Pérez-Álvarez *et al*., (2013); 46: Pérez-Álvarez *et al*., (2015b)

Duration: in years since the beginning of the experiment; C: cold climate (average T > 12 ºC); M: mild climate (average T 12-15 ºC); W: warm climate (average T < 15 ºC); TSS: total soluble solids, TA: titrable acidity; YAN: Yeast assimilable nitrogen; (w): measured in wine.

1.2.7.1. Sugar content and acidity

As mentioned above, the basic grape juice parameters TSS, pH and TA did not show any variation in most of the reviewed studies. Such was the case in several Hungarian wine regions in studies which used a spontaneous cover crop and organic mulching (Varga *et al*., 2012), or spontaneous flowering legumes and grass cover crops in Furmint vineyards (Donkó *et al*., 2017). Similarly, no differences in grape juice parameters were found in Pinot noir vineyards when spontaneous legume or grass cover crops (monocultures or mixtures) were compared in Oregon (USA) (Sweet and Schreiner, 2010). Another experiment performed in the same region with the same variety showed no changes in these parameters with a 3-year *F. rubra* cover crop (Gouthu, Skinkis, Morre, Maier, & Deluc, 2012). The same result was observed in Cabernet Sauvignon in North Carolina (USA) where grassy cover crops were established (Giese *et al*., 2015). In Iowa (USA), where the annual precipitation can reach 700 mm, no differences in TSS or pH values were detected with a *F. rubra* cover crop, although TA showed an upward trend with cv. Maréchal Foch (DeVetter *et al*., 2015). In a trial conducted in a Merlot vineyard in California, no differences in must parameters were found when green manure, annual clover and perennial grass cover crops were used (Ingels *et al*., 2005). For the same variety and region, similar results were reported with an oat (*A. sativa*) cover crop, or a legume/oat cover crop mixture (Steenwerth *et al*., 2016). The TSS content in Cabernet Sauvignon grapes was unaffected by the presence of a native perennial grass cover crop, independently of additional irrigation, although higher irrigation levels appeared to increase yields. Native grasses led to increased tartaric acid contents, while bare soils showed higher levels of malic acid (Daane *et al*., 2018). *S. cereale* cover crops did not alter TSS and pH values in Chardonnay vineyards in California, and neither did *Triticosecale* plant covers. However, the use of *Triticosecale* resulted in grape juices with lower TA (Smith et al., 2008). The presence of a spontaneous cover crop in a Cabernet Sauvignon vineyard in Turkey did not alter TSS, pH or TA parameters, although veraison onset was brought forward by 4 days (Bahar and Semih Yaşain, 2010). Cabernet Sauvignon vineyards in Brasil, managed with *Raphanus raphanistrum*, *Avena strigosa*, *S. cereale*, *L. perenne* and two clover species showed no differences in TSS and pH values (Bettoni *et al*., 2016). In a trial conducted in a Manto Negro vineyard in Majorca (Spain), no significant differences were found in must parameters when spontaneous and mixtures of grass and legume cover crops were established compared to tilled control plots (Pou *et al*., 2011). Similar results were found in Valencia, in Tempranillo and Bobal vineyards with legume cover crops that were tilled at flowering and incorporated into the soil (Pérez-Bermúdez *et al*., 2016). Similarly, a barley cover crop did not alter TSS, pH, TA, tartaric and malic acid content in Tempranillo vineyards in La Rioja (Spain) and neither did *T. resupinatu* plant covers (Pérez-Álvarez *et al*., 2015a).

In contrast to the aforementioned studies, others have reported changes in must composition. For example, spontaneous and *A. sativa* and *Vicia* sp. mixtures led to increased TSS content in Pedro Ximenez variety in Andalucía (Spain) after 3 years, although the remaining grape composition parameters were unaltered (Pérez *et al*., 2018). Ripoche *et al.* (2011) reported a decrease in TSS content in the first year in cv. Aranel vineyards in Montpellier managed with a permanent *F. arundinacea* cover crop. Another experiment with cv. Tempranillo in Alentejo (Portugal) showed that the presence of a spontaneous cover crop increased TSS in one of the two study years, but decreased TA in both years (Lopes *et al*., 2011). In a Cabernet Sauvignon vineyard located in central Portugal, a reduction in TA was observed in the third season in the presence of a spontaneous cover crop and a grass-legume mixture - probably due to a significant reduction in vegetative growth - whereas no significant differences were found for TSS and pH

(Lopes, Monteiro, Machado, Fernandes, & Araújo, 2008). A 10-year spontaneous cover crop study in Fernão Pires vineyards in Portugal showed a reduced TSS content, as pH and TA remained unaltered (Cruz *et al.*, 2012). The presence of natural permanent plant cover in central Italy resulted in an increased TSS content in Canaiolo nero and Trebbiano giallo grape cultivars during ripening; however, TSS was the same as that for control bare soils at the end of the cycle, whereas pH and TA parameters remained unaffected (Muganu *et al.*, 2013). A *F. arundinacea* (70 %) and *L. perenne* (30 %) cover crop resulted in a decrease in TSS content in Grechetto grapevines (Palliotti *et al.*, 2007). The use of natural grass, ground cover with *T. subterraneum* or with *F. arundinacea* resulted in higher TSS content while pH and TA tended to decrease in Sangiovese vineyards. Moreover, tall fescue gave an earlier harvest date due to the increased TSS (Ferrini, Mattii, & Storchi, 1996). However, the aforementioned cover crops also in Italy showed an increase in pH and TA parameters while TSS content remained unaltered in the same grape variety (Mattii *et al.*, 2005). Reynolds *et al.* (2006) observed a delayed grape ripening in Gewürztraminer vineyards in Canada due to the presence of *Agropyum cristatum* and *F. ovina* mixture cover crop, accompanied by a decrease in TSS, while TA was unaffected. Furthermore, a trial conducted in Leon Millot vineyards in Canada managed using different cover crop mixtures of legume and grass did not show significant differences in TSS, or were slightly higher in some cases, but those differences were still smaller in the second growing season (Messiga *et al.*, 2016). In a vineyard planted with Merlot variety in France, bloom and veraison occurred earlier in *F. arundinacea* cover-cropped vines, as berries showed no change in pH and had lower TA and higher TSS content compared to untilled plots (Rodriguez-Lovelle *et al.*, 2000a). Higher TSS content and lower TA were detected in Sauvignon Blanc grape juices from *L. perenne* and *Cichorium intybus* cover cropped vineyards in New Zealand (Caspari, Neal, & Naylor, 1997).

More examples of changes in those parameters have been reported in Sauvignon Blanc vineyards in South Africa, where TSS content increased and acidity decreased in the presence of a cover crop; this effect was more intense when the cover crop was maintained for a longer period of time during the season (Fourie *et al.*, 2007b). In Uruguay, a full *F. rubra* cover in a Tannat vineyard also resulted in higher TSS content (Coniberti *et al.*, 2018b).

1.2.7.2. Yeast assimilable nitrogen (YAN)

The impact of cover crops on nitrogen nutrition has already been reviewed in Abad *et. al.* (2021a). However due to the impact of YAN on must fermentation and wine characteristics (Bell and Henschke, 2005), we present here the results reported in the articles analysed in the systematic review. As a general rule, legume cover crops usually increase soil N content (Fourie *et al.*, 2007c; Messiga *et al.*, 2015; Ovalle *et al.*, 2007; Pérez-Álvarez *et al.*, 2015b; Sulas *et al.*, 2017), but this increment does not always result in a change in N content in grape juice (Sulas *et al.*, 2017). For instance, a legume cover crop did not cause any increase in YAN in Shiraz grapes, probably as a consequence of the water stress in the soil created by the cover crop that limited N fixation and was managed without irrigation (Kazakou *et al.*, 2016). Conversely, Fourie *et al.* (2007b) observed an increase in N in grape juice of Sauvignon Blanc with the use of *Ornithopus sativus* and *Vicia dasycarpa* cover crops, but not in the first years of the study. In this same study, the chemical removal of cover crop before budbreak resulted in a clear increase in must N content.

The general trend for grassy cover crops is the opposite of legume cover regarding nitrogen, as the presence of the crop results in competition for this. In this regard, YAN was observed to decrease in the presence of the following types of cover crops: permanent grass in Cabernet Sauvignon vineyards in North Carolina (Giese *et al.*, 2015), a mixture of *F. arundinacea* (70 %) and *L. perenne* (30 %) in Grechetto vineyards located in central Italy (Palliotti *et al.*, 2007), *F. arundinacea* in Merlot vineyards in Bordeaux (Rodriguez-Lovelle *et al.*, 2000b), and *Chicorium intybus* var. *sativum* in Cabernet Sauvignon vineyards in New Zealand (Wheeler *et al.*, 2005). These differences between YAN in cover vineyard and nake soil sometimes appear during the first years of the cover crop, as reported for *F. arundinacea* in Montpellier (Ripoche *et al.* 2011), and then these get smaller, or can remain unnoticed until the cover crop has been established for several years, as reported with *H. vulgare* in Tempranillo (Pérez-Álvarez *et al.*, 2015a).

In California, YAN in Cabernet Sauvignon berries was unaffected by cover crop management (a spontaneous cover crop followed by tillage, or a barley cover mowed and then tilled) (Lee and Steenwerth, 2011). However, in Pinot noir in Oregon, differences in YAN were observed depending on whether the cover crop comprised legume, winter annuals or permanent grass and legume cover crops, although the effect of each cover crop also differed depending on the year (Sweet and Schreiner, 2010). Gouthu *et al.* (2012), focused their study on the amino acid content of Pinot noir berries in the Finger Lakes region, reporting that the ratio of YAN increased with cover cropping (*F. rubra*); however, the free amino acid content was 40-45 % lower in berries from cover crop treatments compared to that of berries from control plots. In the cold and humid climate of the Finger Lakes region, where excess levels of N can be a problem, this implies that a competitive cover crop can be an appropriate means of alternative managing vineyard soils.

1.2.7.3. Phenolic compound content

There is also a diversity of results in the influence of the presence of a cover crop on phenolic composition of grapes, although the general trend is for observations of an increase in their content associated with yield reductions. For example, an *H. vulgare* mown cover crop resulted in higher anthocyanin content than tillage management with Cabernet Sauvignon in California (Lee and Steenwerth, 2013); this could be linked to decreased berry size. In several experiments conducted in Portugal, phenolics generally increased for at least one of the phenolic compounds measured for Cabernet Sauvignon (Lopes *et al.*, 2008) and Tempranillo (Silvestre *et al.*, 2012; Tomaz *et al.*, 2017), but no changes were reported in other experiments with Tempranillo (Lopes *et al.*, 2011).

Research performed in a cv. Carignano vineyard in the northwest of Italy revealed that only the presence of a *Dactilys glomerate* cover crop increased anthocyanins, but this was not the case for different permanent grass-legume mixtures (Mercenaro *et al.*, 2014). In central Italy, the presence of a spontaneous cover crop in cv. Canailo nero, resulted in increased total polyphenol content, while identical management did not obtain the same result in cv. Trebbiano Giallo (Muganu *et al.*, 2013). A *F. arundinacea* (70 %) and *L. perenne* (30 %) cover crop caused an increase in polyphenols and colour in the Grechetto white wine grape variety in central Italy (Palliotti *et al.*, 2007), whereas cover cropping with a mix made by 20 % *F. rubra*, 20 % *F. ovina* and 60 % *T. subterraneum* decreased the concentration of flavonoids and anthocyanins in southern Italy. In Sardinia the concentration of total polyphenols and anthocyanins in cv. Carginano increased with *D. glomerata* (80 %) and *Lolium rigidum* (20 %), while the spontaneous (*Bromus hordeaceus*, *Avena sterilis* and *Vulpia myuros*) and legume mixture (50 % *Medicago polymorpha* and 50 % *Trifolium yanninicum*) cover crops showed reduced values compared to tillage (E. Muscas et al., 2017).

In Spain, native vegetation and *L. perenne* cover crops increased anthocyanin concentrations to a greater extent than *T. subterraneum* for cv. Mencía in Galicia (Bouzas-Cid *et al.*, 2016; Trigo-Córdoba *et al.*, 2015). In a trial conducted in La Rioja, an *H. vulgare* cover crop resulted in higher levels of polyphenols and colour intensity in Tempranillo, whereas this effect was not observed with a *T. resupinatum* cover crop (Pérez-Álvarez *et al.*, 2015a). In central Spain, no differences were observed in Tempranillo when tillage and *Brachypodium distachyon* and *S. cereale* cover crops were compared (Marques *et al.*, 2010). Similarly, in an experiment carried out in Majorca, the use of spontaneous or grass and legume mixture cover crops did not alter the concentration of total phenolics (Pou *et al.*, 2011).

There are also some reports of research carried out in other countries with varying results depending on the experimental conditions. For instance, a spontaneous cover crop led to reduced tannin and flavonol content, while increased amounts of anthocyanins were observed in Cabernet Sauvignon vineyards in Mendoza, Argentina (Nazrala, 2008). In New Zealand, a *C. intibys* var. *sativum* cover crop resulted in an increased anthocyanin content of Cabernet Sauvignon berries (Wheeler *et al.*, 2005). Phenolic compounds were also increased by the presence of a natural grass cover in Cabernet Sauvignon musts from Turkish vineyards (Bahar and Semih Yaşain, 2010), although anthocyanin content decreased. Cover crops in Cabernet Sauvignon vineyards in China increased total phenols, the highest increase being observed for *F. arundinacea*, followed by *T. repens* and *M. sativa*, while soil tillage providing the lowest values (Xi *et al.*, 2010).

1.2.7.4. Aromas

Comparatively little research has evaluated the impacts of using a cover crop on aromatic compounds. As previously highlighted for the other effects on grape composition, it is important to take into account the fact that the reported effects can vary greatly depending on the research conditions, and that they can frequently be an indirect consequence of changes in yield and vegetative growth.

In a commercial Riesling vineyard in the Finger Lakes region, the use of under-vine cover crops of resident vegetation, buckwheat or *L. multiflorum* resulted in different perceived aroma in wines compared to when herbicide was used, despite vegetative growth or yield having been unaffected (Jordan *et al.*, 2016). A cover crop treatment consisting in a full cover of the vineyard soil with *F. rubra* increased fruit aroma and overall aroma intensity of cv. Tannat in Uruguay (Coniberti *et al.*, 2018a). Conversely, in Canada, the use of a mixture of *A. cristatum* and *F. ovina* resulted in a Gewürztraminer wine with lower quantities of free volatile terpenes, but higher concentrations of potentially volatile terpenes (Reynolds *et al.*, 2006). Meanwhile, no effect was observed in Sauvignon Blanc vineyards in South Africa (Fourie *et al.*, 2007b). In New Zealand, a *C. intibys* cover crop resulted in an increase in ripe fruit aroma (Wheeler *et al.*, 2005), and a higher glycerol content and lower 2,3-butanediol content were reported in wines produced from vines subjected to cover-cropped treatments in Italy (Coletta *et al.*, 2013; De Pascali *et al.*, 2014; Toci *et al.*, 2012). Furthermore, in the Shaanxi Province, north west China, Xi *et al.*, (2011) detected higher levels of volatile components in Cabernet Sauvignon when using cover crops, especially those comprising *M. sativa* and *F. arundinacea*.

1.2.7.5. Yeast populations

It is also possible for cover crops to have an indirect influence on wild yeast populations, which can in turn affect wine characteristics when fermentation is conducted without the inoculation of commercial yeasts. In this regard, Cordero-Bueso *et al.* (2011b) observed changes in the *Saccharomyces* populations in spontaneous fermentations during three seasons in Syrah, depended on whether the vines were grown in the presence of a cover crop or of bare soil. The authors hypothesised that the presence of a cover crop in vineyards reduces *Saccharomyces* populations due to a competitive effect on fungi and grape yeasts populations, reducing yeast quantity and biodiversity in the vineyard, mainly when fermentative strains are used.

1.2.8. Conclusions

The present systematic bibliographic review shows that cover crops tend to result in a reduced vineyard vegetative growth, which can commonly be associated with a reduced incidence of the main fungal diseases. Cover crops generally also reduce the incidence of pests, especially *Cicadellidae* and mite species, as their presence results in an increase in natural enemies.

In general, cover crops result in an increase in water deficit, although this effect is highly variable as it depends on soil and climate characteristics, and on the period of the year in which the covers are active. The increased competition for water that occurs when cover crops are used can be, to some extent, modulated by the fact that cover crops increase water infiltration into the soil, may reduce soil evaporation or can indirectly lead to lower water needs through leaf area and yield reduction.

The impact of cover crop on vineyard yield is relatively variable. In warmer climates, the observed yield reduction is greater, though irrigation practices tend to compensate for these losses. Apart of soil and climate characteristics, rootstock characteristics also appear to influence the effect of cover crops on grape yield: yield was reported to decrease less with increased rootstock tolerance to drought. Berry size is less affected by the presence of cover crops. Similarly, must quality parameters, like TSS or TA tend to stabilise change, whereas anthocyanins and polyphenols are usually the compounds most favoured by using cover crops. Type of cover crop determines the effect on YAN content, which can decrease, except when the cover crop is comprised of legumes, which usually cause an increase that is generally observed once the cover crop has been established for several seasons.

As a final remark, we consider it worth the effort to carry out the intensive work required to perform a systematic review, as it is the best way to minimise the omission of relevant research and biases in the article selection process. Our two companion papers are an example of how such review methodology can be successfully applied to broad agronomic topics on the variety of impacts that can occur when implementing a growing practice.

1.3. Las cubiertas vegetales bajo la línea de cultivo

Son pocos los trabajos que se han desarrollado referentes al empleo de cubiertas vegetales bajo la línea de cultivo en viñedo. En todos los casos el manejo de la calle se realiza con una cubierta vegetal, estudiándose distintos manejos bajo la línea de cultivo. Mayoritariamente estos trabajos se localizan en zonas de climas húmedos y/o frescos, y únicamente tres se desarrollaron fuera de estas condiciones, uno en Cerdeña y otros dos en Australia. Salvo los trabajos realizados en Australia, en todos los casos el objetivo de la utilización de la cubierta en la línea de cultivo es reducir el crecimiento vegetativo del viñedo, equilibrándolo así con la producción. En el caso de los trabajos realizados en Australia, su objetivo no es reducir el vigor, sino encontrar alternativas sostenibles y económicamente viables para el control de la vegetación adventicia.

A continuación, se presenta una breve descripción de estos trabajos agrupados según las características climáticas de la región en la que se han desarrollado.

1.3.1. Regiones frías y húmedas

El objetivo de todos los trabajos realizados en estas zonas era conseguir una disminución del crecimiento vegetativo del viñedo que permita equilibrarlo con la producción. Los trabajos se localizan en Carolina del Norte, Virginia, Pensilvania y en la región de Nueva York. Se trata de zonas con elevadas precipitaciones, en las que en ocasiones los inviernos fríos condicionan el manejo del viñedo, siendo habitual en algunas de estas zonas la protección del punto de injerto mediante aportes de tierra en dicho lugar y posterior retirada una vez pasado el periodo de bajas temperaturas. Esta circunstancia de manejo condiciona que en algunos de los estudios se deba recurrir al empleo de cubiertas anuales en lugar de cubiertas perennes.

En el estado de Nueva York, Jordan *et al.* (2016) evaluaron durante 3 campañas una cubierta espontánea (*Portulaca oleracea*, *Polygunum pensylvanicum*, *Digitaria ischaemum*, *D. sanguinalis*, *Poa annua*, *Setaria pumilia*, *Persicaria maculosa* o *Chenopodium álbum*), una cubierta de trigo sarraceno (*Fagopyrum esculentim*) y una cubierta de raygrass (*Lolium multiflorum*), frente a un manejo con herbicida. La calle estaba ocupada por una cubierta espontánea. La variedad de uva cultivada era Riesling, clon 198, sobre portainjerto SO4, de 15 años de edad, a marco de 2,7 x 1,8 m, sin riego y conducido en sistema Scott Henry. La cubierta de trigo sarraceno y raygrass tuvo una buena instalación, aunque hubo presencia de otras especies. El empleo de cubiertas bajo la línea no alteró el crecimiento vegetativo. Ni los parámetros productivos, ni los de mosto (sólidos solubles totales, pH, acidez total y nitrógeno fácilmente asimilable-NFA) se vieron alterados. Sí existió una percepción diferente de los aromas de los vinos, aunque no quedó clara la preferencia a favor de las cubiertas o del herbicida. Las distintas cubiertas no variaron el contenido de M.O. del suelo, seguramente debido al alto nivel de partida (5,91-6,85 %). Tampoco se vio afectada la estabilidad de agregados en suelo. A nivel nutricional de la planta se observó una reducción del contenido de N en peciolo en el tercer año de estudio. El estado hídrico, estimado mediante medidas de potencial hídrico, no se vio alterado entre los distintos manejos. La falta de competencia por recursos hídricos y nutricionales pueden ser la causa de que estas cubiertas no hayan tenido efecto deseado de disminución del crecimiento vegetativo. En lo que se refiere a las características biológicas del suelo, Chou *et al.* (2018) en el mismo ensayo observaron un incrementa de la comunidad fúngica y bacteriana del suelo cuando se empleaba una cubierta espontánea frente al uso de herbicida, siendo más importante el aumento conforme más años del uso de la cubierta había pasado.

Centinari *et al.* (2016b) estudiaron durante tres campañas una cubierta de raygrass (*Lolium multiflorum*), una cubierta de trigo sarraceno (*Fagopyrum esculentum*) y una cubierta de nabo (*Brassica rapa* var. *rapa*) frente al uso de laboreo bajo la línea de cultivo. La calle se manejaba con cubierta espontánea. Se trataba de un viñedo de Cabernet Franc, clon 214, sobre 3309C, de 6 años, a marco de 1,5 x 3 m. Únicamente la cubierta de raygrass redujo en uno de los años el crecimiento de los pámpanos, mientras que los parámetros productivos y de composición de los mostos no se vieron alterados con el empleo de ninguna de las cubiertas. El estado hídrico, según medidas de potencial hídrico, tampoco se vio afectado. A nivel nutricional, según el análisis peciolar, solo la cubierta de raygrass redujo el K en una de las campañas. Las raíces finas del viñedo se ubicaron a mayor profundidad debido a la presencia de las raíces de raygrass y trigo sarraceno en la capa superficial. Esta redistribución en profundidad incrementó la vida media de estas raíces respecto a las del viñedo laboreado.

En la misma región, Karl *et al.* (2016a, 2016b) estudiaron durante tres años una cubierta espontánea y una cubierta de *Trifolium repens* frente a un manejo con laboreo y a un manejo con herbicida. La calle estaba sembrada con una mezcla de *Festuca duriuscula* y *F. arundinacea.* El viñedo era la variedad Cabernet Franc, clon 1, sobre portainjerto 3309C, de 4 años de edad, a marco de 1.8 x 2.8 m. La cubierta de trébol presentó problemas de instalación al tener que realizar siembras anuales, en lugar de dejarlo trabajar como perenne. Las cubiertas redujeron el crecimiento y la producción respecto al herbicida, pero no tuvieron diferencias con el laboreo. En la composición del mosto ambas cubiertas redujeron el pH, y la cubierta espontánea redujo además el NFA. El estado hídrico, según indicaron las medidas de potencial hídrico no presentó variaciones entre los diferentes manejos. Solo de manera puntual, el potencial a mediodía en el mes de agosto fue más bajo en presencia de la cubierta espontánea. No se apreciaron efectos de las cubiertas ni en los análisis químicos de los vinos, ni en los análisis sensoriales. Los efectos sobre el suelo tras tres campañas de empleo de las cubiertas fueron el incremento de la porosidad y de la respiración. Ni la infiltración, ni la estabilidad de agregados mejoraron significativamente, aunque sí existió una tendencia al incremento de la estabilidad. El carbono orgánico y el nitrógeno total disueltos en el agua de escorrentía disminuyeron con la presencia de las cubiertas vegetales.

También en la región de Nueva York, Chou and Vanden Heuvel (2018) estudiaron durante 3 campañas una cubierta espontánea, una de *Cichorium intybus*, una de *Medicago sativa*, una de *Raphanus sativus* y una de *Festuca arundinacea* frente a un manejo con herbicida. La calle estaba sembrada con festuca, trébol blanco y otras hierbas espontáneas. El estudio se realizó sobre un Cabernet Franc, clon 1, sobre portainjerto 3309C, de 15 años, a marco de 2,13 x 2,74 m. El peso de madera de poda disminuyó para *C. intybus* (65%) > *R. sativus* (54%) > *F. arundinacea* (50%) respecto al herbicida, pero únicamente en la segunda campaña. La cubierta espontánea y de rábano incrementaron la producción en el tercer año debido a un incremento en el número de racimos por cepa. En esa última campaña, para todas las cubiertas, se produjo un incremento del tamaño de baya. Ni los sólidos solubles, ni la acidez total se vieron afectados por el empleo de cubiertas. Por el contrario, la festuca disminuyó el pH en una de las campañas, y *C. intybus* disminuyó el NFA hasta un 40% en la campaña en la que se produjo una disminución del crecimiento vegetativo. Respecto a los parámetros de suelo, en la segunda campaña se produjo una mejora de la estabilidad de agregados cuando se empleó la cubierta espontánea y la alfalfa. La M.O. no presentó diferencias, aunque sí una tendencia a incrementarse con todas las cubiertas. Por último, el empleo de las cubiertas, y de manera más importante el de *F. arundinacea* incremento la respiración microbiana del suelo.

En el estado de Virginia, Hatch *et al.* (2011) y Hickey *et al.* (2016) estudiaron una cubierta de *Festuca rubra* durante 7 años. Las calles estaban sembradas con *F. arundinacea* y *Dactylis glomerata*. El viñedo era de la variedad Cabernet Sauvignon, clon 337, de 3 años, a marco de 3 x 1,5 m. La festuca bajo la línea de cultivo produjo una reducción del crecimiento de brotes, sección de tronco, y peso de madera de poda, disminuyendo la diferencia con el herbicida hasta igualarse en el séptimo año. Los años con menores precipitaciones a inicios de campaña se produjeron menores crecimientos vegetativos y potenciales hídricos puntualmente menores en presencia de cubierta. Conforme pasaron los años, y con precipitaciones dentro de los valores medios, el manejo con herbicida provocó una disminución del estrés hídrico de las plantas, según medida de potencial hídrico. Esta diferenciación se explicaría por la mejora en la infiltración del agua de lluvia, que produce mayores niveles de humedad en los 10-20 cm primeros de suelo. La cubierta redujo la producción por disminución en el número de bayas por racimo y por tamaños de bayas menores, atribuyendo los autores este efecto a la competencia por el N en floración. El contenido de sólidos solubles, medido como grado brix, se incrementó, no viéndose afectados el pH, ni los antocianos y fenoles totales. La producción y los sólidos solubles se igualaron al manejo con herbicida con el paso de los años.

En Carolina del Norte, Giese *et al.* (2014, 2015) estudiaron durante 5 años dos cubiertas de *F. arundinacea*, una de *F. ovina*, una de *Lolium perenne* y una de *Dactylis glomerata* frente al empleo de herbicida. La calle estaba ocupada por *F. arundinacea*. Se trataba de un viñedo de Cabernet Sauvignon, clon 8, sobre portainjerto SO4, de 7 años de edad, a marco de 1,83 x 2,74m. *F. arundinacea*, con una mayor densidad de cubrición del suelo, compitió mejor con la vegetación adventicia espontánea. Todas las cubiertas redujeron el crecimiento vegetativo, de manera especial las de *F. arundinacea* que fueron las que más biomasa produjeron. Ni el contenido de N peciolar en floración, ni el estado hídrico se vieron afectados. Las diferencias en los niveles de reducción del crecimiento vegetativo entre cubiertas disminuyeron cuando los años fueron más secos. La producción no se vio afectada salvo en un año donde la cubierta de *F. ovina* presentó mayor producción que el resto de manejos, incluido el manejo con herbicida. Este año las producciones fueron especialmente elevadas para todos los manejos (7,5-8,34 kg·cepa^{-1} frente a los 3,9-4,9 kg·cepa^{-1} del resto de años). Ni los sólidos solubles ni la acidez total se vieron modificados. El contenido en nitrógeno fácilmente asimilable y la arginina disminuyeron en dos campañas cuando se empleó *F. arunidancea* KY-31, mientras que con el empleo de *L. perenne* y *D. glomerata* solo se observó un descenso en el contenido en arginina en una de las campañas.

Por último, Fleishman *et al.* (2019, 2021) en Pensilvania estudio una cubierta en la línea de cultivo de *F. rubra* frente a un manejo con herbicida. La calle estaba sembrada con *F. brevipila*. El viñedo estaba plantado con el híbrido Noiret (complex *Vitis* sp. insterspecific crossing, NY 65.0467.08 x Steuben), sobre dos portainjertos distintos: Riparia (vigor bajo) y 101-14 Mgt (vigor medio), de 5 años de edad. Tras el primer año de estudio, y coincidiendo con un año húmedo, el crecimiento vegetativo sufrió una mayor disminución debida al efecto de la cubierta cuando se empleó un portainjerto de vigor medio frente a uno de vigor bajo. La producción no se vio alterada en ese primer año. El empleo de la cubierta produjo un incremento de la biomasa radicular en los primeros 40 cm del suelo, lo que tuvo un efecto en el incremento de C y N del suelo tras 3 años cercano al 50%, sin observarse efectos en las capas inferiores del suelo. Este desarrollo radicular de la cubierta produjo una redistribución de las raíces del viñedo, especialmente en los primeros 20 cm, que es donde se concentraban las raíces de la cubierta.

1.3.2. Regiones frías y secas

En lo que hace referencia a regiones con clima frío y seco, sólo se dispone de un estudio (Vukicevich *et al.* (2019)), ubicado en la Columbia Británica, en una zona con una precipitación anual de 320 mm. Al igual que ocurría en los trabajos descritos en el punto anterior, este trabajo pretendía disminuir el vigor del viñedo para equilibrarlo con la producción. En la línea de cultivo se estudiaron seis manejos distintos: cubierta de *Bouteloua dactyloides* Columbus; cubierta de *Festuca rubra* subsp. Communtata; cubierta de *Capsella bursa-pastoris*; cubierta de *Lotus corniculatus*, laboreo y herbicida. La calle estaba sembrada de *Festuca spp.* y *Lolium perenne*. Era un viñedo de Sauvignon Blanc, de 1 año, sobre portainjerto 101-14 Mgt, a marco de 1,22 x 2,44 m. Al año siguiente de la instalación de las cubiertas se observó que las gramíneas y la leguminosa redujeron el crecimiento vegetativo, no así *C. bursa-pastoris*. Esta reducción estuvo relacionada con un menor contenido de N, que fue más marcado para el caso de la *F. rubra*. No hubo diferencias en los potenciales hídricos más que para *L. corniculatus*. La abundancia de hongos de suelo, así como la de hongos micorrícicos no se vio incrementada por el empleo de cubiertas vegetales, aunque sí se produjo un incremento del C microbiano con el empleo de *B. dactyloides*.

1.3.3. Regiones templadas y húmedas

En lo que hace referencia a climas templados y húmedos, se dispone de la información presentada en los trabajos de Coniberti *et al.*, en los que el objetivo de la implantación de la cubierta era, como en los casos anteriores, aumentar la competencia y así reducir el crecimiento vegetativo del viñedo. En un primer trabajo (Coniberti *et al.*, 2017b, 2018a), durante 3 campañas se estudió una cubierta total (calle y espacio bajo las cepas) de *F. arundinacea*, frente a un manejo de calle con *F. arundinacea* y aplicación de herbicida bajo las cepas. Se trataba de un viñedo de la variedad Tannat, sobre portainjerto SO4, con 7 años de edad, en dos marcos de plantación: 2,8 x 0,8 y 2,8 x 1,5 m. El ensayo contaba con riego localizado. El empleo de la cubierta bajo las cepas disminuyó el peso de madera de poda y de la producción debido a la reducción del peso de racimo. Esta reducción del crecimiento vegetativo a su vez disminuyó los daños por botritis. En lo referente a la composición de la uva, se incrementó el contenido de sólidos solubles y la concentración de antocianos totales, aunque esta última no fue constante en el tiempo, y se redujo la acidez total, aunque el pH no se vio afectado. En cuanto a los vinos, aquéllos elaborados con uvas procedentes de cepas con cubierta presentaron unos niveles de aromas frutales superiores y en general una mayor intensidad aromática y de color, siendo mejor valorados por los catadores.

En un segundo trabajo (Coniberti *et al.*, 2018b), durante 3 años, estudiaron el efecto de una cubierta total de *F. arundinacea* frente a una cubierta de esta misma especie ubicada solo en la calle. Nuevamente la variedad estudiada fue Tannat, sobre portainjerto 3309C, con un marco de 1,2 x 2,8 m y aporte de riego. En este caso también observaron una disminución del crecimiento vegetativo debido principalmente a una competencia de la cubierta por el agua. Si se realizaban aportes de agua en momentos tempranos del ciclo estas reducciones de crecimiento vegetativo desaparecían. En cuanto a los efectos agronómicos, desde el primer año se produjo una reducción en el tamaño de baya que tuvo repercusiones en la producción a partir del segundo año. Se incrementó la concentración de azúcares y antocianos totales, mientras que el NFA se redujo a partir de la segunda campaña. A nivel sanitario, se redujo la incidencia de botritis en los racimos, como consecuencia, de acuerdo a los autores, de la reducción en la disponibilidad de agua y de nitrógeno en la planta.

1.3.4. Regiones de clima mediterráneo

El trabajo realizado por Muscas *et al.* (2017) en Cerdeña-Italia, está orientado, como todos los estudios descritos en los apartados anteriores, a conseguir reducir el desarrollo vegetativo del viñedo. En este caso, compararon el laboreo tradicional con el empleo de cubiertas totales de vegetación espontánea (*Bromus hordeaceus*, *Avenas sterilis*, *Vulpia myrus*), sembrada con leguminosas (*Medicago polymorpha* cv. Anglona 50 % y *Trifolium yannunucum* cv. Gosse 50 %); y sembrada con gramíneas (*Dactylis glomerata* cv. Currie 80 % + *Lolium rigidum* cv. Nurra 20 %). El ensayo se realizó sobre un viñedo de la variedad Mazuela, sobre portainjerto 779P, de 17 años de edad, a marco de 2,7 x 1 m, con riego. La cubierta espontánea y la sembrada con gramíneas fueron las que mejor y más rápidamente cubrieron el suelo, presentando la leguminosa una instalación débil. Todas las cubiertas lograron reducir el crecimiento vegetativo, siendo mayor esta disminución cuando se empleó una cubierta de gramíneas. La producción también se redujo con el empleo de las cubiertas. Esta disminución se debió a la disminución del número de racimos por cepa en el caso de la cubierta de gramíneas, mientras que en la cubierta espontánea y en la de leguminosa la disminución del rendimiento obedecía a una disminución del tamaño de baya. El contenido de N en hoja fue mayor para el manejo mediante laboreo y para la cubierta de leguminosas. La cubierta de gramíneas incrementó el contenido de sólidos solubles en baya, mientras que la cubierta de leguminosas disminuyó los polifenoles totales. Por último, la cubierta de leguminosa tuvo un efecto indeseado sobre el insecto plaga *Planococcus ficus* al incrementar su supervivencia, fecundidad y fertilidad.

En Australia, en Nueva Gales del Sur, Tesic *et al.*, (2007) estudiaron durante 4 campañas, en dos localidades con distintos niveles de pluviometría (304 mm y 492 mm entre octubre y abril), un viñedo de Chardonnay, de 4 y 12 años de edad, respectivamente; a marco de 3 x 2 m, con aporte de riego, tres manejos distintos de suelo: suelo completamente desnudo con empleo de herbicida, calle con cubierta espontánea y la línea de cultivo con herbicida y cubierta total espontánea. El empleo de la cubierta total redujo el contenido de humedad del suelo a 40 cm de profundidad en las dos últimas campañas de estudio. Ni en superficie (0-10 cm) ni en profundidad (40 cm) se apreciaron diferencias de C orgánico. El contenido de N peciolar en floración se redujo con el empleo de la cubierta completa en las dos últimas campañas, coincidiendo a su vez con disminuciones del crecimiento vegetativo y de la producción por disminución del número de racimos por cepa. El peso de la baya disminuyó proporcionalmente al incremento de cobertura del suelo. En la zona más cálida también se produjo una disminución del número de bayas por racimo. Los componentes de la baya no se vieron afectados por el manejo en los primeros tres años. En el cuarto año, en la zona más cálida, se produjo un incremento del contenido de sólidos solubles en baya cuando se empleó la cubierta total. En la zona más fresca se produjo una disminución de la acidez total cuando se empleó la cubierta total.

Por último, en Australia meridional se realizó un estudio con el objetivo de encontrar alternativas económicamente rentables al empleo de herbicidas (Penfold *et al.*, 2018). Para ello, en cuatro localidades se estudiaron durante 3 campañas (las dos primeras más secas y la tercera más húmeda respecto de la media) 10 cubiertas diferentes en la línea de cultivo (*Dactylis glomerata*; *Vulpia myuros*; *Rytidosperma geniculata*; *Medicago littoralis* + *M. truncatula*; *M. polymorpha* + *Lolium* *rigidum*; *M. polymorpha* + *M. orbicularis*; *Trifolium fragiferum* + *Festuca ovina*; *T. glanduliferum* + *T. subterraneum*; *Atriplex semibaccata* y *Medicago spp.* + *L. rigidum* + *Trifolium spp.*) frente a un manejo con herbicida. Todas las parcelas disponían de riego por goteo, las calles estaban cubiertas de vegetación y las precipitaciones abarcaban desde los 250 a los 700mm. El crecimiento vegetativo no se vio afectado por el empleo de ninguna de las cubiertas, aunque si se observó una disminución del índice de área foliar (LAI) cuando el año fue seco. Por el contrario, en los años húmedos el LAI fue mayor cuando se emplearon cubiertas de gramíneas o leguminosas anuales. El empleo de leguminosas con gramíneas o de leguminosas solas no redujo la producción e incluso llegaron a aumentarla en 3 de las 4 localidades, especialmente cuando el año fue más húmedo. El empleo de gramíneas perennes (*D. glomerata*, *R. geniculata*, o *F. ovina*) sí produjo una disminución de la producción de hasta un 20%. Los parámetros de baya y mosto no se vieron afectados salvo para el caso de las cubiertas de leguminosas, las cuales incrementaron el NFA. Este incremento estaría relacionado con el incremento también detectado en el N peciolar. Cuando se estudiaron los vinos resultantes, aquellos provenientes de cubiertas de medicagos o de medicagos con gramíneas fueron los mejor valorados. El C de suelo se incrementó para todas las cubiertas, especialmente con el empleo de gramínea o de gramíneas con leguminosas. El empleo de la mezcla de gramíneas con leguminosas produjo el mayor incremento de N total de suelo (15%), incluso superior al del empleo de una leguminosa sola. El N extraíble en suelo se incrementó en presencia de cubierta de leguminosa y en la mezcla de estas con gramíneas en un 75%. El C orgánico de la POM se incrementó con todas las cubiertas en más de un 45% respecto al herbicida. Los incrementos de C estuvieron relacionados con los incrementos de biomasa radicular, siendo la cubierta de gramínea la que más biomasa radicular produjo (69,2 kg·m^2), seguido de la mezcla de gramíneas y leguminosas (10,6 kg·m^2) y de la leguminosa sola (2,2 kg·m^2). Así mismo, se incrementó la abundancia de lombrices con el empleo de las cubiertas. (Ball *et al.*, 2020). El empleo de cubiertas anuales bajo el cordón (medicagos y medicagos con gramíneas) en el estudio que realizaron tuvo beneficios económicos respecto al manejo con herbicidas, al reducir los gastos de manejo e incrementar en algunos casos las producciones.

1.3.5. Consideraciones finales

El empleo de cubiertas vegetales bajo las cepas es una práctica de cultivo novedosa y, como era de esperar, los trabajos científicos desarrollados en diferentes zonas del mundo muestran que la respuesta obtenida es dependiente de la climatología de la zona, el tipo de cubierta empleado y la edad del viñedo sobre el que se instala. En términos generales, y considerando que el número de trabajos recogidos en la literatura científica es todavía muy limitado, se observa en general un elevado interés de esta práctica y pocos aspectos negativos que podrían estar asociados a su uso. En cualquier caso, y tras esta recopilación bibliográfica, queda en evidencia el interés y necesidad de profundizar en el estudio de las cubiertas bajo la línea de cultivo dado sus potenciales beneficios para el suelo, y sus respuestas, más variables, sobre el rendimiento y la composición de la uva y el vino.

2. OBJETIVOS

El objetivo de esta tesis doctoral es evaluar el interés del uso de una cubierta vegetal bajo la línea como estrategia de gestión del suelo en viñedos cultivados en condiciones de clima mediterráneo- Este objetivo general se puede dividir en los siguientes subobjetivos:

- Evaluar el efecto de la cubierta vegetal bajo la línea sobre el comportamiento agronómico del cultivo, incluyendo su interacción con la vegetación adventicia, su competencia con el cultivo por los recursos hídricos y nutricionales, incidencia sobre el riesgo de heladas y su efecto sobre el desarrollo vegetativo, el rendimiento y sobre las características de la uva producida.
- Evaluar el efecto de la cubierta vegetal bajo la línea sobre las características fisicoquímicas y organolépticas de los vinos producidos, así como su influencia sobre la población de las levaduras naturales.
- Evaluar el efecto de la cubierta vegetal bajo la línea sobre la calidad del suelo evaluada a través de una combinación de parámetros físico-químicos y biológicos.

3. MATERIAL Y MÉTODOS

Los capítulos 4, 5, 6 y 7, por su concepción a modo de artículos independientes, presentan su propio apartado "Material y métodos". Sin embargo, se ha considerado de interés incluir en la memoria de la Tesis Doctoral un apartado específico, de manera que el lector disponga de una descripción más detallada de algunos aspectos del ensayo y de una visión global de la metodología empleada.

3.1. Diseño experimental

3.1.1. Ubicación y material vegetal

El dispositivo experimental sobre el que se asientan todos los resultados de esta Tesis se estableció en 2018 en una parcela perteneciente a Bodegas Ochoa. Los trabajos experimentales se mantuvieron durante tres campañas (2018, 2019 y 2020). La parcela empleada se ubica en el término municipal de Traibuenas (Navarra, España), localizado en el valle medio del Ebro (Figura 3-1). La variedad cultivada es Merlot (clon 343) sobre portainjerto 420A, plantada en 2001 con una distancia entre filas de 3 m y de 1 m entre cepas. La conducción del viñedo es en doble Cordón Royat.

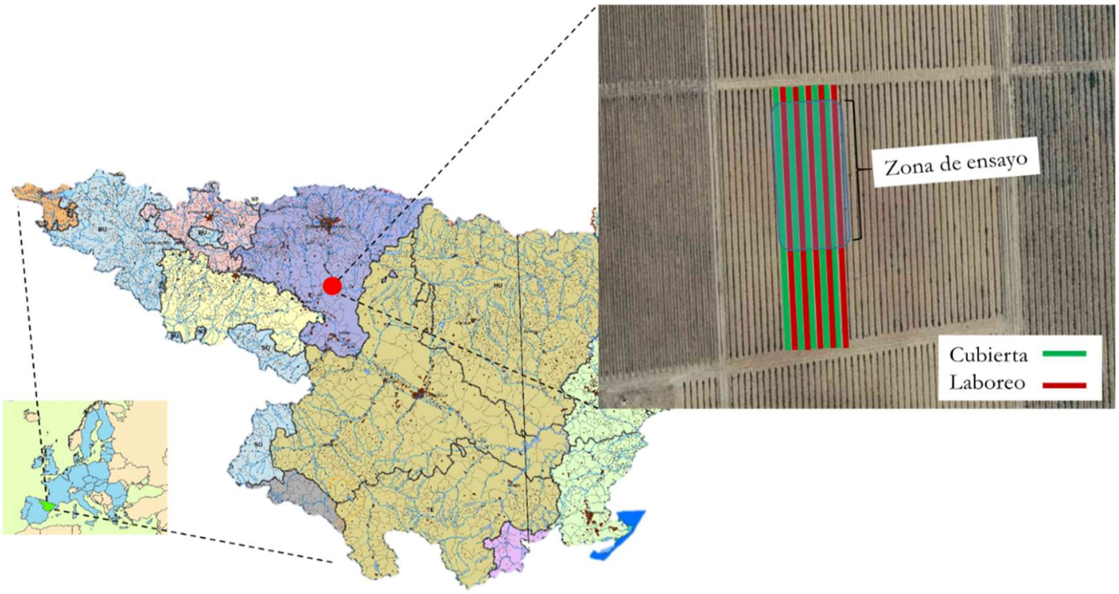

Figura 3-1. Mapa de ubicación y esquema de la parcela de ensayo.
Fuente: SITEbro y SITNA

Dada la elevada pedregosidad de la parcela, y el reparto desigual de ésta a lo largo de la línea de cultivo, el ensayo se centró en la zona norte de la misma, la cual presentaba una menor pedregosidad en toda la superficie (Figura 3-1). Las variantes a comparar fueron una cubierta vegetal de *Trifolium fragiferum* L. bajo la línea de cultivo (UV) y un manejo mediante laboreo intercepas (T), dispuestos en filas alterna entre ellos, con cinco repeticiones para cada tratamiento. Los controles, para cada repetición y variante, se centraron en 60 cepas consecutivas, eliminando las 5 primeras cepas de los bordes de cada fila. Así mismo, los controles destructivos de vendimia se realizaron sobre 20 cepas seleccionadas de entre estas 60 iniciales, La elección de las mismas se basó en la homogeneidad de la sección de sus troncos medidos a 30 cm del suelo, en el espacio comprendido entre dos nudos.

3.1.2. Manejo de la parcela

La parcela se maneja bajo certificación ecológica desde el año 2018. Cuenta con instalación de riego por goteo, con difusores de 3,5 $L \cdot h^{-1}$ distanciados 0,75 m entre sí. El riego comenzó el 6 de junio en 2018, el 16 de junio en 2019 y el 1 de junio en 2020. En los dos primeros años se aplicó un día de riego de 6 horas a la semana hasta el mes de agosto, cuando se cambió a dos días de riegos semanales de 3 horas cada uno hasta finalizar la campaña de riego los días 10 de septiembre en 2018 y 20 de septiembre en 2019. En el año 2020 se hicieron tres riegos semanales de 2 horas hasta el 13 de julio, cambiando a dos riegos semanales de 3 horas hasta el 31 de agosto, cuando nuevamente se cambió a dos riegos semanales de 2 horas hasta terminar el riego el 15 de septiembre.

La parcela se abonó en el año 2020 con 450 $Kg \cdot ha^{-1}$ de un abono orgánico en formato pellet (Fercrisa biosuelo-Crisara) incorporándolo al suelo con pase de semichisel en la primera semana de marzo. Los tratamientos fitosanitarios son los autorizados dentro de la normativa ecológica, controlándose las principales enfermedades criptogámicas a base de cobres y azufres. Por lo demás, el manejo del viñedo fue el habitual en la zona, con supresión de los brotes fuera de los pulgares cuando estos tienen 10-15 cm de longitud, tratando de conservar la poda dejada en invierno de 2 yemas por pulgar, con 3 pulgares por brazo. En el mes de julio se realiza un despunte de la vegetación. La calle se mantiene libre de vegetación mediante pase de cultivador cuatro veces al año (noviembre, marzo, mayo y julio).

3.1.3. Características edáficas de la parcela

El suelo se clasifica como Typic Calcixerepts según se indica en el mapa de suelos de Navarra, perteneciente a la Serie 5. Son suelos moderadamente profundos, desarrollados sobre las terrazas medias de los ríos Cidacos y Aragón. Su principal característica es la elevada pedregosidad, que los incluye en las familias esqueléticas (Figura 3-2.a). La familia mineralógica es generalmente carbonática con contenidos en carbonatos entre un 30-40% en el horizonte superficial. La fertilidad de estos suelos es moderada, el volumen explorable por las raíces es medio, y disponen de buen drenaje. La capacidad de retención de agua por el contrario es limitada.

Concretamente, la calicata realizada en la zona del ensayo el 3 de mayo de 2018 muestra un suelo con una textura franca en los primeros 90 cm y una textura franco-arenosa en la siguiente profundidad. Las raíces del viñedo se desarrollan hasta una profundidad aproximada de 70 cm (Figura 3-2.b). El nivel de materia orgánica en el perfil superior es de 1,26%, el de carbonatos totales del 33% y el de caliza activa del 8,94% (Tabla 3-1).

3.1.4. Características climáticas de la parcela

El clima se clasifica como húmedo-templado mediterráneo según la clasificación de Papadakis. La temperatura media durante el periodo vegetativo (abril-octubre) fue de 19ºC para los tres años, siendo la campaña más húmeda la 2018, con 333mm frente a los 220 y 234 mm de los años 2019 y 2020, respectivamente. Según la clasificación climática multicriterio de Tonietto and Carbonneau (2004), todas las campañas se clasifican como templadas-cálidas, con los años 2018 y 2020 moderadamente secos y el 2019 muy seco. El índice de frescor nocturno en el último mes fue frío en el año 2018 y muy frío en los años 2019 y 2020 (Tabla 4-1 - Apartado 4.2.). La temperatura media mensual y la precipitación acumulada mensual se pueden observar en la Figura 4-2 del Apartado 4.2.

Tabla 3-1. Datos físico-químicos de la calicata realizada en la parcela de ensayo el 3 de mayo de 2018.

	Perfil superior	**Perfil inferior**
Profundidad (cm)	**0-90**	**90-125**
Arena (%)	47,17	59,31
Limo (%)	31,05	30,11
Arcilla (%)	21,78	10,56
Textura USDA	Franco	Franco-arenoso
pH agua (1:2,5)	8,62	8,95
pH KCl (1:2,5)	7,68	8,11
MO (%)	1,26	0,1
Fósforo-P_2O_5 (mg/Kg)	12,93	0,17
Potasio-K_2O (mg/Kg)	166,31	45,53
Nitrógeno Total (%)	0,09	0,02
Relación C/N	8,01	2,72
Carbonatos Totales (%)	33,04	44,35
Caliza activa (%)	8,94	3,78
C.E. (1:1) (dS/m)	0,29	0,27

3.1.5. Descripción de las variantes estudiadas

El ensayo consistió en la siembra de *Trifolium fragiferum* en un espacio de 40 cm de ancho bajo las cepas a lo largo de la línea de cultivo. Previamente a la siembra se realizó un pase de intercepas modelo "Davitronic" de Industrias David. La siembra se realizó el 27 de febrero de 2018 de manera manual (Figura 3-2.c), a dosis de 15g·m^{-2} en filas alternas, con 5 repeticiones por tratamiento. Tras la siembra se produjo una precipitación en forma de nieve que ayudó a asentar la semilla. Posteriormente a la siembra no se hizo ningún manejo de la cubierta en los años siguientes. El tratamiento *Cubierta* (UV) se comparó con el manejo convencional realizado por la bodega, consistente en un *Laboreo* (T) mediante 4 pases de intercepas a comienzos de noviembre, en marzo, mayo y julio (Figura 3-3).

T. fragiferum (Figura 3-2.d), también conocido como trébol fresa, es una planta perenne perteneciente a la familia de las fabáceas, de tallos pilosos de 10-30 cm de longitud. Desarrolla estolones, enraizando en los nudos. Tiene hojas alternas, estipuladas y pecioladas, formadas por tres foliolos, con un tamaño de 8-20 mm, obovados, elípticos o obcordados. Las inflorescencias son capítulos de 10-22 mm de diámetro en la floración, con forma globosa o elipsoide; con pedúnculos de hasta 20 cm, a menudo pilosos. Estas inflorescencias presentan brácteas soldadas a la base y un número de flores de 30-60 por inflorescencia. El cáliz es densamente piloso y se hincha en la madurez, lo que le da un aspecto globoso. La corola es rosada con el estandarte libre. El fruto es una legumbre inclusa, con 1-2 semillas. Las semillas tienen una longitud de 1,1-1,7 mm y 1,0-1,2 mm de ancho, el color de esta es mayoritariamente amarillento-verdoso con manchas o puntos de color violeta oscuro. Tiene una raíz principal profunda. La polinización es cruzada y favorecida por los insectos (Pascual, 1978).

3.2. Controles realizados

3.2.1. Controles agronómicos

3.2.1.1. Competencia con vegetación adventicias

A inicios del mes de agosto se realizó la valoración de vegetación adventicias. Se utilizó una versión modificada de la escala de Horsfall and Barrat (1945). A esta escala se le añadieron dos intervalos adicionales para permitir detallar las especies que aparecen únicamente en una ocasión (0-0,1%) o un par de veces (0,1-0,5%). Se identificaron todas las especies vegetales presentes en la fila y se les asignó un porcentaje de cubrición del suelo.

3.2.1.2. Estado nutricional

En floración e inicio de envero se tomó muestra de 100 peciolos para análisis de N, P, K, Ca, Mg, Na, Fe, Cu, Mn, Zn and B. Los peciolos de floración correspondían a la hoja opuesta al racimo inferior de los pámpanos, mientras que los de envero correspondían a la hoja del segundo racimo del pámpano. Las muestras se conservaron en frío (4-6ºC) hasta su entrega en el laboratorio Nasertic para su análisis. Una vez aquí, las muestras se limpiaron de posibles impurezas adheridas, se secaron y se molieron. Tras esto se realizó una digestión con ácido nítrico concentrado y se procedio a la cuantificación de los diferentes elementos por medio de espectrometría de masas de plasma (ICP-MS).

3.2.1.3. Estado hídrico

El efecto de los tratamientos sobre el contenido de agua en la planta se cuantificó de dos maneras. La primera de ellas a través de los potenciales hídricos en peciolo (Ψm) a mediodía (11-13h) desde inicios del mes de julio hasta vendimia. Cada diez días aproximadamente, sobre 4 hojas sanas y adultas, previamente embolsadas durante 1,5h (Figura 3-4.a), se midió el potencial mediante cámara de presión de Scholander (P3000, Soil Moisture Corp., Santa Barbara, CA, USA). La segunda variable empleada para estimar el estado hídrico fue la determinación del ratio isotópico del carbono ($\delta^{13}C$), medida integral del déficit hídrico de la cepa a lo largo de la maduración (Santesteban *et al.*, 2015). De una muestra de 100 bayas, para cada tratamiento y repetición en el momento de vendimia, se extrajo mosto empleando una licuadora modelo LMU 9018 y se llevó a cabo la determinación del $\delta^{13}C$ empleando un analizador Elemental (NC2500, Carlo Erba Reagents, Rodano, Italy) acoplado a un espectrómetro isotópico de masas (Thermoquest Delta Plus, ThermoFinnigan, Bremen, Germany).

3.2.1.4. Tasa de cuajado

A mediados del mes de mayo en 2019 y 2020 se realizó la valoración de la tasa de cuajado. Para ello se marcaron 10 racimos por repetición, midiendo la longitud del raquis y la longitud de los hombros del racimo. Estos mismos racimos se recogieron e identificaron una vez cuajados, contándose el número de bayas cuajado por racimo (Figura 3-4.c). Estas mismas bayas se emplearon para identificar el número de semillas que contenía cada baya sobre una muestra de 100 bayas. El mismo día del marcaje de los racimos, se muestrearon al azar 20 racimos de distintos tamaños por tratamiento, contando en gabinete el número de glomérulos que presentaban en relación a la longitud de su raquis y sus hombros (Figura 3-4.b). Con esta relación se estimó el número de glomérulos esperables en los racimos marcados en campo. La ratio entre las bayas cuajadas y el número de glomérulos de los racimos nos permitió obtener la tasa de cuajado de los racimos.

3.2.1.5. Rendimiento productivo y composición de baya

Los parámetros de producción, número de racimos por cepa, peso del racimo y kilos de uva por cepa, se tomaron sobre las 20 cepas seleccionadas para medidas destructivas. Las vendimias se realizaron 3-4 días previos a la fecha de vendimia marcada por la bodega, siendo la misma para ambos tratamientos. Las fechas de control fueron el 24 de septiembre de 2018, el 5 de septiembre de 2019 y el 2 de septiembre de 2020. La muestra de 100 bayas empleada para la isotopía del carbono también se empleo para el control del peso de baya, así como para los parámetros de composición de la baya. Se determinaron los sólidos solubles totales, pH, acidez total, ácido málico y nitrógeno fácilmente asimilable (NFA) con un Miura 200 (TDI analysers, Gavá-Barcelona). Los fenoles en uva se estimaron a través del método Cromoenos®. En el mes de diciembre, sobre las 20 cepas en las que se realizaron los controles de vendimia, se llevó a cabo el control de peso de madera de poda (ampliado en el Capítulo 4).

3.2.1.6. Daño por heladas

En el año 2021, el día 15 de marzo, se colocaron sensores de temperatura a la altura de los pulgares para poder valorar el efecto de las cubiertas sobre las heladas primaverales (Figura 3-4.d). El 24 de abril se realizó la valoración del daño por frío sobre los brotes (ampliado en el Apartado 7.2).

3.2.2. Controles enológicos

3.2.2.1. Elaboración de vinos

Para la elaboración de vinos se tomó una muestra de 100 kg de uva de las repeticiones 3, 4 y 5 de cada tratamiento. Únicamente se tomaron tres repeticiones por las limitaciones de capacidad que existían desde EVENA para poder realizar un número mayor de éstas. Las tres repeticiones elegidas se correspondían con aquellas que mejor desarrollo de la cubierta presentaban el primer año. Una vez en bodega, cada muestra se despalilló, estrujó y encubó individualmente. Se adicionó metabisulfito y levaduras *Saccharomyces cerevisiae* (Oenoferm® Be-red). Durante el proceso de fermentación se controló temperatura y densidad de los mostos. Tras la fermentación, se prensó y se adicionaron bacterias lácticas. Se corrigió el sulfuroso, se filtró por filtro de celulosa y se embotelló. Sobre estos vinos se realizó una analítica básica de composición en el laboratorio de Enología del Gobierno de Navarra (grado probable, pH, acidez total, ácido málico, acidez volátil, Ca, Mg, K, densidades ópticas a 420, 520 y 620nm, e índice de polifenoles totales-IPT).

3.2.2.2. Análisis organoléptico

Con los vinos resultantes se realizó una cata con catadores experimentados. La cata se realizó de acuerdo a la metodología Flash Profile, facilitándosele a cada catador una lista de descriptores que pudiera ayudarles en la selección de los descriptores. Los catadores debían ordenar de menor a mayor, para cada uno de los descriptores elegidos por ellos, los 7 vinos a catar (las tres repeticiones de cada tratamiento, más un vino repetido de uno de los dos tratamientos, a fin de poder comprobar la validez del juicio de los catadores) (ampliado en capítulo 5).

3.2.2.3. Elaboración de vinos con levaduras autóctonas

Además de estos vinos, en el año 2020 se realiza una elaboración de vinos sin levaduras exógenas para comprobar si existe alteración en la población de levaduras autóctonas de las uvas. Se tomó una muestra separada de 25 kg para las repeticiones 3, 4 y 5 de cada tratamiento. Todo el material (cajas de vendimia, tijeras, tanques) fue previamente desinfectado. La vinificación, con despalillado y estrujado manual, se realizó en un espacio donde nunca se ha elaborado vino, para evitar contaminaciones de levaduras exógenas. Los tanques se colocaron en una nevera industrial a temperatura de 22 ºC, removiendo el sombrero del vino diariamente y controlando su temperatura y densidad. Una vez terminada la fermentación, se envió una muestra al laboratorio de INCAVI-IRTA para el análisis de la población de levaduras *S. cerevisiae* según la metodología propuesta en la resolución de la OIV-OENO 408-2011 (ampliado en apartado 7.3).

3.2.3. Controles edafológicos

3.2.3.1. Carbono orgánico del suelo (SOC) y particulado (POC)

El 7 de mayo de 2019, tras un año de permanencia de la cubierta, se realizó un muestreo de suelo a dos profundidades (0-15 cm y 15-30 cm). La primera profundidad corresponde al área de desarrollo de las raíces de la cubierta de *T. fragiferum*, mientras que en la segunda se localizan las raíces del viñedo (Figura 3-5.a). La muestra se tomó con azada tratando de alterar lo mínimo posible la estructura del suelo. Posteriormente se secaron a temperatura ambiente y una parte se tamizó a 2 mm. Sobre esta muestra tamizada se analizó el carbono orgánico del suelo (SOC) y el carbono orgánico particulado (POC) mediante oxidación húmeda (Nelson and Sommers, 1996).

3.2.3.2. Estructura de suelo y agregación

Sobre la muestra anterior sin tamizar, y siguiendo la metodología propuesta por Oliveira *et al.*, (2019), se realizó dispersión de la estructura en distintos tamaños de agregación. Una muestra de suelo inalterado tamizado a 6 mm se separó en macroagregados (Magg), fracción POM de los macroagregados (cPOM), microagregados de los macroagregados (magg), arena fina de los microagregados (Fine sand), y arcilla y limo (S+c). Se calculó el diámetro medio ponderado de los agregados (MWD) y la estabilidad de los agregados en agua (WSA). Sobre cada una de las fracciones se determinaron el C mediante oxidación húmeda y N mediante el método Kjeldahl.

3.2.3.3. Conductividad hidráulica

El 30 de octubre de 2019, tras el final de la campaña, se realizó un nuevo muestreo de suelo mediante anillos de 100 cm^3 para la determinación de la densidad aparente del suelo, la porosidad y la conductividad hidráulica. La conductividad se mido mediante permeámetro Eijkelkamp.

3.2.3.4. Actividad biológica

A. Respiración basal

La actividad biológica del suelo se determinó a través de diversas mediciones. Una medida indirecta mediante respiración de suelo empleando un medidor portátil SCR-1 EGM-4 de PP System. Al mismo tiempo se control la temperatura a ras de suelo, y la temperatura y humedad de suelo a 10 cm de profundidad (Figura 3-5.b). Estas medidas se realizaron en brotación, floración, envero y vendimia para los años 2019 y 2020, mientras que en el año 2018 solo se realizaron en envero y vendimia.

B. Biomasa microbiana

Se midió el C y N de la biomasa microbiana del suelo (MBC y MBN, respectivamente) empleando el método de fumigación por cloroformo (Brookes *et al.*, 1985; Vance *et al.*, 1987). Las muestras se tomaron en floración y vendimia en el año 2019 y 2020, y únicamente en vendimia en el año 2018. Para ello se empleó un tubo de PVC de 35 cm de longitud, con un diámetro de 50 mm, cortado en bisel en su extremo inferior. Dicho tubo constaba de dos incisiones a 15 cm del extremo inferior para poder separar las dos profundidades de estudio: 0-15 cm y 15-30 cm (Figura 3-5.c).

Junto a los muestreos anteriores se enterró otro tubo de iguales características, pero con las incisiones cubiertas por cinta aislante y tapado su extremo superior para evitar infiltraciones de agua en el mismo (Figura 3-5.d), que permaneció en el suelo por espacio de un mes. Una vez extraído, se midió en ambas muestras el contenido de nitrato y amonio, con lo que se calculó el balance de ambos.

C. Diversidad bacteriana

Por último, sobre las muestras de suelo empleadas para la medición de la biomasa microbiana, se realizó la determinación de la diversidad funcional de bacterias del suelo empleando el método Biolog EcoPlates^{TM}. Este método permite la identificación de la diversidad de bacterias heterótrofas cultivables. Se valora la presencia de distintos grupos funcionales de bacterias según cómo éstas son capaces de atacar las 31 fuentes de C (sustratos) diferentes que presenta el EcoPlate. La cuantificación se realiza mediante la diferencia entre la absorbancia a 750 nm y a 559 nm. Con estos datos se construye una curva en función del tiempo. Analizando dicha curva con el programa Sigmaplot se determina el punto medio donde la curva tiene un crecimiento lineal, que es considerado el punto más representativo para la evaluación de la diversidad de la comunidad bacteriana. Así, en dicho punto se calcula el número de sustratos utilizados (NSU) y el índice de diversidad de Shannon (H') (ampliado en capítulo 6).

3.2.4. Análisis estadístico

El análisis estadístico de los distintos parámetros se realizó mediante t-test, revisando que los parámetros cumpliesen los condicionantes de normalidad y homogeneidad mediante el test de Shapiro y el test de Barlett. Si los parámetros no cumplían con dichos condicionantes se realizaron las correcciones necesarias. El análisis se realizó empleando el programa informático R (R Development Core Team, 2016). El análisis de cata se realizó por el método de Análisis factorial múltiple (MFA) con el programa estadístico XLSTAT.

Figura 3-2. a) Detalle pedregosidad superficial; b) Detalle distribución de raíces en el perfil del suelo; c) Siembra manual de la cubierta; d) Detalle *T. fragiferum*.

Figura 3-3. Aspecto del ensayo a fecha del 14 de julio de 2020 a) Aspecto general del ensayo; b) Detalle del tratamiento con cubierta (UV); c) Detalle del tratamiento laboreado (T).

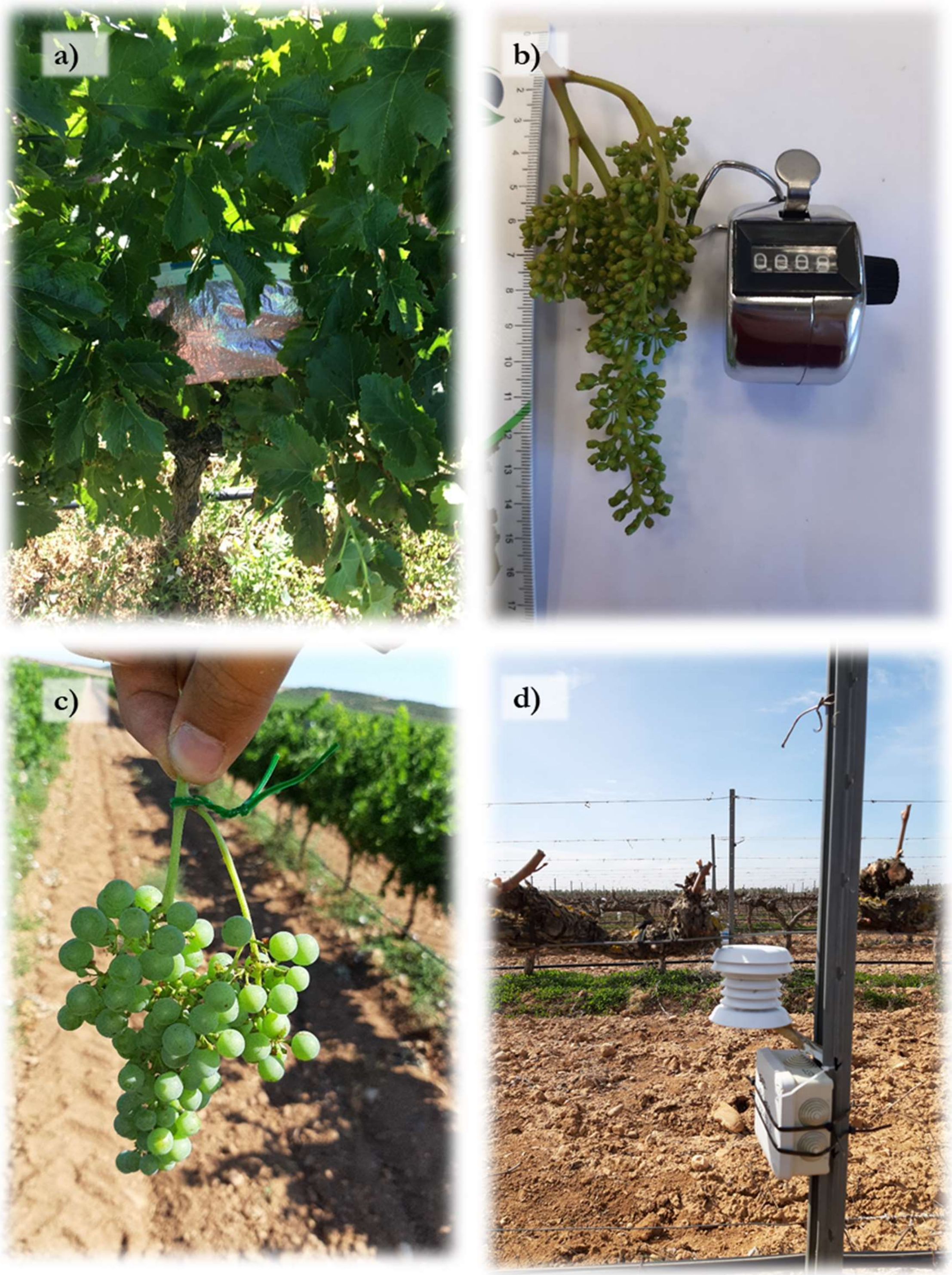

Figura 3-4. a) Hoja embolsada previa a la medición del potencial hídrico; b) Racimo preparado para conteo de glomérulos; c) Racimo marcado para medición de la tasa de cuajado una vez cuajado; d) Sensor de temperatura en brotacióna.

Figura 3-5. a) Muestreo de la primera profundidad de suelo para análisis de agregación; b) Medición de respiración y temperatura ambiente y de suelo; c) Tubo de PVC empleado para muestreos de biomasa microbiana y lecturas de nitratos y amonios, con la separación para las dos profundidades de muestreo; d) Tubos para la medición del balance de nitrato y amonio, dcha. tubo con tapa que permanecerá enterrado durante un mes.

4. UNDER-VINE COVER CROPS: IMPACT ON WEED DEVELOPMENT, YIELD AND GRAPE COMPOSITION

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* This article has been slightly modified compared to the original to include the information related to the third year of study.

4.1. Introduction

Many vineyards worldwide use cover crops in the inter-row as a soil management strategy as, under many circumstances, their benefits outnumber the potential drawbacks they may have (Steenwerth and Guerra 2012). However, the space under the vines (i.e., the rows) are frequently kept free of vegetation, at least in Mediterranean climate conditions, to avoid high competition with the crop, both for nutritional and water resources. This bare area is commonly maintained through mechanical tillage or using herbicides. As herbicides are currently being questioned for their environmental impact, and legal constraints to their use increase (AFP, 2019), there is a great interest in reducing or eliminating their use. Additionally, focusing on the wine sector, some studies have demonstrated that herbicides can reduce grapevine root mycorrhization and soil microorganism populations or alter nutrient composition in grapevine roots, leaves, grape juice and xylem sap (Chou and Vanden Heuvel*.,* 2018; Donnini *et al.,* 2016; Zaller *et al.,* 2018), and that these changes may affect wine fermentation (Morozova *et al.,* 2017). Although less questioned, mechanical tillage of the row area to keep it free of vegetation also has some drawbacks as it generally results in an increased cost associated with the higher frequency of tilling operations compared to herbicides, while it also requires specific equipment. Additionally, soil disturbance associated with mechanical tilling enhances organic matter degradation and, in turn, alters the population of soil microorganisms, decreases water infiltration and increases the soil susceptibility to erosion (Ben-Salem *et al.*, 2018; Ruiz-Colmenero *et al.*, 2011; Virto *et al.*, 2012).

Given the drawbacks of using herbicides or mechanical tillage for soil management under the vines, the establishment of cover crops, with low competition potential, appears as an appealing alternative (Jordan *et al.,* 2016; Karl *et al.,* 2016b). This option has been evaluated in a very reduced number of research works (Chou and Vanden Heuvel 2018; Coniberti *et al.,* 2018a; Hickey *et al.,* 2016; Jordan *et al.,* 2016; Karl *et al.,* 2016b; Penfold *et al.,* 2018), out of which nearly none has been conducted in Mediterranean conditions. In this context, this work aims to evaluate the interest of using under-vine cover crops as a feasible and sustainable management option for vineyards in Mediterranean areas. As a preliminary test, six different cover crop mixes (*Lotus corniculatus*, *Trifolium fragiferum*, *L. Corniculatus* + *T. fragiferum*, *Festuca ovina*, *F. ovina* + *T. fragiferum* and *Lolium rigidum* + *L. corniculatus*) were evaluated during 2016–2018 to check their adaptation to the vineyard and competition with other adventitious species (Figure 4-1). Among them, *T. fragiferum* was selected as the most suitable species due to its ability to compete with other species, its reduced cost of the establishment (it is perennial) and to its ability to supply nitrogen to the crop through nitrogen fixation (Abad *et al.,* 2019).

Figure 4-1. The appearance of the different under-vine covers in their second evaluation season in a preliminary test: a) *Lotus corniculatus*, b) *Trifolium fragiferum*, c) *L. Corniculatus* + *T. fragiferum*, d) *Festuca ovina*, e) *F. ovina* + *T. fragiferum*, f) *Lolium rigidum* + *L. corniculatus.*

4.2. Methods

The trial was carried out in a vineyard belonging to Bodegas Ochoa winery, located in the village of Traibuenas (Navarra-Spain) during 2018 and 2019. The cultivated variety is Merlot (clone 343) on rootstock 420A, planted in 2001, with a distance between vines of 1 m and between rows of 3 m, and trained as a vertical shoot positioned double Cordon Royat. The plot has a drip irrigation system, with 3.5 $L·h^{-1}$ drippers spaced 0.75 m. The irrigation started the 6th of June in 2018 and the 16th of June in 2019, applying 6-hour watering once a week until August, when it was changed to two 3-hour irrigation days per week until the 10th September in 2018 and until the 20th of September in 2019. In 2020, the irrigation started the 1st of June applying 6-hour watering three times a week until 13th of July, when it was changed to two 3-hour irrigation days per week until 31th of August, and later 2-hour watering twice a week until the 15th of September.

The soil can be classified as Typic Calcixerepts, with a loam texture up to the first 90 cm and a sandy loam in depth. The level of organic matter is 1.26 %, the total carbonates are 33 % and the active limestone is 8.94 %. The climate, according to Papadakis classification, can be defined as a humid temperate Mediterranean climate. Climate data for the 2018, 2019 and 2020 seasons were obtained from an automatic weather station belonging to the regional meteorological network, located close to the vineyard (1,500 m in a straight line), and are summarized in Table 4-1 and Figure 4-2.

Table 4-1. Mean temperature, rainfall, Heliothermal Index (HI), Cool Night Index (CI) and Dryness Index (DI) calculated for both season, according to (Tonietto and Carbonneau, 2004).

	2018	**2019**	**2020**
Mean temperature (Apr-Oct,° C)	19	19	19
Rainfall (Apr-Oct, mm)	333	220	234
Heliothermal Index, HI	2378	2391	2361
Cool night Index, CI (°C)	13.5	11.9	11.7
Dryness Index, DI (mm)	45	-113	-35

Data obtained from Traibuenas meteorological station belonging to the regional network. Sensor models are Vaisala HMP45C for temperature and Campbell ARG100 for rainfall.

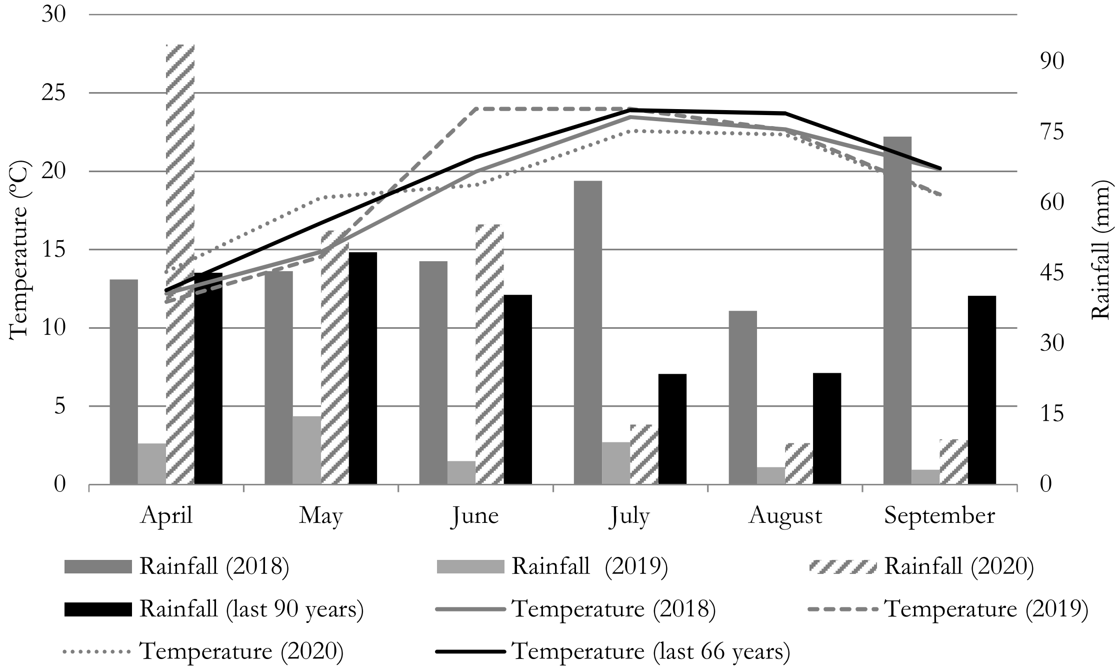

Figure 4-2. Climate conditions of the three seasons, 2018, 2019 and 2020 (April–September): mean monthly temperature (°C) and accumulate monthly rainfall (mm).

The experimental design included two treatments: cover-crop under the vines (UV) and mechanically tilled (T) control, with five replicates per treatment in alternate rows. The first six vines in each row were not considered for sampling, and out of the 60 vines available per replicate, 20 vines were selected for homogeneity using measurements of the trunk cross-sectional area, estimated measuring two orthogonal diameters 30 cm above the ground. These 20 vines were marked and all the measurements performed in them.

For cover cropped vines (UV), seeds of *Trifolium fragiferum*, at a 15 $g \cdot m^{-2}$ dose were sown on a 40 cm-wide strip. Sowing was done manually on late February in 2018 after an inter-vine cultivator operation. Two days later, there was a light snowfall which helped to settle the seed on the ground. In the bare rows (T), inter-vine tilling work was carried out on the same sowing preparation dates. The area was kept relatively free of vegetation tilling four dates: at the beginning of November, March, May and July in 2018, and beginning of November, March, May and end of July in 2019 and 2020 (Figure 4-3).

Figure 4-3. Soil conditions with cover crop under-vine (left) and tillage (right) on June 18, 2019.

A petiolar sampling was carried out in flowering and another when the veraison was reached for analysis of N, P, K, Ca, Mg, Na, Fe, Cu, Mn, Zn and B. For the flowering samplings, random vines were taken at along each repetition 100 petioles corresponding to the leaf opposite the first bunch of the shoot. In the veraison sampling, the petioles corresponded to the leaves opposite the second bunch of the shoot. The identified samples were kept in a cold (4-6ºC) until they were delivered to the Nasertic Laboratory for analysis.

At the beginning of August, approximately coinciding with the mid-veraison, a detailed visual assessment of the presence of adventitious vegetation was carried out. All the species present were identified, and a percentage of soil cover for each was visually quantified. To perform this quantification, a modified version of the Horsfall and Barrat (1945) scale was used, where two additional intervals were added to allow detailing when certain species appeared only once (0-0.1 %), twice (0.1-0.5 %), etc. Although this scale was initially conceived to evaluate the incidence of plant diseases, it is also very useful for cover crop diversity evaluation, as it allows reporting with greater detail at both ends in the scale, i.e., scarce and very frequent species.

The effect of cover crop on plant water status was estimated through the measurement of midday (11 am to 1 pm) stem water potential (Ψm) between early July and harvest. Determinations were carried out on four healthy leaves per replicate, each one in different vines, which had been bagged 1.5 hours prior to measurement using ziplock-bags covered with a metallic high-density polyethylene reflective film (SonocoRF, Sonoco Products Co., Hartsville, South Carolina, USA). Measurements were carried out with a Scholander pressure chamber (P3000, Soil Moisture Corp., Santa Barbara, CA, USA). Sampling and measurements were performed according to Turner and Long (1980). Additionally, a 100-berry sample at harvest from each replicate was collected. The harvest was September 24, 2018, September 5, 2019, and September 2, 2020, according to the criteria of the winery. To determine the carbon isotope ratio ($\delta^{13}C$) using an Elemental analyzer (NC2500, Carlo Erba Reagents, Rodano, Italy) coupled to an Isotopic Mass Spectrometer (Thermoquest Delta Plus, ThermoFinnigan, Bremen, Germany). Carbon isotope ratio allows for an integration of the water deficit experienced by grapevines along the ripening into a single value (Santesteban *et al.,* 2015).

The agronomical implications of the under-vine cover crop were evaluated by determining yield components and grape composition at harvest, which was performed on the same date both in UV and T vines. The yield was determined by counting and weighing all the clusters produced in the 20 vines at each replicate, whereas grape composition was determined in one 100-berry sample per replicate. The berry samples were formed by 5 berries per vine, picked from 1 cluster per vine and taken from each part in the cluster (shoulder, middle, and tip; outside and inside). Samples were carried to the lab at low temperature (4-6 ºC) for analysis, weighed to determine mean berry weight (BW), and immediately homogenized with an LMU 9018 American blender (Man, México) for 10 s at full speed part of this homogenate (100 g approx.) was filtered with a gauze tissue and used to measure total soluble solids (TSS), pH, titratable acidity (TA), malic (MalA) concentrations and yeast assimilable nitrogen (YAN). All measures were made with a Miura 200 (TDI analysers, Gavá-Barcelona). The phenolic parameters were measured according to Cromoenos® method. This method consists of a fast extraction of phenolics following a procedure and reagents provided by Bioenos company (www.bioenos.com) and has been proved to predict wine colour and phenolic composition similarly or even better than other classical procedures (Kontoudakis *et al.,* 2010). In December, the 20 selected vines were pruned, counting the number of shoots and the total weight of shoots per vine.

In the middle of May in 2019 and 2020, 10 clusters are marked per repetition, in which the length of the rachis and the length of the shoulders of the clusters were measured. The cluster was taken from the arm that faces the inside of the plot when coming from the north, the second spur, the first shoot (the lowest), the first cluster (the lowest). These same clusters were collected and identified once they had set and the number of berries they presented was counted in the cabinet. These same berries were used to count the number of seeds present per berry on 100 of them. In turn, and on the same day as the bunch marking, 20 random cluster of different sizes were taken, on them in the cabinet the length of the spines and the shoulders are measured and the glomeruli that appear on the shoulders and in the rachis. With this, the rachis length / glomeruli relationship was obtained, which was used to estimate the number of glomeruli that the marked clusters will have in the viness. After controlling the number of berries set per cluster and knowing the estimate of the glomeruli of each cluster, the fruit set rate was obtained.

Data were compared through t-tests, all analyses being performed using the R computing environment (R Development Core Team, 2016).

4.3. Results

In 2018, 21 plant species were identified on UV treatment, covering 82 % of the under-vine surface, though just nine reached representativeness of more than 2 % of the total surface. The clover that had been sown occupied only 26 % of the surface in this first year. In 2019, 25 plant species were identified, and in this second year, clover covered around 70 % of the surface, with eight species with more than 2 % of the Surface. In 2020, 36 plant species were identified on UV covering 100% surface. Only 4 species occupied more than 2% of the total surface. In T vines, in 2018, 19 plant species could be identified, occupying 66.2 % of the surface, and only four represented more than 2 %. The presence of *Convolvulus arvensis* covered the majority of the surface at 40 %. In 2019, 23 weeds were identified, with only three occupying more than 2 %, and once again, *Convolvulus arvensis* covered the greatest surface area, reaching 27.5 %. In 2020, 37 weeds were identified, covering 51% of surface, and with 9 species of more than 2% of surface. In this last year the presece of *C. arvensis* was reduced (Table 4-2).

Table 4-2. Surface covered with adventitious vegetation species under the vines for each treatment and season.

Species	2018		2019		2020	
	UV	T	UV	T	UV	T
Trifolium fragiferum	26		67.5		85	
Convolvulus arvensis	27.5	40	10.5	27.5		4.5
Aster squamatus	5		3.25		4	3
Chenopodium album	5	5				
Sonchus oleraceus	5	7.5	1,5			
Amaranthus retroflexus	3	5				
Coniza sp.	3		5			4
Salsola kali	3					
Stellaria media	2					8
Picris echioides			3,25			
Lactuca serriola			3.25		2,5	
Picnomon acarna			2.5			
Rubia peregrina				5		6
Setaria viridis				2,5		
Crepis foetida			3.25			6
Bromus rubens					2,1	2,5
Kickixia elatine						6
Silene nocturna						4.1
Total	79.5	57.5	100	35	93.6	44.1

Only species with more than 2 % presence are reported

The nutritional levels in the plant according to petiolar analyzes do not show any difference between treatments for the 2018 and 2020 season. Only in 2019, there was a 5% decrease in N in veraison in UV.

The presence of the cover crop under the vines resulted in differences in stem water potential for some of the dates of measurement. The greatest impact was observed in August for all seasons. UV plants showed lower water potential (Figure 4-4). Nevertheless, the water deficit was not severe at any moment due to the contribution of irrigation. The carbon isotope ratio

($\delta^{13}C$) did not show differences between treatments in 2018 and 2019. Only in 2020 existed significant differences in $\delta^{13}C$ (Table 4-3).

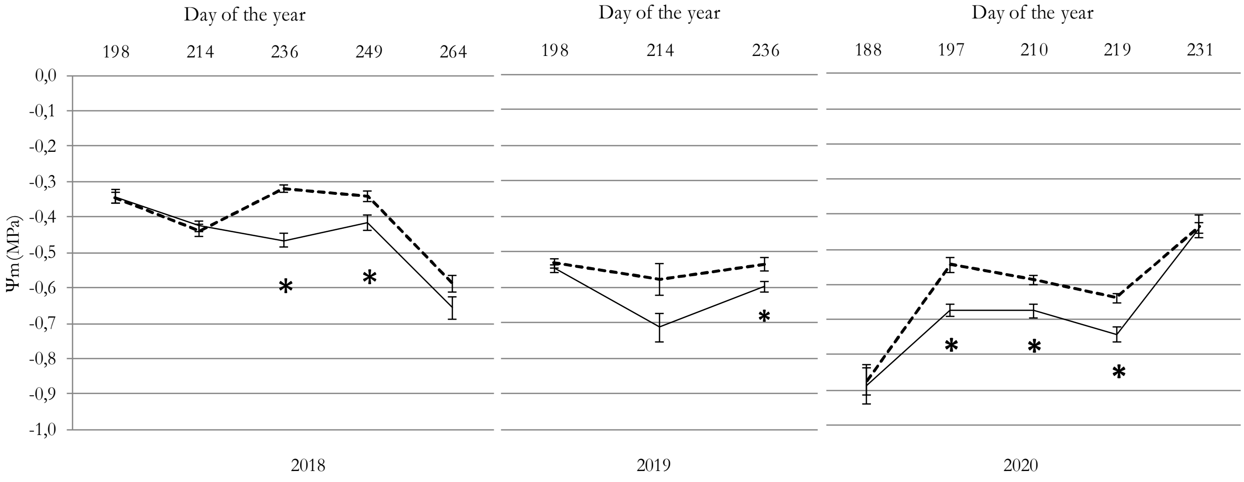

Figure 4-4. Effect of under-vine cover crop on the evolution of midday stem water potential (Ψm).

Vertical bars for each date and treatment correspond to the standard error, n = 5, and significant differences at $p < 0.05$ have been marked with *.

Table 4-3. Effect of the cover crop under-vine on the carbon isotope ratio ($\delta^{13}C$).

Year	**Treatment**	**$\delta\ ^{13}C$ (‰)**
	UV	-26.87
2018	T	-26.85
	p	*0.838*
	UV	-27.11
2019	T	-27.38
	p	0.058
	UV	-25.86
2020	T	-26.44
	p	***<0.001***

The fruit set rate presented a reduction of 14 % in UV, being significant in 2019. The number of seeds per berry did not show differences between the treatments, with one or two seeds per berry as the most repeated values (Table 4-4).

The use of *T. fragiferum* as under-vine cover did not impact the yield components and the vegetative development was also unaffected by soil management strategy, except for a slight difference in shoot number in the second season (Table 4-5). No differences were found between treatments for grape composition, although a trend towards higher levels of phenolic maturity parameters was observed for UV vines (Table 4-6).

Table 4-4. Fruit set rate and number of seed per berry

Year	Treatment	Fruit set rate (%)	Seeds·Berry^{-1} (%)			
			1	2	3	4
2019	UV	38.0	55.4	36.3	7.4	0.6
	T	44.1	51.9	40.4	7.6	0.2
	p	***0.04***	*0.425*	*0.215*	*0.953*	*0.244*
2020	UV	47.7	44.3	46.1	8.2	1.4
	T	55.8	42.2	46.3	11.1	0.4
	p	*0.134*	*0.700*	*0.960*	*0.300*	*0.240*

Table 4-5. Effect of under-vine cover crop on yield components, pruning wood components and Ravaz Index for each season.

P-values < 0.05 have been highlighted in bold.

Year	Treatment	Cluster no.	Yield (kg vine^{-1})	Cluster weigth (g)	Berry weight (g)	Shoot no.	Pruning wood weight (kg·vine^{-1})	Ravaz Index
2018	UV	20.2	2.8	139	1,35	11.6	0.446	6.96
	T	20.1	2.9	145	1,49	11.9	0.431	6.87
	p	*0.838*	*0.554*	*0.41*	*0,12*	*0.637*	*0.646*	*0.916*
2019	UV	19.7	2.01	102.1	1.04	12.04	0.270	8
	T	20.7	2.2	105.8	1.04	13.18	0.295	7.8
	p	*0.349*	*0.415*	*0.499*	*0.898*	***0.017***	*0.213*	*0.813*
2020	UV	18.5	2.1	115.2	1.14	12.91	0.271	7.8
	T	18.9	2.4	128.4	1.15	13.3	0.304	8.1
	p	*0.675*	*0.249*	*0.088*	*0.848*	*0.390*	*0.071*	*0.709*

Table 4-6. Effect of under-vine cover crop on berry composition: Total Solid Soluble (TSS), Total Acidity (TA), Malic Acidity (MalA), Yeast Assimilable Nitrogen (YAN) and on phenolic parameters of berry for each season.

Year	Treatment	TSS (°Brix)	TA (g TarA·L^{-1})	pH	MalA (g·L^{-1})	YAN (mg·L^{-1})	Phenolic maturity index	total polyphenol index	Anthocyanin berries (mg·L^{-1})	Tannins berries (mg·L^{-1})
2018	UV	15.3	5.5	3.27	0.58	128	1.31	42.67	2209	1151
	T	15.2	5.54	3.26	0.46	102.6	1.38	41.41	2031	1120
	p	*0.443*	*0.731*	*0.61*	*0.372*	*0.323*	*0.382*	*0.086*	*0.237*	*0.085*
2019	UV	14.4	6.62	3.21	0.66	100	1.602	54.1	2275.3	1429
	T	14.4	6.68	3.20	0.62	97.2	1.653	51.02	2078	1354.1
	p	*0.998*	*0.707*	*0.49*	*0.587*	*0.533*	*0.741*	*0.463*	*0.16*	*0.463*
2020	UV	14.1	6.78	3.21	0.58	124	1.69	56.7	2257	1492
	T	14.4	6.68	3.20	0.58	109.6	1.68	56.25	2236	1481
	p	*0.256*	*0.419*	*0.77*	*1*	*0.205*	*0.972*	*0.752*	*0.864*	*0.757*

4.4. Discussion

Sowing *T. fragiferum* clover under the vines (UV) resulted in a progressive increase in its presence, with a larger covered area in the second year of growth. McGourty *et al.* (2008) reported a similar increase over time with subterranean clover. Regarding the presence of adventitious vegetation, this was similar both seasons for UV. In T treatment, the conventional management of the soil through mechanical tilling under the vines did not provide the complete elimination of adventitious vegetation, as growers tolerate certain presence before repeating tillage. Mechanical tillage proved to favour the presence of some summer species such as *Convolvulus arvensi*, a species that becomes dominant in summer in many vineyards, as reported in Steinmaus *et al.* (2008).

The water potential was somewhat lower when the cover crop was used, especially after veraison, both treatments being in general under light to moderate water deficit conditions, and just a little more stressful in the year 2020 (Carbonneau and Ojeda, 2013). In one studies using a legume cover (*Lotus corniculatus*) -in British Columbia- lower leaf water potential was also observed in the vineyard where crops under the vines were used (Vukicevich *et al.,* 2019). However, in other studies where coverage under the vineyard was used, no differences appeared, maybe due to the fact the climate was more humid (Coniberti *et al.,* 2018a; Karl *et al.,* 2016b) or, if there were variations, they did not occur for all the cover-crop species used (Chou and Vanden Heuvel, 2018a). The fact our experiment was performed in an irrigated vineyard has undoubtedly favoured clover survival and decreased differences between treatments. McGourty *et al.* (2008) detected greater water deficit whit a legume cover crop when water potential was measured just before irrigation, but differences attenuated after irrigation had been applied. The carbon isotope ratio that indicates the water stress accumulated in the plant throughout the season did not show the specific differences observed for water potential in August for 2018 and 2019, and confirms as weak the water stress suffered by the vineyard of both seasons, and it is significant lower in 2020 (Brillante *et al.,* 2020; Santesteban *et al.,* 2015).

The yield was not affected in the three years of the study. This effect does not agree with what was observed by Hickey *et al.* (2016) with a *Festuca rubra* cover in Virginia, or by Karl *et al.* (2016b) with *Trifolium repens* and native vegetation in Finger Lake. Conversely, Chou and Vanden Heuvel (2018a) in the Finger Lake district, did not observe yield variations, and even in one of the seasons, they obtained higher yield with a spontaneous cover crop compared to glyphosate-maintained bare soil. Penfold *et al.* (2018), in Australia, obtained increased yield in wetter years with a cover crop that combined *Trifolium fragiferum* and *Festuca ovina*. In our case, with warmer climatic conditions, the lack of variations in yield may be due to the use of irrigation, which diminishes the competition between the cover crop species and the vines, as indicated by Steenwerth *et al.* (2013) with the using of cover crops in an alleyway in California.

Berry composition was nearly unaffected, which agrees with the overall effects observed for water status, and yield, and in accordance to the results observed for TSS and phenolics in Hickey *et al.* (2016). There is only a certain trend to observe higher phenolics content in UV treatment, which needs to be confirmed. Concerning nitrogen, some additional effect could be expected due to the fact an N-fixing legume was being used. Nevertheless, YAN was not increased due to the presence of the cover crop, agreeing with the results by Chou and Vanden Heuvel (2018a), who did not find variations with respect to this parameter, nor with the use of legumes or grasses with respect to management with herbicide or tillage. Sulas *et al.* (2017) reported that a cover of *Medicago polymorpha* in an alleyway, despite being able to assimilate 125 kgN·ha^{-1}·year^{-1}, it just contributed 10 % to the vineyard N use. Under our experimental conditions, a longer-term cover crop establishment is probably required to generate noticeable effects of legume N-fixation on the crop.

Last, concerning vegetative development, the pruning weight, although not significantly reduced, showed a trend to decrease in the second and third year due to a reduction in the number of shoots per vine. This decrease in the pruning wood weight is usually the most remarkable effect of the use of cover crops. Vukicevich *et al.* (2019), with a cover of *Lotus corniculatus* in British Columbia, experienced decreased vine growth. There was also a decrease of 26 % with the use of a *Fescue rubra* cover in Virginia (Hickey *et al.,* 2016) or 49 % with *Trifolium repens* in Finger Lake (Karl *et al.,* 2016b). These vigour reductions could help to reduce thinning (Hickey *et al.,* 2016), or to decrease the incidence of fungal diseases (Valdés-Gómez *et al.,* 2008).

4.5. Conclusions

The use of *T. fragiferum* as an under-vine cover crop has proved to be a potentially useful tool for soil management in Mediterranean conditions, causing nearly no changes in vine performance, and competing well against the installation of undesired adventitious species. This first experiment under such conditions shows that management free of herbicides and mechanical tilling is feasible, and further research is required to fully explore the potentiality and limitations of under the vine cover-cropping.

5. CUBIERTAS VEGETALES BAJO LA LÍNEA DE CULTIVO: IMPACTO EN LA ELABORACIÓN DE VINOS Y SU CALIDAD

5.1. Introducción

A diferencia de otros cultivos frutales, donde la fruta puede considerarse por sí misma el producto final que mayoritariamente llega a mercado, en la viticultura, el producto final que llega al consumidor no es la uva sino el vino, por lo que toda modificación que se realice en alguno de los parámetros productivos de la uva puede influenciar en el producto final y debería ser tenido en cuenta. De manera general, los diversos factores que afectan al vino podrían agruparse en cuatro grandes bloques: el medio donde crece el cultivo (suelo y clima), el material vegetal (variedad y clon de la vinífera y del portainjerto), las prácticas culturales (sistemas de conducción, riegos, abonados…), y las prácticas enológicas (tipos de levaduras, remontados, etc.).

El manejo del suelo es una de las prácticas culturales que más está cambiando en los últimos tiempos, pudiendo influir estos cambios en la composición de los vinos. Se sabe que las cubiertas vegetales mayoritariamente producen una reducción de crecimiento vegetativo en la planta, principalmente por la competencia que ejercen ésta por los recursos hídricos y nutricionales (Abad *et al.*, 2021b). Esta reducción del crecimiento vegetativo repercute de manera directa en la producción de carbohidratos, la cual tiene una repercusión negativa en la acumulación de sólidos solubles en las bayas (Basile *et al.*, 2015; Parker *et al.*, 2016b). Así mismo, una mayor exposición de las bayas incrementa la temperatura del racimo, lo que puede producir un incremento del contenido de sólidos solubles, o una reducción de la acidez o de los antocianos (Coombe, 1987). Los aromas también pueden verse alterados por variaciones en la temperatura. Así, la concentración del 2-isobutil-3-metoxipirazina (responsable de los aromas a pimiento verde) disminuye con el incremento de temperatura, al igual que le ocurre al linalool. Por el contrario, la producción de aromas herbáceos en los vinos se incrementa en viñedos con elevados niveles de vegetación. La competencia por los recursos hídricos puede producir un incremento del contenido de taninos y antocianos. Los precursores de tioles se reducen cuando se produce un estrés hídrico en la planta, mientras que se pueden ver incrementados cuando el déficit hídrico es moderado (Van Leeuwen y Darriet, 2016). A pesar de lo anterior, en la mayoría de los casos en los que se emplea una cubierta vegetal no se producen disminuciones en el contenido de sólidos solubles, ni en los parámetros de acidez total y pH. Solo en un tercio de las ocasiones en las que se emplea una cubierta vegetal, el tamaño de la baya se ve disminuido, lo que ayudaría a incrementar la concentración de polifenoles en los mostos al modificar la relación pulpa/hollejo (Abad *et al.*, 2021b).

Otro de los aspectos por los que la presencia de una cubierta vegetal puede modificar las características del vino es su influencia sobre el nitrógeno disponible en el mosto para las levaduras. A esta fracción se le suele denominar nitrógeno fácilmente asimilable (NFA), y de su nivel dependerán, no solo la duración del proceso fermentativo, sino también la formación de precursores aromáticos en los vinos. Como se señaló en el capítulo 1 de esta tesis, cuando se emplea una cubierta vegetal el NFA se ve reducido en la mitad de las ocasiones, y únicamente se incrementa su contenido en un 11% de las ocasiones, coincidiendo en estos casos con cubiertas de leguminosas. (Abad *et al.*, 2021b). Un bajo contenido de NFA suele implicar poblaciones de levadura bajas y a un vigor de fermentación deficiente, incrementando el riesgo de fermentaciones lentas y mayor producción de tioles (p. ej. sulfuro de hidrógeno) y alcoholes superiores indeseables, y baja producción de ésteres y ácidos grasos volátiles de cadena larga. Un alto contenido de NFA en mosto conduce a un aumento de la biomasa de levaduras y una mayor producción de calor máximo debido a un mayor vigor de fermentación, con una mayor formación de acetato de etilo, ácido acético y acidez volátil (Bell y Henschke, 2005).

Así pues, se observa como varias características del vino podrían verse afectadas por las modificaciones que se realicen en el manejo del suelo. En este trabajo se ha evaluado la repercusión que el empleo de una cubierta vegetal de *T. fragiferum* bajo la línea de cultivo pude producir en los vinos obtenidos.

5.2. Metodología

Los días 24 de septiembre de 2018, 5 de septiembre de 2019 y 2 de septiembre de 2020, se realizó la vendimia de tres repeticiones para cada uno de los dos tratamientos ensayados (cubierta vegetal bajo la línea de cultivo-UV, y laboreo bajo la línea de cultivo-T) para la elaboración de vinos. La vinificación se realizó en la Bodega Experimental del Gobierno de Navarra (EVENA), en Olite. La dimensión del ensayo para la vinificación se redujo de cinco a tres repeticiones por tratamiento debido a cuestiones de logísticas. Las repeticiones seleccionadas (la 3, 4 y 5 de acuerdo al esquema incluido en el capítulo 3) fueron aquellas que presentaban un desarrollo de la cubierta más homogéneo en el primer año, manteniéndose las mismas repeticiones para los dos años siguientes. De cada una de las repeticiones se vendimiaron manualmente entre 100 y 120 kg. La uva se transportó en un tiempo breve (<2 h) a las instalaciones de EVENA, donde se realizó el despalillado y estrujado, transfiriendo la uva de cada repetición a un depósito. En este momento se añadieron levaduras (Oenoferm® Be-red, 25 $g·hL^{-1}$) y metabisulfito (6 $g·hL^{-1}$). Diariamente se bazuqueó manualmente y se tomaron medidas de temperatura y densidad de los mostos. Cuando los mostos estuvieron por debajo de 1000 $kg·m^{-3}$ de densidad se dio por terminada la fermentación alcohólica, hecho que se confirmó mediante medida de azúcares libres. Posteriormente, se prensó en prensa neumática a presión de 2 atmósferas, se corrigió el sulfuroso y se adicionaron bacterias lácticas (dosis 0,63 $g·hL^{-1}$) para asegurar el inicio de la fermentación maloláctica. Los vinos obtenidos se embotellaron tras pasar por un filtro de celulosa.

Los bajos niveles de NFA encontrados en el mosto (139-106 $mg·L^{-1}$) el primer año provocaron que las fermentaciones fueran excesivamente largas, en torno a 31 días (Figura 5-1). En años sucesivos, y para tratar de evitar estos problemas, los mostos se corrigieron, hasta alcanzar un nivel mínimo de NFA. El NFA necesario para la fermentación se calculó como $NFA_{necesario}$ = Grado probable x 16. Así pues, conociendo el NFA que presentaba la muestra, la diferencia con el $NFA_{necesario}$ indicaba el NFA que requería aportarse. Con el fin de alterar lo menos posible las muestras, se procedió a incrementar el NFA para todas las repeticiones según el menor incremento que fuera necesario en el conjunto de muestras, de manera que las diferencias en NFA que presentaban los mostos iniciales se mantuvieran en igual proporción una vez adicionados los nutrientes, pero sin comprometer la fermentación. Las correcciones se llevaron a cabo añadiendo fosfato biamónico a dosis máxima de 40 $g·hL^{-1}$, y en los casos en que las necesidades fueran superiores, dicho aporte se complementó a mitad de fermentación con nitrógeno orgánico (autolisado de levadura-FERMAID™ O). Las correcciones aportadas permitieron que en 2019 y 2020 las fermentaciones tuvieran una duración mucho más adecuada, entorno a quince días, siempre manteniéndose la temperatura entorno a los 20ºC (Figura 5-1).

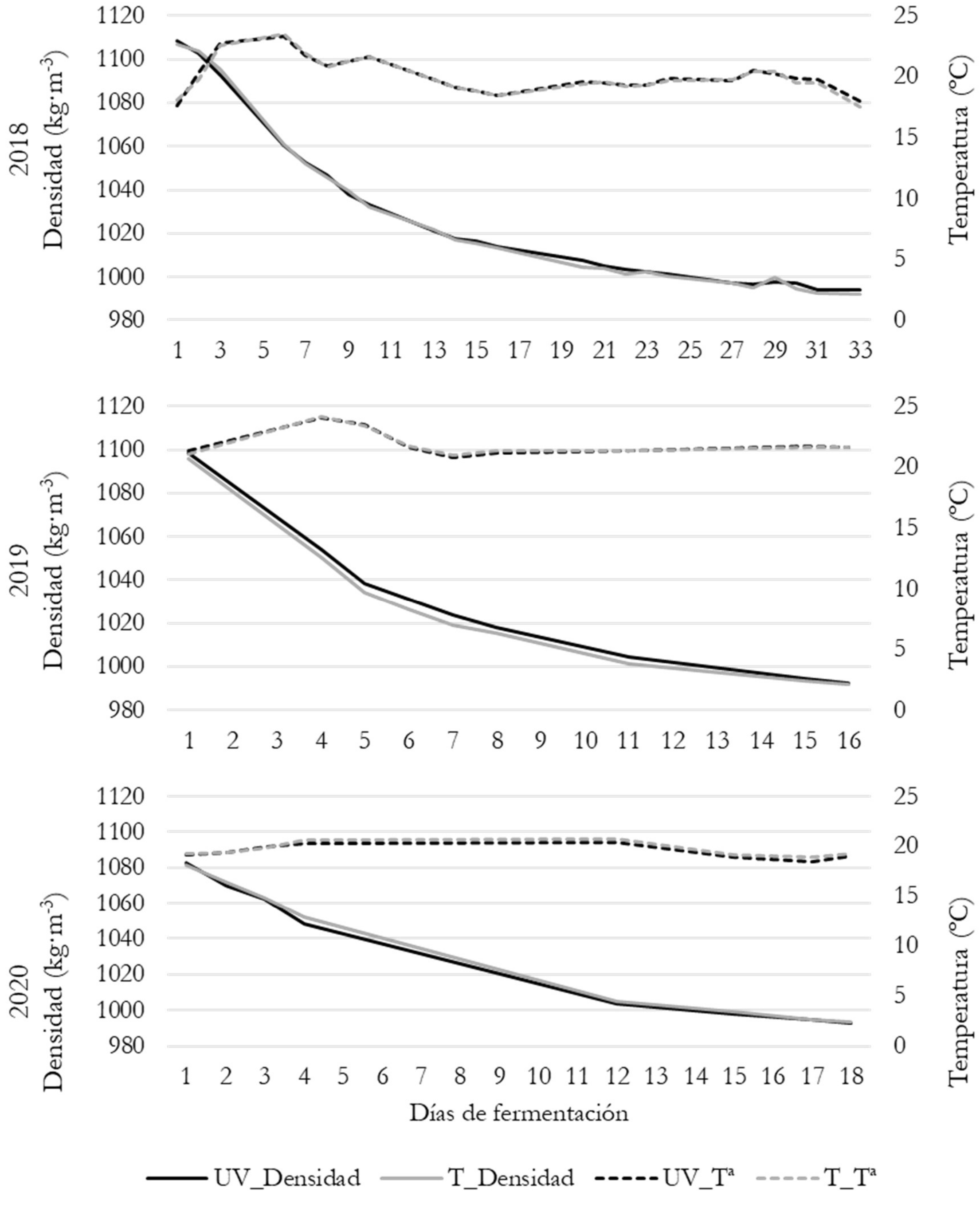

Figura 5-1. Dinámica de fermentación. Densidad media de los mostos y temperatura.

Todos los vinos fueron analizados para los parámetros de grado alcohólico adquirido (20/20 %Vol.), pH, acidez total (g·L^{-1} ác. Tartárico), Acido L-málico (g·L^{-1}), acidez volátil (g·L^{-1} ác. Acético), azúcares reductores (g·L^{-1} glucosa), Ca (mg·L^{-1}), Mg (mg·L^{-1}), K (mg·L^{-1}), densidad óptica a 420, 520 y 620 nm (Un Abs·cm^{-1}), e Índice de Polifenoles Totales (IPT) (Un Abs·cm^{-1}) en el Laboratorio Enológico de Gobierno de Navarra.

En cuanto a la evaluación sensorial, ésta se llevó a cabo empleando el método Flash-Profile (Delarue, 2015). Los jueces contaban con siete vinos, las 3 repeticiones elaboradas para cada uno de los tratamientos y una repetición de uno ellos, a fin comprobar la validez del panel de cata. Los vinos estaban identificados en el pie de la copa con un código numérico de tres cifras. Cada juez tenía ordenados los vinos de izquierda a derecha en un orden distinto, según un modelo de cuadrados latinos de Williams. Cada juez disponía de una lista preseleccionada de 17 descriptores como ayuda (Tabla 5-1). El juez podía elegir, a voluntad, los descriptores que considerase de la lista u otros que no aparecieran en ella. Para cada descriptor seleccionado debían ordenar los vinos de mayor a menor intensidad, pudiendo coincidir en un mismo nivel de intensidad más de un vino. En el Anexo I se presenta la ficha de cata a rellenar por los jueces.

Tabla 5-1. Lista de descriptores preseleccionados para la cata.

Descriptores fase visual	**Descriptores fase gustativa**
Intensidad del color	Acidez
Limpidez	Amargor
Descriptores fase olfativa	Astringencia
Aromas especiados	Franqueza
Aromas florales	Intensidad tánica
Aromas frutales	Persistencia
Aromas herbáceos	Volumen
Aromas lácteos	**Otros** (Incluir a criterio del catador)
Aromas químicos	
Finura	
Franqueza	
Intensidad del aroma	

Los vinos de las campañas 2018 y 2020 fueron catados en el mes de febrero-marzo del año siguiente. Los vinos de 2019, debido a las restricciones del Covid-19, vieron retrasada su cata hasta diciembre del año siguiente. Las catas se realizaron en las salas de catas de EVENA (Olite) y de la Universidad Pública de Navarra-UPNA (Pamplona). En conjunto, en el año 2018 participaron 15 jueces, en el 2019 participaron 23 jueces y en el 2020 participaron 20 jueces. Los catadores respondían en todos los casos a un perfil concreto, siendo personas acostumbradas a la cata de vinos y conocedores de la terminología empleada, pero no formaban parte de ningún panel entrenado. Las edades estaban comprendidas entre los 25 y los 70 años, siendo el 62 % mujeres y el 38 % hombres.

El análisis estadístico de los parámetros analíticos de los vinos se realizó mediante t-test empleando el programa R (R Development Core Team, 2016). El análisis de cata se realizó por el método de Análisis factorial múltiple (MFA) con el programa estadístico XLSTAT. Los jueces que inicialmente presentaron un valor del coeficiente RV inferior a 0,65 fueron eliminados del análisis.

5.3. Resultados y discusión

5.3.1. Parámetros químicos

Los mostos de partida para la elaboración de los vinos no presentaron diferencias entre tratamientos. Los mostos del año 2018 presentaron un grado probable superior y una acidez total inferior a los de los años 2019 y 2020. (Tabla 5-2).

Empleo de cubierta vegetal produjo un incremento del IPT de los vinos en el año 2018, manteniéndose dicha tendencia en las otras dos campañas. Los vinos del año 2018 fueron vinos con un grado probable superior, resultado de los mostos de los que procedían. La acidez total se situó en niveles de entre 7,40 y 8,50 g·L^{-1} ác. tartárico, niveles que pueden considerarse algo elevados para tratarse de vinos tintos. La acidez volátil, presentó niveles elevados en el año 2018 (0,95-0,97g·L^{-1} ác. acético) y 2020 (0,85-0,87 g·L^{-1} ác. acético) (Tabla 5-2).

Tabla 5-2. Parámetros de los mostos previos al inicio de la fermentación.
UV: Cubierta bajo la línea; T: Laboreo; NFA: Nitrógeno fácilmente asimilable

Año	Tratamiento	Grado Probable (% vol.)	pH	Acidez total (g·L^{-1} ác. tartárico)	Ácido málico (g·L^{-1})	NFA (mg·L^{-1})
2018	UV	15,1	3,20	4,43	0,500	138,7
	T	15,0	3,16	4,67	0,633	106,0
	p	*0,580*	*0,354*	*0,378*	*0,469*	*0,331*
2019	UV	14,4	3,14	5,43	0,800	104,0
	T	13,9	3,10	6,00	0,900	94,3
	p	*0,274*	*0,202*	*0,123*	*0,288*	*0,431*
2020	UV	14,3	3,16	5,83	0,600	118,3
	T	13,8	3,14	6,03	0,733	114,3
	p	*0,282*	*0,713*	*0,566*	*0,275*	*0,684*

Estos resultados muestran que el empleo de la cubierta vegetal bajo la línea de cultivo del viñedo no produjo cambios en los vinos en comparación con un manejo con laboreo. Este hecho estaría en línea con la falta de diferencias en parámetros productivos y de los mostos resultantes (Capítulo 4).Estos resultados coinciden con los de Penfold *et al.* (2018) quienes tampoco observaron variaciones en los parámetros de los mostos al emplear una cubierta bajo la línea de cultivo de *T. fragiferum* + *F. ovina*.

El empleo de la cubierta de una especie leguminosa no ha conseguido en tres años aumentar los niveles de NFA como habría sido esperable. De manera similar, Penfold *et al.* (2018) solo observaron incrementos en el NFA con una cubierta de *T. fragiferum* + *F. ovina* en una de las cuatro localizaciones de estudio, en un único año. Quizás el espacio limitado que ocupa la cubierta (un 13 % de la superficie), y el hecho de que la cubierta suele ser quien se beneficia directamente de la fijación de nitrógeno que genera, no han permitido un aporte de N que se refleje en el viñedo. Es probable que se requiera de más tiempo para que los aportes de N sean apreciables sobre el viñedo, como apunta Sulas *et al.* (2017). El bajo nivel de NFA en los mostos ha condicionado la fermentación, lo que ha repercutido en la elaboración de los vinos.

5.3.2. Análisis sensorial

Con carácter previo al análisis de los resultados, se procedió a depurar los resultados, eliminando a los catadores que no cumplían con un coeficiente RV inferior a 0,65 al estudiar los datos en un primer momento. Este análisis llevó a prescindir de dos catadores en 2018, cuatro en 2019 y otros cuatro en 2020, de modo que quedaron 13, 19 y 16 juicios válidos en cada uno de los tres años. La representación de los resultados de la cata mediante análisis de componentes principales permite comprobar que las muestras repetidas cada año se presentan juntas, dentro de los mismos cuadrantes, lo que confirmaría la validez de los juicios de los catadores mantenidos (Figura 5-2. Superior).

En el año 2018, los componentes F1 y F2 representaron el 62 % de la variabilidad (F1 45 % y F2 17 %). Valores negativos en F1 se asociaron a aromas químicos, astringencia o amargor; mientras que valores positivos en este eje se asociaron a limpidez, franqueza olfativa, aromas frutales y lácteos. En la componente F2, los valores negativos estaban vinculados a aromas frutales y acidez; mientras que los positivos se relacionaban más con una mayor intensidad colorante y astringencia. En 2019, las componentes F1 y F2 representaron una proporción mucho más reducida de la variabilidad total (un 47 %, F1 27,5 % y F2 19,5 %). Los valores negativos para la F1 se asociaban con una mayor intensidad colorante, aromas químicos, astringencia, amargor y acidez; mientras que los valores positivos estaban vinculados con la franqueza e intensidad olfativa, aromas frutales y especiados. En la componente F2 los valores negativos se correspondían con vinos de más intensidad de color y aromas especiados, mientras que los valores positivos en esta componente se asociaban con aromas herbáceos y mayor acidez. Por último, en el año 2020, las componentes F1 y F2 representaron el 48 % (F1 25 % y F2 23 %), de modo que los valores negativos de la componente F1 se correspondían con aromas químicos y herbáceos, astringencia y acidez; y los positivos con franqueza olfativa, aromas frutales y florales, y franqueza gustativa. En cuanto a la componente F2, los valores negativos se correspondían con una percepción elevada de la acidez, astringencia y persistencia; mientras que en su parte positiva se encontrarían la intensidad de color y aromática, y aromas florales y frutales (Figura 5-2. Inferior).

Únicamente en el año 2020 existió un mayor agrupamiento de los vinos por tratamientos, presentando los vinos con cubierta vegetal (UV) un mayor peso en la componente positiva de F1, vinos más frutales-florales; mientras que los manejados bajo laboreo (T), irían en la dirección opuesta, vinos con peores componentes aromáticas. En cambio, tanto en el año 2018 como en el año 2019 no se observó agrupamiento en los vinos. En el año 2018, mientras dos de los vinos con cubierta y uno de los vinos de laboreo presentaron valores positivos de la componente F1, la otra repetición con cubierta se agrupa con los otros dos vinos de laboreo. En el año 2019, un vino con cubierta y un vino de laboreo presentan un mayor peso en la componente F1 positiva, frente a los otros que se agruparían en la parte negativa (Figura 5.2. Superior).

A la vista de los resultados presentados, puede afirmarse que la cata no ha permitido separar las dos estrategias de manejo de suelo hasta el tercer año. Este tercer año, si bien se aprecia una cierta tendencia, las diferencias no son tampoco muy notables. Esta falta de diferenciación se corresponde con el comportamiento observado en campo y con la ausencia de diferencias relevantes entre los mostos iniciales. En este trabajo, aunque el empleo de la cubierta produjo una alteración en varios parámetros de suelo (Capítulo 6), el tratamiento de cubierta apenas modificó las condiciones hídricas del cultivo debido al apoyo del riego durante la campaña estival (Apartado 4. 3- Figura 4-4 y Tabla 4-3), lo que podría ayudar a explicar esa homogeneidad de los parámetros de composición de los mostos. Estos resultados estarían en la línea de los observados por Penfold *et al.* (2018), los cuales, en condiciones de clima mediterráneo con riego, no observaron separación entre vinos manejados con una cubierta de *T. fragiferum* + *F. ovina* y vinos manejados con herbicida bajo la línea de cultivo.

En general, y partiendo de la premisa de que las diferencias entre tratamientos han sido limitadas, sí que se observa una tendencia a que los vinos procedentes de las uvas producidas por las cepas con cubiertas presentaran perfiles aromáticos algo más elevados, con un mayor peso de las componentes florales y frutales. Este hecho podría deberse a una diferenciación en la composición de los aminoácidos de los mostos, si bien los defectos aromáticos presentados en todos los vinos parecen estar relacionados con el bajo nivel de NFA de los mostos (Torrea *et al.*, 2011).

El método de cata empleado (Flash-Profile) permitió clasificar los vinos entre sí de manera sencilla, si bien habría resultado interesante acompañar esta cata de una cata descriptiva que hubiese permitido, no solo comparar los vinos entre sí, sino observar las particularidades que presentaba cada vino. Así, los jueces detectaron durante la cata aspectos aromáticos y gustativos negativos en los vinos que no quedan reflejados en la comparación entre vinos que proporciona el método. Otro aspecto que no queda correctamente resuelto es la valoración de los vinos para un mismo parámetro. Según el ejemplo que se muestra en la Figura 5-3, para un mismo atributo y con una misma ordenación entre sí de los vinos, en la clasificación superior se marca un distanciamiento importante entre los vinos A-C, y C-B; mientras que en la parte inferior el conjunto de los vinos presentaría una diferenciación para dichos atributos muy reducida. Pese a ello, los valores que se emplean para el análisis factorial múltiple (MFA) considerará ambas catas iguales.

Tabla 5-3. Parámetros analíticos de los vinos terminados.

Año	**Tratamiento**	**Grado alcohólico adquirido (%vol)**	**pH**	**Acidez total (g/l ác. Tartárico)**	**Ácido málico (g/l)**	**Acidez volatil (g/l ác. acetico)**	**Ca (mg/l)**	**Mg (mg/l)**	**K (mg/l)**	**420nm**	**520 nm**	**620 nm**	**IPT (Abs/cm)**
2018	UV	15,3	3,12	7,80	0,400	0,950	64,3	93,0	447,3	3,12	5,97	0,914	39,0
	L	15,1	3,07	8,23	0,433	0,973	64,7	101,0	403,3	2,88	5,46	0,965	35,3
	p	*0,375*	*0,259*	*0,217*	*0,374*	*0,450*	*0,879*	*0,166*	*0,107*	*0,355*	*0,284*	*0,816*	*0,051*
2019	UV	14,1	3,11	7,43	0,363	0,457	96,7	99,3	508,3	3,61	7,13	1,448	38,0
	L	13,9	3,12	7,40	0,260	0,450	90,0	105,7	497,0	3,48	6,89	1,402	36,7
	p	*0,451*	*0,920*	*0,956*	*0,420*	*0,907*	*0,449*	*0,184*	*0,772*	*0,771*	*0,765*	*0,893*	*0,676*
2020	UV	14,1	2,99	8,30	0,533	0,873	75,3	104,3	344,0	3,40	7,02	1,105	39,3
	L	13,5	2,97	8,50	0,467	0,850	78,7	111,7	302,3	3,28	6,70	1,163	36,0
	p	*0,085*	*0,509*	*0,527*	*0,230*	*0,677*	*0,301*	*0,471*	*0,149*	*0,643*	*0,543*	*0,706*	*0,100*

UV: Cubierta bajo la línea; T: Laboreo; IPT: Índice de polifenoles totales

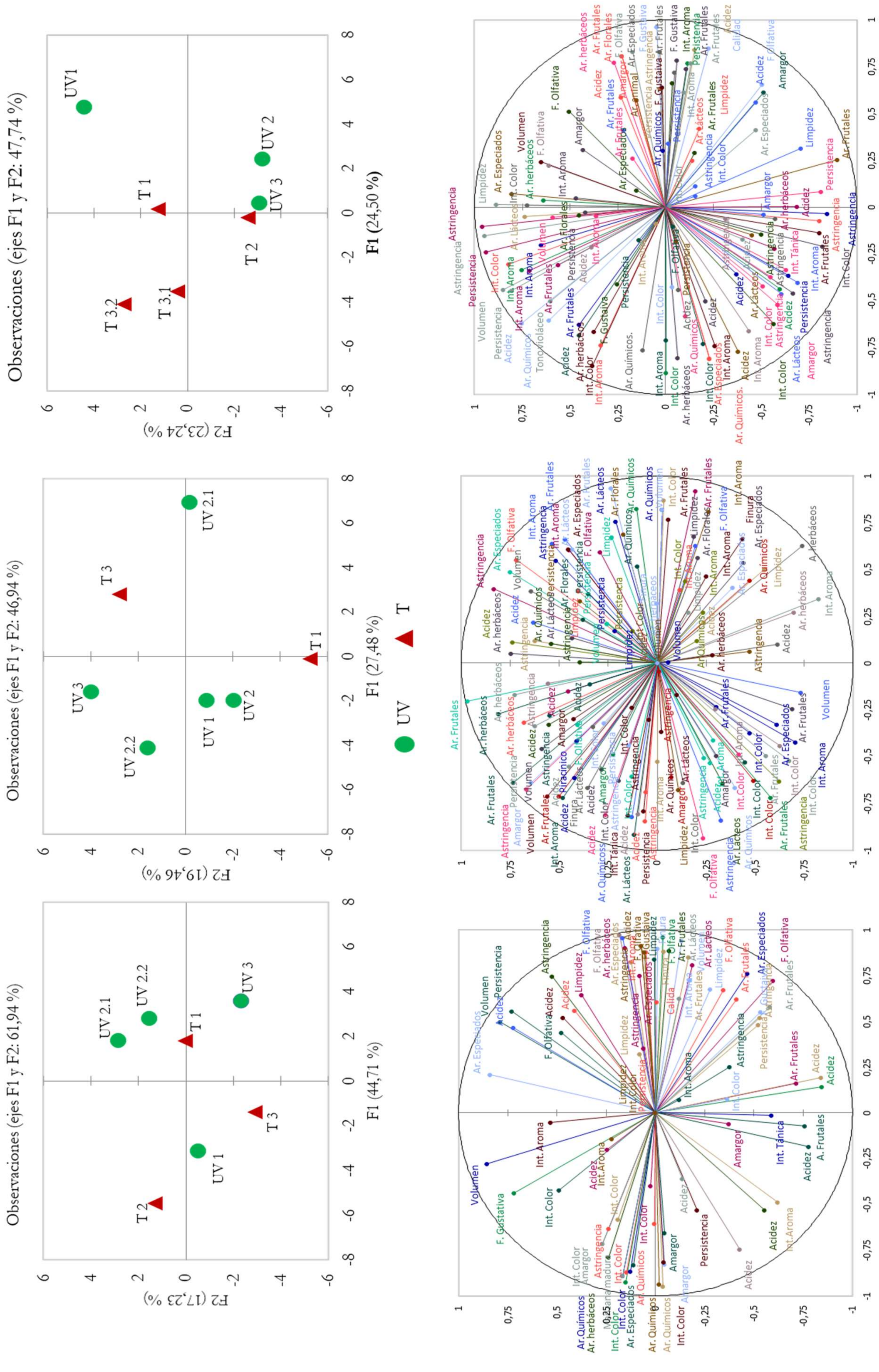

Figura 5-2. Análisis de factor múltiple de los vinos. Ordenación de los vinos (superior) y composición de cada factor según los distintos catadores (inferior).

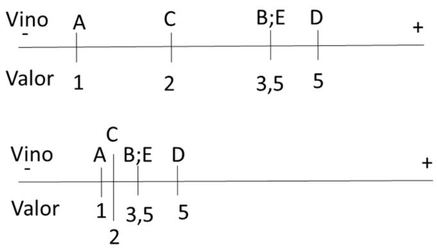

Figura 5-3. Ejemplo de valoración de un vino según la ficha de cata Flash-Profile. Superior: vinos valorados para un atributo más distantes entre sí; Inferior: vinos para un atributo con menores diferencias entre sí; Letras: vinos; Números: Valor que recibe cada vino en función de la posición que ocupa con respecto al resto de vinos de la cata.

Lo anteriormente expuesto deja ver las limitaciones que, tanto la elaboración, como la cata de vinos, pueden presentar. En el proceso de elaboración, riesgos como paradas de fermentación, contaminaciones bacterianas u oxidaciones pueden condicionar los vinos resultantes. En el proceso de cata, la metodología empleada, aun tratando de usar aquella que más se ajuste al objetivo buscado, puede conllevar la pérdida de cierta información que permita entender otras cuestiones, además de estar sujeta siempre a una valoración subjetiva que, aunque se trate de reducir siempre permanece (Khalafyan *et al.*, 2021). Analizar otros parámetros en los mostos como aminoácidos o precursores aromáticos permitiría estimar el comportamiento de los mostos en los procesos de vinificación sin asumir los riesgos de la misma o la subjetividad de las catas (Bell y Henschke, 2005; Guitart *et al*, 1999).

5.4. Conclusiones

El empleo de una cubierta vegetal de *T. fragiferum* bajo la línea de cultivo en condiciones de clima mediterráneo y con soporte de riego no produce vinos diferenciados respecto de un manejo con laboreo, si bien parece que la tendencia al incremento del NFA en los mostos podría contribuir a mejorar la componente aromática de los vinos.

6. UNDER-VINE COVER CROPS: IMPACT ON PHYSICAL AND BIOLOGICAL SOIL PROPRIETIES

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6.1. Introduction

Cover crops are nowadays one of the most appealing soil management options in viticulture. Until some decades ago, the use of cover crops in vineyards was nearly restricted to rainy regions, but their use is currently experiencing an increasing trend in drier regions. For instance, in Spain, the country with the biggest vineyard acreage in the world, the surface vineyards that use cover crops increased nearly a 15 % between 2009 and 2019, whereas the area of sown covers increased ten-fold for the same period (MAPA, 2009, 2019). Although the acreage of vineyards using cover crops in Mediterranean countries is still small (≈50,600 ha in Spain, only 5.2% of the total acreage), these data show a change in soil management rationale. Cover crops are frequently a good choice from an environmental point of view, since they generally increase soil organic carbon (SOC), improve water infiltration and aggregate stability, reduce soil erosion and greenhouse gas emissions, and increase biodiversity in vineyards (Abad *et al.*, 2021a). Nevertheless, as vines and cover crops coexist in the same space, they can compete for nutrients and water at certain moments in the season, which can affect vineyard performance. Such competition can result in relevant changes from the grower's point of view, e.g. the cover crop can modify shoot growth, bud fertility, fruit set, berry development, yield, and grape composition (Abad *et al.*, 2021b). These pros and cons need to be examined on a case-by-case basis, in order to determine which cover crop, if any, is convenient for each specific vineyard.

Since cover crop benefits frequently outnumber their potential drawbacks (Steenwerth and Guerra, 2012), it is becoming more and more common to establish cover crops in the alleys between the rows. Nonetheless, the space under the vines themselves (i.e., the rows) is normally kept free of vegetation (weed-free) through mechanical tillage and/or herbicide application, despite both methods may present several adverse consequences. On the one hand, many herbicides are strongly being questioned for their potential environmental impact and, in consequence, legal constraints and society´s disapproval to their use are currently increasing (AFP, 2019). Additionally, for the wine sector itself, the use of herbicides can have a negative impact on soil bacteria and mycorrhizae, plant nutritional status, and wine fermentation (Chou *et al.*, 2018; Donnini *et al.*, 2016; Morozova *et al.*, 2017; Zaller *et al.*, 2018). On the other hand, the mechanical tillage of the row area may also have some drawbacks: it can increase economic costs, affect soil structure, accelerate the degradation of organic matter, alter soil microbial communities, decrease water infiltration, and increase soil susceptibility to erosion (Ben-Salem *et al.*, 2018; Ruiz-Colmenero *et al.*, 2011; Virto *et al.*, 2012).

The establishment of low-competing under-vine cover crops constitutes an interesting alternative to herbicide application and mechanical tillage (Jordan *et al.*, 2016; Karl *et al.*, 2016b) which has been, nevertheless, scarcely studied (Chou and Vanden Heuvel, 2018; Coniberti *et al.*, 2018a; Hickey *et al.*, 2016; Jordan *et al.*, 2016; Karl *et al.*, 2016b; Penfold *et al.*, 2019). In particular, to the best of our knowledge, only the work of Penfold *et al.* (2019) in Australia has evaluated this soil management practice (i.e., under-vine cover crop) under Mediterranean climate conditions.

In this context, we carried out a field experiment to evaluate the implementation of under-vine cover crops as a feasible and sustainable management option for vineyards located in Mediterranean areas, with special emphasis on its effects on soil quality (the agronomic implications of this experiment have already been reported in Abad *et al.*, 2020). Despite the difficulty of providing a consensus definition of soil quality, this concept is acknowledged as critical for ensuring the sustainability of the terrestrial environment and the biosphere (Bastida *et al.*, 2008), and, hence, needs to be carefully considered when evaluating the implications of agricultural practices. FAO has recently defined soil quality as "*the ability of the soil to sustain the productivity, diversity, and environmental services of terrestrial ecosystems*" (ITPS, 2020), and, although there is no consensus on which parameters suit best its assessment (Bünemann *et al.*, 2018), it is generally agreed that variables related to physical, chemical and biological characteristics should be simultaneously considered (Bünemann *et al.*, 2018; Riches *et al.*, 2013; Virto *et al.*, 2012). For our experiment, *Trifolium fragiferum* was selected as the most suitable plant species after some preliminary experiments, due to its ability to compete with other species, the reduced cost of its establishment (it is perennial), and its ability to supply nitrogen to the crop through nitrogen fixation.

This cover crop was successfully established under the vine, maintained during three consecutive seasons, and its agronomic implications reported (Abad *et al.*, 2020), having been proved to be a good choice for weed control, conveying a slight reduction of vegetative growth, a slight increase in water deficit, no changes in yield and grape composition were observed. In this article, we present complementary results from that experiment, in regards of the implication of the cover crop in vineyard soil characteristics associated to its quality.

The aim of this work was to evaluate the effect of the establishment of a *T. fragiferum* under-vine cover crop on the soil quality of a vineyard subjected to Mediterranean climate conditions, as reflected by the values of a variety of soil physical, chemical and biological properties. We hypothesized that the *T. fragiferum* under-vine cover crop would lead to soil quality improvement in our Mediterranean climate vineyard.

6.2. Material & methods

6.2.1. Site and experimental design

The trial was carried out in a commercial vineyard located in Traibuenas (Navarra, Spain) between 2018 and 2020. This vineyard, which belongs to Bodegas Ochoa winery, was planted in 2001 and, since 2018, is being managed under organic certification. The soil is a Typic Calcixerept ((S.S.S.) Soil Survey Staff, 2014), with a loam texture (47.2 % sand, 31.0 % silt and 31.8 % clay) down to the first 90 cm. In the initial soil sampling, before the experiment started, the content of soil organic carbon (SOC) was 0.73 %, the content of total carbonates was 33.04 %, and the percentage of active limestone was 8.94 % to the first 90 cm. Climate in the area, according to Papadakis (1952), is humid temperate Mediterranean. The *Vitis vinifera* L. variety cultivated is Merlot (clone 343) grafted onto rootstock 420A, with a planting distance of 3 x 1 m, a North-South row orientation, and trained as a vertical shoot positioned double Cordon Royat. The experimental field has a drip irrigation system, with 3.5 $L \cdot h^{-1}$ drippers spaced 0.75 m, which is used according to a deficit irrigation strategy (average irrigation in 2018-20 accounted for 61 $mm \cdot yr^{-1}$). More details on site climate and vineyard characteristics can be found in Abad *et al.* (2020).

The experimental design included two treatments, i.e. (1) UV = under-vine cover crop; and (2) T = mechanically tilled control, with five replicates per treatment located in alternate rows. For the UV treatment, seeds of *T. fragiferum* were sown at a 15 g·m^{-2} dose on a 40 cm-wide strip. Sowing was done manually on February 27th, 2018, after inter-vine cultivation (Davitronic model of ID-David, 5 cm depth). Two days later, there was a light snowfall, which helped to settle seeds on the ground. In the control treatment (T), tilling work (5 cm deep) was carried out on the same sowing preparation dates. The inter-rows were kept free of vegetation (weed-free) by tilling four times every year: early November, March, May, and late June or early July.

6.2.2. Total and particulate organic carbon

Soil samples for the analysis of soil organic carbon (SOC) and particulate organic carbon (POC) were taken in May of 2019, approximately one year after under-vine cover crop establishment. One sampling point was randomly defined at each treatment replicate. At each of these sampling points, soil was sampled at two depths (0-15 cm and 15-30 cm), in order to be able to separately analyse the soil occupied by cover-crop roots from that occupied by vine roots. Soil samples were taken with a hoe, disturbing the surrounding soil as little as possible. Samples were then allowed to dry off at room temperature and, subsequently, ground and sieved at 2 mm, except for a fraction of each sample that was separated for the study of aggregate stability (see below).

Soil organic carbon stock (SOC_{Stock}) in the 0-15 and 15-30 cm depth (D) layer was calculated from SOC and bulk density (BD) measurements (Equation 1) (Rodríguez Martín *et al.*, 2016), as suggested by the FAO (Lefèvre *et al.*, 2017).

Equation 1

$$SOCStock = SOC \times BD \times D$$

Organic C in the fraction of soil organic matter defined as particulate organic matter (POM) based on its size (>50 µm; referred to as POC) was determined by chemical dispersion with $(NaPO_3)_6$ 1N, and sieving of 20 g of air-dried soil, as described in Marriott and Wander (2006). After shaking overnight at room temperature, and three washes with deionized water, samples were left to dry at room temperature, weighed and, finally, ground to <200 µm in an agate mortar to ensure homogeneity before analysis. Total SOC and POC were determined by wet oxidation on air-dried, sieved samples (Nelson and Sommers, 1996).

6.2.3. Hydraulic conductivity, bulk density and porosity

Measurements of the soil saturated hydraulic conductivity (Ks) and bulk density were performed 20 months after the onset of the experiment, in October, after all cultivation and harvesting operations in the cropping season had finished. Undisturbed core samples were collected under vine, between two vines, using bevel-edged steel rings (Ø = 5 cm, total volume = 100 cm^3) for the 0-15 cm and 15-30 cm depth increments to determine soil bulk density (BD). Porosity was calculated as 1-BD/RD (RD: real density 2.65 g·cm^{-3}) The soil cores sampled to calculate bulk density (BD) were used to study soil permeability. By this stage, the under-vine cover crop had been established for two full seasons. Permeability was measured using a laboratory Eijkelkamp (Eijkelkamp Soil and Water, Giesbeek, The Netherlands). Soil cores were previously saturated with deionized water under vacuum before placing them in the permeameter water tank. The K-factor was calculated according to Equation 2:

Equation 2

$$K_s = \frac{V.L}{A.t.h}$$

where

Ks: Saturated permeability coefficient or K-factor

V: Volume of water flowing through the sample

L: Length of the soil sample

A: Cross-section surface of the sample

t: Time used for the water volume V to pass through the core

h: Water level difference inside and outside ring holder

6.2.4. Soil structure and aggregation

Soil structure and aggregation were evaluated (in the same 0-15 cm soil samples used for the determination of SOC) following a protocol similar to that described in Oliveira *et al.* (2019). To this purpose, field-moist soil samples were gently forced to pass a 6-mm opening mesh and, subsequently, three stable aggregate size-fractions were separated: macroaggregates (Magg; >250 µm), microaggregates (magg; 50-250 µm), and clay-size fraction (s+c; <50 µm). Macroaggregates were further separated into two additional fractions: coarse POM (cPOM >250 µm) and microaggregates within macroaggegates (mMagg <250 µm). Likewise, microaggregates were separated from the fine sand (50-250 µm) by sonication. Macroaggregates (Magg) and microaggregates (magg) were expressed as weight proportion, after previously correcting aggregate-size fractions according to Equation 3 (Six *et al.*, 2002a):

Equation 3

$$\text{Sand} - \text{corrected aggregation} - \text{size fraction (g aggregate/ Kg soil)} = \left((size - fraction\ weight) - \left(\frac{sand\ weight\ of\ same\ fraction{-}size}{\sum (total\ sample\ mass - total\ sand\ mass)} \right) \right) \times 100$$

The aggregates mean weighted diameter (MWD, µm) was calculated according to Equation 4, where Wi is the weight of the different fractions, φi is the mean diameter of the mesh size, and W is the weight of the total sample. Water stable aggregates (WSA, %) was calculated as the proportion of Magg over total soil mass, according to Equation 5, where $WMagg$ is the weight of the Macroaggegate fraction and W is the weight of the total sample.

Equation 4

$$MWD = \frac{\sum Wi\, x\, \varphi i}{W}$$

Equation 5

$$WSA = \frac{WMagg}{W} x100$$

The total content of carbon (wet oxidation) and nitrogen (Kjeldahl method) associated to each of these fractions was also calculated. Since no organic matter was associated with sand, a correction was made to avoid "dilution" of the C content by sand (Equation 6) (Six *et al.*, 2002a):

Equation 6

$$Sand - corrected\ C\ in\ fraction\ (g\ C\ /kg\ fraction) = \frac{C\ content\ in\ fraction\ (g\ C\ /Kg\ fraction)}{1 - proportion\ of\ sand\ in\ same\ fraction}$$

6.2.5. Effect of under-vine cover crop on soil microbial communities

The effect of the under-vine cover crop on soil microbial communities was evaluated through the determination of the following soil parameters: basal respiration, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), nitrate (ΔNO_3^-) and ammonium (ΔNH_4^+) balance, and community-level physiological profiles (CLPPs) with Biolog EcoPlates^{TM}.

Soil microbial activity was estimated through the quantification of basal respiration using a portable SCR-1 EGM-4 of PP System. Measurements were carried out at four phenological stages: budburst, flowering, veraison and harvest (the first two stages were studied in 2019 and 2020, whereas the latter two were studied in 2018, 2019 and 2020). Five random measurement points were defined at each treatment. In parallel, soil surface temperature was measured, as well as soil temperature and humidity at 10 cm soil depth.

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the chloroform fumigation method (Brookes *et al.*, 1985; Vance *et al.*, 1987), assuming a fumigation efficiency of 0.45 for both parameters (Jenkinson *et al.*, 2004; Joergensen *et al.*, 2011). Soil samples were taken at flowering in 2019 and 2020, and at harvest time in 2018, 2019 and 2020. Sampling points were randomly selected along the five lanes of treatment. A PVC tube (50 mm outside diameter and 35 cm long), cut in bevel at its end and with two slits at 15 cm, was made to be able to divide soil samples into two sampling depths. In the year 2020, at harvest it was not possible to sample the soil in depth given the hardness of the soil.

Nitrate and ammonium balances were determined by comparing values taken one month apart. The first measurement was performed in the same soil samples used for the determination of microbial biomass. One month later, additional samples were obtained collected out of a similar tube that had been left nearby for one month, with the slits covered with electrical tape and covered with a lid. The difference between the nitrate and ammonium content before and after a month is the balance, and provides an estimation of net nitrification and ammonification. Immobilization of nitrogen happens when the net value is negative (Robertson *et al.*, 1999). Nitrate (NO_3^-) and ammonium (NH_4^+) contents were determined adding 50 mL of 2 M KCl to 10 g of soil. The mixture was then stirred for 1 h at 150 rpm on a rotary shaker. Analytical determinations were carried out by segmented flow colorimetry (AA3, Braun+Luebbe, SEAL Analytical, Norderstedt, Germany).

The same soil samples were used for the estimation of bacterial functional diversity, as reflected by community-level physiological profiles (CLPPs) data obtained with Biolog EcoPlates^{TM}. This method provides CLPPs of cultivable, fast-growing, heterotrophic bacterial populations. Measurements were carried out in samples collected at flowering (2019 and 2020) and harvest (2018, 2019 and 2020). Sample preparation was carried out following Epelde *et al.* (2008). In short, 1 g of soil was diluted into 9 mL of sterile water, and then shaken at 125 $rpm \cdot h^{-1}$. Then, 0.12 mL were taken and diluted in 11.88 mL of sterile water. Finally, 300 µL of this diluted solution was pipetted to each Biolog EcoPlate^{TM} well. All samples were analysed in duplicate. Absorbances at 559 nm (A_{559}) and 750 nm (A_{750}) for each well were measured twice a day for one week, and once a day for an additional week. Between measurements, plates were incubated at 30 ºC in darkness, inside zip bags to prevent evaporation. In order to eliminate the potential confounding effect of turbidity, (A_{750} - A_{559}) values were calculated, and only absorbances above 0.25 were considered meaningful. Absorbance values over time for each pair of duplicates were used to draw average well colour development (AWCD) curves. Curves were analysed with SigmaPlot software to determine the midpoint of the period where the curve showed a linear growth. At this point, corresponds to the most representative moment to assess the bacterial community diversity, for which the number of substrates used (NSU: a proxy for species richness) (Zak *et al.*, 1994) and the Shannon's diversity index (H') were calculated.

6.2.6. Statistical analysis

Treatment means were compared using t-test, previously verifying that data distribution fulfilled the requirements of normality and homogeneity through Shapiro's and Barlett's tests. When these requirements were not met, data were transformed using logarithmic or inverse functions prior to analysis. Significance was considered for a 95% confidence level, unless otherwise indicated. All analyses were performed using R computing environment (R Development Core Team, 2016).

6.3. Results

6.3.1. Effect of under-vine cover crop on soil physical and chemical parameters

In the 0-15 cm soil layer, the establishment of the cover crop produced an increase SOC and POC, as well as in their relative proportion: 33 % for SOC, 74 % for POC, 30 % for the POC/SOC ratio, and 8 % for the SOC/N$_{total}$ ratio. N$_{total}$ stock was not affected (Table 6-1). In the 15-30 cm soil layer, there was an increase in POC (+78 %) and POC/SOC ratio (+59 %), but no significant differences were detected for SOC (Table 6-1).

Table 6-1. Effect of treatments on soil organic carbon stock (SOC$_{Stock}$), particulate organic carbon stock (POC$_{Stock}$), and POC$_{Stock}$ /SOC$_{Stock}$ ratio. UV: under-vine cover crop. C: tilled control. P-values <0.05 appear highlighted in bold.

Soil depth	**Treatment**	**SOC$_{Stock}$ (Mg.ha^{-1})**	**POC$_{Stock}$ (Mg.ha^{-1})**	**POC$_{Stock}$/SOC$_{Stock}$**	**N$_{TotalStock}$ (Mg.ha^{-1})**	**SOC/N$_{Total}$**
0-15 cm	UV	27.575	4.752	0.172	1.852	11.65
	T	20.714	2.730	0.132	1.669	10.77
	p	***0.008***	***0.001***	***0.006***	*0.145*	***0.006***
15-30 cm	UV	24.738	3.690	0.148	-	-
	T	21.884	2.063	0.093	-	-
	p	*0.291*	***0.010***	***<0.001***	**-**	**-**

In the first 0-15 cm, similar values of BD and Ks were observed in UV *vs.* T soils (Table 6-2). However, in the 15-30 cm layer, significantly lower (p<0.05) values of Ks were found in T *vs.* UV soils (actually, Ks values were close to zero in T soils).

Tabla 6-2. Effect of treatments on soil hydraulic conductivity (K$_s$), bulk density and porosity. UV: under-vine cover crop. C: tilled control. P-values <0.05 appear highlighted in bold.

Soil depth	**Treatment**	**K$_s$ (cm·min^{-1})**	**Bulk density (g·cm^{-3})**	**Porosity**
0-15 cm	UV	0.006	1.515	0.428
	T	0.009	1.460	0.449
	p	*0.586*	*0.401*	*0.401*
15-30 cm	UV	0.008	1.618	0.390
	T	0.001	1.743	0.342
	p	***0.009***	*0.074*	*0.074*

The recovery values at the end of the aggregates size-fractionation process, compared to the mass in the initial soil samples, were 98.26 % and 98.30 % for UV and T samples, respectively. In UV, the cover crop resulted in an increase in the percentage of Magg (+15% compared to T), and a decrease in the percentage of magg (53 % lower compared to T)

(Table 6-3). The percentage of non-aggregated smaller particles (s+c) also decreased under UV treatment (35% lower compared to T). Higher values of MWD (+17%) and WSA (+18%) were observed in UV soils (Table 6-3).

Tabla 6-3. Effect of treatments on the size-distribution of water-stable aggregates and water stability in the surface layer (0-15 cm), after one year. p-values <0.05 appear highlighted in bold.

Soil depth	Treatment	Magg (%)	magg (%)	s+c (%)	MWD (µm)	WSA (%)
0-15 cm	UV	88.28	8.375	2.649	1.819	84.68
	T	76.73	17.821	4.047	1.558	71.53
	p	***0.027***	***0.025***	*0.073*	***0.020***	***0.020***

Magg: Macroaggregates (> 250 µm); magg: microaggregates (50-250 µm); s+c: Silt + clay (< 50µm); MWD: mean weight diameter; WSA: water stable aggregate.

Within aggregate size-fractions, C and N contents did not show statistically significant differences between treatments in Magg (Table 5). By contrast, statistically significant higher values of C and N were found in magg and s+c in UV were observed compared to T: +17% and +14 %, respectively, for C, and +14 % and +11 % for total N, respectively. The C/N ratio did not present any statistically significant differences (Table 6-4).

Tabla 6-4. Effect of treatments on organic C and total N contents, and C/N ratios, in the different soil aggregate-size fractions after one year. p-values <0.05 appear highlighted in bold.

	Fractions	UV	T	*p*
Carbon (mg C·g fraction^{-1})	Magg	13.67	11.36	*0.114*
	magg	18.34	15.67	***0.046***
	s+c	14.71	12.89	***0.017***
Nitrogen (mg N·g fraction^{-1})	Magg	1.58	1.10	*0.246*
	magg	1.89	1.66	***0.048***
	s+c	1.50	1.35	***0.011***
C/N	Magg	10.92	10.34	*0.246*
	magg	9.70	9.44	*0.559*
	s+c	9.95	9.53	*0.087*

6.3.2. Effect of under-vine cover crop on soil microbial communities

The presence of the cover crop resulted in an increase in soil respiration values from harvest time of the second year until the end of the experiment (Figure 6-1.a). The presence of the cover crop led to decreases in soil temperature from the second season onwards, particularly during the warmer periods of the year (Figure 6-1.c and 6-1.d). No clear statistically significant effects were detected regarding soil moisture (Figure 6-1.b).

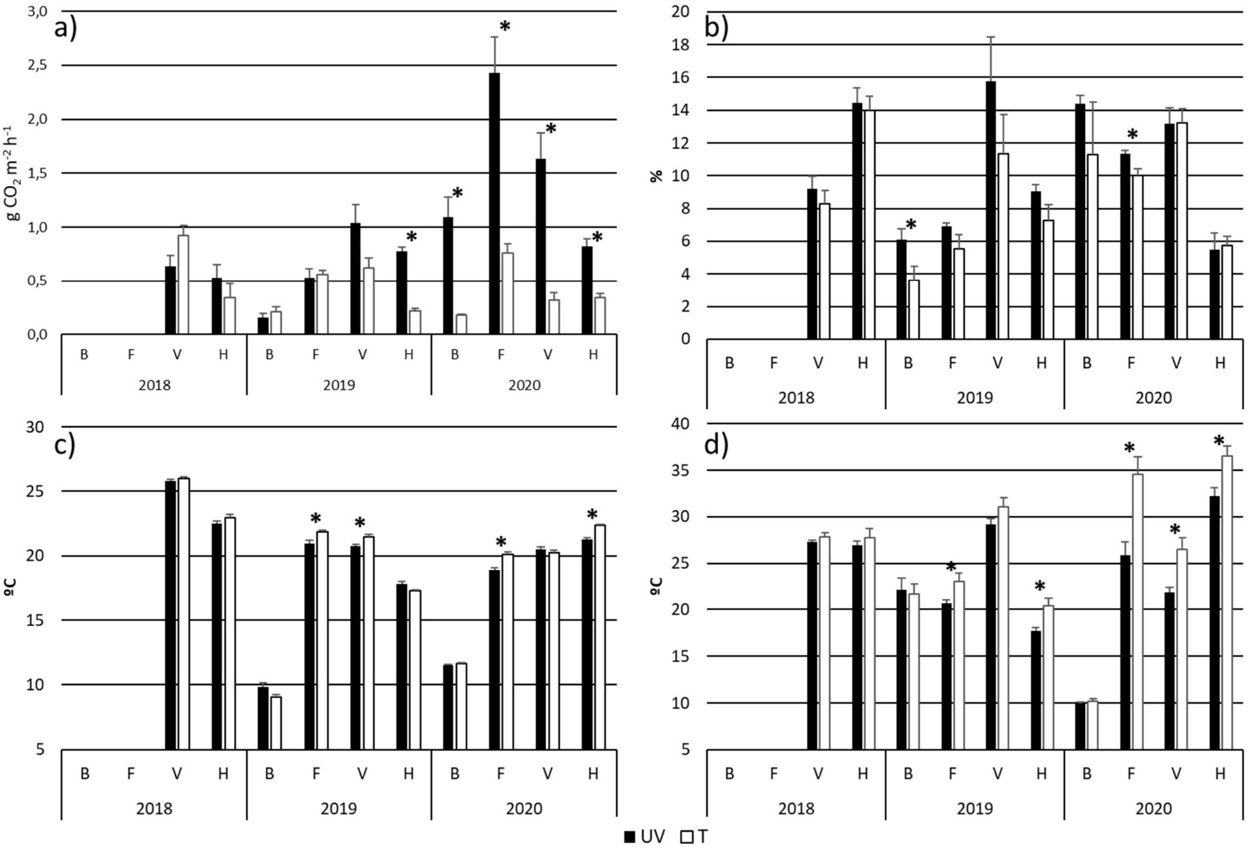

Figure 6-1. Effect of treatments on soil respiration, moisture and temperature at 10 cm soil depth, and surface temperature at budburst (B), flowering (F), veraison (V) and harvest (H) times.

a) Soil respiration (g CO$_2$ m^{-2} h^{-1}); b) Soil moisture at 10 cm soil depth (%); c) Soil temperature at 10 cm soil depth (ºC); d) Surface temperature (ºC). *Significant differences 95%.

In the 15-30 cm soil layer, the cover crop caused no changes in microbial biomass, neither at flowering nor at veraison. In the upper soil layer (0-15 cm), the presence of the cover crop resulted in higher MBC and MBN values, although most of the observed differences were not statistically significant. Actually, the only statistically significant difference were MBC at flowering 2019 (+120 % under UV *vs.* T), MBC/MBN ratio at flowering 2019 (+100 %), and MBN at harvest 2020 (+59 %) (Figure 6-2).

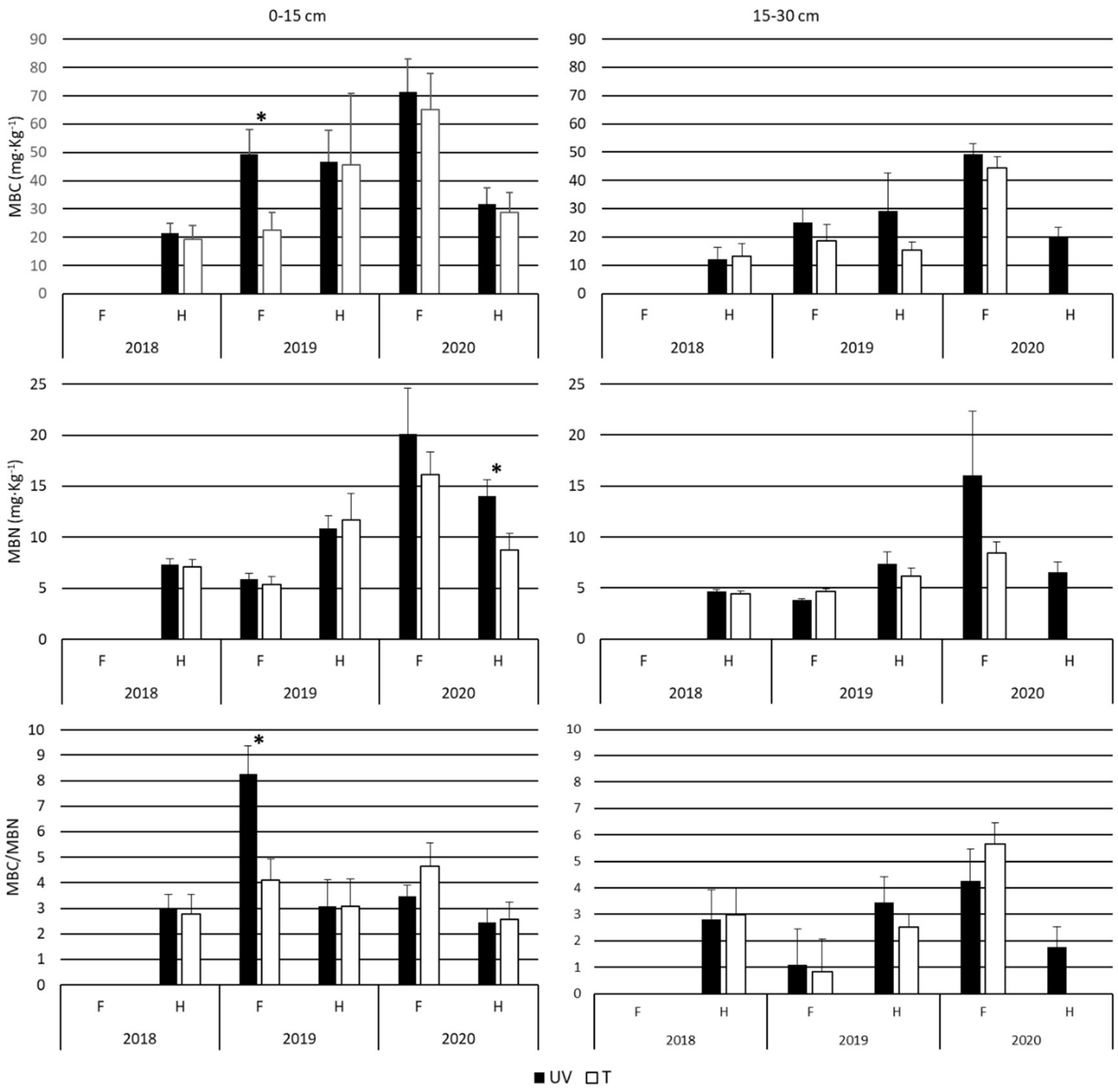

Figure 6-2. Effect of treatments on soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and MBC/MBN ratio at flowering (F) and harvest (H), for the two soil layers (0-15 and 15-30 cm depth) studied here. *Significant differences 95 %.

In UV soils at a 0-15 cm depth, the nitrate and ammonium balances could suggest an immobilization of NH_4^+ at flowering (this effect was not observed at a 15-30 cm soil depth). At harvest, the cover crop produced a greater immobilization of NO_3^-, with respect T soil, in the last year of the experiment. In the 15-30 cm soil layer, the behaviour was different, being the T soils where the nitrate was the least available (significant differences were only observed in 2019) (Figure 6-3).

At harvest time, in all the studied years, the cover crop showed a general trend toward a greater immobilization of ammonium at 0-15 cm soil depth, differences being statistically significant in 2019 and 2020. At this harvest time, a similar behaviour was detected, in this case with statistically significant differences only in 2019 (Figure 6-3).

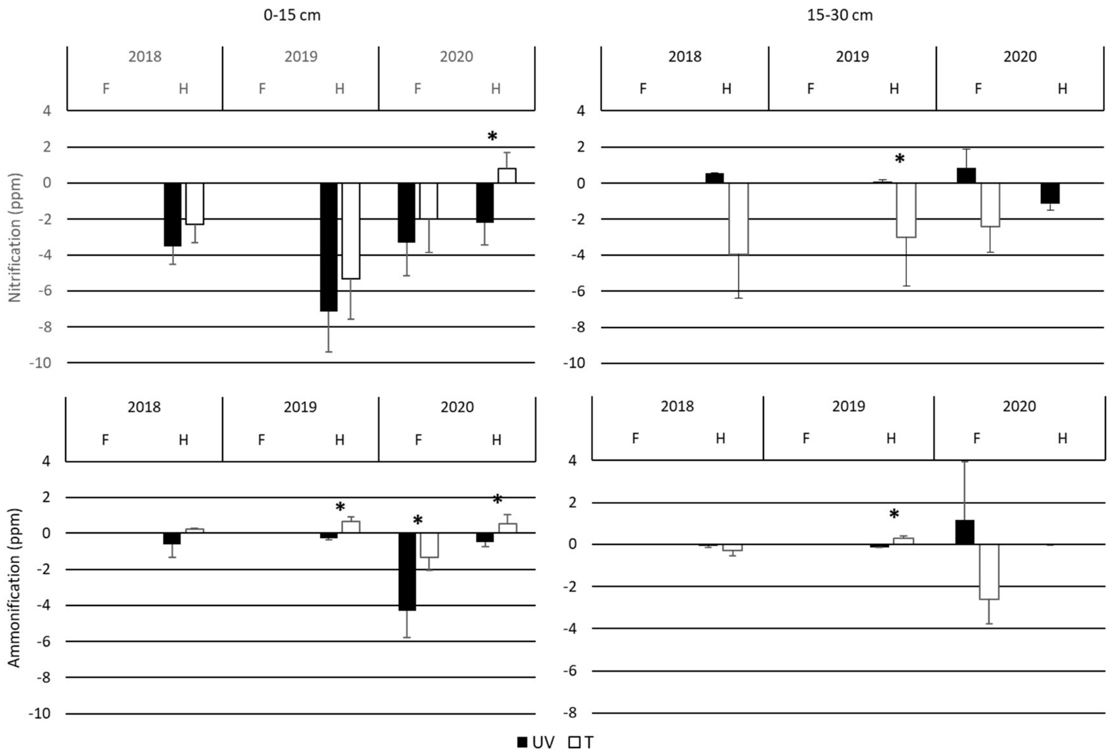

Figure 6-3. Effect of treatments on nitrate and ammonium balance at flowering (F) and harvest (H). * Significant differences 95 %.

The cover crop also resulted in limited changes in the soil bacterial functional diversity (Figure 6-4). The cover crop only increased, in the upper soil layer (0-15 cm) at harvest, the NSU in 2019 and 2020, and the Shannon's diversity in 2020 (Figure 6-4).

6.4. Discussion

The implementation of the *T. fragiferum* under-vine cover crop resulted in significant changes in most of the soil indicators considered in this study.

All the results presented need to be contextualized to the characteristics of the cover crop used (*T. fragiferum*) and to the agroecosystem where they were obtained, the soil under the vines in a semiarid climate drip irrigated vineyard. Similarly, their consequences have also to be considered in terms of the particularities of the crop. First, it is necessary to consider that the initial soil organic carbon content is low (0.73 % SOC) which may explain having detected changes in such relatively short time (one year) after the onset of the experiment. In soils with higher starting levels of organic carbon are high (3.4-3.9 % SOC), it has been observed that the use of cover crops does not necessarily result in gains in SOC or improvements of the soil structure even after three years (Jordan *et al.*, 2016).

The first observation was that the cover crop-induced an increase in the amount of SOC stored at the two depths considered (33 and 13 % at 0-15 and 15-30 cm soil depth, respectively), similar to that reported by Tarricone *et al.* (2020) with a subterranean clover crop in the row after two seasons. A more marked response was observed for POC values, as well as in the POC/SOC ratio (Table 6-1), indicating that the increase in SOC was mainly associated to the most labile fraction (Abiven *et al.*, 2009; Six *et al.*, 2002b), likely issued from the cover crop biomass. The stability of this increase in organic matter, though relevant, should be evaluated at a longer term, since this lability may imply also a faster mineralization of these organic inputs, as suggested by the higher respiration rates observed in UV (Figure 6-1) in the second, and especially, third year of study.

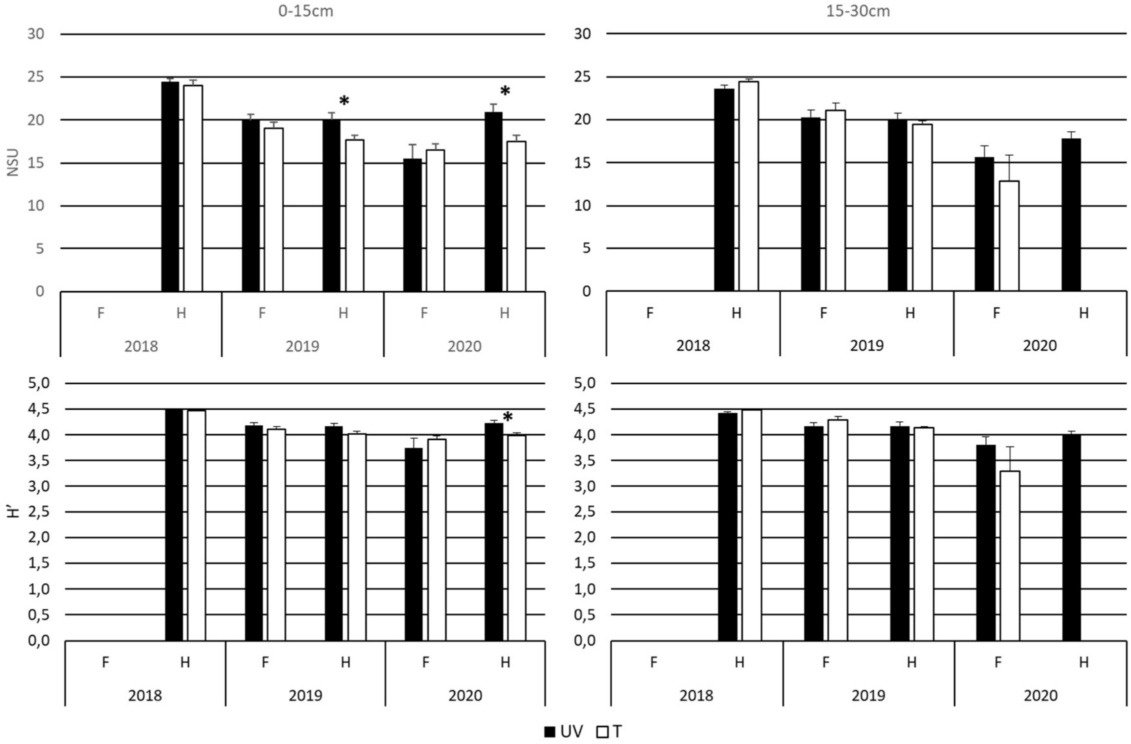

Figure 6-4. Effect of treatments on of bacterial functional diversity as reflected by community-level physiological profiles (CLPPs) obtained with Biolog EcoPlates^{TM}. NSU: Number of substrates used. H': Shannon's diversity index. * Significant differences 95 %.

The observation that the increase in SOC was accompanied by an upward trend in total N in the soil, provides also evidence supporting that most of the new SOC stored originated from the N-fixing cover crop, rather than from vines roots of aerial residues. However, the presence of the cover crop resulted in an increase of the carbon to nitrogen ratio (Table 6-4). The reasons behind this result could be mainly two. First, the relatively short period of time considered and the lack of incorporation of the vegetal residues into the soil, may have hindered N gain as pointed out by Dick (1983) for a cover of the same clover. Second, the observed increase in the soil microbial biomass may have implied a high degree of N capture from the soil, a fact that would explain with the changes in the nitrate and ammonium balances observed in the upper layer. Additionaly, it can also be hypothesized that the increase in Ks facilitated the washing of N into deeper layers.

One of the most interesting results of our work is the responsiveness of soil physical properties and structure to the installation of the cover crop, as we observed relevant changes after only one year, whereas in other studies on the effect of cover crops in vineyards, it has been observed that these changes may occur at least four or five years after the implementation of the cover crop (Karl *et al.*, 2016b; Virto *et al.*, 2012), even if some beneficial effects of the cover crop on the soil biological properties could be observed from the first year (Virto *et al.*, 2012). This earliness in the response is probably due to the fact that, unlike in the works cited, in our case, the addition of water and nutrients was localized directly below the vines where the cover crop was implemented.

Aggregation and aggregates size-distribution showed that cover cropping resulted in an improvement of the two indicators considered (MWD and WSA) already in the first year of study, with an increase of the proportion of Magg, and the concomitant decrease in the smaller size-fractions. This suggests an activation of the soil aggregation cycle, as described by Tisdall and Oades (1982), and later developed in detail for temperate soils by Six *et al.* (2004), in which smaller aggregates can incorporate into stable larger aggregates when organic matter inputs grant a sustained biological activity, as suggested again by the higher respiration rates observed in UV in the second and third year (Figure 6-1). In terms of soil functioning, these results can be understood as an improvement of soil quality at different levels in the soil under the vines with cover crops. In addition to the provision of a better physical environment for soil biological processes, water retention and infiltration, also the resistance to physical degradation can be associated to a more stable soil structure (Rabot *et al.*, 2018). Higher infiltration rates were indeed observed in UV than in T at 15-30 cm (Table 6-2), and the observed values of BD and porosity at this depth also suggested an improvement of porosity in the deeper layer (15-30 cm at $p<0.10$). The impact of this change on roots development is beyond the scope of this study, but can be indirectly estimated considering that in the third year of the study, the hardness of the deeper layer in T hindered the insertion of the PVC tube, as the soil was dry due to lack of precipitation and the irrigation season was over. Finally, although erosion cannot be a relevant issue in the study plot because of its relatively flat slope, it is known that Mediterranean soils are especially susceptible to erosion because of the characteristics of summer rainfall (Ben-Salem *et al.*, 2018; Gómez *et al.*, 2011; Le Bissonnais *et al.*, 2007). Under-vine cover crops would therefore, in the conditions of this study, contribute to reduce erosion, not only by physically protecting the soil from the impact of rain (and irrigation) drops, but also by providing a lower soil erodibility. Reduced soil crusting can also be expected from higher macroaggregtates stability.

The presence of the cover crop UV also resulted in an improvement of porosity in the deeper layer (15-30 cm), remaining unchanged in the upper one. Therefore, the beneficial effects of the cover regarding infiltration and porosity are maintained at depth, with a remarkable decrease in compaction compared to under the vine mechanically tilled areas. The impact of this change even affected the soil sampling process itself, since in 2020 harvest the hardness of the deeper layer hindered the insertion of the PVC tube, as the soil was dry due to lack of precipitation and as irrigation was over.

Concerning the parameters associated to soil biology, the presence of the cover crop resulted in a relevant increase of respiration, microbial carbon and functional diversity of soil bacteria. Soil respiration, as indicated by West *et al.* (1987), cannot be used directly to estimate microbial biomass or activity. In our case, respiration would be certainly increased due to the presence of roots from the cover crop in the under-vine, but the increase in soil microbial activity could also been slightly benefited by the observed increase in soil moisture, and by a certain acidification that might have occurred in the rhizosphere, as reported in other works (Luo and Zhou, 2006). In our research conditions, where deficit irrigation was applied at a regular basis, it was observed that the surface layer of the soil the humidity was higher where there is cover (Figure 6-1), which could be due to the decrease in temperature on the soil surface caused by cover shading, and to the protection against the strong prevailing North winds in the area that contribute to dry out the soil surface when bare.

The changes that occurred in microbial biomass associated to the presence of the cover crop are relevant from a soil quality point of view as it carries out many critical functions in the soil ecosystem, as source and sink for nutrients, or the formation of soil structure (Gil-Sotres *et al.*, 2005). In our case, the MBC values observed (15-50 g MBC·kg^{-1} of soil) were within the range of those observed by Virto *et al.* (2012) in a vineyard near the trial area, but in this case using a grass cover between the rows (46.4 g MBC·kg^{-1} without grass cover -*F. arundinacea* + *Lolium multiflorum*- for 26.1 g MBC·kg^{-1} without grass cover, after 10 years), and somewhat lower than those observed in a vineyard in Southern Italy (51.3 g MBC·kg^{-1} without soil cover for 109 g MBC·kg^{-1} under cover crops -Festuca arundinacea-, after 7 years) (Gattullo *et al.*, 2020).

The effect of the cover crop on soil bacterial functional diversity measured using the Biolog EcoPlates™ was also relevant. This technique works with the functionality of bacteria in the soil and not with their taxonomic composition, which can be more useful in understanding the functioning of complex communities such as those in the soil (Baraza *et al.*, 2019; Van Der Heijden *et al.*, 2008). The positive impact of the cover crop on the bacterial diversity was particularly relevant in the measurements made at harvest of the last two seasons, when, for instance, the number of substrates used (NSU) by bacteria in samples from the 0-15 cm layer increased from 17.7 to 20.0, and from 17.5 to 21 in the second and third year of the study, respectively. The observed changes in SOC, POC, humidity and soil structure can be related to these observations of greater diversity. In relation to soil structure, which has been found to be of greater effect on fungi than on bacteria (Likar *et al.*, 2017), it is likely that the improvement of soil structure in UV would also have had an effect on these microorganisms.

Nevertheless, considering the average low SOC levels in the studied soil, the bacterial diversity indices estimated through Biolog EcoPlates™ corresponded to a soil with *good health* according to the model of Soil Health Cards developed locally by Mijangos *et al.* (2016). In this scale, the observed values were found to be only slightly below those found for lettuces with high organic amendments doses (Urra *et al.*, 2020). This result is probably related to the fact that water was applied localized through drip irrigation under the vines and nutrient inputs were also located in the area near the vine by means of fertilizer spreaders with a fertilizer localization system, both in UV and T, which can favour bacterial richness compared to other areas such as the alleys between the rows (Holland *et al.*, 2016). In this case, even in these relatively favourable conditions for bacterial development, the implementation of the cover crop resulted in an increase in the soil bacterial functional diversity. In fact, the higher increase was observed for the upper soil layer (0-15 cm), corresponding with the area showing a higher cover crop root density, supporting the idea that these cover crops rhizosphere would be a hotspot for biological diversity (Mommer *et al.*, 2016; Steenwerth *et al.*, 2008).

Another relevant point in this study are the implications of the cover crop on nitrogen cycling and availability, since this element is particularly relevant in red grapes cultivated for winemaking. Although as, in any crop, there is a positive association between nitrogen availability and yield, in the case of red grapevine growers usually prefer to avoid high nitrogen contents at certain key phenological stages, since they can result in a decrease of grape quality (Verdenal *et al.*, 2021). In this vineyard, base nitrogen content was relatively low, and the nitrogen available to the plant was lower in the presence of the cover. This fact may be due to various factors: its use by the microorganism communities, which was however in this case not supported by the evolution of MBN, which were highly variable between the different sampling moments (Figure 6-2). Another explanation is the fact that, as mentioned by Cheng and Baumgartner (2004), a different composition of arbuscular mycorrhizal (AM) fungi in the presence of a legume, could result in a greater sequestration of N (Scandellari, 2017). Although AM were not considered in this study, they can be expected to have increased in the presence of vegetation cover as indicated (Cheng and Baumgartner, 2006; Trouvelot *et al.*, 2015), particularly in the case of a legume as pointed out by Rutto *et al.* (2003). Thus, although certain N contributions from the cover to the crop could be expected, it appears that only a very small part is used by the crop (Sulas *et al.* 2017). If an increase in soil nitrogen were required, it could be more efficient to use a mixed cover of legumes with grasses, which could increase the N in the soil in a more important way than using a single legume (Ball *et al.*, 2020; Blesh, 2019).

Altogether, the results obtained in this work highlight the potentiality of introducing cover crops under the vines as an effective method to improve vineyard soil health, compatible with good agronomic results (Abad *et al.*, 2020). As it can allow a faster improvement of the soil condition than what could be reached with cover crops in the alleys, it seems a particularly effective tool for soil improvement in grape growing areas where cover crops cannot be used between the rows due to climatic or soil depth restrictions. In the roadmap to increase vineyard sustainability by enhancing biological diversity, organic growing is certainly a praiseworthy cornerstone. However, it has been shown to favor mainly macrofauna and nematodes, rather than microorganisms (Henneron *et al.*, 2014), since switching to organic viticulture does not necessarily result by itself in an improvement of the soil structure. Even more, under some circumstances, the use of copper as a fungicide in organic farming has been shown to reduce populations of fungi and bacteria (Naveed *et al.*, 2014; Corneo *et al.*, 2013). Therefore, conservation agriculture practices, such as the installation of a cover crop in the row may be the key tool to increase fungi and bacterial diversity, and thus strengthen the contribution of grape growing to soil health in agroecosystems.

Conversely, some of the potential agronomic drawbacks of the cover crop appeared as negligible, as reported in Abad *et al.* (2020). On the one side, the impact of the nitrogen fixing cover crop did not cause any unbalance in nitrogen nutrition, since the potential of the cover crop as donor is limited, does not appear to compete or to provide excess of the nutrient in a mid-term, and its impact could be easily balanced with adjustments in fertigation. On the other side, competition for irrigation water was shown to be modest, and the improvement in soil porosity associated to the presence of the cover will favor the infiltration towards the vine roots of both rain and irrigation water.

In conclusion, the presence of the *T. fragiferum* under-vine cover crop resulted in an improvement of soil quality, as reflected by the values of physical, chemical and microbial properties with potential as indicators of soil functioning. Therefore, considering its implications on agronomic performance and soil quality, the use of under the vine cover crops has been proved to be a feasible and beneficial tool that can be incorporated to the portfolio of soil management options for vineyards in Mediterranean areas, being probably a good option for most conditions where support irrigation is available. There is, nevertheless, a necessity of evaluating their implications at a longer term in soil, agronomic, oenological features, and to study the potential of other cover crop species that could be better suited for other climates and soils.

7. OTRAS IMPLICACIONES DEL EMPLEO DE CUBIERTAS VEGETALES BAJO LAS CEPAS

7.1. Declaración de intenciones

Toda memoria de Tesis doctoral se sustenta sobre un amplio trabajo de investigación, en el que frecuentemente se abordan aspectos que finalmente no tienen cabida en el trabajo final, bien sea por su falta de relevancia, por cambios en los planteamientos iniciales de los trabajos, o por tratar de condensar de la manera más adecuada la información más importante, sin que con ello se convierta el documento de tesis en un material de difícil lectura.

En mi caso, y para dar coherencia a los resultados obtenidos, he querido agrupar la información en tres puntos: aspectos agronómicos (capítulo 4), aspectos enológicos (capítulo 5) y aspectos edafológicos (capítulo 6). Esta ordenación de la información no me permitía introducir dos aspectos que, si bien podrían tener cabida dentro de los capítulos anteriormente mencionados, distorsionaban la estructura de los mismos al constar de resultados de una sola campaña. Sin embargo, la información generada puede resultar de interés para el lector, por lo que se ha optado por elaborar un apartado final en el que se recojan. En concreto, estos trabajos hacen referencia al efecto de la cubierta bajo las cepas sobre el riesgo de heladas de primavera y, al efecto de estas cubiertas sobre la población de levaduras *Saccharomyces cerevisiae* en vinos fermentados sin adición de levaduras exógenas.

Un tercer apartado, al cual tampoco conseguía darle cabida en los capítulos anteriores es el estudio de la viabilidad económica del empleo de la cubierta de trébol fresa frente a los manejos más habituales en el viñedo, como son el laboreo intercepas y el uso herbicida. Este punto podría considerarse transversal y de gran importancia de cara a considerar recomendar la instalación de cubiertas bajo las cepas de manera real en el cultivo del viñedo.

7.2. Efecto de las cubiertas sobre daños por heladas de primavera

Aunque inicialmente no se planteó el estudio del efecto que la presencia de esta cubierta bajo las cepas podría tener sobre el incremento de los daños por heladas primaverales, esta cuestión ha aparecido en diversas ocasiones en los debates generados en la presentación de los resultados parciales del ensayo en distintos foros. Por ello, en el año 2021 pusimos en marcha un dispositivo experimental que permitiera evaluar el efecto que la cubierta de *T. fragiferum* bajo la línea de cultivo pudiera tener sobre heladas de primavera.

Durante la primavera, coincidiendo con el momento de brotación de la viña, se pueden producir heladas de distinto tipo, que pueden afectar a los nuevos brotes y reducir la cosecha. Las heladas más habituales en la zona de estudio son las heladas de radiación, y es en este tipo de heladas en las que la presencia de una cubierta puede tener un efecto por las razones que se señalan a continuación. Todos los cuerpos en la naturaleza emiten radiación, si bien durante el día el balance de radiación emitido por los cuerpos y el recibido de la radiación solar es positivo, produciéndose una acumulación de calor. Durante la noche, sin embargo, al no existir la radiación solar, se produce una cesión de calor de los cuerpos a la atmósfera, que por contacto enfría las capas bajas de la atmósfera. Esta cesión de calor se emite en una longitud de onda larga que no puede ser absorbida por el aire atmosférico ni por los cuerpos. En esta cesión participa el suelo, pero también la vegetación. Cuanto más desarrollo presenta la vegetación, más superficie radiante presenta, por lo que el enfriamiento de las capas bajas de aire es mayor (Urbano Terron, 2001).

El día 15 de marzo de 2021 se instalaron sensores de temperatura (Talk Thermistor Probe PB-5005-0M6) dentro de una pantalla protectora (Onset Solar Radiation Shield RS3) en tres de las repeticiones de cada tratamiento. Los sensores se situaron a la altura de los brazos de las cepas, y estaban conectados a un datalogger (Tinytag talk 2 TK -4023) que recogía la información horaria de la temperatura máxima y mínima (Figura 7-1). Trascurridos dos meses y tras pasar el periodo de riesgo de heladas de primavera, el día 27 de mayo se realizó la descarga de los datos.

El día 24 de abril se realizó un control visual de los daños por heladas en los brotes. Sobre un total de 10 cepas para cada tratamiento y repetición, 5 a la derecha y 5 a la izquierda del sensor de temperatura, se evaluaron todos los brotes, clasificándolos en tres niveles de daño: Sin daños, daños leves (algún daño de hielo, pero con partes verdes en el brote) y daños severos (todo el brote dañado por hielo) (Figura 7-2).

El estudio estadístico de los daños de heladas se realizó mediante t-test con el programa R (R Development Core Team, 2016) para todos los periodos horarios de aquellos "días" con un salto térmico superior a 20ºC entre la temperatura máxima y la mínima. El "día" se consideró entre las 11h de un día y las 10h del día siguiente, de manera que se abarque el momento de máxima y mínima temperatura.

Figura 7-1. Sensores de temperatura colocados a la altura de los brazos de la cepa para evaluar los efectos de las bajas temperaturas en la brotación

Figura 7-2. Daños de heladas en el momento de la evaluación. Izda. daño leve; Dcha. daño severo.

La identificación de los días en los que se ha podido producir un fenómeno de radiación más importante, con un salto térmico de más de 20ºC han sido 13 (Figura 7-3). Estos días se han considerado como situaciones de interés para el estudio de las implicaciones de la presencia de la cubierta sobre el riesgo de heladas ya que, independientemente de que las temperaturas estuvieran o no por debajo de 0ºC, la dinámica de enfriamiento de la atmósfera en el entorno de las cepas sería comparable. En términos generales, el empleo de la cubierta vegetal bajo las cepas no tuvo un efecto claro sobre la evolución de las temperaturas a la altura de los brotes (Tabla 7-1), si bien cuando se observa en detalle los 4 días en los que las temperaturas se situaron por debajo de 0ºC (Figura 7-4) se observa cómo, aunque no existen diferencias si hay una tendencia a tener temperaturas algo inferiores cuando hay presencia de cubierta.

Fecha y hora	UV (ºC)	T (ºC)	Δ Temperatura (ºC)
24/03/2021 08:00	-2,603	-2,627	-0,024
25/03/2021 09:00	4,480	4,574	0,094
26/03/2021 08:00	4,381	4,474	0,093
27/03/2021 06:00	5,062	5,218	0,156
28/03/2021 09:00	1,011	1,209	0,197
29/03/2021 08:00	2,729	2,821	0,092
31/03/2021 08:00	3,918	3,942	0,024
02/04/2021 08:00	4,294	4,406	0,111
05/04/2021 08:00	-0,675	-0,442	0,233
13/04/2021 08:00	-2,735	-2,528	0,207
15/04/2021 03:00	4,132	4,181	0,049
16/04/2021 07:00	2,289	2,386	0,097
19/04/2021 08:00	-0,853	-0,773	0,081
Media	1,956	2,065	0,109

Tabla 7-1. Temperaturas mínimas para los días con salto térmico de más de 20ºC, y diferencia de temperaturas mínimas entre la cubierta (UV) y el laboreo (T).

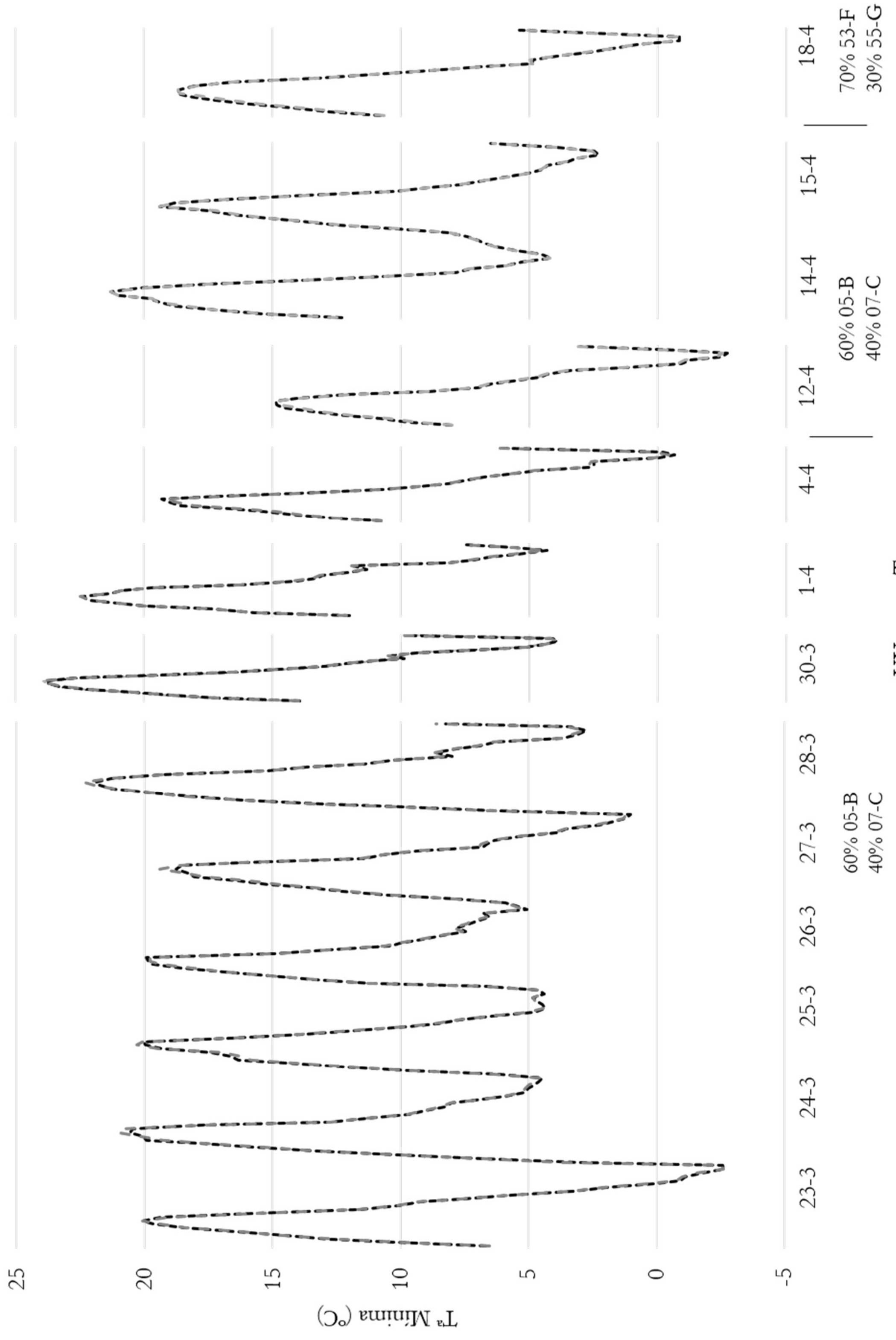

Figura 7-3. Evolución de las temperaturas para los días con un salto térmico de más de 20ºC entre la temperatura máxima y la mínima para el periodo de 11h de la mañana a 10h del día siguiente.

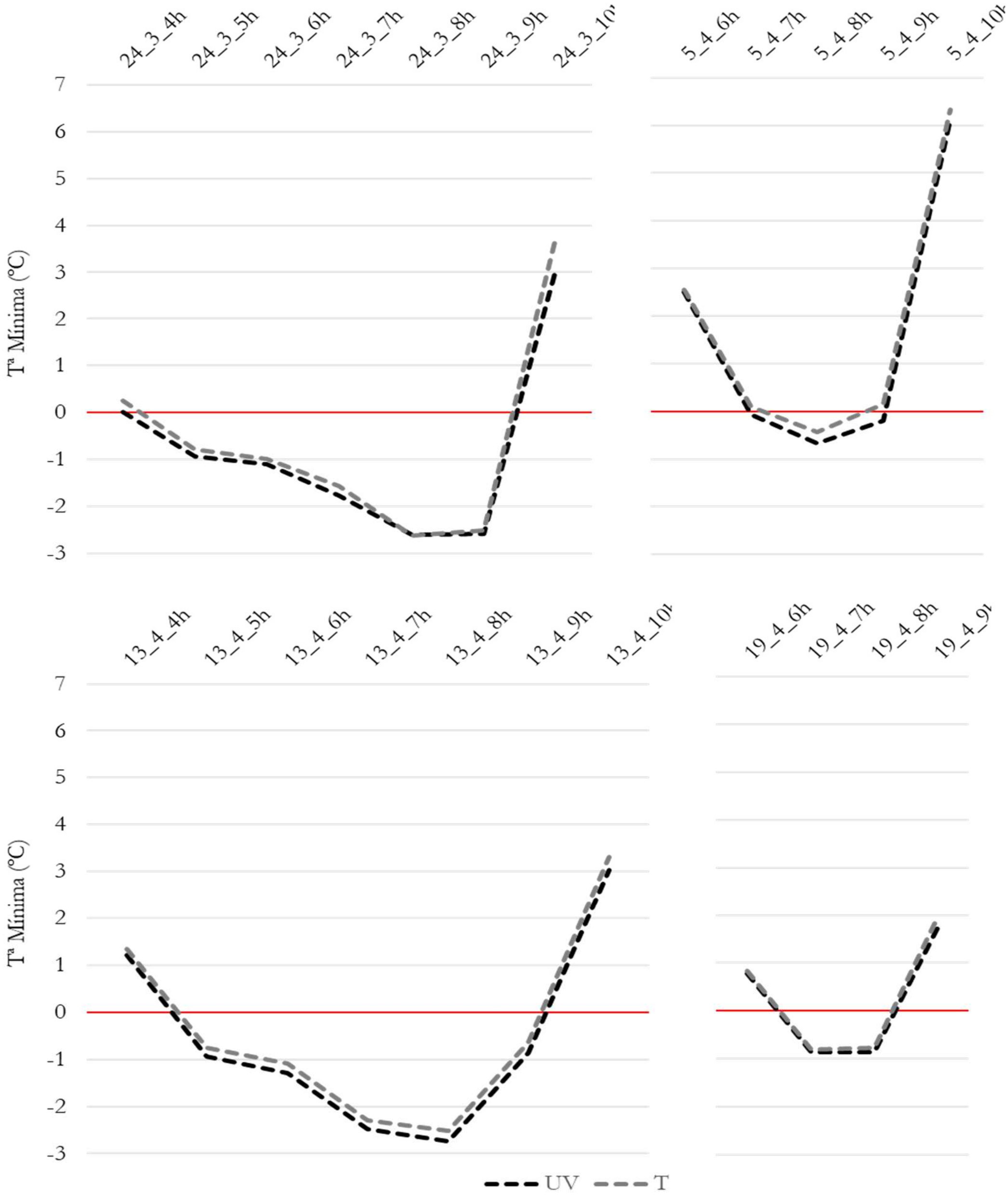

Figura 7-4. Detalle de la evolución en el periodo más frio del día para los cuatro días con temperaturas inferiores a los 0ºC con posible incidencia de helada de radiación.

Tabla 7-2. Valoración de daño por frío en los pámpanos a fecha de 24 de abril de 2021.

		Valoración del daño por hielo (%)			
Tratamiento	**Nº medio pámpanos·cepa^{-1}**	**Sin daños**	**Daño leve**	**Daño Severo**	**Suma daño leve y severo**
Cubierta	12,6	78,8	8,5	12,7	21,2
Laboreo	12,3	88,5	4,1	7,4	11,5
P	*0,671*	*0,512*	*0,230*	*0,155*	*0,103*

El análisis cuantitativo de los daños muestra también una ligera tendencia hacia daños ligeramente superiores (Tabla 7-2) en el caso de las cepas con cubierta, si bien las diferencias no han sido muy elevadas y tendrían que confirmarse con más datos.

Así pues, y con datos de una única campaña, no puede decirse que la presencia de la cubierta de *T. fragiferum* bajo las cepas incremente el riesgo de heladas de radiación, si bien en algunas situaciones el efecto de la presencia de una cubierta bajo las cepas en el momento de brotación del viñedo podría tener un impacto negativo.

7.3. Efecto de las cubiertas sobre la población de levaduras

Presentado en comunicación oral en el XVI Congreso Nacional de Ciencias Hortícolas. 17 al 22 de octubre de 2021. Córdoba

Las levaduras que llevan a cabo la fermentación condicionan las características del vino obtenido, y es habitual emplear, como práctica enológica, levaduras secas activas (LSA) que han sido seleccionadas por presentar atributos específicos y garantizar una buena cinética de la fermentación. Sin embargo, en los últimos años, existe un creciente interés por conocer mejor e implementar el uso enológico de las levaduras que, de manera natura, vienen asociadas a la uva en una zona o explotación. Algunas bodegas han puesto en marcha procesos de selección de levaduras propias, de modo que pueden encontrar un equilibrio entre la tipicidad que puede proporcionar una levadura propia y las garantías de repetibilidad y seguridad de la fermentación que confiere el uso de una levadura seleccionada.

Incluso cuando se emplea LSA, las levaduras autóctonas también tienen un papel, habiéndose observado que en los primeros estadíos del proceso fermentativo (3-6 días) el nivel de levaduras naturales puede ser mayor que el de la LSA, por lo que los aromas de los vinos resultantes estarán influenciados por las levaduras autóctonas de la parcela (Querol *et al.*, 1992). Incluso dentro de una misma especie de levadura, las diferentes cepas pueden aportar matices diferenciadores en los vinos, jugando por tanto un papel muy importante en la expresión de la tipicidad de los vinos (Romano y Capece, 2007). Se han constatado como la distribución geográfica de los viñedos determina la comunidad de levaduras en el viñedo (Castrillo *et al.*, 2019; Drumonde-Neves *et al.*, 2017), así como el manejo que se haga de él (Cordero-Bueso *et al.*, 2011a; 2011b; Grangeteau *et al.*, 2017; López-Piñeiro *et al.* 2013b; Martins *et al.*, 2014).

El objetivo de los trabajos recogidos en este capítulo es evaluar el efecto que una cubierta de *T. fragiferum* bajo la línea de cultivo ejerce sobre la población de levaduras fermentativas presentes en los vinos elaborados sin inoculación de LSA.

Para ello, en el año 2020, al día siguiente de realizar los controles de vendimia, se procedió a vendimiar manualmente 25 kg de uva para las mismas tres repeticiones por tratamiento que las empleadas en la vinificación expuesta en el Capítulo 6. En este caso, la vinificación se llevó a cabo sin aporte de LSA, habiendo sido todo el material empleado (tijeras, cajas de vendimia, tanques de fermentación) desinfectados con ASEP150-Proquimia (ácido peracético 15 %) a dosis de 0,5 % previo a su uso. Posteriormente, las muestras se trasladaron a una planta piloto de la Universidad Pública de Navarra donde nunca se ha realizado ninguna vinificación, y se procedió al despalillado y estrujado de manera manual. Se emplearon guantes de nitrilo en la manipulación de las uvas y se sustituyeron entre cada muestra para evitar contaminaciones de levaduras. Los tanques de fermentación fueron colocados en una cámara fría industrial que se mantuvo a 22ºC durante todo el proceso de fermentación. Cada día se midió la temperatura y la densidad de los mostos. Cuando los mostos alcanzaron una densidad por debajo de 1 kg·L^{-1} durante tres días se consideró terminada la fase de fermentación alcohólica. Para cada uno de los depósitos de fermentación, se reservó una muestra de 300 mL en frascos previamente esterilizados, y se enviaron al laboratorio de

INCAVI-IRTA de Vilafranca del Penedés (Barcelona) para el estudio de las cepas de *Saccharomyces cerevisiae*. A la llegada, para cada muestra se sembraron dos placas de Petri con medio agar Sabourad con cloranfenicol. Las placas se incubaron durante 48h a 28ºC con la finalidad de obtener colonias separadas, cada una de ellas procedentes de la multiplicación de una cepa de levadura presente en la fermentación. En total se analizaron 74 cepas para cada vino, pertenecientes todas ellas a *S. cerevisiae* al tratarse de muestras de final de fermentación. Posteriormente se realizó su análisis mediante polimorfismo de longitud de los fragmentos de restricción del ADN mitocondrial (adaptado de Querol *et al.*, 1992) según las propuestas de la OIV-OENO (408-2011). A partir de la placa conservada anteriormente, se tomó una colonia con el asa de siembra para cada vial con 0,8 mL de medio YPD y se incubó durante 48h a 28ºC. De aquí, y tras varios procesos de centrifugados, concentración e incubaciones de las muestras, se extrajo el ADN. Posteriormente se realizó una digestión con la enzima de restricción Hinf I que reconoce unas dianas específicas en el ADN (mitocondrial y nuclear) y se procedió a la electroforesis con un gel de 10 cm de longitud con agarosa al 1 % (100 mL TBE + 1 g agarosa), con marcador de peso molecular λ-EcoRI Hind III (Marcador III de Roche Diagnostics) a 90 voltios. A cada perfil de bandas distintos se les asignó una letra del abecedario, correspondiendo cada perfil con una cepa diferente de *S. cerevisiae*.

Una de las muestras enviadas a INCAVI-IRTA se malogró en el viaje, por lo que únicamente se pudo realizar el estudio de las cepas de *S. cerevisiae* sobre 3 repeticiones de UV y 2 repeticiones de T, lo que no permite la confirmación estadística de los resultados obtenidos. Los resultados muestras como en UV la cepa A (71,4 %) es predominante, seguida de la cepa B (18,4 %). En el caso de T, si bien A presenta una mayor proporción (42,9 %), existe una presencia destacable de más cepas, como la B (22,7 %) o la F (23,5 %) (Tabla 7-3).

Tabla 7-3. Distribución de las diferentes cepas de *S. cerevisiae* en la elaboración de vinos sin aporte de levaduras secas activas (LSA).

UV: cubierta bajo la línea de cultivo; T: laboreo; N: Número de cepas identificadas dentro de un mismo perfil.

Cepa	**UV**		**T**	
S. cerevisiae	N	%	N	%
A	56	71,4	51	42,9
B	14	18,4	27	22,7
C	1	1,3		
D	1	1,3		
E	3	3,8	2	1,7
F			28	23,5
G			4	3,4
H	1	1,3		
I	1	1,3		
J			5	4,2
K			2	1,7
L	1	1,3		

Los resultados anteriores, si bien únicamente son resultado de un año de análisis, parecen indicar que la presencia de la cubierta vegetal podría reducir la diversidad poblacional de *S. cerevisiae*. Cordero-Bueso *et al.* (2011a) en un viñedo de Madrid, también observó una reducción en la diversidad de especies de levaduras cuando se había presencia de una cubierta en la calle respecto a un manejo con herbicidas o con laboreo era menor. Incluso se llegó a detectar la ausencia de *S. cerevisiae* cuando se empleó la cubierta vegetal, lo que impidió la fermentación espontánea de algunos de los mostos. Según el autor, esta pérdida de diversidad estaría relacionada con una competencia entre hongos y levaduras. En nuestro caso, esta ausencia de *S. cerevisiae* no se ha producido, y no se ha abordado el estudio del efecto de la cubierta en la población de otras especies de levaduras. En el caso del viñedo empleado en esta Tesis doctoral, podría argumentarse que la presencia de *Saccharomyces* podría estar favorecida porque la parcela se maneja bajo certificación ecológica, lo que podría generar un ambiente más propicio para dicha especie de levaduras (Cordero-Bueso *et al.*, 2011b).

En conclusión, parece que el empleo una cubierta vegetal sí permite el desarrollo de la levadura fermentativa *S. cerevisiae*, pero incrementa la dominancia de un menor número de cepas frente a un manejo sin cubierta, donde la diversidad dentro de la especie es mayor.

7.4. Estudio económico del empleo de cubiertas frente a manejo con laboreo o herbicida

Además de todas las implicaciones que esta cubierta tiene sobre factores agronómicos, enológicos y edafológicos, resulta igualmente interesante conocer qué implicaciones económicas presenta su uso respecto de los manejos habituales que se vienen realizando hasta ahora: empleo de herbicidas o laboreo intercepas. Las opciones habituales de laboreo y herbicida requieren de intervenciones anuales, en ocasiones en más de una ocasión por campaña. El empleo de la cubierta requiere únicamente de las labores preparatorias para la siembra y de la propia siembra en su primer año, sin que posteriormente sean necesarias más intervenciones. En ocasiones, si acaso, podría resultar interesante un repaso manual en el primer año, en época estival, para suprimir las adventicias que más destaquen en la cubierta.

A continuación, se presenta una estimación económica para una hectárea de viñedo, con calles de cultivo de 2,8 m, empleando tres manejos del suelo bajo las cepas distintos: a) Cubierta vegetal de *T. fragiferum*; b) Laboreo intercepas; c) Herbicida. Se han estudiado cuatro periodos de duración de la cubierta: 1, 2, 3 y 4 años. Se estima una pérdida de rendimiento de uva por el empleo de la cubierta respecto del empleo de herbicida o de laboreo de 580 kg·ha^{-1}, para una producción media de 7.500 kg·ha^{-1}. Se han planteado cuatro escenarios de precios de la uva: 0,35; 0,45; 0,60 y 0,80 €·kg^{-1}. El coste de mano de obra cualificada (tractorista) se ha estimado en 12 €·h^{-1} y la no cualificada (peón de campo) en 9 €·h^{-1}. Todos los datos de precios son orientativos, y están elaborados a partir de consultas a la Sección de viticultura y enología de Gobierno de Navarra, a Bodegas Ochoa, al distribuidor de maquinaria agrícola Agriauto Remón S.A. y a la Sección Económica de la empresa pública INTIA (Tabla 7-4). Para estos cálculos, se ha considerado una tendencia a la baja en la producción del viñedo cuando se emplea la cubierta, más marcada la reducción conforme más años desde su instalación han pasado.

A continuación, se presenta la descripción de las principales labores de los distintos manejos:

a) **Cubierta de *T. fragiferum*:** se siembra una banda de 40 cm de ancho bajo la cepa, con una dosis de siembra de 15 g·m^{2}. El coste de la semilla es de 10,5 €·kg^{-1}. Previa a la siembra, se realizan dos pases de intercepas para dejar un suelo de siembra adecuado, estimándose un rendimiento horario de la labor de 0,9 h·ha^{-1}. La siembra se realiza manualmente y el tiempo de siembra requerido se estima en 2,5 h·ha^{-1} para una sola persona. Posteriormente a la siembra no se requiere de ninguna actuación sobre la misma, aunque podría ser interesante una labor de deshierbe manual en los meses de verano del primer año de instalación de la cubierta si existiera una alta presencia de otras hierbas. Esta labor supondría un tiempo aproximado de 3 h·ha^{-1}·persona^{-1}. El mantenimiento de la calle se estima en 4 pases de cultivador en los meses de noviembre, marzo, mayo y julio, con un rendimiento de 0,68 h·ha^{-1}.

b) **Laboreo intercepas:** se requieren de 4 pases de cultivador para el mantenimiento de la calle, más la labor de intercepas. El pase de cultivador de noviembre se realiza únicamente con el cultivador con un rendimiento horario de 0,68 h·ha^{-1}, mientras que los pases de marzo, mayo y julio se realizan con el intercepas acoplado al cultivador, por lo que el rendimiento horario sube a 0,93 h·ha^{-1}.

c) **Herbicida:** se realizan 4 pases de cultivador para el mantenimiento de la calle, con rendimiento de 0,68 h·ha^{-1}. Se estiman dos pases de herbicida, uno a la salida de invierno con un herbicida residual y otro en verano con un herbicida de contacto. Se estima el rendimiento para la aplicación de herbicida de 0,75 h·ha^{-1}, con un coste de los productos herbicidas de 35 €·ha^{-1}.

Tabla 7-4. Datos de referencia de la maquinaria empleada para el estudio económico.

	Tractor (80CV)	Intercepas de cuchilla hidráulico	Cultivador 2,5m	Barra de herbicida hidráulica
Inversión (€)	45.000	10.500	7.500	1.200
Trabajo anual (h·año^{-1})	400	40	44	20
Vida útil (años)	12	15	15	15
Valor residual (%)	10	20	20	20
Tipo interés (%)	0,5	0,5	0,5	0,5
Alojamiento (m^{2})	10	3	7	4
Coste alojamiento (€·m^{-2})	8	8	8	8
Coste seguro (€·año^{-1})	150	-	-	-
Consumo combustible (L·h^{-1})	7,5	-	-	-
Precio combustible (€·L^{-1})	0,7	-	-	-
Reparaciones (€·h^{-1})	0,28	1,07	1,07	1,07

La viabilidad económica del empleo de este tipo de cubiertas frente a otros manejos agronómicos de esta zona del suelo estará condicionada a asegurar una supervivencia de la cubierta de entre 2 y 5 años. Tal y como se puede observar en la Tabla 7-5, las posibles mermas de producción debidas a la competencia de la cubierta podrían incrementar el periodo de amortización de la cubierta requerido para que el gasto inicial no afectara a la rentabilidad de la explotación. En ese sentido, y como es de esperar, dichas mermas productivas tendrán un impacto económico mayor cuando los precios percibidos por la uva sean más elevados. Esta situación de reducción en el rendimiento es muy posible que no se produjera, o que, en caso de producirse, se pudiera corregir con un leve incremento en las aportaciones de riego y fertilización, las cuales apenas tendría repercusión económica. La necesidad o no de realizar un repaso manual para la eliminación de hierbas adventicias en el primer año tras la instalación de la cubierta si ésta no ha logrado una buena cubrición del suelo, no afecta a los plazos de retorno de la inversión.

Tabla 7-5. Años teoricos necesarios para equiparar los costes del empleo de la cubierta de *T. fragiferum* a un manejo con laboreo intercepas o con herbicida, frente a tres escenarios de producción.

Valores en negrita corresponden a la cubierta sin repaso manual. Valores en cursiva corresponden a la cubierta con repaso manual.

	COMPARATIVA CON LABOREO INTERCEPAS				COMPARATIVA CON HERBICIDA			
	Precios percibidos por kg de uva (€·kg^{-1})				Precios percibidos por kg de uva (€·kg^{-1})			
	0,35	0,45	0,6	0,8	0,35	0,45	0,6	0,8
Rendimiento 100% (7.500 kg·ha^{-1})	**1,7**/*1,8*	**1,7**/*1,8*	**1,7**/*1,8*	**1,7**/*1,8*	**1,3**/*1,4*	**1,3**/*1,4*	**1,3**/*1,4*	**1,3**/*1,4*
Rendimiento 95% (7.125 kg·ha^{-1})	**2,3**/*2,3*	**2,5**/*2,5*	**2,9**/*2,9*	**3,2**/*3,2*	**2,0**/*2,0*	**2,1**/*2,2*	**2,4**/*2,5*	**2,8**/*2,8*
Rendimiento 90% (6.750 kg·ha^{-1})	**2,9**/*3,0*	**3,4**/*3,4*	**4,1**/*4,2*	**5,1**/*5,1*	**2,3**/*2,4*	**2,7**/*2,7*	**3,1**/*3,2*	**4,0**/*4,0*

En el caso de la evaluación económica realizada por Karl *et al*., (2016b) con cubiertas anuales, los menores costes de manejo fueron para el manejo con una cubierta vegetal espontánea (84 $\$\cdot ha^{-1}$) seguida de una cubierta de *Trifolium repens* (169 $\$\cdot ha^{-1}$), del manejo con herbicida (548 $\$\cdot ha^{-1}$) y del laboreo (1036 $\$\cdot ha^{-1}$). Pero cuando se le resta la pérdida de ingresos por la reducción de producción, el manejo económicamente más rentable fue el herbicida, presentando la cubierta espontánea una reducción de beneficio de 1190 a 6496 $\$\cdot ha^{-1}$, la cubierta de *T. repens* de 491 a 5467 $\$\cdot ha^{-1}$, y el manejo con laboreo de 1590 a 6891 $\$\cdot ha^{-1}$. Por su parte, Nordblom *et al.* (2020) en el estudio económico que realizó sobre el trabajo de Penfold *et al.* (2018) observó como las cubiertas vegetales bajo la línea de cultivo pueden ser económicamente rentables dependiendo de la zona de cultivo donde se implanten, ya que esta condicionará en muchas ocasiones el precio percibido por la uva. En su caso los costes de instalación de las cubiertas los asumía para una vida útil de las mismas de cinco años. El rendimiento productivo que se obtenga con cada manejo tendrá repercusiones en la rentabilidad de la técnica. Así, las cubiertas que no merman el rendimiento o que consiguen incrementarlo, como el caso de los medicagos, resultan ser las más interesantes.

Por tanto, el empleo de una cubierta de *T. fragiferum* bajo la línea de cultivo puede considerarse económicamente rentable con respecto a otros manejos cuando se asegura una permanencia de la cubierta entre 2 y 5 años. La rentabilidad estará condicionada a su vez por los rendimientos productivos que se obtengan, así como el precio de la uva que se perciba.

Existen otros elementos difícilmente cuantificables desde el punto de vista económico, como serían los ambientales, mejora de la calidad del suelo o la reducción de la erosión, los cuales no deberían perderse de vista cuando se opte por un tipo de manejo de suelo. Así mismo, otras labores agronómicas, como despuntes o aclareos de racimos en viñedos muy productivos podrían llegar a no ser necesarias. Por último, la sanidad del cultivo también podría llegar a verse mejorada, obteniéndose uvas de mayor calidad.

8. CONCLUSIONES

- El empleo de una cubierta vegetal de *Trifolium fragiferum* bajo la línea de cultivo en condiciones de clima mediterráneo y con aporte de riego, supone una alternativa interesante para el manejo del suelo de viñedo frente al empleo de herbicidas o de laboreos intercepas. Dicha cubierta logra un control adecuado de las plantas adventicias, si bien requiere de un repaso manual si existe presencia de especies de elevado porte como *Coniza* sp., *Crepis foetida* o *Aster squamatus*.

- A nivel agronómico, la implantación de esta cubierta no causa variaciones muy relevantes en los parámetros productivos, ni en la composición de baya, si bien podrían presentar problemas en viñedos de zonas propensas a heladas primaverales. En cualquier caso, y aunque la competencia por recursos hídricos es reducida, sería recomendable un ligero aporte suplementario de riego durante los meses centrales del verano que compensara la ligera tendencia observada hacia una reducción del rendimiento. Asímismo, podría resultar interesante incrementar ligeramente la nutrición del viñedo en los primeros años de la cubierta, lo que podría contribuir también a minimizar posibles mermas en el rendimiento.

- Los vinos resultantes han marcado unauna tendencia a aumentar las notas florales y frutales en los vinos provenientes de las cepas con cubierta, lo que podría estar asociado al aumento observado para los niveles de nitrógeno fácilmente asimilable, consecuencia de la capacidad de fijación de la especie de cubierta empleada. Resultaría de interés estudiar en detalle aspectos analíticos no abordados en esta tesis, como aminoácidos y/o precursores aromáticos, de modo que se pudiera confirmar esta tendencia sin estar limitados por las incertidumbres del proceso de elaboración a pequeña escala ni por la subjetividad intrínseca a una evaluación por cata.

- En cuanto al impacto ambiental, los parámetros de calidad de suelo se han visto incrementados de manera significativa. El secuestro de carbono, así como la mejora de la estructura del suelo se han visto incrementados de manera importante a partir de la primera campaña, mientras que las mejorías sobre los aspectos ligados con la biología del suelo se observaron tras un periodo de tiempo algo mayor. En cualquier caso, se considera que el efecto favorable observado es rápido, lo que podría estar explicado por el tipo de cubierta empleda, por las condiciones de partida del suelo y por la utilización de riego, por lo que sería adecuado valorar dicha cubierta en otras condiciones edafoclimáticas, así como estudiar el impacto de otro tipo de especies vegetales como cubiertas. El alcance limitado de esta tesis no ha permitido abarcar aspectos muy relevantes relacionados con las condiciones agroecológicas del sistema, siendo de mucho interés acometer la evaluación del impacto de este tipo de actuaciones sobre la entomofauna y sobre la actividad de pequeños mamíferos y aves.

- La rentabilidad de esta práctica de cultivo frente al manejo con herbicidas o laboreo, en términos meramente económicos, estará ligada a que se pueda lograr una supervivencia de la cubierta lo más prolongada posible en el tiempo, sin necesidad de resembrar, así como minimizar o evitar las pérdidas productivas del viñedo. En cualquier caso, en un contexto más amplio de rentabilidad social y ambiental, que debería ser reconocido por las políticas públicas y por los consumidores, habría que considerar también otros aspectos como la mejora paisajística, mejora de la calidad del suelo y aumento de la biodiversidad, más complicados de evaluar en términos económicos, pero que tendrían que redundar también en un beneficio del viticultor.

- Los resultados presentados en esta Tesis se corresponden con un estudio en un único viñedo y durante un periodo de evaluación relativamente corto, por lo sería necesario obervar el comportamiento a largo plazo de esta cubierta y sus implicaciones, así como ampliar los ensayos a otros viñedos.

CONCLUSIONS

- The use of a plant cover of *Trifolium fragiferum* under-vine in Mediterranean climate conditions and with irrigation is an interesting alternative for vineyard soil management compared to the use of herbicides or inter-vine tillage. This cover achieves an adequate control of adventitious plants, although it does require a manual tillage if tall species such as *Coniza sp*., *Crepis foetida* or *Aster squamatus* are present.

- At an agronomic level, the establishment of this cover does not cause significant variations in production parameters or berry composition, although it could present problems in vineyards in areas prone to spring frosts. In any case, and although competition for water resources is reduced, a slight supplementary irrigation during the central summer months would be advisable to compensate for the slight tendency observed towards a reduction in yield. It might also be worthwhile to slightly increase the nutrition of the vineyard in the first years of canopy, which could also help to minimise possible yield losses.

- The resulting wines have shown a tendency to increase floral and fruity notes in the wines from the vines with canopy, which could be associated with the observed increase in the levels of yeast assimilable nitrogen, a consequence of the fixation capacity of the cover species used. It would be of interest to study in detail analytical aspects not addressed in this thesis, such as amino acids and/or aromatic precursors, so that this trend could be confirmed without being limited by the uncertainties of the small-scale winemaking process or the subjectivity intrinsic to an evaluation by tasting.

- In terms of environmental impact, soil quality parameters have increased significantly. Carbon sequestration as well as soil structure improvement have increased significantly from the first season onwards, while improvements on soil biology aspects were observed after a slightly longer period of time. In any case, the favourable effect observed is considered to be rapid, which could be explained by the type of cover used, by the initial soil conditions and by the use of irrigation, so it would be appropriate to evaluate this cover under other soil and climatic conditions, as well as to study the impact of other types of plant species as covers. The limited scope of this thesis has not allowed us to cover very relevant aspects related to the agroecological conditions of the system, being of great interest to undertake the evaluation of the impact of this type of actions on the entomofauna and on the activity of small mammals and birds.

- The profitability of this cultivation practice compared to management with herbicides or tillage, in purely economic terms, will be linked to achieving the longest possible survival of the canopy over time, without the need for replanting, as well as minimising or avoiding production losses in the vineyard. In any case, in a broader context of social and environmental profitability, which should be recognised by public policies and consumers, other aspects should also be considered, such as landscape improvement, improved soil quality and increased biodiversity, which are more complicated to evaluate in economic terms, but which should also benefit the vine grower.

- The results presented in this Thesis correspond to a study in a single vineyard and during a relatively short evaluation period, so it would be necessary to observe the long-term behaviour of this cover and its implications, as well as to extend the trials to other vineyards.

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10. ANEXO I-FICHA DE CATA

Nombre del Juez: ______________________________ Nº Cabina: ___ Fecha: __________

Instrucciones:

Se le presenta una serie de muestras identificadas con tres cifras: 357, 821, 974…

1. *Escriba el nombre de cada uno de los atributos que va a proceder a valorar*
2. *Evalúe las muestras y ordénelas, según su intensidad, para cada uno de los atributos seleccionados, marcando sobre la línea correspondiente con una barra (/) e indicando encima de la señal las cifras que corresponden a cada muestra (si no encuentra diferencias entre dos muestras, pueden colocarse en la misma posición).*

Atributo 1:

Bajo____Alto

Atributo 2:

Bajo____Alto

Atributo 3:

Bajo____Alto

Atributo 4:

Bajo____Alto

Atributo 5:

Bajo____Alto

Atributo 6:

Bajo____Alto

Atributo 7:

Bajo____Alto