





Using a trait-based approach to characterize weeds in a Chianti classico vineyard and their effect on grapevine nutrition

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Summary

Characterizing the spontaneous vegetation in perennial systems is a relatively novel field of study. This work applies the trait-based approach to study the effect of different soil management practices on weed functional traits in a Mediterranean vineyard. The effect of weed functional traits on grapevine stress indicators is also studied. The study was conducted in a commercial vineyard in the Chianti classico territory in Tuscany (Italy) and compared: conventional tillage, mown grass-legume cover crop, tilled grass-legume cover crop and mown spontaneous vegetation. Midday stem water potential (Ψ stem) and SPAD values were taken as stress indicators to measure the effect of the inter-row vegetation on the vines.

Treatment had a significant effect on both SPAD and Ψ stem. Vines in the conventional tillage treatment were generally the least stressed. The % of competitiveness of the weed community had a significant effect on Ψ stem. Treatment had no significant effect on weed functional traits, although the RLQ analysis showed a greater presence of creeping perennials, grasses and annuals in the tilled treatments.

Overall, the associations found between weed functional traits and management practices are in agreement with the current weed science literature, despite there not being a significant effect of the treatment on the traits, possibly because of the recent establishment of the experiment. Tillage and the sown cover crops seemed to be the strongest drivers for selection of weed functional traits. The small differences observed between treatments in terms of effects on vine stress may favor the adoption of no-till practices, providing that early vine stress indicators are used, and annual weather patterns are duly considered.

Key words: Functional traits, Spontaneous vegetation, Cover crops, Mediterranean, Stem Water Potential, Vine stress

Introduction

In perennial systems spontaneous vegetation is not seen as a primary threat to the crop as arboreous and herbaceous plants may have different temporal and spatial niches of resource use (Celette et al., 2008). In some cases, in orchards spontaneous vegetation is welcomed as it can improve trafficability and reduce the use of external pest control inputs by, for example, attracting beneficial predators of pests (Muscas et al., 2017; Garcia et al., 2018). In vineyards sown and spontaneous cover crops are used in both temperate and Mediterranean climates to reduce grapevine vegetative vigor, limit soil erosion and improve grape and wine quality (Monteiro & Lopes, 2007; Giese et al., 2014). Interestingly, spontaneous vegetation has shown to offer a cost-effective solution to such problems without major impacts on grape yield and quality (Lopes et al., 2011; Kazakou et al., 2016).

Despite this evidence, growers in Mediterranean areas often prefer to maintain bare inter-rows to minimize the risk of competition as water is a limiting resource (Celette *et al.*, 2008; Garcia *et al.*, 2018). Mediterranean vineyards are often rain-fed due to difficult access to irrigation water or because they are managed under certain production specifications (Garcia *et al.*, 2018), but studies which do not adopt irrigation in vineyard experiments are scarce (see Monteiro & Lopes, 2007). Vineyards have relatively low nitrogen requirements compared to most crops and usually a moderate water and nitrogen stress is favored to improve grape and wine quality (Celette *et al.*, 2009). In the variable Mediterranean climate, maintaining this delicate equilibrium of moderate stress could be easily compromised if the ground cover vegetation is not managed properly, leading to negative consequences for production even in following years. Therefore, studies on spontaneous vegetation in Mediterranean vineyards must foreground the likely dis-service of resource competition by the inter-row vegetation on the grapevines.

Characterizing the spontaneous vegetation in vineyards is important to provide farmers with information on the degree of competition between inter-row vegetation and grapevines. Recent literature has characterized spontaneous vegetation based on the species' functional traits, i.e. "a species feature that could be related to the expression of a given agroecosystem service or disservice" (Garnier & Navas, 2012) (Fried *et al.*, 2012; Garnier & Navas, 2012; Colbach *et al.*, 2014; Armengot *et al.*, 2016; Bàrberi *et al.*, 2018). By applying this definition, the trait-based approach focusses on functional diversity rather than taxonomic diversity. This increases awareness of keeping biodiversity in agro-ecosystems and increase the systems' capacity of delivering agro-ecosystem services (Moonen & Bàrberi, 2008).

In agro-ecosystems, weed community composition can be modified through management practices (e.g. tillage or conservation agriculture) in order to favor species that express functional traits related to ecosystem service provision (Lavorel & Garnier, 2002; Damour *et al.*, 2014, 2015; Kazakou *et al.*, 2016). Studying weeds based on their functional traits allows to understand the associations between the weed community and management practices to obtain general information on how the latter could reduce functional traits related to dis-services (Trichard *et al.*, 2013; Colbach *et al.*, 2014; Damour *et al.*, 2014, 2015; Armengot *et al.*, 2016).

Literature on the characterization of spontaneous vegetation in Mediterranean vineyards is scarce. It appears to be that only Kazakou *et al.* (2016) have identified spontaneous species in vineyards using functional traits and have studied the effect of weed community functional traits on grapevine stress indicators. Other studies have used functional traits (e.g. life history, primary classification) to explain the effect of resident vegetation or permanent cover crops on the grapevine, although it was not the primary aim of the research (Monteiro & Lopes, 2007; Celette *et al.*, 2008; Giese *et al.*, 2014).

The main goal of the study is to assess how the weed community responds to different soil management practices and how the functional traits affect grapevine stress indicators (based on Kazakou et al., 2016). Specifically, the study aims to test the following hypotheses 1) different soil management practices affect weed functional traits, in particular cover crops will select a more competitive weed community compared to the spontaneous vegetation and conventional tillage treatments 2) the presence of legumes in the sown cover cropped treatments lead to less nitrogen stress (i.e. higher recorded SPAD values) in the grapevines compared to the spontaneous vegetation and conventional tillage treatments 3) grapevines in the intercropped treatments show higher water stress compared to the vines in CT due to competition between the ground cover and the crop.

Materials and Methods

Experimental Site

The Chianti classico territory (Tuscany, Italy) fit the purposes of this research as it is renowned for the production of high-quality wine. The climate is typically Mediterranean, with dry hot summers and mild rainy winters. The average annual rainfall is 801 mm and the annual temperature 14.4°C average is (http://www.sir.toscana.it, accessed 18 September 2018). Producers are exposed to high risks if soil management practices are not tailored to support the vineyard growth and grape quality in the variable Mediterranean climate As an example, the common soil management practice (mown spontaneous vegetation) was put to test in 2017, when the area experienced an exceptionally dry and hot summer, with recorded precipitations of just 21 mm in July and August (http://www.sir.toscana.it, accessed 18 September 2018). In 2017 the annual precipitation in the area of study was 632.4 mm, 169 mm below the regional average (http://www.sir.toscana.it, accessed 18 September 2018). The severe drought experienced in 2017 put at risk both grape production and wine quality. Since then, the conversion to conventional tillage has been an open option for farmers even if it implies increased erosion from water run-off, compaction by machinery and loss of organic matter (Novara et al., 2011; Pou et al., 2011; Prosdocimi et al., 2016).

The topography of the Chianti classico territory (between the provinces of Siena and Firenze, Italy) is characterized by sloping land ranging between 0% and 122% and an average altitude of 233 m a.s.l. (http://www.soilmaps.it, accessed 30 January 2018).

The commercial vineyard in which the study was carried out is located in San Giusto a Rentennano (43°22'14.1"N 11°25'19.4"E), which is characterized by sandy loam soils originated from tuff stone.

Experimental Design

The grapevines (*Vitis vinifera*, L. var. Sangiovese R10, rootstock 420A) are planted at a density of 5000 plants ha^{-1} (2.50 x 0.8 m). The orientation of the vineyard is S-W (year of establishment: 1991). The training system of the grapevines is in transition from spurred cordon to the "guyot" trellis system.

Five soil management practices have been studied during the growing season of 2018 (Fig. 1):

1) Conventional tillage (CT); tillage was done in autumn, at the time of termination of the ground cover (late May) and in late August with a rigid tine cultivator at 15 cm depth.

2) Cover crop of *Vicia faba minor* L. (pigeon bean) sown at 90 kg seeds ha⁻¹, tilled in late May (L);

3) Cover crop of *Hordeum vulgare* L. (barley) and *Trifolium squarrosum* L. (squarrosum clover) sown at 85 and 25 kg seeds ha⁻¹ respectively, mown in late May and left as surface mulch (GLM);

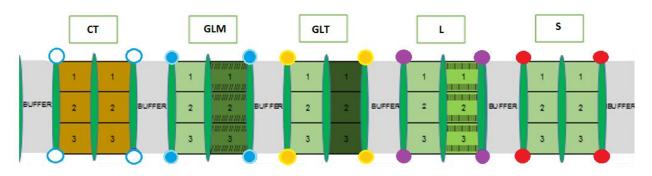


Figure 1. Layout of the experimental design. The colors were used to identify each treatment in the field. Numbers indicate pseudo-replicates.

4) Cover crop of barley and squarrosum clover sown at 85 and 25 kg seeds ha⁻¹ respectively, tilled in late May (GLT);

5) Spontaneous vegetation mown in late May and left as surface mulch (S).

Cover crops were sown in November 2017. In autumn, the spontaneous vegetation rows were left undisturbed. In all treatments, the soil under the rows was kept clean with an in-row ventral plough. Each treatment consists of one vine row of about 100 m and one inter-row, divided in three pseudo-replicates ("blocks") according to the slope of the vineyard, to account for the soil heterogeneity between and within treatments. Treatments were displayed in alternate rows, separated by a buffer strip.

Data Collection

Spontaneous Vegetation

The ground cover vegetation was collected in mid-May, right before cover crop termination. Measurements were taken in one 0.5 m x 0.5 m quadrat, with four sub-replicates per block (n=12 per treatment, n=60 per plot). The total plant cover per quadrat was assessed visually and expressed as percentage of bare soil. Species were identified and their relative cover was estimated visually. A second ground cover sampling of the inter-row vegetation was performed at the beginning of August.

Selection of plant functional traits

The functional traits chosen for this study were: Grime strategy, life form, Specific Leaf Area (SLA) and primary classification (grass or dicotyledon). Grime strategy indicates whether a species is competitive, stress-tolerant or ruderal (Grime, 1977). SLA is used to position species along the "Leaf Economic Spectrum", indicating whether a species' has an acquisitive or conservative strategy (Tribouillois *et al.*, 2015). Life history (annual, perennial) indicates how fast species regenerate after

the winter. Perennial species regenerate faster than annuals in the spring, competing earlier with the crop. Functional traits were primarily retrieved from the database developed by Bàrberi et al. (2018) and the LEDA database (Kleyer et al., 2008). The Grime strategy of each weed was expressed as % of competitiveness (%C), using the database developed by Pierce et al. (2017). The BIOLFLOR database (Klotz et al., 2002) and the "TR8" package in R Studio (Bocci, 2017) were also used to retrieve traits for some of the species. When species could not be found in the databases, traits were either taken from the most similar species in the database or were obtained as an average of the trait at genus level. Species which were found three or less times were eliminated from the statistical analysis. SLA values were log-transformed prior to data analysis.

Grapevines

Measurements on the grapevines were done from flowering (May) until veraison (August) at a 15-day time step. SPAD measurements were done from late May using a SPAD-502 chlorophyll meter (Konica Minolta Sensing Europe B.V.) (Prost & Jeuffroy, 2007); three exterior and fully expanded median leaves per stock were chosen (Taskos et al., 2015) and on each leaf three points were measured and averaged (five stocks per block, 675 measurements per plot per date). From late July, Midday Stem Water Potential (Ustem) of the grapevines was measured using a pressure chamber (PMS 600D, PMS Instrument Company, USA) (Scholander, 1965); one undamaged leaf per stock was selected (three stocks per block, 45 measurements per plot per date). One hour before measuring, the leaves were enclosed in plastic bags covered with aluminum foil to stop transpiration (Williams & Araujo, 2002). Measurements started at midday and were taken following the wrapping order.

Data Analysis _{Soil} Analyses on soil data were done on a baseline dataset which contained chemical and physical soil information of 15 sampling points in the vineyard (one per block). Three soil depths per sampling point were analyzed (n=45) (0-10, 10-30 and 30-60 cm). Prior to analysis the values of the three depths per point were weighted and averaged to obtain one set of values per block (n=15). Given the high soil heterogeneity within the experimental plot, K-means clustering was performed on the soil physical and chemical data to identify relatively uniform "zones" in the experimental plot (package "fpc", function "kmeans") (Henning, 2018).

Spontaneous vegetation

Data from the second ground cover sampling was used to perform analyses on spontaneous vegetation. Non-Metric Multidimensional Scaling (NMDS) was used as a preliminary analysis to study species' distribution in relation to treatment and block (package "vegan", function "metaMDS") (Oksanen, 2013). Treatment and block effect were studied with a Permutational multivariate analysis of variance (Permanova) using the "adonis" function ("vegan" package) (Oksanen, 2013). When the effect was found significant pairwise comparisons were performed (function "pairwise.adonis") (Arbizu, 2017). Species' cover data were then analyzed using the RLQ method (Kleyer et al., 2012; Bàrberi et al., 2018). This method uses three tables: the R table contains the environmental data (treatments, blocks and quadrats); the L table contains species abundances (or cover in this case) per each site; the Q table contains species traits. A fourth corner analysis (function "fourthcorner" in package "ade4") was also performed to assess the correlation between the environmental variables and the functional traits (Dray et al., 2014).

Effect of functional traits on grapevine stress indicators The effect of the functional traits on the grapevine SPAD and Ψ stem values was assessed using a Mixed Effect Model (package "nlme"). The random part of the model included sampling time and clusters (nested within the blocks). Given the high level of data heterogeneity, statistical results indicating a p value <0.10 were also discussed, although these data should be treated with caution. All statistical analyses were performed in RStudio (version 3.4.3, 2017).

Results

Soil

The k-means clustering performed on the soil data revealed a total of four clusters. No differentiation was made between blocks (except for CT); the strongest gradient was transversal to the slope of the vineyard (Fig. 2).

Spontaneous vegetation

At the first cover sampling 28 species were found. After the ground cover vegetation was terminated, a total of 23 species were found (Tab. 1).

Table 1. List of species found during the second cover sampling performed at the beginning of August.

Species		
Adonis foemina L.	Heliotropium europaeum L.	Polygonum aviculare L.
Agropyron repens (L.) P. Beauv.	Hypericum perforatum L.	Potentilla reptans L.
Alopecurus mysuroides Huds.	Lactuca perenne L.	Rumex crispus L.
Anagallis arvensis L.	Lactuca serriola L.	Senecio vulgaris L.
Cirsium arvense (L.) Scop.	Lolium perenne L.	Sonchus arvensis L.
Convolvulus arvensis L.	Medicago lupulina L.	
Cynodon dactilon (L.) Pers.	<u>Mercurialis</u> annua L.	
<u>Daucus</u> carota L	<u>Picris echioides</u> L.	

The first two axes of the RLQ analysis explained 75.54% of total inertia (i.e. variance). The first axis of

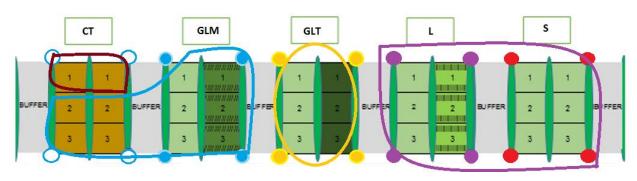


Figure 2. Zones with similar soil characteristics in the commercial vineyard, obtained with k-means clustering. Soil variability along the slope is less strong than the variability across the slope.

the RLQ (Fig. 3) slightly discriminated between management types, i.e. tilled vs. mown treatments. Block 1 (at the highest point of the slope) was also distinctly separated from blocks 2 and 3. The second axis discriminated between grasses and forbs.

The Permanova performed on the species' cover data indicated that there was no significant effect of treatment or block (p > 0.1). Moreover, the NMDS plot (stress=0.14) showed no particular linkages between species distribution and treatment or block indicating that other factors may have played an important role in determining species distribution within the vineyard.

The strongest association was found between *Cynodon dactylon (L.)* and *Agropyron repens (L.)* and the CT treatment, especially in the second and third blocks, which are the creeping perennial grasses found in the RLQ plot (Fig. 3). With respect to the functional traits, the fourth corner analysis showed no significant effect of treatment or block. 0.007392). Post-hoc Tukey test showed that vines in CT had significantly higher SPAD values than vines in S (Es-timate= -0.1124317, Pr(>|z|)= 0.0110) and vines in GLT had significantly higher SPAD values than vines in S (Es-timate= - 0.1114709, Pr(>|z|)= 0.0697) (Fig. 4).

Grapevine Water Potential

Both treatment and the % of competitiveness of the weed community had a significant effect on Ψ stem (Fig. 5) (Pr(>Chisq)= 0.027328 and Pr(>Chisq)= 0.006846 respectively). Post-hoc Tukey test showed that vines in CT had significantly lower Ψ stem values (in absolute value) than vines in GLM (Estimate=0.09141, Pr(>|z|)= 0.0526) and that vines in L had significantly lower Ψ stem values (in absolute value) than vines in GLM (Estimate= -0.11090, Pr(>|z|)=0.0620).

Discussion

Effects of inter-row soil management practices on the spontaneous vegetation.

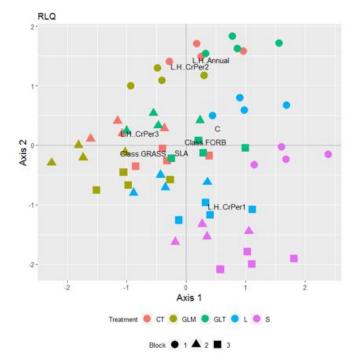


Figure 3. RLQ plot (n=60). Functional traits expressed as factors (Life history, primary classification) are coded as the functional trait type followed by the level of the trait (e.g. life history, creeping perennial with stolons and above-ground roots= LI.HI.CrPer1). CrPer2= under-ground plagiotropic shoots, rhizomes. CrPer3= co-presence of two or more structures.

Effects of weed functional traits on vine stress indicators.

Grapevine SPAD

From the mixed effect model it resulted that treatment had a significant effect on the SPAD values (Pr(>Chisq)=

Despite the soil heterogeneity indicated by the cluster analysis, the results found in this study are in line with the available literature. After one year of applying new management practices in the inter-rows, the RLQ plot already showed distinct associations between some traits and soil management practices. In general, tillage

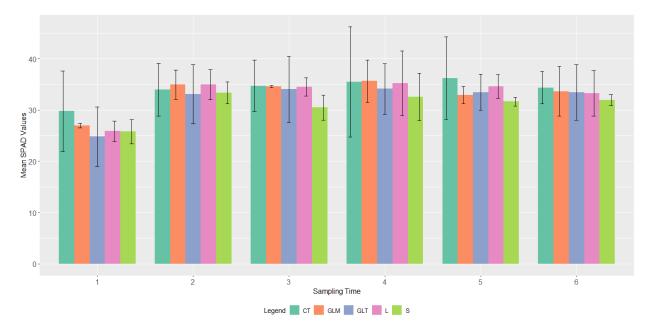


Figure 4. Mean SPAD values at each sampling time (from late May to late August, n=4050). Vines in the CT and GLT treatments had significantly higher SPAD values than vines in the S treatment (p=0.0110 and p=0.0697 respectively).

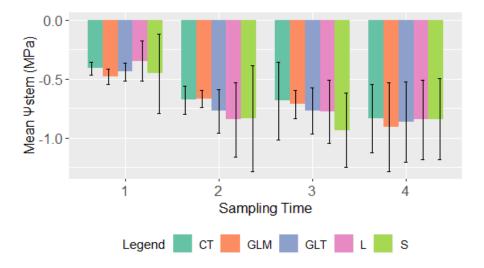


Figure 5. Mean Ψ stem values at each sampling time (from late July to late August, n=180). Vines in the CT were less stressed than vines in GLM and vines in L were less stressed than vines in GLM (p=0.0526 and p=0.0620 respectively).

and cover cropping showed evident effects on the weeds' functional traits compared to the spontaneous vegetation treatment.

Tillage has been shown to select less diverse communities dominated by annual species with higher SLA and more competitive strategies (Trichard *et al.*, 2013; Armengot *et al.*, 2016; Kazakou *et al.*, 2016). In this study annual dicots were associated with the tilled treatments, but the higher %C was found in the cover crop treatments. Tillage treatments were also strongly associated with creeping perennials, which is expected as tillage facilitates fragmentation and dispersal of rhizomes in the soil (Armengot *et al.*, 2016). Creeping perennials were found also in the tilled cover crop treatments (GLT and L) but showed more competitive traits (e.g. *Potentilla reptans* (L.) and *Convolvulus arvensis* (L.)). The rapid development of the barley/clover and of the pigeon bean is likely to have selected for weeds more competitive for light and space (Kazakou *et al.*, 2016).

In CT the weed community was not associated with a high %C. Instead it was dominated by species with more ruderal and stress-tolerant strategies, such as *Cynodon dactylon*, which was mostly found in the lower parts of the vineyard (Fried *et al.,* 2012; Kazakou *et al.,* 2016; Bàrberi *et al.,* 2018).

Clearer effects are likely to manifest in the future years. Most studies published on the response of weed functional traits to management often considered a time span of at least three years since the beginning pf the experiment (e.g. Garnier et al., 2006; Trichard *et al.*, 2013; Armengot *et al.*, 2016; Kazakou *et al.* 2016), or have analyzed long-term datasets with 30 years of information about land use and weed composition (e.g. Fried *et al.* 2012).

Effects of weed functional traits on vine stress in-dicators.

Grapevine SPAD

Functional traits did not have an effect on the SPAD values recorded on the vine leaves. Treatment had the strongest effect on the SPAD values, with vines in the CT treatment being significantly less stressed than vines in the S treatment. The cluster analysis performed on the soil data showed that the two spatially opposite treatments (CT and S) belonged to two different clusters and indeed they were the only significantly different treatments regarding leaf SPAD values at all sampling times. The CT treatment was located on a generally clayey soil with a lower C/N ratio compared to the S treatment. The cumulated rainfall by August 2018 was 674.6 mm, with higher precipitations in the summer period (144.2 mm) compared to the average summer rainfall of the area (126 mm, years 1981-2010). The pedoclimatic factors combined with the improved water infiltration provided by tillage, vines in the CT treatment were more favored in comparison to the other treatments.

With respect to the cover crop treatments, the effect of introducing a legume in the inter-row did not seem beneficial in increasing the SPAD values after the termination of the cover crop, compared to the other treatments. Moreover, the probable sub-optimal density of the cover crop did not produce sufficient biomass to release enough organic nitrogen in the soil. Although the rhizobium nodules were not checked, there is also a possibility that the nitrogen-fixing ability of the legumes was not optimal.

Berry quality in vineyards is not correlated to biomass production. Vine growers tend to limit nutrient uptake from the soil in order to maintain low biomass production and promote grape (and wine) quality (Celette *et al.*, 2009). This can be done with cover cropping, but it is important to regulate the nitrogen fluxes to avoid the vine using nitrogen stored in its ligneous parts.

In the vineyard under study different spontaneous *Medicago* species were found. Spontaneous leguminous species could be favored in the less fertile parts of the vineyard by inoculating Rhizobium bacteria. This could help improve the soil structure as to better retain moisture and nutrients, acting as a buffer during dry years.

Grapevine Water Potential

In general, vines in the CT treatment showed lower Wstem values throughout the summer season compared to the other treatments. The aeration of the soil through tillage at the beginning of the summer likely helped water infiltration at deeper soil levels (30-60cm) where the vines actively take up water (Morlat & Jacquet, 2003). The L and GLM treatments induced higher stress on the grapevines compared to CT as they had a higher % of competitiveness. Vines in the intercropped treatments have also likely suffered the competition from the sown cover crops earlier in the season.

The inter-rows' effect on the vines was likely mitigated by the relatively high precipitations of 2018. Inter-annual rainfall variability was suggested as a primary factor in determining the inter-row's vegetation effect on the vines (Pou *et al.*, 2011; Steenwerth *et al.*, 2016). In particular, the rainfall during the phase of *veraison* (berry ripening) was 16.8 mm and was preceded by above-average June precipitations (http://www.lamma.rete.toscana.it, accessed 18 September 2018; http://www.sir.toscana.it, accessed 18 September 2018).

The overall density of the inter-row vegetation also mitigated the vegetation effect on the grapevines. The vine rows were regularly kept clean with a mechanical ventral plough, so the vegetation developed approximately within a 1m space between rows.

Following Delpuech & Metay (2018), the vegetation in the inter-rows kept by the vine growers in the Chianti district corresponds to a 60% cover crop density. The intercropped treatments also followed this design which probably set the vines in these treatments at a higher stress level compared to CT.

The small differences between treatments regarding SPAD and Ψ stem would lead to favor a 60% spontaneous vegetation density design combined with mowing. Vegetation in the S treatment was not associated with high %C, compared to the spontaneous vegetation in the cover crop and CT treatments. Moreover, the cover crops need to be sown each autumn which leads to detrimental effects on the soil, due to erosion caused by heavy autumn rains (Celette *et al.*, 2008; Celette & Gary, 2013; Garcia *et al.*, 2018). Favoring the S treatment would be beneficial in reducing soil losses and improving soil structure (Monteiro & Lopes, 2007; Celette *et al.*, 2008; Pou *et al.*, 2011). Despite this, early indicators of stress such as Fraction of Transpirable Soil Water (Delpuech & Metay, 2018) would be recommended to help deal with the inter-row vegetation management, especially in dry years.

Conclusions

This study has made a step forward in research on soil management practices in Mediterranean vineyards by using a functional trait approach and assessing competition of the ground cover on the vines. After only one year of study clear patterns were already visible. Regarding the vine stress indicators, environmental variability mitigated the effects of the functional traits related to competition on the grapevines. Legumes were not beneficial in increasing grapevine SPAD values compared to other treatments; on the other hand CT was beneficial in maintaining higher Ψ stem values compared to the other treatments.

Ironically, the main limitation to this study was an unusually rainy season, which has led to optimistically favor a more agro-ecological practice (S) instead of CT in the commercial vineyard under study. Even though vines in CT were less stressed, the data collected in this first experimental year are not enough to make strong conclusions about whether conventional tillage is indeed a justified solution to prevent excessive stress in Mediterranean vineyards. Mature vineyards are susceptible to competition with the inter-row vegetation, but effects on the grapevines are usually observed after three years of experimentation (Tesic *et al.*, 2007; Steenwerth *et al.*, 2016; Delpuech & Metay, 2018).

Studies which operate in Mediterranean areas need to acknowledge environmental limitations such as soil heterogeneity and inter-annual weather variability. Taking into consideration the whole system would allow to seek zone-specific solutions in vineyards and give more realistic and tailored agronomic advice to local farmers. In this sense, carrying out research in commercial vineyards contributes to adopt more systemic approaches to research and improve the reliability of scientific findings.

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