IMPLICATIONS OF HERBICIDE, CULTIVATION OR COVER CROP UNDER-VINE SOIL MANAGEMENT ON THE BELOWGROUND MICROBIOTE

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Context and purpose of the study. Soil management through cover crops in the lines of the vineyards is a common practice in viticulture, since it improves the characteristics of the soil. It has been shown that the cover crops can influence the cycle of nutrients, promote infiltration, decrease erosion, and enhance the soil microbiota biodiversity improving the grapevines. However, the area under the vines tends to be left bare by applying herbicides or tillage to avoid competition with the crop in hot climates. The use of cover crops under the vines might be a plausible alternative to the use of herbicides or cultivation, improving grapevine quality and soil characteristics. The aim of this research was to study the implications of different management of the soil under the vines (herbicide, cultivation or cover crops) on grapevine growth, water and nutritional status and belowground microbial communities.

Material and methods: Experimental design consisted in 4 treatments applied on potted Tempranillo grapevines with 10 repetitions each grown in an open-top greenhouse in 2022. Treatments consisted in two species of cover crops (*Trifolium fragiferum* and *Bromus repens)*, herbicide (glyphosate al 36%) and an untreated control. The total biomass of covers and the vine growth were measured throughout the season. Water status was monitored by measuring the stem water potential. In Autumn, three plants per treatment were collected to record fresh and dry masses of the different organs (roots and shoots) and water content was estimated as the relationship between the fresh and dry masses. Soil microbial diversity and physiological profiles were measured using the plates Biolog Ecoplates^{TM} from soil samples collected at 25 cm.

Results. According to our results, T. *Fragiferum* was the cover crop under the vine that obtained the highest biomass. In spite of the enhanced vegetative development of *T. fragiferum,* preliminary results did not show differences on grapevine performance and growth compared to other treatments. However, the use of cover crops under the vine affected soil microbial communities enhancing their diversity and their activity. In general, the cover crops obtained better results, in comparison with the use of herbicide, *T. Fragiferum* being the one that had the greatest effect on the biological quality of the soil. The lack of effect on cover crops under the vines on the grapevine performance might indicate a minimum competition between the grapevine and the studied cover crops. Therefore, the use of these covers under-vine could be an alternative to the use of herbicides to control the growth of adventitious vegetation. In addition, the improvement of the biological quality of the soil would also affect positively the performance of grapevines.

Keywords: Bacterial diversity, functional diversity, soil health, Tempranillo, *Trifolium fragiferum*, water content.

1. Introduction

Soil management through cover crops in the lines of the vineyards is a common practice in viticulture, since it improves the characteristics of the soil (Adad et al., 2021). As these authors reviewed, cover crops can influence the cycle of nutrients, promote infiltration, decrease erosion, and enhance the soil microbiota biodiversity improving the physiologic state of grapevines. However, the area under the vines tends to be left bare by applying herbicides or tillage to avoid competition with the crop in hot climates. The use of cover crops under the vines might be a plausible alternative to the use of herbicides or cultivation, improving grapevine quality and soil characteristics (Abad et al., 2020; Nogales et al., 2021), as well as increase soil organic matter and biodiversity (Kim et al., 2020; Vukicevich et al., 2016). Furthermore, a recent research pointed out that the introduction of an arbuscular mycorrhizal fungi (AMF) through rye donor cover crops under the vines was an efficient method to inoculate adult grapevines by promoting the establishment of grapevine root mycorrhizal communities that were able to increase plant adaptability to extreme weather events (Nogales et al., 2021).

Therefore, the aim of this research was to study the implications of different management of the soil under the vines (herbicide, cultivation or cover crops) on grapevine growth, water and nutritional status and belowground microbial communities.

2. Material and methods

Plant material and growing conditions

Plant materials - Experiment was conducted at experimental installations of the Public University of Navarra (UPNA, Pamplona, Navarra, Spain) in 2022. Two-year old Tempranillo grafted onto 110 Richter were grown in 35 L pots filled with peat in an open greenhouse. Plants were drip irrigated to fit maximal evapotranspiration. Experimental design consisted in 4 treatments applied on potted Tempranillo grapevines with 10 repetitions each. Treatments consisted in two species of cover crops (*Trifolium fragiferum* and *Bromus repens)*, herbicide (glyphosate at 36%) and an untreated control.

Plant measurements - The total biomass of covers were weighted and dried to estimate water content. At the end of the growing season, leaves were counted and total length of shoots was measured. Total cross-sectional area was estimated after measuring the shoot areas with a digital caliper (CD67-S15PP, Mitutoyo Corp., Japan). Water status was monitored by measuring the stem water potential (Ψ_S) of a fully expanded leaf exposed to sun and without signs of disease and/or damage per treatment-replicate. Leaves were then covered before measurements with a reflective foil-lined zip-top plastic bag to suppress transpiration. The Ψ_S was measured with a Scholander pressure chamber (P3000, Soil Moisture Corp., Santa Barbara, CA, USA). Then, three plants per treatment were collected to record fresh and dry masses of the different organs (roots and shoots) and water content was estimated as the relationship between the fresh and dry masses. Root samples were collected and stained for the determination of the mycorrhizal colonization according to Torres et al. (2016). Soil microbial diversity and physiological profiles were measured using the plates Biolog Ecoplates^{TM} from soil samples collected at 25 cm.

Statistical analysis - Statistical analyses were conducted with R studio version 3.6.1 (RStudio Team, 2020). Grapevine growth, water status and bacterial diversity parameters were analyzed by using the one-way analysis of variance (ANOVA) after assessing the normality of the data. Means ± standard errors (SE) were calculated and, when the F ratio was significant ($P \leq 0.05$), a Duncan *posthoc* test was executed using “agricolae” 1.2–8 R package (de Mendiburu, 2016).

3. Results and discussion

3.1. *Cover crop biomass and water content differed between studied species.*

Cover crops used in this experiment (i.e., or natural growing) strongly differed in their growth, thus, *T. fragiferum* growth was *to ca*. four times greater than the one of *B. repens* and a hundredfold increase compared to natural growing before cultivation. This came with a higher water retention of the system (Table

1). This result could account for increasing the net ecosystem carbon balance (NECB), which estimates the C inputs and outputs in commercial production settings at vineyard scale. Our results suggested that the use of *T. fragiferum* as cover crop is linked with a higher storage of C in the system given that cover crops that produce greater biomass increased NECB (Zumkeller et al., 2022).

3.2. *Cover crops had no effect on pot-grapevine vegetative growth*

Table 1 shows vegetative growth of grapevines grown in pots where no effect due to soil management was observed. Previous researchers have shown that grapevine vegetative growth is impaired by cover crops compared to conventional tillage (Steenwerth et al., 2013; Wolff et al., 2018). However, in accordance with recent studies, cover crops might have no effect on it (Abad et al., 2020; Zumkeller et al., 2022). Grapevines water status was not affected by different soil managements (data not shown), accordingly, several studies have reported no effect of cover crops on grapevines water status given that these effects are largely driven by the climatic conditions and irrigation regime at a given site (reviewed by Zumkeller et al., 2023).

3.3. *Soil management with cover crops affected microbes associated with grapevine roots and rhizosphere.*

Under our experimental conditions, cover crop soil management tended to increase mycorrhizal colonization compared to herbicide use (Figure 2A). Similarly, previous studies showed that the establishment of cover crops promotes the proliferation of natural mycorrhizal communities (Brígido et al., 2017; Soti et al., 2016; Nogales et al., 2021).

On the other hand, the analysis of heterotrophic bacteria diversity showed that the use of *T. fragiferum* as cover crop resulted in a higher number of substrates used by soil bacteria and higher Shannon and Simpson indexes (Figure 2B). Similarly, Likar et al. (2017) demonstrated that soil microbial diversity varied considerably between vineyards under conventional and ecological management, with bacterial communities strongly affected by tillage.

4. Conclusions

Although current research presents data from a single-year experiment, data are promising and the use of cover crops as a tool for managing the soil under vines seems to have benefits compared to the conventional soil management with herbicide and tilling.

5. Acknowledgments

This study was supported by the collaborative regional project “Ayudas a Agentes del SINAI para la realización de proyectos de I+D colaborativos” funded by Navarra Government (project PC044-045_CUALVID). N. Torres is beneficiary of a Ramón y Cajal Grant RYC2021-034586-I funded by MCIN/AEI/ 10.13039/501100011033 and by “European Union NextGenerationEU/PRTR”.

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Table 1. Fresh and dry mass, and water content of cover crops in the Tempranillo potted experiment.

	Fresh mass (g)	**Dry mass (g)**	**Water content**
Control	9,686 ± 2,299 **c**	2,886 ± 0,570 **c**	0,678 ± 0,278 **b**
B. repens	244,625 ± 20,444 **b**	23,696 ± 2,487 **b**	0,899 ± 0,136 **a**
T. fragiferum	811,350 ± 41,365 **a**	118,031 ± 4,645 **a**	0,854 ± 0,003 **a**
ANOVA	*	*	*

Values are means ± EE. Within each column different letters represent significant difference according to the Duncan posthoc test.

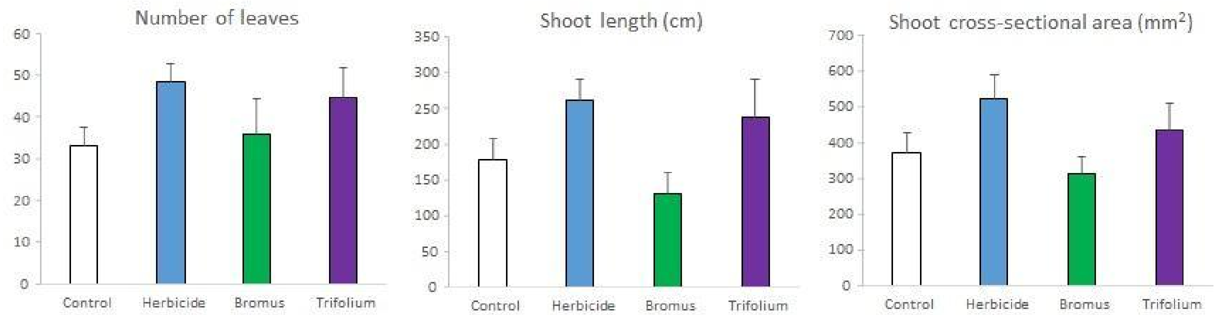

Figure 1: Vegetative growth (number of leaves, shoot length and shoot cross-sectional area) of Tempranillo/110R vines grown in pots with cover crops (*Trifolium fragiferum* and *Bromus repens*), herbicide or untreated (control) in 2022.

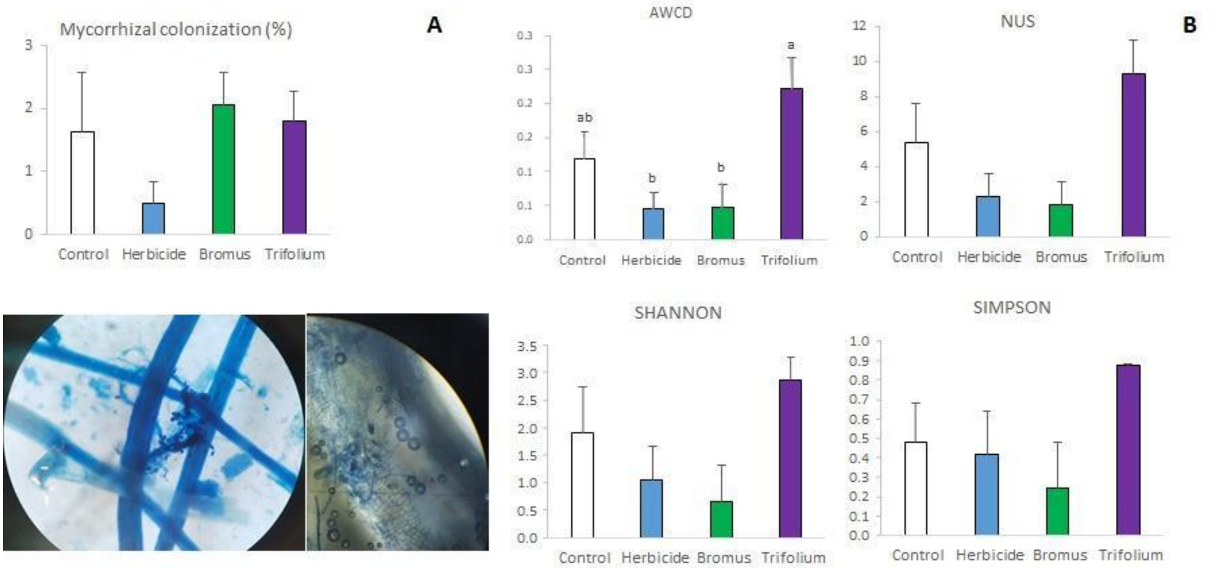

Figure 2: (A) Mycorrhizal colonization (%), and **(B)** heterotrophic bacterial parameters obtained with the Biologs EcoplatesTM of the Tempranillo/110R vines grown in pots with cover crops (*Trifolium fragiferum* and *Bromus repens*), herbicide or untreated (control) in 2022.