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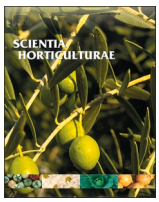

Under-vine cover crops: Impact on physical and biological soil proprieties in an irrigated Mediterranean vineyard

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ABSTRACT

Despite their potential benefits, cover crops in vineyards under to Mediterranean climate conditions are still not widespread, especially in the vine row. This under-vine space is normally kept weed-free through the application of herbicides and/or tillage. In this work, we evaluate the effect of a *Trifolium fragiferum* L. under-vine cover crop (UV) on soil quality, as reflected by a variety of physical, chemical, and biological soil indicators. 15 months after under-vine cover crop implementation, total (SOC) and particulate (POC) organic carbon storage, and soil aggregation (abundance (WSA) and mean weight diameter (MWD) of water stable aggregates) were compared with a control without cover (T) at 0–15 cm. In addition, for three consecutive years after under-vine cover crop implementation, the evolution of soil microbial communities was monitored through the determination of soil basal respiration, soil microbial biomass carbon and nitrogen (MBC and MBN), and community-level physiological profiles (CLPPs). The establishment of the under-vine cover crop resulted in higher values of SOC (27.78 Mg·ha^{-1} in UV *vs.* 20.71 Mg·ha^{-1} in T, +33%) and POC (4.75 Mg·ha^{-1} in UV *vs.* 2.73 Mg·ha^{-1} in T, +74%), as well as aggregation parameters (MWD: 1.82 μm in UV *vs.* 1.56 μm in T, +17% and WSA: 84.68% in UV *vs.* 71.58% in T, +18%). An evolution towards progressively greater values of biological activity (basal respiration) and microbial biomass was detected in under-vine cover crop soils as time elapsed. It was concluded that the *Trifolium fragiferum* L. under-vine cover crop led to soil quality improvement in our Mediterranean climate vineyard. Interestingly, this positive effect was noticed shortly after its implementation.

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., 2021b). 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. Although some alternative can be used to alleviate this competition (Bedbabis et al., 2014; Boselli et al., 2019), it can result in relevant changes from the grower's point of view, *e.g.* the cover crop can modify shoot growth, fruit set, berry development, yield, cluster number (caused by a decrease in bud fruitfulness as a carryover effect for the following season), and grape composition (Abad et al., 2021a). 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.

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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, especially in Mediterranean climate conditions, even though both methods may present several adverse consequences. For one side, many herbicides are strongly being questioned for their potential environmental impact and, in consequence, legal constraints and society's disapproval of their use are currently increasing (Wang et al., 2022). Besides, 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). Additionally, some organic compounds can also play a role to reduce the negative effects of chemicals used in agriculture (Ferrara et al., 2000, 2004). 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-competitive under-vine cover crops constitutes an interesting alternative to herbicide application and mechanical tillage (Jordan et al., 2016; Karl et al., 2016b), that however, it still requires a greater number of investigations in different soil conditions and with different plant species used as cover, especially in Mediterranean climate conditions. Normally, its use is located in regions with high rainfall in order to control excessive vegetative growth. (Chou and Vanden Heuvel, 2018; Coniberti et al., 2018; Hickey et al., 2016; Jordan et al., 2016; Karl et al., 2016b; Penfold et al., 2019; Vanden Heuvel and Centinari, 2021). In particular, to the best of our knowledge, only a few studies, such as the one conducted by Penfold et al. (2019) or the one conducted by Tesic et al. (2007) 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* L. was selected as the most suitable plant species after some preliminary experiments (Abad et al., 2020), 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 effects recently 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 (the main results summarised in Table S1). 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* L. under-vine cover crop on the soil quality of a vineyard under Mediterranean climate, through the examination of various soil physical, chemical and biological properties. We hypothesized that the *T. fragiferum* L. under-vine cover crop would lead to soil quality improvement in a Mediterranean vineyard.

2. Materials & methods

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 (lat.42.37946, long. −1.61312), belongs to Bodegas Ochoa winery, was planted in 2001 and, since 2018, has been managed under organic certification. The soil is a Typic Calcixerept (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 (pooled sample from trial area), 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%. The *Vitis vinifera* L. variety cultivated is Merlot (clone 343) grafted onto rootstock 420A, with a planting distance of 3 × 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 $mm{\cdot}yr^{-1}$). The climate of the area corresponds to Bsk class (hot semi-arid steppe) in Koppen's classification, according to Papadakis (1952), is humid temperate Mediterranean. Meteorological conditions during the trial seasons are summarized in Fig. 1.

The experimental design included two treatments, *i.e.* (1) UV = under-vine cover crop; and (2) T = mechanically tilled control (standard practice), with five replicates per treatment located in alternate rows. For the UV treatment, seeds of *T. fragiferum* L. were sown at a 15 $g{\cdot}m^{-2}$ dose on a 40 cm-wide strip. Broadcast 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 rows were kept free of vegetation (weed-free) by tilling four times every year: early November, March, May, and late June or early July.

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 15 months after under-vine cover crop establishment. One sampling point was randomly defined at each treatment replicate, always between two vines and between two drip irrigation emitters. 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 analyze 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 (Eq. (1)) (Rodríguez Martín et al., 2016), as suggested by the FAO (Lefèvre et al., 2017).

$$SOCStock = SOC \times BD \times D \quad (1)$$

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

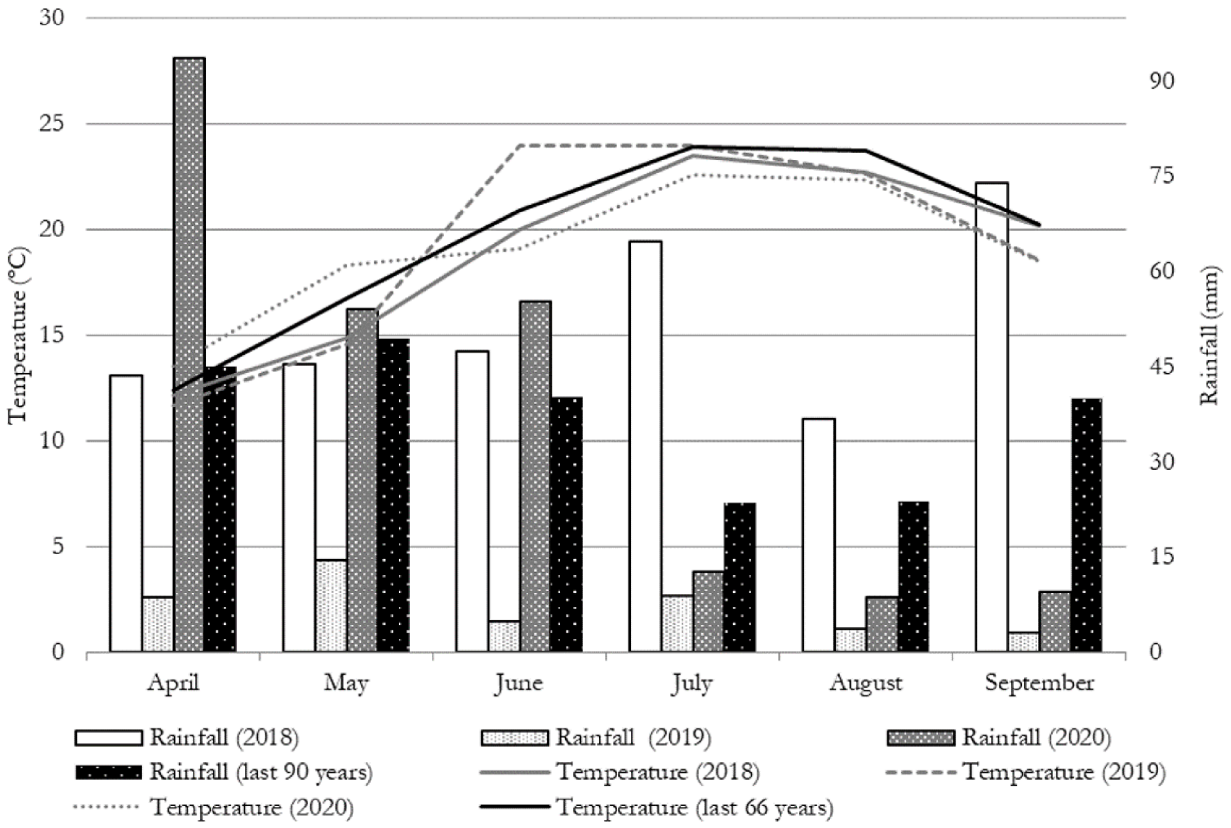

Fig. 1. Weather conditions of the three seasons compared to the mean values in the area (April–September): mean monthly temperature (°C) and accumulate monthly rainfall (mm).

determined by chemical dispersion with $(NaPO_3)_6$ 1 N, 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).

2.3. Hydraulic conductivity, bulk density and porosity

Measurements of the soil saturated hydraulic conductivity (K_s) 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 $g{\cdot}cm^{-3}$) 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 Darcy's Law described in Eq. (2):

$$K_s = \frac{V.L}{A.t.h} \quad (2)$$

where

K_s: Saturated permeability coefficient or K-factor ($cm{\cdot}d^{-1}$)

V: Volume of water flowing through the sample ($cm{\cdot}d^{-1}$)

L: Length of the soil sample (cm)

A: Cross-section surface of the sample (cm^2) t: Time used for the water volume V to pass through the core (d) h: Water level difference inside and outside ring holder (cm)

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). Specifically, 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); using a device (microaggregate isolator) adapted from Six et al. (2002a, 2002b), which consists of a 250 μm sieve placed on top of a shaker, connected by a tube to a 50 μm sieve away from the shaker. 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 Eq. (3) (Six et al., 2002a):

$$\text{Sand} - \text{corrected aggregation} - \text{size fraction (g aggregate / Kg soil)} = \left((size - fraction\ weight) - \left(\frac{sand\ weight\ of\ same\ fraction - size}{\sum (total\ sample\ mass - total\ sand\ mass)} \right) \right) \times 100 \quad (3)$$

The aggregates mean weighted diameter (MWD, μm) was calculated according to Eq. (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,%) were calculated as the proportion of Magg over total soil mass, according to Eq. (5), where *WMagg* is the weight of the Macroaggegate fraction and W is the weight of the total sample.

$$MWD = \frac{\sum Wi\ x\ \varphi i}{W} \quad (4)$$

$$WSA = \frac{WMagg}{W} x100 \quad (5)$$

The total content of carbon (wet oxidation) and nitrogen (Kjeldahl method) associated with 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 (Eq. (6)) (Six et al., 2002a):

$$Sand - corrected\ C\ in\ fraction\ (g\ C\ /kg\ fraction) = \frac{C\ content\ in\ fraction\ (g\ C\ /Kg\ fraction)}{1 - proportion\ of\ sand\ in\ same\ fraction} \quad (6)$$

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™.

Soil microbial activity was estimated through the quantification of basal respiration using a portable SCR-1 EGM-4 of PP System (Amesbury, USA). 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). No collars were used. The total volume of the SRC-1 soil respiration chamber was 1171 mL and the area covered 78 cm^2. After placing the SRC-1 chamber on the soil there was an equilibration period of approximately 5 s. The time for which the change in the chamber CO_2 concentration was monitored was 120 s, unless a maximum change in CO_2 concentration of 50 ppm was reached before and the final measurement was made. During the measuring period, data was collected every 4.8 s. Five random measurement points were defined at each treatment, being these points different every year. In parallel, soil surface temperature was measured, as well as soil temperature at 10 cm soil depth, as well as soil humidity from 0 to 6 cm depth which was measured with a Theta Probe ML3 and HH2 moisture meter (Delta-T Services).

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 rows 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 (NO_3^-) and ammonium (NH_4^+) 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 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 and ammonium 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™. 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™ 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 °C 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 color 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.

Table 1
Effect of treatments on soil organic carbon stock (SOC_{Stock}), particulate organic carbon stock (POC_{Stock}), and POC_{Stock} /SOC_{Stock} ratio after 15 months. UV: under-vine cover crop. T: 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***	–	–

Table 2
Effect of treatments on soil hydraulic conductivity (K_s), bulk density and porosity after 20 months. UV: under-vine cover crop. T: 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*

Table 3
Effect of treatments on the size-distribution of water-stable aggregates and water stability in the surface layer (0–15 cm) after 15 months. UV: under-vine cover crop. T: tilled control. *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 (< 50 μm); MWD: mean weight diameter; WSA: water stable aggregate.

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).

Table 4
Effect of treatments on organic C and total N contents, and C/N ratios, in the different soil aggregate-size fractions after 15 months. UV: under-vine cover crop. T: tilled control. *p*-values <0.05 appear highlighted in bold.

	Fractions	UV	T	*p*
Carbon	Magg	13.67	11.36	*0.114*
(mg C·g fraction^{-1})	magg	18.34	15.67	***0.046***
	s+c	14.71	12.89	***0.017***
	Magg	1.58	1.10	*0.246*
Nitrogen	magg	1.89	1.66	***0.048***
(mg N·g fraction^{-1})	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*

3. Results

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 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 1).

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

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 macroaggregates (Magg, +15% compared to T), and a decrease in the percentage of microaggregates (magg, 53% lower compared to T). 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 3).

Within aggregate size-fractions, C and N contents did not show statistically significant differences between treatments in Magg and the C/N ratio (Table 4). 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.

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 (Fig. 2a). No clear statistically significant effects were detected regarding soil moisture (Fig. 2b). 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 - flowering and veraison period- (Fig. 2c and d).

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 tended to present higher values of MBC and MBN values only for MBC at flowering 2019 (+120% under UV *vs.* T), MBC/MBN ratio at flowering 2019 (+100%), and MBN at harvest 2020 (+59%) (Fig. 3).

In UV soils at a 0–15 cm depth, the nitrate and ammonium balances could suggest an immobilization of NH_4^+ at flowering in 2020 (this effect was not observed at a 15–30 cm soil depth). At harvest, the cover crop resulted in a greater immobilization of NO_3^-, with respect to T treatment, in 2020. In the 15–30 cm soil layer, the behavior was different. Soils treated with UV presented a greater availability of nitrate (though significant differences were only observed in 2019) (Fig. 4). At harvest time, in all years, the cover crop showed a general trend toward a greater immobilization of NH_4^+ at 0–15 cm soil depth, with differences observed

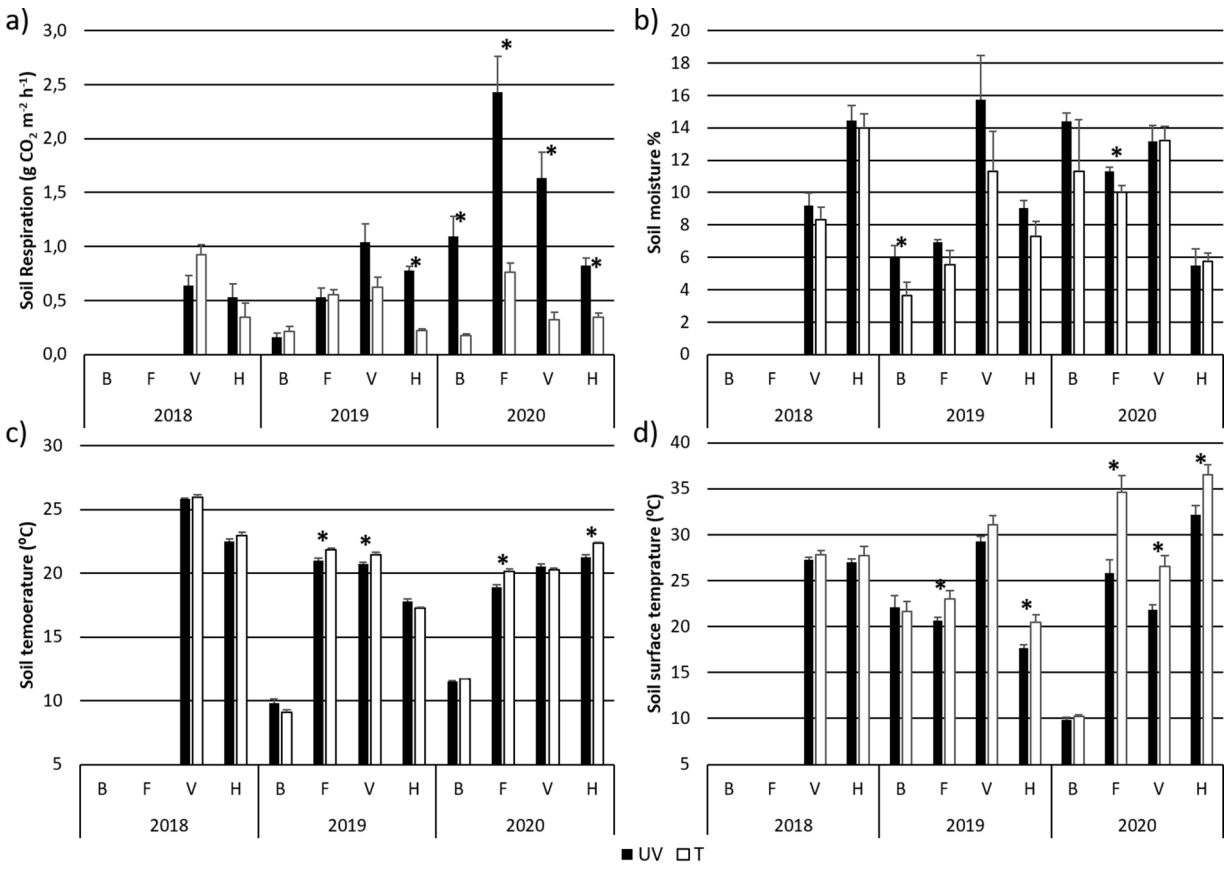

Fig. 2. Effect of treatments on soil respiration, moisture and temperature at 10 cm soil depth, and surface temperature at budburst (B), flowering (F), veraison (V) and harvest (H) times.a) Soil respiration (g CO_2 m^{-2} h^{-1}); b) Soil moisture at 6 cm soil depth (%); c) Soil temperature at 10 cm soil depth (°C); d) Surface temperature (°C). UV: under-vine cover crop. T: tilled control. *Significant differences 95%.

in 2019 and 2020. At this harvest time, a similar behavior was detected, in this case with statistically significant differences only in 2019 (Fig. 4).

The cover resulted in no differences in soil bacterial functional diversity except for increasing number of substrates used (NSU) in the surface depth at harvest in 2019 and 2020, and Shannon's diversity index (H′) in surface depth at harvest in 2020 (Fig. 5).

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* L. is a legume with a period of senescence in winter and a relatively trailing size, and to the agroecosystem where they were obtained, the soil under the vines in a semiarid climate drip irrigated vineyard. Similarly, their effect has also to be considered in terms of the particularities of the crop. In this regard, the initial soil organic carbon content was low (0.73% SOC) which may explain having detected changes in a relatively short period of time (15 months) after the onset of the experiment. Jordan et al. (2016), in soils with higher starting levels of SOC (3.4–3.9%), reported no gains in SOC or improvements of the soil structure even after three years.

In our vineyard, 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, see-Table 1), similar to that reported by Tarricone et al. (2020) with a subterranean clover crop in the inter-row after two seasons. We observed a more marked response for POC values, as well as in the POC/SOC ratio (Table 1), indicating that the increase in SOC was mainly associated to the most labile fraction (Abiven et al., 2009; Six et al., 2002b). The stability of this increase in organic matter, though relevant, should be evaluated over a longer term. This lability may imply also a faster mineralization of these organic inputs, as suggested by the higher respiration rates observed in UV (Fig. 2a) in the second, and especially, third year of study.

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 in the carbon to nitrogen ratio (Table 4). Two possible explanations for this results are (1) the relatively short period of study 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 *Trifolium fragiferum* L. and (2) the observed increase in the soil microbial biomass, that 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. Additionally, it is also possible that the increase in K_s 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 due to the cover crop, as we observed relevant changes after 15 months. In other studies with cover crops in alleys, it has been observed that the changes in soil parameters may occur as late as four or five years after the implementation of the cover crop (Karl et al., 2016a; Virto et al., 2012), although some beneficial effects on soil biological properties has been reported from the first year (Virto et al., 2012). This earliness in the response is probably due to the fact that, in this study, the addition of water and nutrients was localized directly below the vines where the cover crop was planted, maintaining a high degree of biological activity in the soil during the summer.

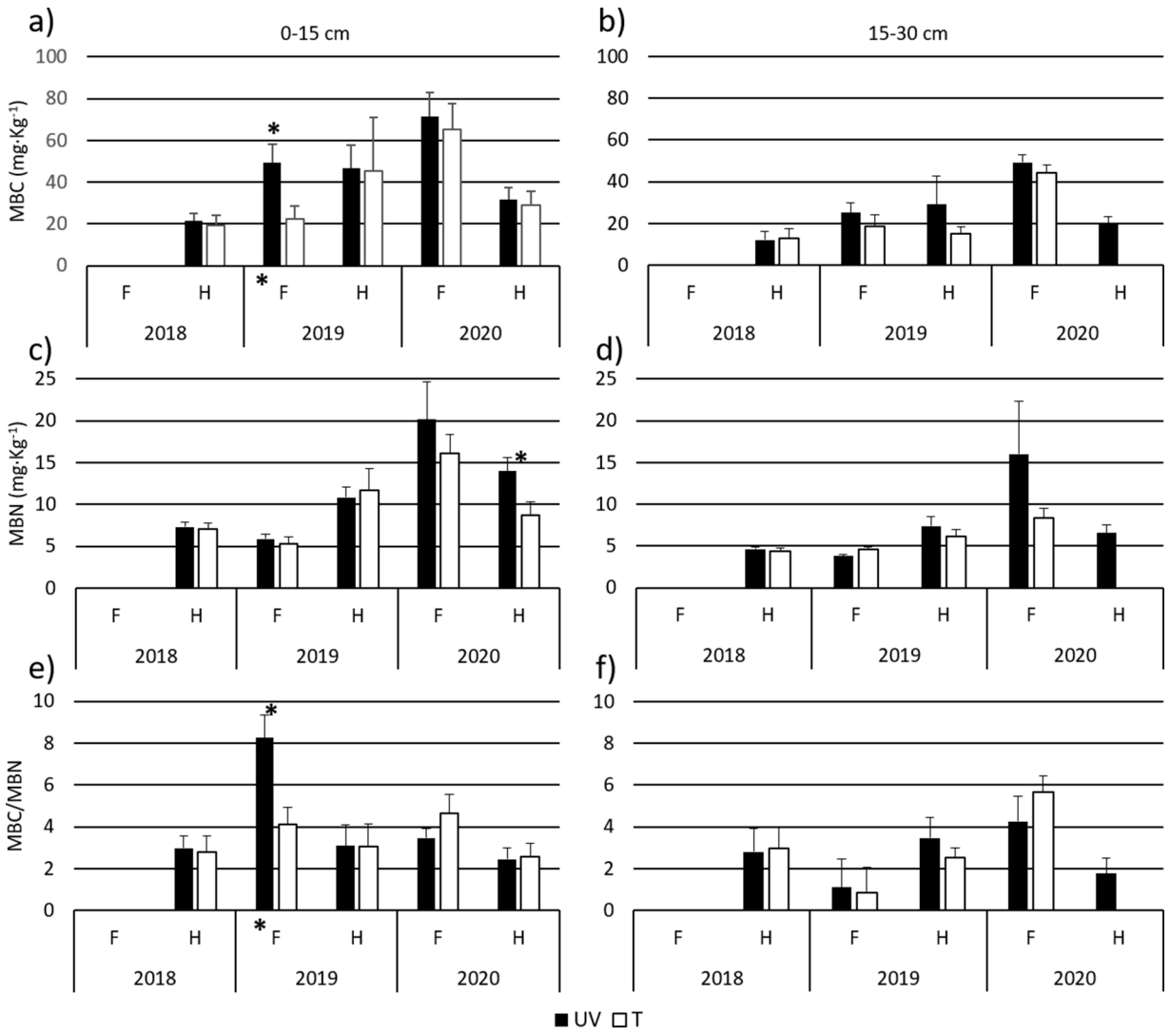

Fig. 3. Effect of treatments on soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and MBC/MBN ratio at flowering (F) and harvest (H), for the two soil layers (0–15 and 15–30 cm depth) studied here. MBC in layer 0–15 cm; (b) MBC in layer 15–30 cm; (c) MBN in layer 0–15 cm; (d) MBN in layer 15–30 cm; (e) MBC/MBN in layer 0–15 cm; (f) MBC/MBN in layer 15–30 cm. UV: under-vine cover crop. T: tilled control. *Significant differences 95%.

Aggregation and aggregates size-distribution showed that cover cropping resulted in an improvement of the two indicators considered (MWD and WSA), with an increase in 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 sustained biological activity, as suggested again by the higher respiration rates observed in UV in the second and third year (Fig. 2a).

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, and also the resistance to physical degradation, can be associated to a more stable soil structure (Amézketa, 1999; Rabot et al., 2018). Higher infiltration rates were observed in UV than in T at 15–30 cm (Table 2), and the observed values of BD and porosity at this depth also suggest an improvement of porosity in the deeper layer (15–30 cm at $p < 0.10$). Finally, although erosion cannot be a relevant issue in the study plot because of its nearly 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, contribute to reduce erosion, by physically protecting the soil from the impact of rain drops. Reduced soil crusting can also be expected from higher macroaggregtates stability, as Vanden Heuvel and Centinari (2021) suggests.

The presence of the under-vine cover crop 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 of T treatment hindered the insertion of the PVC tube, as the soil was dry due to lack of precipitation and since irrigation was over.

Concerning the soil biological parameters, the presence of the cover crop resulted in greater 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 this study, 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 have been slightly benefited by the

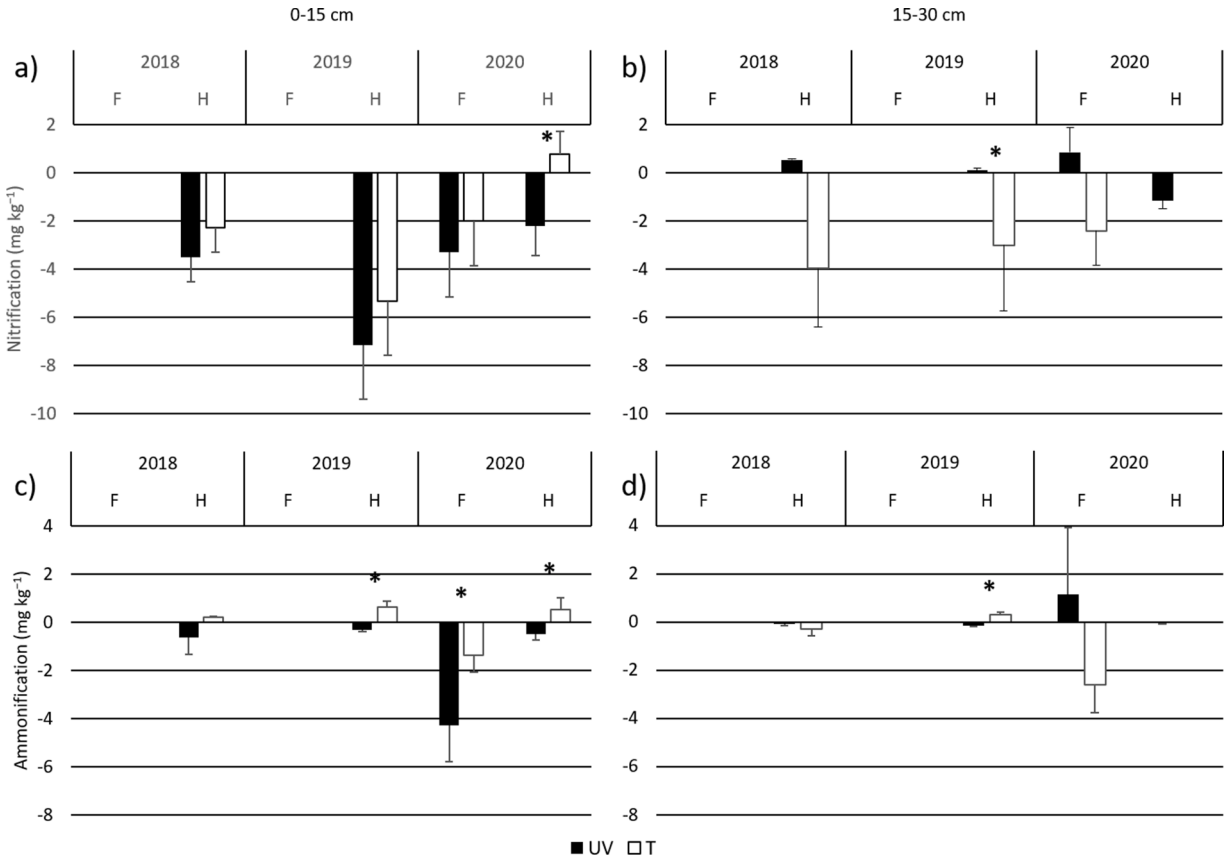

Fig. 4. Effect of treatments on nitrate and ammonium balance at flowering (F) and harvest (H).
(a) Nitrification in layer 0–15 cm; (b) Nitrification in layer 15–30 cm; (c) Ammonification in layer 0–15 cm; (d) Ammonification in layer 15–30 cm. UV: under-vine cover crop. T: tilled control. * Significant differences 95%.

observed increase in soil moisture, and by a certain acidification that might have occurred in the rhizosphere, as reported in other studies (Luo and Zhou, 2006). In our research conditions, where deficit irrigation was applied at a regular basis, it was observed that in the surface layer of the soil the humidity was higher where there was a cover crop (Fig. 2b). This effect could be due to the decrease in temperature on the soil surface caused by cover shading (Fig. 2d), and to the protection against the strong prevailing North winds in the area that contribute to dry out the soil surface when left bare.

The changes that occurred in microbial biomass associated with 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 general, there is a tendency for soils with cover crop to present a higher level of MBC as reported by Virto et al. (2012) in a vineyard near the trial area or to Gattullo et al., 2020 in Southern Italy but in both cases using a grass cover between the rows.

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 in 2019 and 2020, 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 2019 and 2020, respectively. The observed changes in SOC, POC, humidity and soil structure due to this cover crop can be related to these observations of greater diversity.

In any case, 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 which received high organic amendments regularly (Urra et al., 2020). This result is probably related to the fact that water, as it frequently occurs in many perennial crops, 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 favor bacterial richness compared to other areas such as the inter-row area (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; Nogales et al., 2021; 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 important in grapes cultivated either for winemaking or as table grapes (Bell and Henschke, 2005; Ferrara et al., 2018). 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 vine was lower in the presence of the cover, the

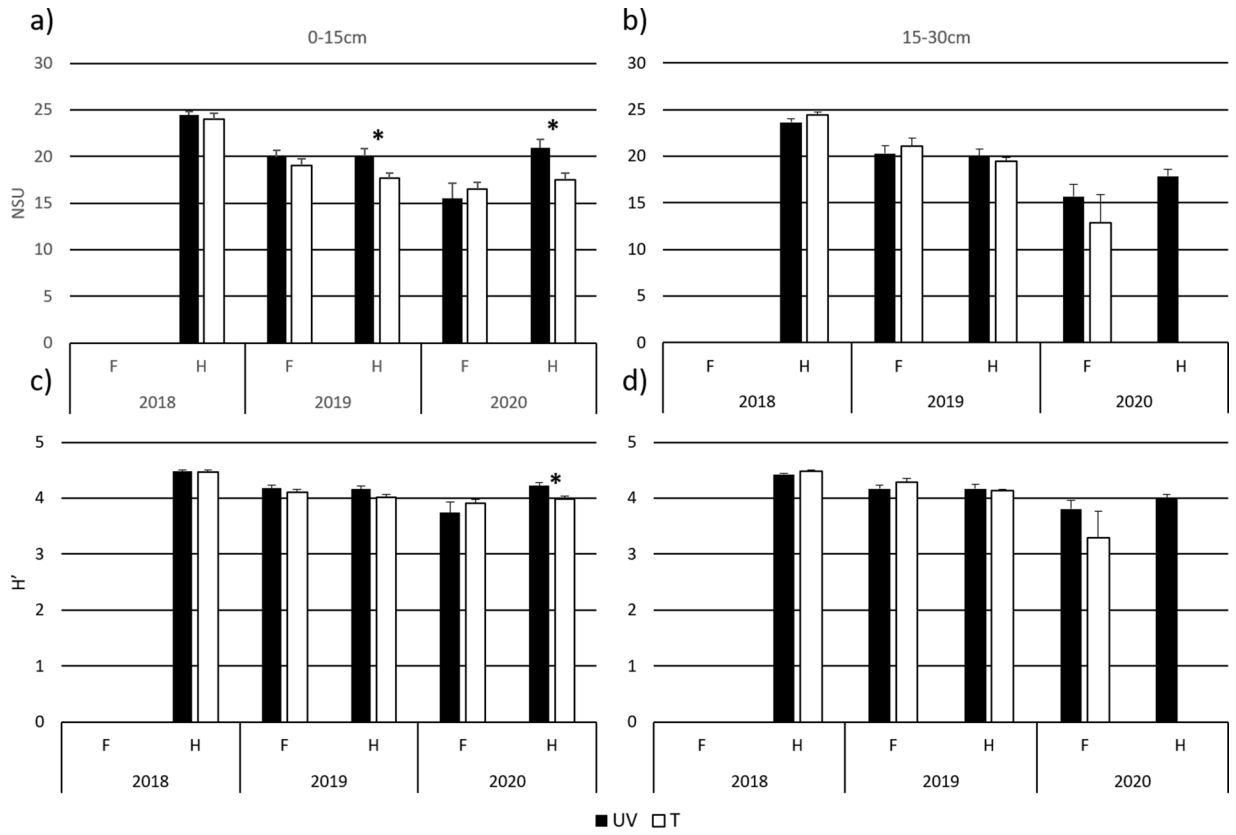

Fig. 5. Effect of treatments on of bacterial functional diversity as reflected by community-level physiological profiles (CLPPs) obtained with Biolog EcoPlates™. NSU: Number of substrates used. H': Shannon's diversity index. UV: under-vine cover crop. T: tilled control. * Significant differences 95%.
(a) Number of substrates in layer 0–15 cm; (b) Number of substrates in layer 15–30 cm; (c) Shannon's diversity index in layer 0–15 cm; (d) Shannon's diversity index in layer 15–30 cm.

competition effect outcoming the fixing ability of the cover. This fact may be due to various factors: N use by the microorganism communities, however in this case not supported by the evolution of MBN, which were highly variable between the different sampling moments (Fig. 3). 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. 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 in Mediterranean climate conditions as an effective method to improve vineyard soil health, compatible with good agronomic results (Abad et al., 2020). The contribution of water during the summer season, coinciding with warm-hot weather, suggests that the increases in the different parameters measured in this study could be faster than those that could occur in the alleys of the vineyard. Therefore, this cover 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; Corneoet al., 2013). Therefore, conservation agriculture practices, such as the installation of a cover crop in the row in Mediterranean conditions 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 under rhe vines 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 with the presence of the cover will favor the infiltration towards the vine roots for both rain and irrigation water.

In conclusion, the presence of the *T. fragiferum* L. under-vine cover crop resulted in an improvement in soil quality, as reflected by the values of the physical, chemical and microbiological properties measured., considering its implications on agronomic performance and soil quality, the use of under-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 where support irrigation is available. Under rainfed conditions, the suitability of this technique is probably more limited, and therefore should be used

cautiously. In any case, there is a necessity of evaluating their implications over a longer term on soil, agronomic, oenological features, and to study the potential of other cover crop species that could be better suited for Mediterranean climate and other soils.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by FJA, BI, IV and CG. The first draft of the manuscript was written by FJA and LGS all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Ethics approval/declarations

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material/ data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2022.111797.

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