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The role of cover crops in enhancing soil health and biodiversity in Mediterranean vineyards

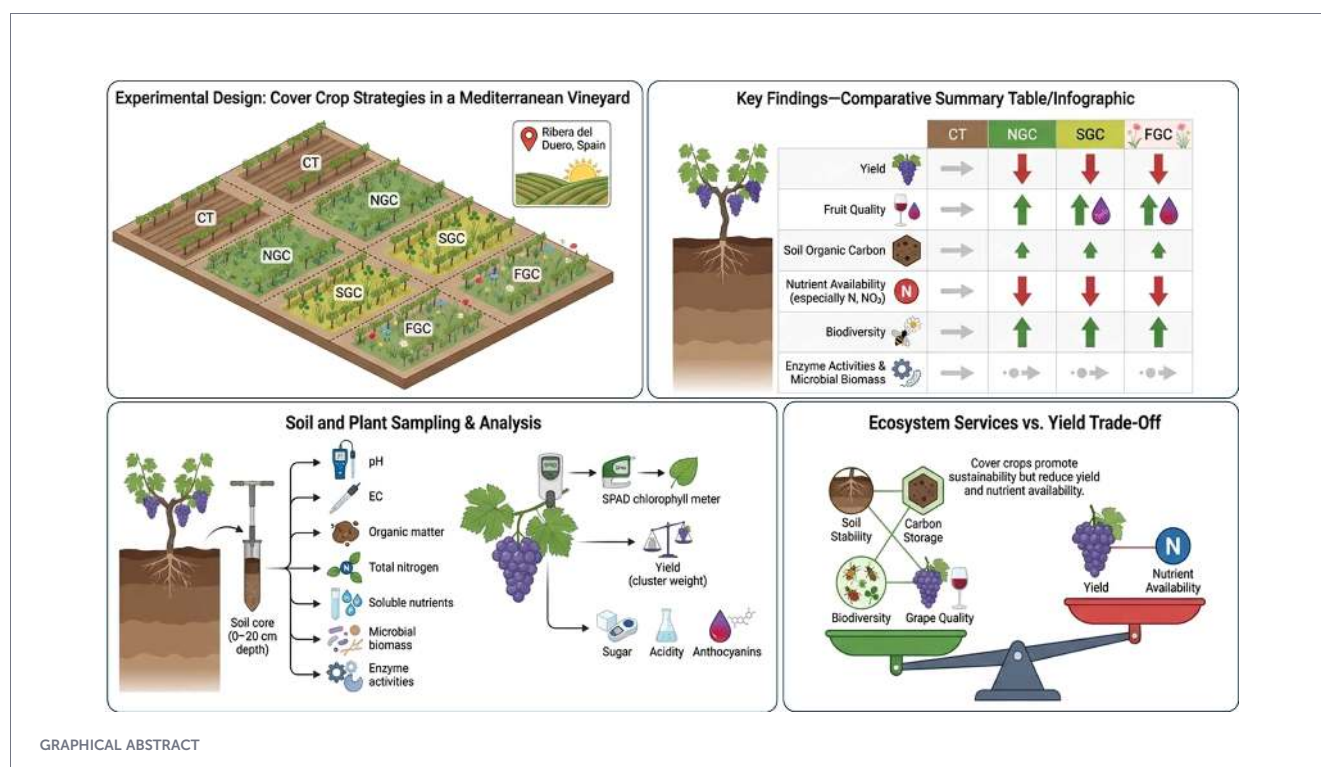
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Soil health is a priority in European policies, such as the EU Soil and Biodiversity Strategies for 2030, owing to land degradation and climate change, with Mediterranean vineyards being especially vulnerable because of their low soil organic matter content and risk of erosion. To improve sustainability, cover crops are increasingly used as alternatives to conventional tillage with bare soils. This study assessed the effects of different ground cover strategies on soil health, grape production, and biodiversity in *Vitis vinifera* cv. Tempranillo vineyard (D. O. Ribera del Duero, Spain). Four soil management systems were compared in 2021–2022: conventional tillage (CT), natural green cover (NGC), a sown grass–legume mixture (SGC), and a sown grass–flower mixture (FGC), in a randomized block design with four replicates. Surface soils (0 cm–20 cm) were sampled in spring 2022 and analyzed for pH, EC, organic matter, total nitrogen, soluble nutrients, microbial biomass, and enzymatic activity, as well as SPAD measurements to monitor plant development, yield, and grape quality. The yield decreased by 75% under FGC compared to that under CT. Soil Organic Carbon (SOC) increased by 57% in SGC compared to CT. Cover crops reduced grape yield but improved quality, notably by increasing anthocyanin content in the must. They also improved the biodiversity of the vineyards and increased the organic carbon content of the soil compared to CT. However, they depleted available nutrients, particularly nitrates, and showed only limited effects on soil biological properties, such as enzyme activities. Overall, cover crops promoted ecosystem services but required trade-offs with yield and soil nutrient availability.

KEYWORDS

agroecology, ecosystem services, grape quality, regenerative viticulture
vineyard sustainability



Highlights

- Cover crop management strongly decreases grapevine yield, but increases must quality.
- Soil organic matter and soil biological activity respond positively to cover crops.
- Species composition of cover crops is critical for ecosystem services.
- Cover crops increase arthropod abundance and enhance predator and parasitoid presence.

1 Introduction

Grapevine cultivation (*Vitis vinifera* L.) is one of the most economically important crops globally, accounting for an estimated area of 7.3 Mha in 2022 (1). The European Union is responsible for 65% of global wine production, with most wine-growing areas concentrated in France, Spain, and Italy. In addition to grapevine production being well adapted to marginal areas with poor soils and variable climates, the present context of climate change poses significant challenges for 21st-century viticulture, particularly in the Mediterranean Basin (2). Rising average temperatures during the growing season, an increased frequency of heatwaves, and a moderate decline in rainfall volume and frequency, coupled with a trend toward more intense and torrential rain events, have observable effects on viticulture (3). In this context, growers are transitioning toward soil-conserving and biodiversity-enhancing cover crops and regenerative practices (4). Short-term adaptation measures can be considered the first protection strategy and should

focus on specific threats, mostly changes in crop-management practices, such as irrigation and sunscreens for leaf protection. In the long term, however, a wide range of adaptation measures should be considered, such as varietal and land allocation changes (5). Associated with this, there is a growing imbalance between the alcoholic and phenolic ripening of grapes (6, 7), which threatens the typicity and *terroir* that define many wine-producing regions (8).

Adapting to climate change also requires a substantial shift in vineyard and soil management practices. Conventional viticulture has led to severe erosion and soil compaction, resulting in decreased fertility, reduced aggregate stability, and diminished biological activity (9). Cover cropping is a well-established practice in vineyard management that is gaining increasing attention for its ecological, agronomic, and environmental benefits, including the increase in biodiversity and ecosystem services, such as carbon sequestration, erosion control, soil fertility, and pest regulation (10). Cover crops are planted between vineyard rows, either across all rows or in alternating rows, to enhance soil fertility (11). This practice can significantly improve soil health through mechanisms such as organic matter input, soil erosion control, enhancement of microbial activity, and pest suppression (12–14).

The selection of suitable plant species is essential for improving soil properties and promoting ecosystem services, such as increased biodiversity, natural pest control, and greater resilience to climate change effects. Benefits include enhanced soil water retention, improved porosity, carbon sequestration, and elevated microbial activity (15). Cover crops also significantly affect soil biochemical properties, which influence soil fertility and vineyard productivity. One of the most notable effects is the addition of organic matter to the soil, which improves soil structure, increases water retention, and enhances nutrient availability (16). Organic matter inputs from

cover crops, such as roots and decaying plant material, provide a steady source of carbon to soil microorganisms, which in turn decompose organic matter and release essential nutrients such as nitrogen, phosphorus, and potassium (17). Recent studies have shown that the set of practices included in regenerative viticulture (cover crops, organic amendments, reduced tillage, and livestock integration) can increase soil organic carbon (SOC) levels in vineyards from 2.8 to more than 11 Mg CO₂ ha⁻¹ yr⁻¹, depending on climate, soil type, and practices used (18). This increase in SOC can improve soil aggregation, enhance soil porosity, and reduce soil erosion, thereby contributing to improved soil health and long-term vineyard sustainability. Furthermore, crop residue additions from cover crops may enhance the availability of P, K, Ca, Fe, and Mg in some soil types under specific climatic conditions (19).

Nevertheless, proper cover crop management is critical to avoid competition with grapevines for water, particularly during summer (20). In areas with fertile soils and high precipitation rates, under-trellis vegetation can reduce excessive plant vigor through competition, resulting in reduced canopy density and pruning weight (21). However, there are also other areas with strong aridity periods, which could lead to excessive competition during the dry seasons, resulting in a significant reduction in grape yield (20, 22). Cover crops can also produce moderate competition that improves the sensory attributes associated with reduced vegetative growth and canopy size, such as increased fruit Brix and anthocyanin concentrations (23). Less dense, more aerated canopies and less compacted bunches are associated with a lower incidence of Botrytis or bunch rot disease (24–26).

The success of permanent vegetation cover is contingent on local environmental conditions. To ensure plant persistence and stability, species should be chosen according to their adaptability to local environmental conditions and specific management practices, mainly their ability to compete with weeds under low-input management conditions (27). In some cases, the establishment of inter-row vegetation negatively affects vine vigor and grape quality parameters important for vinification such as yeast available nitrogen (YAN), which can counterbalance the positive effect of this practice in terms of biodiversity and soil health (28).

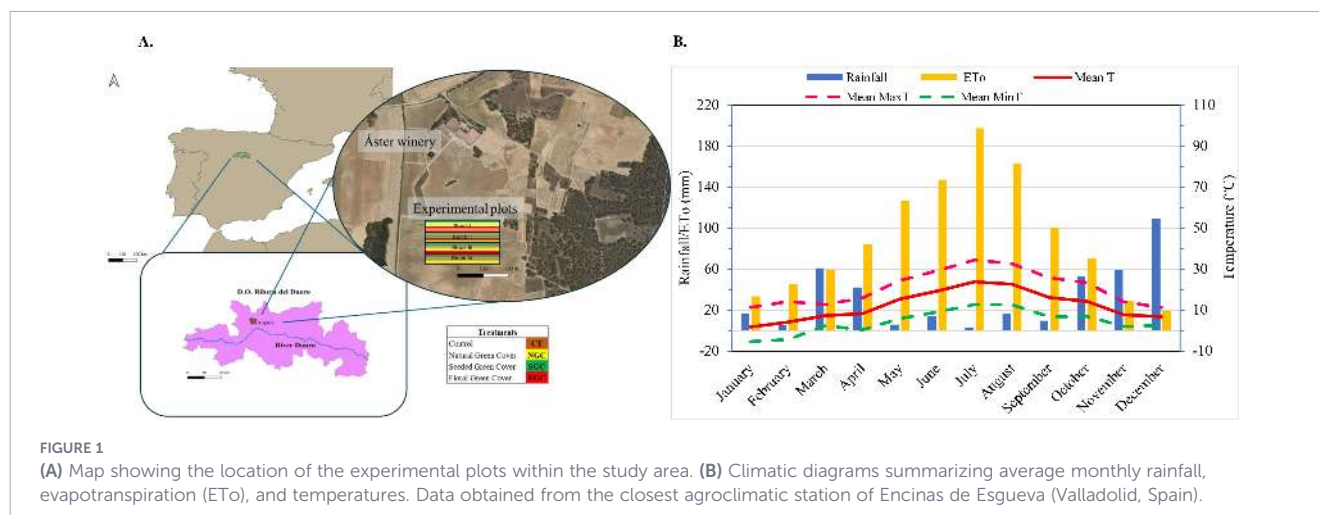
Considering these contrasting effects of intercropping systems, this study aims to evaluate on one hand the impact of different cover crop mixtures on vineyard production and grape quality, and their effect on soil health parameters and floral and faunal diversity, all of which are considered in a holistic way to improve vineyard management in one of the most important wine producing areas in Spain, such as the D. O. Ribera del Duero.

In this study, we hypothesize that cover crop mixtures act as key drivers of soil multifunctionality, thereby modulating vineyard productivity, grape quality, and biodiversity. The specific objectives of the study are: (i) to evaluate the effects of different cover crop mixtures on soil health and functioning in vineyards by assessing changes in physical, chemical, and biological indicators compared with conventional bare-soil management; (ii) to determine how improvements in soil health associated with cover crop mixtures influence soil-plant interactions and affect vine growth, yield, and grape quality; (iii) to assess the impact of cover crop mixtures on above and belowground biodiversity, including plant and faunal diversity, and their contribution to ecosystem functions such as nutrient cycling and biological regulation; and (iv) to analyze the combined effects of cover crop mixtures on soil health, biodiversity, and vine performance in order to evaluate their contribution to the resilience and sustainability of vineyard agroecosystems in the Ribera del Duero.

2 Materials and methods

2.1 Description of the study area and experimental sites

The study was conducted in a vineyard that is in the municipality of Anguix (Burgos), Northern Spain (41° 45' 22.6" N, 3° 54' 10.4" W), belonging to the D. O. Ribera del Duero (Figure 1A). The soil is a Calcaric Cambisol (CMc) with a silty-clayey texture, located at 809 m above sea level (masl), with a dry-summer Mediterranean climate (Csb) according to Köppen, an average annual temperature of 11.6 °C, and an average annual rainfall of 420 mm. The



plantation was of *Vitis vinifera* L. var. Tempranillo on a 1103P rootstock, with a spacing of 3 m × 1.3 m (row × vine), in trellis with double cordon conduction and east to west orientation.

2.2 Treatments and experimental design

Different treatments were established in the interrow spaces in October 2020: natural weed vegetation (NGC, Natural Green Cover); introduction of a mixed cover of grass (80%) and legumes (20%) called Seeded Green Cover (SGC): *Brachipodium distachyum* (L.) P. Beauv., vetch (*Vicia sativa*), ervil (*Vicia ervilia*), pea (*Pisum sativum*), and red pea (*Lathyrus cycera*); introduction of a cover with a mixture of grasses (90%) and floral species (10%) called Floral Green Cover (FGC): *Festuca arundinacea*, *Bromus rubens*, *Brachypodium distachyum*, *Poa pratensis*, *Lolium perenne*, *Medicago polymorpha*, *Trifolium repens*, *Trifolium pratense* as grasses and *Centaurea erythraea*, *Omphalodes linifolia*, *Anthemis arvensis*, *Anagallis* ssp., *Papaver rhoeas*, *Bellis perennis*, *Taraxacum officinale*, *Bartsia trixago*, *Chrysanthemum* ssp. and *Calendula arvensis*; as well as a controls in which the interrow was maintained with bare soil and traditional tillage (CT, Control) with chisel plow and compacting roller at the beginning of the season. Vineyard weeding was performed to maintain a clear row without any vegetation. The experimental design was a randomized complete block with four experimental replicates, with three rows of grapevines in each plot. Each treatment was introduced in its inter-row spaces, and 20 control grapevines, all with similar vigor and development, were located on each central row. Additionally, an interrow space was maintained with traditional tillage between treatments. The cover crops were reseeded in October 2021.

2.3 Floral diversity analysis

Four areas of 1 m² were randomly selected in the inter-row spaces of each treatment for floral and faunal diversity determination and soil characterization. In mid-May 2022, plant species abundance and diversity were assessed using the Braun–Blanquet methodology in each of the four 1 m² areas selected along each inter-row. Plant cover was estimated as the product of the frequency of each species and its % cover as a numerical transformation of the 7-step version of the Braun–Blanquet scale (Table 1) (29). Plant cover diversity was analyzed using the free software program Past 4.09 (30).

2.4 Faunal diversity analysis

Soil arthropod diversity was studied by installing four pitfall traps in each treatment and in each of the four 1 m² selected areas in May and June. The traps consisted of a plastic cup filled with 150 mL of a mixture of water and polypropylene glycol (3:1) and three drops of soap. This mixture preserves the arthropods long enough for identification in the laboratory. Pitfall traps were placed in the center of the vineyard interrow, separated by at least 25 m, and remained installed for 7 days. Entomofauna was identified to at least the order level. Faunal diversity was analyzed using Past 4.09.

2.5 Soil chemical and biochemical properties

A soil sample was collected using a soil auger (Eijkelkamp, The Netherlands) at the center of each of the four areas at a depth of 0 cm–0 cm and combined into a composite soil sample. These samples were kept refrigerated until they were transported to the laboratory, sieved to 2 mm, and subdivided: one portion was immediately frozen at –20 °C for biochemical determinations, and the other portion was air-dried for physicochemical determinations.

2.5.1 Physicochemical properties

Air-dried soil samples were used for physicochemical analyses, including: soil pH and electrical conductivity, determined in a soil–water suspension (1:5 w/v); total C and N, measured by dry combustion in a TruSpec (LECO, USA) autoanalyzer; organic carbon, analyzed by digesting 0.2 g of soil with 1 mL 1 N K₂Cr₂O₇ and sulfuric acid, followed by addition of phosphoric acid and back-titration with Mohr's salt using an automated titrator equipped with a Pt-electrode (Eco Titrator, Metrohm, Switzerland); lime content, quantified by volumetry after acidic attack, and soluble NO₃-N, exchangeable NH₄-N, and available PO₄-P (P-Olsen) extracted with 1 M KCl and 0.5 M NaHCO₃, respectively, and analyzed colorimetrically using a segmented flow analyzer San ++ (SKALAR, The Netherlands). All chemical measurements were performed in duplicate, and for statistical treatment, these values were averaged to obtain one value per sample.

2.5.2 Basal soil respiration

Basal soil respiration (BSR) was determined according to the protocol described by Alef and Nannipieri (31). Frozen soil samples (20 g) were attemperated for 72 h at 22 ± 0.5 °C, placed in 1-liter jars with hermetic sealing caps, and a beaker containing 4 mL of 0.5 M NaOH was used as a gas trap. After 24 h of incubation, 2 mL of 0.5 M BaCl₂ was added to precipitate barium carbonate-adsorbed CO₂, and the remaining NaOH was measured with 0.1 M HCl using an automatic titrator (EcoTitrator, Metrohm, Switzerland). BSR was expressed as mg CO₂-C kg^{–1} dry soil h^{–1}.

TABLE 1 Numerical transformation of cover-abundance Braun–Blanquet scale.

Braun–Blanquet code	Description	Approx. % cover	Numeric value
r	Solitary, rare, <5 individuals	<0.1%	0.1
+	Few individuals, small cover	<1%	0.5
1	Abundant but <5% cover	<5%	2.5
2	5%–25% cover	5%–25%	15
3	25%–50% cover	25%–50%	37.5
4	50%–75% cover	50%–75%	62.5
5	75%–100% cover	75%–100%	87.5

2.5.3 Enzyme activities

Enzyme activities were measured using fluorogenic MUF or AMC-substrates in 96-microtiter plates (32). Acid and alkaline phosphatases (EC 3.1.3.2/AcPA and EC 3.1.3.3/AlkPA, respectively), α - and β -glucosidases (EC 3.2.1.20/aGA and EC 3.2.1.21/bGA, respectively), N-acetyl- β -glucosaminidase (EC 3.2.1.30/bNAG), β -xylosidase (EC 3.2.2.37/bXyl), leucyl-aminopeptidase (EC 3.4.11.1/LeuAMP), and arylsulfatase (EC 3.1.6.1/AS) were determined using 4-methylumbelliferone (MUF) or amino-4-methylcoumarin (AMC) derivatives as their substrates. A frozen sample (equivalent to 1 g oven-dried material) was weighed in a sterile beaker and suspended in 20 mL of sterile deionized water. A homogenous suspension was obtained by pulsed sonication for 2 min (40 W) of the sample placed in an ice water bath. Aliquots (50 μ L) were withdrawn and dispensed into a 96-well microplate (eight analytical replicates of each sample). Buffers (50 μ L) at pH 5 for AcPA and bGA, pH 6 for aGA, bXyl, bNAG, and AS, pH 9 for LeuAMP, and pH 10 for AlkPA and 1 mM substrate solution (100 μ L) were added, giving a final substrate concentration of 500 μ M. The plates were sealed to prevent transpiration and incubated for 180 min at 30 °C under agitation (150 rpm). The reaction was stopped by the addition of 50 μ L of Tris buffer at pH 12 and immediately read in a fluorometric plate-reader (GENios, TECAN, Switzerland) using excitation and emission filters of 360 nm and 450 nm, respectively. Fluorescence was converted into an amount of MUF or AMC according to calibration standards (0 pM–1,500 pM) prepared on each plate to account for fluorescence quenching through soil particles and organic matter and expressed as pmol MUF or AMC g⁻¹ dry soil h⁻¹. Total hydrolytic enzymatic activity is estimated as a sum of substrates hydrolyzed by soil and expressed in the same units.

2.6 Vigor, yield, and quality parameters

At the end of July, corresponding to the fruit development phenological stage (BBCH 70–79), 20 leaves from the grape-growing zone were collected from each control vine, all with similar development and without apparent illness symptoms, placed in paper bags, refrigerated, and transported to the laboratory for Soil Plant Analysis Development (SPAD) measurement using a Chlorophyll Concentration Meter (CCM-200 plus, Opti-Sciences Inc., Hudson, USA).

At harvest, the following production parameters were measured: number of shoots per vine, number of clusters per vine, cluster weight (kg/cluster), average vine production (kg/vine), and total yield (t/ha). Two bunches of grapes per vine stock were used for the quality control. Additional measures, such as the number of bunches per shoot and shoots per vine, were also taken in the field. At the end of harvest and during the dormancy stage, pruning values (kg/vine) were determined by on-site weighing with a hanging scale, and the Ravaz Index was calculated as the ratio of grape production to vine wood.

Two portions of grapes from the apical and lateral parts of each cluster were destemmed and homogenized with a commercial blender (Moulinex) to obtain a grape homogenate. The analyzed wine quality parameters were determined according to OIV (33): total weight of 100 berries, number of berries per cluster, and must density using a 25 mL pycnometer (g/cm³). The juice was

centrifuged for 5 min at 3,500 rpm in a 50 mL Falcon tube and filtered (Whatman filter 520 A 1/2). In the clarified must, total soluble solids were determined using a hand-held refractometer (TTS, °Brix), total titratable acidity (TA g L⁻¹), and yeast assimilable nitrogen (YAN, mg L⁻¹), measured as alpha-amino nitrogen and ammonia by titration after formaldehyde addition.

Spectrophotometric measurements were performed using a 1-mm cell path length at A280 nm, A420 nm, A520 nm, and A620 nm in a spectrophotometer (Genesis 20, Milton Roy, USA). The total polyphenol index (TPI) was calculated as A280 $\times$ dilution. Color intensity (CI) was calculated as the sum of A420 nm, A520 nm, and A620 nm, and the color ratio (CR) was determined as the quotient of A420 nm and A520 nm. Anthocyanins were determined after preparing a stock solution containing 1.0 mL of centrifuged grape must, 1.0 mL of ethanol, and 20.0 mL of 2% (v/v) HCl. From this stock solution, a sample (A) was prepared by mixing 5.0 mL of the stock solution and 2.0 mL of distilled water, and a sample blank (B) was prepared by mixing 5.0 mL of the stock solution and 2.0 mL of 16% (w/v) NaHCO₃. After 15 min, the absorbance of both samples was measured at A520 nm. Anthocyanin content was calculated using the following formula:

$$[\text{Anthocyanins}] \left(\frac{\text{mg}}{\text{L}} \right) = 875 \cdot (A_{520}(\text{A}) - A_{520}(\text{B}))$$

Tannin content was measured by adding 0.3 mL of concentrated HCl to 1.0 mL of centrifuged grape must, previously diluted 1:10 (sample A), and incubating it for 30 min in a water bath at 90 °C in the dark. A sample blank (B) was prepared in the same manner but without any incubation. After 30 min, 0.5 mL of ethanol was added, and the absorbance was measured at A550 nm.

$$[\text{Tannins}] \left(\frac{\text{mg}}{\text{L}} \right) = 19,33 \cdot \frac{(A_{550}(\text{A}) - A_{550}(\text{B}))}{5}$$

2.7 Statistical analysis

Statistically significant differences between treatments were obtained using ANOVA after checking for normality and homogeneity of variances using Shapiro–Wilk and Levene’s tests, respectively. *Post-hoc* Tukey’s test with 95% confidence intervals was used to display differences between treatments. A Q–Q plot was used to check for the normality of the residuals of the model. In the case of non-normally distributed values, the Kruskal–Wallis’ test was performed, followed by Dunn’s *post-hoc* test to display statistically significant differences. Statistical analyses were performed using JASP v. 0.19.3 (34). Redundancy analyses (RDA) were conducted using Canoco v 4.5 after variable normalization. Significance was evaluated using a Monte Carlo permutation test with 500 permutations (35).

3 Results

3.1 Botanical survey of cover crops

Cover crops were established in October 2020 and reseeded in November 2021 at a rate of 25 kg ha⁻¹, in both cases following the

recommendations of the seed provider (Cantueso Natural Seeds, Córdoba, Spain). In both years, the cover crops were mowed at the end of May to prevent competition with grapevines for water and nutrients.

Figure 1B shows a climate diagram highlighting extremely dry conditions with a pronounced water deficit from May to September. This period coincides with the physiological development of the vine, including fruit formation and ripening. The harsh climatic conditions limited vine productivity in areas with sandy-textured soils and influenced the growth and composition of the cover crops. Specifically, they favored early germinating grasses over legumes and herbaceous floral species.

As shown in Figure 2A, the Floral Green Cover (FGC) treatment was dominated by grasses of the Poaceae family, mainly perennial ryegrass (*L. perenne* L.) and tall fescue (*F. arundinacea* Schreb.). In some observation points, crimson clover (*Trifolium incarnatum* L., Fabaceae) was present. However, most of the floral species initially introduced did not establish successfully, except for field marigold (*C. arvensis* L.).

In the Seeded Green Cover (SGC) treatment, the most abundant species was stiff brome (*Brachypodium distachyon* L.), consistent with its high proportion (80%) in the seed mixture. Perennial ryegrass and peas (*P. sativum* L.) were also present with substantial coverage. Interestingly, a non-introduced species, shepherd's purse (*Capsella bursa-pastoris* L., Brassicaceae), was also dominant.

In the Natural Green Cover (NGC) treatment, where no seeding was performed and spontaneous vegetation was allowed to grow, *C. bursa-pastoris* was the most abundant species. Other commonly observed species included false barley (*Hordeum murinum* L.), London rocket (*Sisymbrium irio* L.), herb-Robert (*Geranium robertianum* L.), and henbit deadnettle (*Lamium amplexicaule* L.).

The biodiversity indices of the plant communities under each cover treatment are presented in Table 2. Significant differences

were observed in the number of taxa in the following order: SGC > NGC > FGC. Margalef's, Shannon's, and Simpson's biodiversity indices also showed clear distinctions, with the FGC treatment differing markedly from the SGC and NGC treatments, both of which exhibited higher plant diversity and lower dominance (Supplementary Table 1). No statistically significant differences were found in the number of individuals (estimated as % total cover) or in evenness values. The redundancy analysis (RDA) of plant species abundance (Figure 2B) showed considerable variability among treatment replicates. Samples from the FGC treatment were more widely dispersed across the ordination space, whereas NGC and SGC samples tended to cluster toward the left side of the plot. Some species vectors (e.g., *Papaver* and *Trifolium*) were associated with the positive side of the first axis, whereas several grass and ruderal species were oriented toward the negative side of the ordination. Overall, the ordination indicated limited separation among treatments, suggesting a relatively low differentiation in plant species abundance among FGC, NGC, and SGC plots.

3.2 Soil properties

The variations in chemical soil properties across the treatments are shown in Table 3. Our results showed that soil pH was slightly lower under the cover crop treatments than under conventional tillage (CT = 8.51 vs. SGC = 8.18), although the differences were not significant. The most notable change was observed in the nitrate content, which was six times higher in CT than in the cover crop treatments. A similar trend was observed for available phosphate, although the differences were smaller and non-significant. The lowest EC values were recorded for FGC, whereas SGC exhibited significantly higher EC values. Total soil N was generally low (<0.1%) but showed significant differences, with the highest

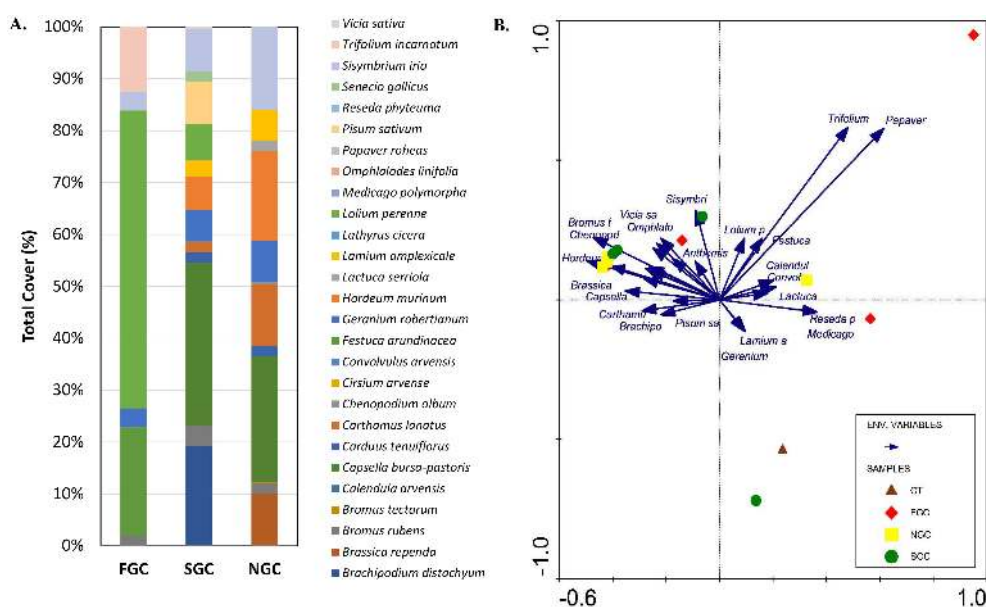


FIGURE 2 Botanical diversity of interrow spaces estimated as percentage of total cover by species: (A) Total cover percentage of different plant species in the assayed treatments. (B) RDA biplot of plant species abundance constrained by treatment.

TABLE 2 Biodiversity indices of vegetation in cover crops: FGC, floral green cover; SGC, seeded green cover; NGC, natural green cover. Mean values ($\pm$ SD).

Treatments	Individuals				Taxa_S				Dominance				Simpson_1-D				Shannon_H				Evenness_e^H/S				Margalef			
FGC	52	$\pm$	3	a	7	$\pm$	2	a	0.408	$\pm$	0.094	b	0.592	$\pm$	0.094	a	1.130	$\pm$	0.225	a	0.521	$\pm$	0.187	a	1.396	$\pm$	0.490	a
NGC	45	$\pm$	22	a	9	$\pm$	1	ab	0.187	$\pm$	0.039	a	0.813	$\pm$	0.039	b	1.797	$\pm$	0.178	b	0.681	$\pm$	0.121	a	2.180	$\pm$	0.282	b
SGC	48	$\pm$	23	a	10	$\pm$	2	b	0.207	$\pm$	0.071	a	0.793	$\pm$	0.071	b	1.766	$\pm$	0.325	b	0.601	$\pm$	0.124	a	2.391	$\pm$	0.234	b

Treatments with different letters display significant differences at $p < 0.05$.

TABLE 3 Variation of the chemical properties in the different cover crops assayed: FGC, floral green cover; SGC, seeded green cover; NGC, natural green cover. Mean values ($\pm$ SD).

Treatments	pH				EC dS m ⁻¹				Total N % DW				C-org g kg ⁻¹ DW				NO3-N mg kg ⁻¹ DW				NH4-N mg kg ⁻¹ DW				PO4-P mg kg ⁻¹ DW			
CT	8.51	$\pm$	0.38	a	0.365	$\pm$	0.032	ab	0.067	$\pm$	0.007	ab	7.240	$\pm$	3.331	a	23.627	$\pm$	2.555	b	3.656	$\pm$	0.775	a	25.910	$\pm$	2.718	a
FGC	8.47	$\pm$	0.09	a	0.357	$\pm$	0.020	a	0.053	$\pm$	0.016	a	7.237	$\pm$	3.309	a	5.833	$\pm$	1.319	a	3.019	$\pm$	0.962	a	21.500	$\pm$	3.954	a
NGC	8.22	$\pm$	0.07	a	0.388	$\pm$	0.030	ab	0.082	$\pm$	0.010	b	6.253	$\pm$	2.719	a	4.801	$\pm$	1.335	a	3.461	$\pm$	1.344	a	19.150	$\pm$	2.569	a
SGC	8.18	$\pm$	0.26	a	0.416	$\pm$	0.019	b	0.060	$\pm$	0.010	ab	11.390	$\pm$	3.699	a	6.746	$\pm$	3.800	a	4.061	$\pm$	0.948	a	22.380	$\pm$	4.750	a

Treatments with different letters display significant differences at $p < 0.05$.

values in NGC and the lowest in FGC. Soil organic carbon (SOC) under seeded green cover (SGC) reached 11.39 g kg^{-1} , compared with 7.24 g kg^{-1} under conventional tillage (CT), corresponding to an increase of approximately 57%, which was not observed in the other cover crop treatments.

Regarding biochemical properties, only a few significant differences were detected, mainly for proteolytic and α -D-glucosidase activity, reflecting the high variability among replicates (all the statistical information is presented in [Supplementary Table 3](#)). Basal soil respiration (BSR) was highest in FGC, slightly above CT, and lowest in NGC ([Figure 3B](#)). Among the soil enzymatic activities, alkaline phosphatase (AlkPA), proteases (LeuAMP), and β -D-glucosidase (bGA) predominated, all of which are hydrolytic enzymes associated with the P, N, and C cycles ([Figure 3B](#)). In SGC, proteolytic and cellulolytic activities were clearly higher than those in CT, whereas the lowest enzyme activity values were found in FGC ([Figure 3A](#)).

3.3 Faunal composition

Arthropods collected in pitfall traps at the end of spring 2022 were identified at least to the order level and, when possible, to family or genus. [Figure 4A](#) shows faunal abundance across treatments, with the highest mean abundance observed in the FGC treatment. Conventional tillage (CT) plots exhibited higher arthropod abundance than the NGC and SGC treatments, largely due to greater numbers of *Collembola*, *Diptera*, and *Hymenoptera*.

Redundancy analysis (RDA), using arthropod abundance as a response variable and plant species and treatments as explanatory variables, revealed considerable variability among replicates ([Figure 4B](#)). The RDA analysis indicated that the first axis explained 63.0% of the variance in arthropod abundance, while the first two axes together accounted for 81.1% of the total variance, indicating that the two-dimensional ordination captured most of the explainable variation. The Monte Carlo permutation test for the first

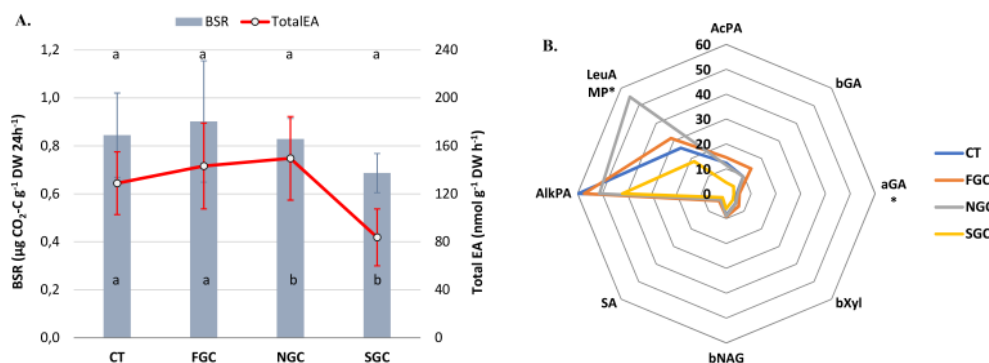


FIGURE 3

Biochemical soil properties variations in the different interrow management treatments: (A) Basal soil respiration (BSR) and total enzymatic activity (TotalEA). Treatments with different letters display significant differences at $p < 0.05$. (B) Radar graph of enzymatic activities (in $\text{nmol g}^{-1} \text{ DW h}^{-1}$). *Asterisk indicate significant differences at $p < 0.05$.

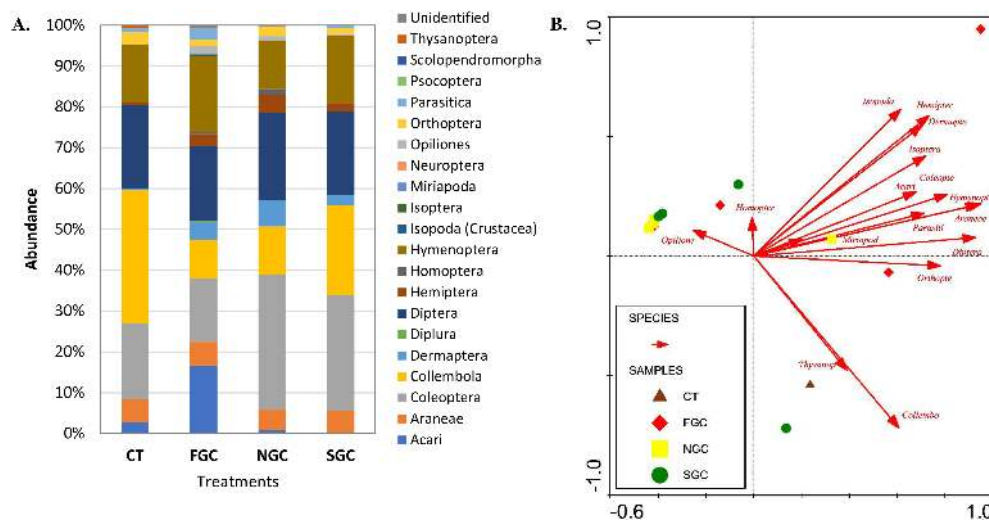


FIGURE 4

Faunal abundance in the interrow spaces of the different treatments: (A) Abundance of insect species. (B) RDA biplot of arthropod abundance constrained by treatment.

canonical axis yielded an F-ratio of 3.411 ($p = 0.052$), indicating a near-significant relationship between arthropod abundance and the explanatory variables.

FGC samples were widely dispersed in the ordination space, and the separation between FGC_I and FGC_II suggests a strong site-related effect influencing arthropod distribution. This pattern indicates that the main gradient represented by RDA1 was largely associated with the FGC treatment, which structured the distribution of several arthropod groups. In contrast, most CT, NGC, and SGC samples were grouped closer to the origin, indicating lower differentiation among these treatments. The triplot displayed in Figure 5 indicated associations between plant species such as *T. incarnatum* and *P. rhoeas* and the abundance of several arthropod groups, including *Hemiptera*, *Dermaptera*, and *Orthoptera*. Several

arthropod groups with longer vectors, such as *Diptera*, *Coleoptera*, *Hymenoptera*, and *Acari*, contributed strongly to the ordination pattern. Nevertheless, FGC tended to show higher Shannon, Simpson, and Margalef indices, whereas NGC and SGC exhibited comparatively lower values (Supplementary Table 4).

3.4 Vine vigor, yield, and quality parameters

Vine production, pruning weights, and Ravaz index values are presented in Table 4. Significant differences in yield were observed between the CT and cover crop treatments. Cover crops markedly reduced vine productivity, with decreases of 75% (FGC) and 50% (SGC), compared with only 5% in NGC, where no seeding was

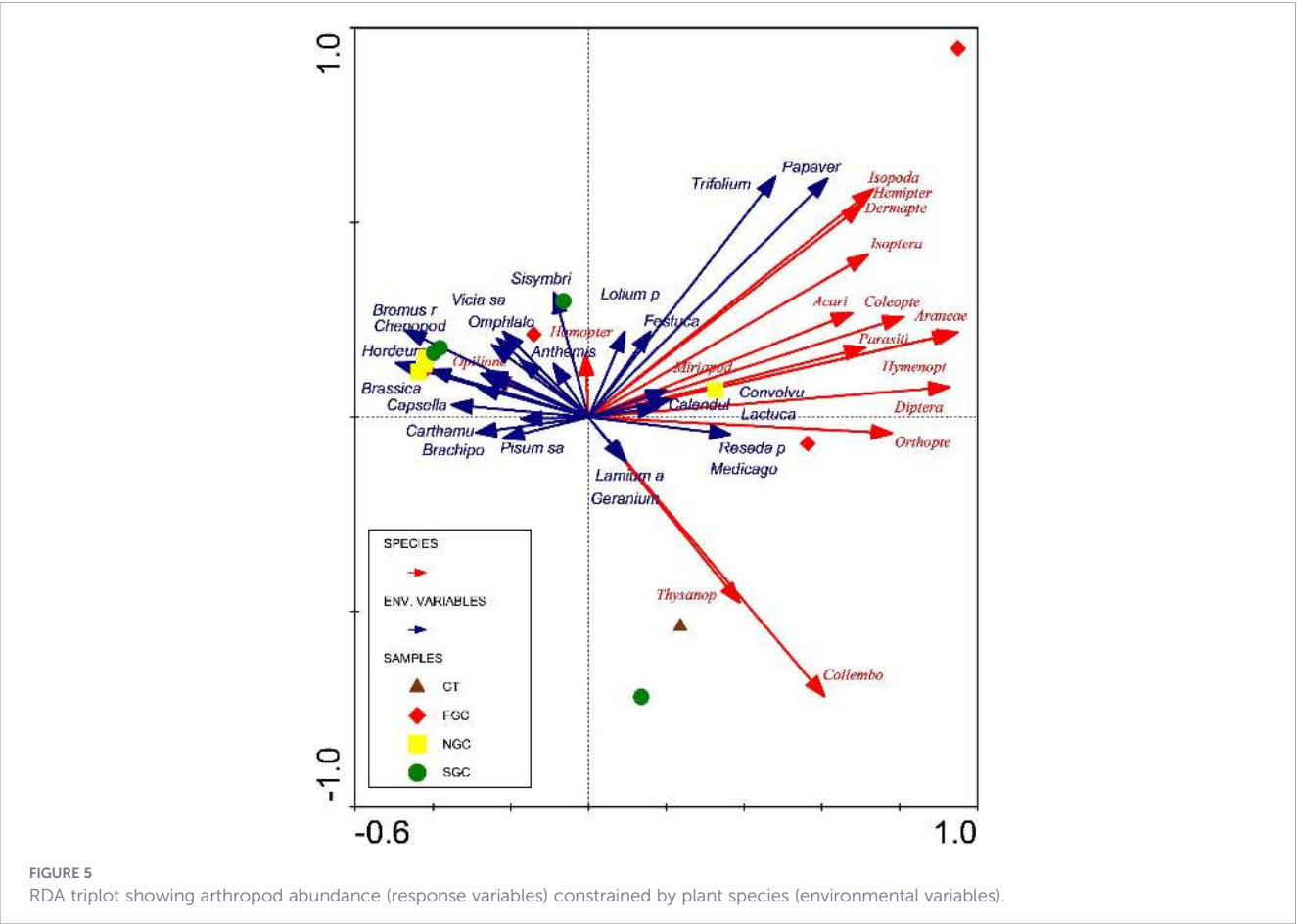


TABLE 4 Treatments with different letters display significant differences at $p < 0.05$. Mean production and pruning per vine, and Ravaz's index variation in the vineyard in function of the introduced cover crops. Mean values ($\pm$ SD).

Treatments	Yield				Pruning				Ravaz's index			
	t/ha				t/ha				Value			
CT	3.423	$\pm$	0.460	b	1.413	$\pm$	0.110	c	3.163	$\pm$	1.520	a
FGC	0.915	$\pm$	0.223	a	0.686	$\pm$	0.216	a	2.256	$\pm$	0.397	a
NGC	3.268	$\pm$	0.339	b	1.041	$\pm$	0.364	bc	3.410	$\pm$	0.460	a
SGC	1.663	$\pm$	0.352	ab	1.084	$\pm$	0.222	ab	3.529	$\pm$	1.325	a

Treatments with different letters display significant differences at $p < 0.05$.

performed (Supplementary Table 6). Similar reductions were observed in pruning weights, which decreased by 51%, 26%, and 23% in FGC, NGC, and SGC, respectively. In NGC, pruning decreased more than yield, whereas the opposite was observed in SGC. Consequently, the Ravaz Index was higher in NGC and SGC than in CT and FGC treatments.

The must quality parameters showed few significant differences among the treatments (Supplementary Tables 7A, B). However, some patterns are noteworthy. The weight of 100 berries was higher in CT and SGC than in FGC and NGC. The soluble sugar content and potential alcohol degree were higher in NGC and SGC than in CT and FGC. Yeast-available nitrogen (YAN) followed a similar pattern to °Brix, with higher values in CT and FGC than in NGC and SGC treatments. Color-related indices were lower in CT than in the cover crop treatments, with significant differences in the color index and anthocyanin content between CT and FGC/SGC, which displayed the highest values.

4 Discussion

4.1 Vine vigor, yield, and soil quality parameters

Viticulture, predominantly practiced in Mediterranean and temperate regions, is highly sensitive to climatic stressors, such as rising temperatures, declining precipitation, and progressive soil degradation. These pressures have become increasingly severe under the current trajectory of global climate change (18). Therefore, it is essential to understand crop responses under climate change scenarios, in which the frequency of heatwaves and prolonged periods of aridity are projected to increase (36). These conditions are expected to significantly affect key wine-producing regions in Spain, including D.O. Rioja and D.O. Ribera del Duero, among others (37).

Traditional viticulture, whether using bush or trellis systems as practiced in most Mediterranean countries, typically leaves part of the soil surface uncultivated. This bare soil is mechanically maintained without vegetation to avoid competition for water and nutrients, thereby enhancing vegetative growth, yield, plant nutrition, and water status. However, it has significant negative consequences, including depletion of organic carbon, loss of soil structure, and increased erosion (38). Therefore, the use of cover crops in vineyard soil management is becoming increasingly widespread, as they contribute to higher soil organic carbon, improved water infiltration, enhanced aggregate stability, drastic reductions in soil erosion and greenhouse gas emissions, and greater biodiversity within vineyards (13).

Nevertheless, in Mediterranean environments, the adoption of cover crops is challenging because of water scarcity, making it necessary to balance their benefits and disadvantages (39). This study, conducted in sandy soil of the D.O. Ribera del Duero under semi-arid conditions (Aridity Index = 0.366), provides an illustrative case: cover crops protected the soil from erosion but also had

marked impacts on yield, vine physiology, soil health, and biodiversity. Similar findings have been reported in other Mediterranean vineyards. For instance, Marques et al. (40) investigated soil erosion and infiltration dynamics using a rainfall simulator in a Tempranillo vineyard in Campo Real (central-southern Spain), comparing conventional tillage with a *B. distachyon* cover crop over two years. Their results showed that cover crops increased infiltration and soil moisture during wet periods; however, under dry conditions, conventional tillage enhanced porosity and deeper water infiltration.

In sandy soils, such as those in this experiment, these dynamics contributed to the substantial reductions in yield and pruning weight observed in the cover crop treatments (FGC and SGC) relative to the control, particularly during a year with severe drought, such as 2022. In this production area, cover crops are usually mowed by late May to reduce competition for water and nutrients with the vines. However, the irregular rainfall distribution in 2022, with a pronounced spring drought, suggests that earlier mowing (e.g., late April) may have been advisable.

This study revealed substantial yield reductions under cover crop treatments, with up to a 75% decrease for floral green cover (FGC) and a 50% decrease for seeded green cover (SGC) compared to conventional tillage. Such reductions represent a significant economic constraint, as lower grape production can directly affect vineyard profitability, particularly in high-value wine regions, where yield stability is critical. Although cover crops may provide long-term benefits for soil health, carbon sequestration, and ecosystem services, immediate economic trade-offs could limit their practical adoption unless these systems are coupled with management strategies that mitigate competition for water and nutrients, such as temporal mowing, selective species mixtures, or targeted irrigation (22). Moreover, combining cover crops with soil amendments (e.g., biochar or compost) or alternative management practices (e.g., alternating tilled and vegetated interrow, interrow rolling, or sub-row mulching management) may mitigate yield penalties by increasing nutrient availability and water retention, ultimately enhancing system resilience and productivity (41). In some cases, tillage-based termination, although less conservation-oriented, effectively stops cover crop transpiration and increases soil water and nitrogen stocks in Mediterranean conditions (42). Partial cover cropping on sloped or shallow soils is another option that may balance water conservation with erosion control and fruit quality (43). Overall, while cover crops offer ecological advantages, their widespread adoption will depend on strategies that balance soil conservation goals with vineyard productivity and economic viability, particularly in a European context where market demand increasingly favors wine quality over quantity (44).

This effect was also reflected in lower berry weights, although no major differences were observed in must quality parameters, except for anthocyanin content, which was higher in vines with cover crops than in those under conventional tillage. It is well established that water deficit enhances the accumulation of secondary metabolites, such as anthocyanins and terpenes, in grapevines (45, 46). This trade-off is highly relevant in viticultural regions where grape quality is prioritized over yield, and agronomic practices, such as

green pruning or controlled water stress, are deliberately applied to enhance quality (47). Therefore, cover crop management—including species composition, timing, and intensity—is a key factor in viticulture, with contrasting impacts on yield and must quality.

In a systematic review (13), it was reported that cover crops generally increase soil organic carbon (SOC), improve aggregate stability, and reduce greenhouse gas emissions. However, in other published studies, SOC accumulation was variable, depending on cover crop type, weeding regime, and the number of years since establishment. SOC increases are gradual; previous studies have shown that spontaneous grasses or leguminous cover crops require approximately 3–5 years to significantly increase SOC (48, 49). In general, grass-based cover crops, including *B. distachyon*, tend to generate larger SOC gains than legume-only cover crops (40, 50). *B. distachyon* is an interesting grass to use as a cover crop, particularly promising for Mediterranean vineyards and orchards, because its early maturation and self-seeding capacity which require minimal management (51). The pronounced SOC accumulation under SGC, in addition to its strong variability between replicates, highlights that living cover systems provide synergistic benefits for carbon sequestration and soil health, reinforcing their potential as climate-resilient soil management strategies.

Nutrient dynamics are also an important aspect of this study. The observed decrease in soil pH, although minor, may have implications for nutrient availability, as even small pH shifts can influence the solubility and uptake of key macronutrients, such as phosphorus and potassium, and micronutrients, such as iron and zinc (52). Cover crops can produce localized acidification in the rhizosphere through organic matter inputs and root exudates, enhancing nutrient mobilization without substantially altering the bulk soil pH. Consequently, slight decreases under seeded (SGC), floral (FGC), or natural (NGC) cover crops may promote improved nutrient cycling compared to CT, potentially supporting vine growth and grape quality over time. These findings underscore that soil chemical changes under cover crops are subtle but can have cumulative effects on nutrient dynamics, particularly when integrated with other soil amendments such as compost or biochar to enhance fertility and microbial activity. Further monitoring of soil solution chemistry and nutrient availability indices would clarify the agronomic significance of these pH trends.

Cover crops consistently affect vineyard nitrogen dynamics, with the strongest impact on soil nitrate ($\text{NO}_3\text{-N}$). Nitrate levels are lower in cover-cropped soils than in bare or tilled controls. Rocha et al. (14) identified $\text{NO}_3\text{-N}$ as the main factor differentiating treatments, with higher levels in bare soil and clear reductions under Phacelia and rye. These findings agree with Tiwari et al. (22), who reported reduced nitrate leaching and improved nitrogen retention under cover crops. In contrast, ammonium ($\text{NH}_4\text{-N}$) and phosphate ($\text{PO}_4\text{-P}$) show no consistent response (14), a pattern also observed by Vrignon-Brenas et al. (53). Overall, cover crops reduce soil $\text{NO}_3\text{-N}$, while $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ remain relatively stable. However, this decrease did not appear to affect vine physiology, as no significant differences were detected in SPAD values, which are early indicators of chlorophyll and leaf nitrogen content (53). Similarly, no differences were observed in yeast-assimilable

nitrogen (YAN) in must samples, which is a critical parameter for fermentation and wine quality.

Our results indicated that soil proteolytic activity increased following the introduction of cover crops, particularly under the NGC and FGC treatments. This enhancement may be linked to the substantial reduction in soil nitrate concentrations induced by cover crops, as higher proteolytic turnover can accelerate nitrogen cycling and promote microbial immobilization processes (53).

4.2 Botanical and faunal biodiversity

The cover crops used in this study were grass-based: *B. distachyon* in the SGC and *L. perenne* plus *F. arundinacea* in the FGC. These species establish well in early spring but can suppress the growth of legumes and flowering plants included in seed mixtures. Consequently, only a few floral species (e.g., *C. arvensis*) and occasional legumes, such as *T. incarnatum*, were detected. This limitation is important for the development of beneficial insect communities, which play a central role in ecologically based pest management (EBPM) through habitat enhancement, natural enemy conservation, and biological control (54). *C. bursa-pastoris* was the dominant species, along with other weeds that are usually present in adjacent crops. Future seed mixtures should therefore be tailored to management objectives—whether erosion control, carbon storage, soil health improvement, or EBPM—and consider stress tolerance and soil texture when designing optimal species compositions.

The introduction of cover crops is also expected to enhance soil biological activity, as greater SOC stimulates microbial metabolism (9, 13, 55). In this study, microbial and enzymatic activities were higher in the FGC treatments and lower in SGC. This may be linked to denser root systems in spontaneous grasses of NGC or the introduced species such as *L. perenne* and *F. arundinacea* compared with *B. distachyon*, resulting in stronger rhizosphere effects. Conversely, the low soil moisture observed in spring likely reduced microbial activity in species that were less adapted to semi-arid conditions.

Vegetation cover positively influenced soil fauna diversity, particularly spiders and functional groups such as detritivores, omnivores, and predators. Litter cover was the primary variable influencing soil fauna diversity and composition, highlighting the importance of retaining organic matter in vineyards (56). Blaise et al. (57) found that in Mediterranean vineyards, arthropod abundance was strongly influenced by soil management and related vegetation-associated habitat structure (e.g., inter-row vegetation cover and disturbance intensity), with plant species richness and associated microhabitat conditions showing greater explanatory power for arthropod abundance than vegetation composition alone.

Arthropod abundance was higher in FGC than in the other treatments, indicating a positive effect of cover crops, although site variability was high. The composition of arthropod communities appeared to depend more on local microhabitat conditions within inter-row areas than on the specific vegetation present. For example, in some control plots, groups such as *Collembola*, *Diptera*, and *Hymenoptera* were more abundant, suggesting that factors other than vegetation (e.g., microclimate and soil structure) strongly

influence biodiversity. Despite its lower plant diversity indices, the FGC treatment provided high ground cover that sheltered diverse arthropod groups, including predator families such as *Coccinellidae* (aphid predators), *Apidae* parasitoids (larval predators), *Syrphidae* (hoverflies), and predatory *Hemiptera* (Anthocoridae, Miridae). Additional predators and parasitoids, such as *Mutilla* sp. (parasitic wasps) and *Coranus aegyptius* (predatory bugs), were also recorded in the NGC and SGC treatments.

The associations observed in the RDA triplot suggest that certain plant species, such as *T. incarnatum* and *P. rhoeas*, can provide structural habitat, alternative food resources such as pollen and nectar, and microhabitats that favor the presence of several arthropod groups. In particular, the association with *Dermaptera* and predatory *Hemiptera* may indicate potential contributions to biological control, as these taxa include generalist predators known to prey on soft-bodied vineyard pests (58). Although *Isopoda* are primarily detritivores, their presence may reflect increased litter availability and improved soil conditions associated with vegetated ground cover. Overall, these patterns suggest that flowering ground cover may promote a more functionally diverse arthropod community in vineyard ecosystems.

Winter et al. (59) published a comprehensive meta-analysis summarizing the effects of vineyard management on biodiversity and associated ecosystem services (ES) across the globe. Across studies, extensive vegetation management resulted in a 20% increase in biodiversity and ES, such as C sequestration, pest control, and soil fertility; however, the response was climate- and management-dependent, with aridity and the introduction of ecological practices without pesticide application being the most important factors, resulting in weaker or stronger responses in terms of biodiversity and ES. In a study of cover crops in vineyards in Northern Italy (60), reported a higher abundance of predators, particularly *Carabidae* and *Staphylinidae*, in treatments with legumes (faba bean and vetch), while soil *Araneae* did not appear to be affected, as in our case, and no vineyard pests seemed to be positively affected by cover crops.

One objective of this study was to assess the potential of cover crops for controlling *Lobesia botrana* (Den. & Schiff.) (Lepidoptera: Tortricidae), which is the most serious vineyard pest in the Mediterranean region (61). Monitoring with delta-type pheromone traps, one in the control and another in the FGC treatment, detected the first moth emergence, but no clear treatment effects were observed. Due to the experimental design, which did not allow effective monitoring of flying insects, and the short distances between inter-row spaces without vegetation, it was not possible to isolate treatment-specific effects.

4.3 Limitations and future research

Other management practices, such as organic amendments, biochar application, and mulching, are widely recognized as effective strategies in viticulture for enhancing soil health and agroecosystem resilience under climate-induced extremes such as drought and intense rainfall (62, 63). It is well documented that

biochar and mulches improve soil organic carbon (SOC) stocks, aggregate stability, water retention, and erosion control across diverse conditions (64, 65). Similarly, cover crops consistently increase SOC, microbial biomass, structural stability, and infiltration capacity, particularly over time and in water-limited environments (66). Future research involving the integration of cover crops with organic amendments offers strong potential for synergistic effects. Cover crops provide continuous organic inputs and root-derived carbon that stimulate biological activity and soil structure formation, while amendments such as biochar and compost enhance carbon stabilization, nutrient retention, and surface protection. This combined approach may improve SOC persistence, soil aggregation, and water regulation more effectively than single practices alone. Such integrated soil management strategies are especially relevant for Mediterranean vineyards, which are facing increasing climatic variability and water scarcity (13, 67).

5 Conclusions

- Cover crops improve soil health but reduce vine productivity in semi-arid Mediterranean vineyards. They enhance soil organic carbon, structure, and infiltration, and reduce erosion; however, they also decrease yield and pruning weight in drought-prone sandy soils.
- Cover crop management strongly influences grapevine physiology and must quality. Water competition reduced berry weight but increased anthocyanin concentration, highlighting a trade-off between yield and grape quality.
- Soil biological activity responds positively to cover crops but depends on species and water availability. Grasses with dense root systems (e.g., *L. perenne* and *F. arundinacea*) enhanced microbial activity more than *B. distachyon*; however, drought limited the overall effect.
- The species composition of cover crops is critical for ecosystem services. Grass dominance limited legumes and flowering plants, reducing potential benefits for beneficial insects and EBPM and highlighting the need for carefully designed seed mixtures.
- Cover crops increase arthropod abundance, but their effects on pest control remain unclear. Although predator and parasitoid diversity increased (e.g., *Coccinellidae*, *Syrphidae*, and *Hymenoptera*), the experimental design did not permit a clear assessment of their impact on *Lobesia botrana*, a pest with higher incidence in Mediterranean vineyards.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: Zenodo, <https://doi.org/10.5281/zenodo.17427053>.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

AM-P: Conceptualization, Methodology, Writing – original draft, Data curation, Formal analysis, Visualization. MN: Writing – review & editing, Data curation, Formal analysis, Visualization. DL-R: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Investigation, Resources. DP-A: Data curation, Formal analysis, Visualization, Writing – review & editing. RB: Investigation, Resources, Writing – review & editing. CR: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. OA: Investigation, Resources, Writing – review & editing. GS-P-M: Conceptualization, Funding acquisition, Methodology, Project administration, Writing – original draft, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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