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Long-term soil functional differences between spontaneous cover cropping and tillage in a semi-arid vineyard

Juan Emilio Herranz-Luque[1]*, Juan Pedro Martín-Sanz[2], Javier González-Canales[2,3], Marco Antonio Jiménez-González[1], Pilar Carral[1], Blanca Sastre[2] and María José Marqués[1]

[1]Departamento de Geología y Geoquímica, Universidad Autónoma de Madrid, Madrid, Spain, [2]Área Investigación Aplicada y Extensión Agraria, Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Madrid, Spain, [3]Universidad de Alcalá (UAH) Escuela de Doctorado, Madrid, Spain

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Vijay Singh Meena,
ICAR - Mahatma Gandhi Integrated Farming Research Institute, India

*CORRESPONDENCE
Juan Emilio Herranz-Luque
✉juan.herranz@uam.es

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Context: Soil degradation and water scarcity pose critical challenges for vineyard sustainability in semi-arid regions.
Objectives: This study evaluates the long-term effects of spontaneous cover cropping (CC) on soil health in a rainfed vineyard in central Spain, managed without irrigation or pesticides for over two decades.
Methodology: By comparing soils under CC and conventional tillage (TILL), we assessed changes in soil physical properties (porosity and water retention), nutrient content, and microbial function up to 30 cm depth. Stepwise regression analysis was used to explore management-driven relationships among CC, soil properties and nutrient dynamics.
Results: Soils under CC showed significantly higher organic matter content (1.74 ± 0.37% in the topsoil with CC vs. 0.83 ± 0.24% in TILL) and porosity (51.2 ± 2.5% vs. 44.0 ± 1.8% at 10–30 cm depth). Available phosphorus tended to be higher under CC (19.13 ± 0.60 mg/kg in CC vs. 14.13 ± 4.52 mg/kg in TILL), and this trend was further supported by stepwise analysis, which identified P availability as a variable influenced by management practices. Enzymatic activities were consistently elevated under CC, particularly in the topsoil; β-glucosidase (25 ± 9 mU/g) nearly doubled the value observed under tillage. Although soil water availability showed a non-significant trend in the topsoil (10 cm), it was higher in the subsoil (30 cm) under CC (29.0 ± 0.97% vs. 25.1 ± 0.32% in TILL). The stepwise regression analysis supported a management-driven model where CC influence soil organic matter (SOM) and nutrient availability. SOM and soil moisture strongly influenced extractable phosphorus (R^2 = 0.790, p =0.0047) and mineral nitrogen (R^2 =0.907, p =0.00018), with moisture at the time of sampling emerging as the dominant driver of nitrogen dynamics.
Conclusions: The analysis supports a management-driven pathway in which long-term vegetation inputs under CC enhance SOM accumulation. Soil water availability tended to increase in the subsoil under CC, while SOM together with short-term moisture conditions emerged as the main regulators of nutrient dynamics under semi-arid conditions.

Implications: By aligning ecological processes with practical agronomic outcomes such as nutrient retention and soil structure, these findings offer compelling evidence to support the broader adoption of sustainable ground cover practices in Mediterranean viticulture.

KEYWORDS

calcareous soils, cover cropping, groundcovers, phosphorus availability, semi-arid vineyards, soil enzymatic activity, soil organic carbon, sustainable viticulture

1 Introduction

Vineyards located in semi-arid regions face numerous challenges related to soil degradation, which ultimately affect vineyard sustainability and productivity. A common issue in these areas is the continued use of intensive tillage practices, which over time lead to soil erosion, the deterioration of soil structure, the reduction of organic matter content, and the compaction of the soil profile (1). These alterations negatively impact essential soil functions, such as water infiltration and retention, gas exchange, root development, and biological activity, all of which are crucial for maintaining vine health and grape quality. In these degraded soils, the limited capacity to retain moisture becomes particularly problematic under rainfed conditions, where water availability is already a limiting factor due to low and irregular precipitation patterns.

In response to these issues, there is increasing interest in adopting sustainable soil management practices that improve or restore soil health. One such practice is the use of cover crops in the inter-row spaces of vineyards. Cover crops can help mitigate erosion, enhance soil organic matter, and improve soil structure and biodiversity, and to a certain extent reduced disease incidence (2). When properly managed, they can also contribute to greater soil resilience under climatic stress. Research in recent years has demonstrated the potential of cover cropping to improve both soil quality and vineyard productivity in various agroecological contexts (2–8). Despite these benefits, the adoption of cover crops in semi-arid vineyards remains limited, especially in rainfed systems (9, 10). Many winegrowers express concerns about potential competition for water between the cover crop and the vines, particularly during the establishment of young vineyards. Additionally, a general lack of knowledge and experience in cover crop management continues to hinder their implementation (11).

This study seeks to contribute to the growing body of knowledge on sustainable vineyard management by evaluating the long-term effects of spontaneous cover cropping in a semi-arid, rainfed vineyard in Castilla-La Mancha, Spain. Specifically, it compares this management strategy with conventional tillage over a sufficiently long period—more than two decades—to capture meaningful changes in the soil's physical, chemical, and biological properties. The extended timeframe allows us to assess whether spontaneous cover vegetation can offer a viable alternative to traditional tillage in terms of enhancing soil health, and thus, the overall sustainability of vineyard systems in water-limited environments.

Although much scientific and policy attention is often directed toward increasing soil carbon stocks as a strategy for climate mitigation and soil restoration, farmers often perceive limited short-term economic returns and are typically more concerned with immediate agronomic benefits (12). In this context, our study places special emphasis on the long-term effects of cover cropping. To address this, we analyzed both physicochemical soil properties and key enzymatic activities involved in nutrient cycling (e.g., urease, phosphatase), providing a comprehensive view of the biological mechanisms supporting nutrient availability. Any increase in extractable phosphorus and total mineral nitrogen in soils under long-term cover cropping will represent tangible agronomic advantages that are more readily valued by growers than changes in carbon stock alone.

This study aims to evaluate the long-term effects of cover cropping on soil properties in Mediterranean vineyard systems, with a focus on parameters relevant to farmers such as nutrient availability, water management, and soil health. It is based on a vineyard under farmer management where spontaneous vegetation has been maintained for approximately 20 years. Such conditions are uncommon, as most existing studies of CC in vineyards are limited to shorter durations and are typically conducted in experimental settings (13), and are not usually covering more than 10 years (2). In this case, soil disturbance has been minimal, with occasional tillage applied only during extreme climatic events, such as drought or heat waves, to mitigate vine water stress. This context allows for the assessment of the long-term impact of cover cropping on the physical, chemical, and biological properties of the soil. This comparison is conducted at the plot scale between two adjacent vineyards with comparable soil conditions, allowing management effects to be evaluated independently of regional soil variability.

Based on this context, we hypothesize that long-term spontaneous cover cropping in semi-arid, rainfed vineyards enhances soil organic matter accumulation, improves soil physical structure, and strengthens biological processes involved in nutrient cycling when compared to conventional tillage. Accordingly, the objectives of this study are to (i) compare the long-term effects of cover cropping and tillage on soil physical, chemical, and biological properties down to 30 cm depth, and (ii) assess how vegetation-

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Abbreviations: CC, cover cropping; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; FAO, Food and Agriculture Organization of the United Nations; ISO, International Organization for Standardization; NH_4^+, ammonium; NO_3^-, nitrate; NPK, nitrogen–phosphorus–potassium fertilizer; P_2O_5, extractable phosphorus (as phosphorus pentoxide); SOC, soil organic carbon; SOM, soil organic matter; SD, standard deviation; TILL, conventional tillage; WRB, World Reference Base for Soil Resources driven changes in soil properties influence nutrient availability under semi-arid Mediterranean conditions.

2 Materials and methods

2.1 Study site

The study was conducted in two vineyard plots located near Mota del Cuervo, in the province of Cuenca, central Spain (39° 30’09.5”N 2°54’02.8”W; ~750 m a.s.l.). The region lies within the southern Meseta Central, a broad plateau characterized by gently undulating terrain developed on Neogene and Quaternary sediments which overlie a Mesozoic basement. The studied vineyard has been planted on the crest of an anticline where Upper Jurassic limestones and Lower Cretaceous sandstones outcrop. Geomorphologically, the area forms part of the transitional zone between the La Mancha plain and the foothills of the Iberian Range, exhibiting a predominance of low-relief landforms such as fluvial terraces, pediments, and gently sloping alluvial fans.

The climate is classified as cold semi-arid (BSk) according to the Köppen-Geiger system, with a marked Mediterranean influence. Mean annual precipitation ranges from 350 to 450 mm, concentrated mainly in spring and autumn. The mean annual temperature is approximately 13–14 °C, with summer maxima frequently exceeding 35 °C and winter minima occasionally falling below 0 °C. The potential evapotranspiration (ET$_0$) is estimated at 850–950 mm per year (14). This imbalance between precipitation and evapotranspiration defines the region as semiarid, with long periods of water scarcity that constrain plant growth and influence soil development.

The parent materials where both vineyards are located consist of calcareous breccias, forming a colluvium of very gentle relief associated with the core of an anticline of the Chelva Group (15); the dominant soil types in the area are Calcisols and Luvisols, typically with loamy to clay-loam textures and high base saturation (16). These soils often exhibit a weak to moderate structure and a subsurface accumulation of secondary carbonates. They are moderately well drained and are widely used for dryland and irrigated agriculture.

The two vineyard plots sampled in this study (Figure 1) growing over Calcaric Cambisol (17) differ in management: one is maintained under cover cropping, while the other is managed with conventional tillage. The vines, of the white grape variety *Airén*, are 70–80 years old and trained as free-standing bushes (*gobelet* system). Vegetation control in the vineyard under CC, covering 0.5 ha, is limited to the area directly around each vine, where it is manually removed using a hand hoe. Inter-vine vegetation is left unmanaged to maintain ground cover; however, in the event of prolonged or severe drought, the farmer may resort to plowing the entire vineyard as a water conservation strategy. According to information provided by the farmer, the vineyard has been managed under non-irrigated, organic conditions with spontaneous cover cropping for over two decades, without the use

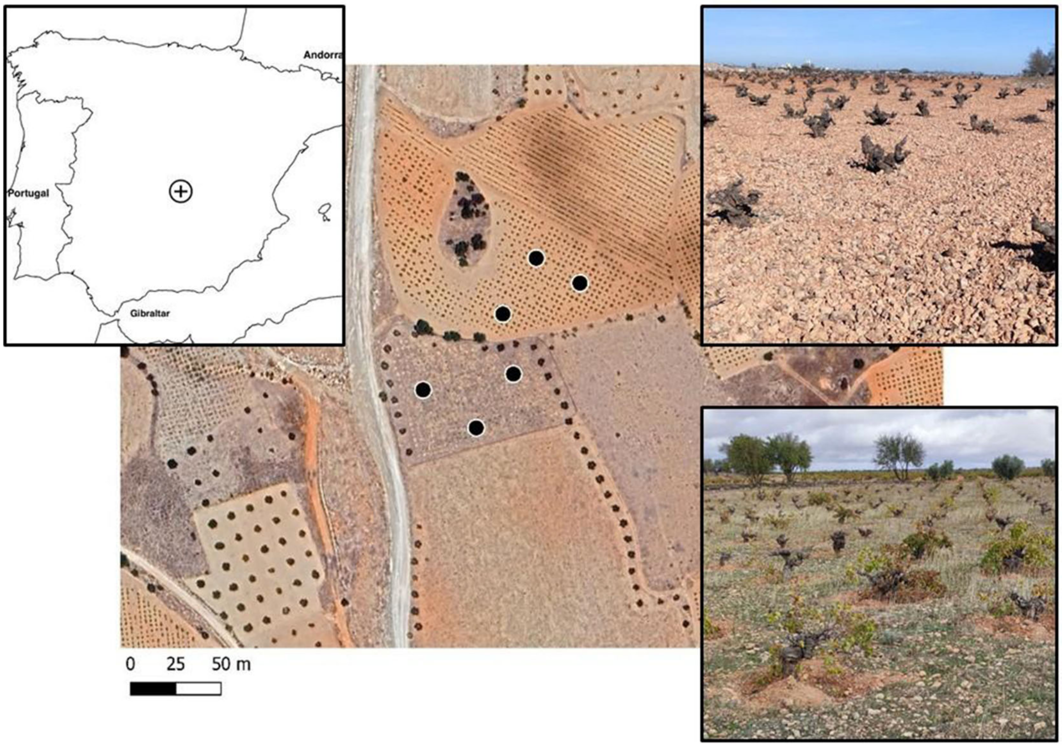

FIGURE 1
Location of the study vineyards in central Spain and layout of sampling points (black dots). The two vineyards are adjacent; the lower plot is managed under spontaneous cover cropping and the upper plot under conventional tillage. Photographs show typical surface conditions, including a stony surface layer common to both vineyards.

of fungicides, herbicides, or any other external inputs whatsoever. This long-term, low-input management likely supports a well-structured and biologically active soil microbial community, offering a favorable context for evaluating soil enzymatic activity and other biological indicators. The second adjacent vineyard, covering 1 ha, is managed with annual tillage. The conventional vineyard management in this region typically involves 2 to 4 tillage operations per year to control weeds and maintain bare soil. Herbicides can be applied under the vine rows, while fungicides, including copper sulfate (Bordeaux mixture), are routinely used to control mildew. Mineral fertilizers are applied during winter, after the autumn rains, when the vineyard is in vegetative dormancy, at typical rates of approximately 150–300 kg ha^{-1} of NPK fertilizer in traditional low-yield rainfed vineyards of the region. Soil sampling in this study was conducted in autumn, prior to fertilizer application.

2.2 Sampling and analytical methods

Three soil samples were randomly collected from each vineyard, with approximately 1 kg of soil taken separately from the surface (0–10 cm) and subsurface (10–30 cm) layers, considering that this is the depth typically affected by tillage and following the FAO recommendation to obtain samples for SOC concentration determinations from these specific depths (3). Each sample was composed of six subsamples: three taken from the inter-rows (between vine rows), and the other three from beneath the vines.

The variables studied were: Soil texture was determined by the hydrometer method (18), following USDA particle-size classification (clay < 2 µm, silt 2–50 µm, sand > 50 µm). Soil organic matter was measured by the Walkley–Black wet oxidation method using potassium dichromate and sulfuric acid (19). Total porosity was calculated from bulk density measurements (20), and soil water retention was determined using pressure plates (21), with available water calculated as the difference between field capacity (2.54 pF) and permanent wilting point (4.2 pF, 20). Nitrate and ammonium were extracted with distilled water (1:5, w/v) by shaking for 1 h, filtered (20 µm), and quantified by UV–visible spectrophotometry. This extraction targets readily available inorganic nitrogen in soil solution (22).At each site, vegetation biomass was estimated by harvesting all vegetation within a 25 cm × 25 cm quadrat, with three replicates per site. Samples were oven-dried to constant weight prior to obtain above ground vegetation biomass. Root biomass was estimated using a cylindrical metal gouge auger (30 cm length, 2.5 cm internal diameter). Two cores were collected per quadrat, each sampled at two depths (0–10 cm and 10–30 cm), resulting in four root samples per quadrat. In the laboratory, soil samples were washed and filtered, and the roots were collected, oven-dried at 60 °C, and weighed, following the root separation procedure described by Frasier et al. (23). Enzymatic activities of soils were also measured by UV-Visible spectrophotometry (24–27). Enzymatic activities were measured in mU/g, where Unit (U) of enzyme activity, is the amount of enzyme that catalyzes the conversion of 1 micromole (µmol) of substrate per minute.

Potential differences were analyzed using Kruskal-Wallis non-parametric test (28). To evaluate the sequence of soil functional recovery processes under long-term vegetation cover, we employed a stepwise regression approach grounded in ecological plausibility and management logic. The analysis proceeded in three stages. First, vegetation metrics—root biomass, aboveground biomass, and percent of living vegetation cover—were included as independent variables in multiple linear regressions to test their influence on soil organic carbon (SOC) and soil water availability, treated as dependent variables. Second, SOC, water availability and soil moisture the day of sampling (g/g) were used as predictors in separate regressions for mineral nitrogen and extractable phosphorus (P_2O_5) to assess how improvements in soil structure and moisture conditions affected nutrient availability. This approach follows the logic of mechanistic modeling in soil ecology (29), where sequential regressions approximate causal relationships in the absence of experimental manipulation. All regressions were performed using standard multiple linear regression procedures in STATISTICA 8.0.

3 Results and discussion

Both vineyards are contiguous and therefore subjected to the same climatic and seasonal conditions. As a result, short-term seasonal variability in soil moisture and nutrient availability affected both management systems simultaneously and does not constitute a confounding factor between treatments.

3.1 Physical chemical effects of cover cropping

Soil texture in both vineyards was classified as sandy loam, with only minor variations in the proportions of clay, silt, and sand (Table 1). This uniformity in particle size distribution across both management systems and depth layers confirms that textural differences are not a confounding factor in this study. As such, the contrasting results in organic matter, porosity, and water retention can be reliably associated with the long-term effects of spontaneous cover cropping as compared to repeated tillage, rather than to intrinsic soil properties.

The comparison between soil management practices in Table 1—cover cropping (CC) versus traditional tillage (TILL)—reveals several differences in key indicators of soil health. Organic matter content was significantly higher in soils under CC management, with values more than twice those observed in tilled soils. In the 0–10 cm layer, CC soils had an average organic matter content of 1.74%, compared to 0.83% in TILL soils ($p = 0.0495$). This difference persisted in the 10–30 cm layer, where CC soils had 1.35% organic matter versus 0.69% in TILL soils ($p = 0.0495$). The increase at greater depths is particularly noteworthy, as many studies report organic matter enrichment only in the uppermost centimeters of soil due to shorter study durations or insufficient biomass input (8). These findings suggest that long-term cover

TABLE 1 Texture, organic matter content (SOM, %), total porosity (%), and total available water (%) under the two management systems (CC: cover cropping; TILL, tillage), considering the layers separately (0–10 cm and 10–30 cm depth).

Texture						
CC 0–10 cm	Clay 15.0; Silt 15.0; Sand 70.0%		CC 10–30 cm	Clay 13.0; Silt 18.3; Sand 68.7%		
TILL 0–10 cm	Clay 16.0; Silt 14.3; Sand 69.7%		TILL 10–30 cm	Clay 12.3; Silt 17.6; Sand 70.0%		
SOM (%) Kruskal-Wallis						
Management	Depth	Mean	SD	Median	Quartiles (25–75%)	
CC	0–10 cm	1.74	0.37	1.65	1.42 - 2.14	H (1. N = 6) =3.8571 p =0.0495
LAB	0–10 cm	0.83	0.05	0.81	0.79 - 0.88	
CC	10–30 cm	1.35	0.06	1.36	1.29 - 1.40	H (1. N = 6) =3.8571 p =0.0495
LAB	10–30 cm	0.69	0.09	0.70	0.59 - 0.77	
Total Porosity (% vol)						
		Mean	SD	Median	Quartiles (25–75%)	
CC	0–10 cm	46.21	0.19	46.29	45.9 - 46.3	H (1. N = 6) =0.047619 p =0.8273
LAB	0–10 cm	46.60	1.62	46.15	45.2 - 48.4	
CC	10–30 cm	51.22	2.52	52.66	48.3 - 52.7	H (1. N = 6) =3.0000 p =0.0833
LAB	10–30 cm	44.05	0.04	44.05	44.0 - 44.1	
Available Water (% vol; Field Capacity- Permanent Wilting point)						
		Mean	SD	Median	Quartiles (25–75%)	
CC	0–10 cm	30.1	2.42	31.0	27.4- 32.0	H (1. N = 6) =0.4280 p =0.5127
LAB	0–10 cm	31.1	1.54	31.3	29.4- 32.5	
CC	10–30 cm	29.6	0.97	30.2	28.5- 30.2	H (1. N = 6) =3.0000
LAB	10–30 cm	25.1	0.32	25.1	24.8- 25.3	p =0.0833
Soil moisture day of sampling (g/g)						
		Mean	SD	Median	Quartiles (25–75%)	
CC	0–10 cm	0.075	0.005	0.075	0.070 - 0.080	H (1. N = 6) =1.190476 p =0.2752
TILL	0–10 cm	0.079	0.001	0.080	0.078 - 0.081	
CC	10–30 cm	0.113	0.011	0.108	0.105 - 0.126	
TILL	10–30 cm	0.119	0.015	0.125	0.102 - 0.131	H (1. N = 6) =.0476190 p =0.8273
Above ground Biomass (Mg/ha)						
		Mean	SD	Median	Quartiles (25–75%)	
CC	0–30 cm	4.71	1.15	4.98	3.31- 5.84	H (1. N = 6) =8.4900 p =0.0036
TILL	0–30 cm	0.29	0.19	0.17	0.16- 0.55	
Living vegetation cover (%)						
		Mean	SD	Median	Quartiles (25–75%)	
CC	0–30 cm	53.7	3.5	52.0	51.3- 57.7	H (1. N = 6) =8.485714 p =.0036
TILL	0–30 cm	1.1	0.5	1.0	0.7- 1.7	
Root Biomass (Mg/ha)						
		Mean	SD	Median	Quartiles (25–75%)	
CC	0–10 cm	5.19	1.69	4.88	3.67 - 7.02	H (1. N = 6) =3.8571 p =.0495
TILL	0–10 cm	1.31	0.52	1.47	0.74 - 1.73	
CC	10–30 cm	2.73	1.05	3.29	1.51 - 3.38	H (1. N = 6) =2.3333
TILL	10–30 cm	1.48	1.39	0.85	0.51 - 3.06	p =0.1266

SD, standard deviation. n = 3. Kruskal-Wallis test compares management within the same depth.

cropping supports organic matter accumulation at depth, most likely driven by root development and *in situ* carbon inputs; however, a limited contribution from vertical transport of organic compounds may also occur and is the subject of ongoing investigation. This would align with the work of Wooliver and Jagadamma (8), who emphasize the importance of long-term trials to capture subsoil improvements under conservation practices.

Based on bulk density–derived porosity, no evidence of increased soil compaction was observed under long-term cover cropping. Soil porosity followed a similar pattern. While surface porosity (0–10 cm) was comparable between the two systems (~46% porosity), significant improvements were observed in the subsurface layer (10–30 cm) under CC management. Soils in this layer exhibited a mean total porosity of 51.2% in CC plots, compared to 44.0% in TILL plots ($p = 0.0833$). This enhancement suggests that long-term CC reduces subsurface compaction, a common problem in tilled vineyards where annual mechanical disturbance disrupts soil aggregates and contributes to compaction (30–32). The increased porosity under CC not only reflects better soil structure but also creates more favorable conditions for root development and microbial activity (33), both critical for nutrient cycling and water uptake (19), that may be particularly important in rainfed systems.

The improvement in soil structure and organic matter under CC was also reflected in the data on available water content. In the 10–30 cm layer, CC soils had an average available water capacity of 29.0% by volume, compared to 25.1% in TILL soils ($p = 0.0833$). Although this difference did not reach conventional statistical significance, it indicates a positive trend and is agronomically meaningful in semi-arid environments, where even small increases in soil water retention can impact crop resilience and productivity; other authors have found in vineyards meta-analysis, the capacity of mulching and cover crop practices to improve green water (34). In contrast, surface layers showed similar values under both treatments, likely due to the rapid drying of topsoil and the shorter exposure to cumulative changes in structure and organic matter.

Soil moisture measured on the sampling day showed no significant differences between cover cropping (CC) and tillage systems at either depth. At 0–10 cm, moisture was slightly higher in TILL (0.079 g/g) than in CC (0.075 g/g), but the difference was not significant ($p = 0.2752$). At 10–30 cm, both systems had higher moisture, with LAB again slightly exceeding CC (0.119 vs. 0.113 g/g; $p = 0.8273$). Overall, moisture was greater at depth, but management effects were minimal.

The aboveground biomass found in this study can be considered high compared to figures found in literature of 2 Mg/ha in grasslands (35–37), although it is line with measured aboveground biomass in vineyards with different cover crop types, including spontaneous vegetation and sown species, which typically exceeded 0.5 Mg ha^{-1}, with values approaching 5 Mg ha^{-1} during the peak of the vegetative period (38).This semiarid environment under study features a managed cover cropping system, where sparse grass vegetation grows between vine rows. In response to drought conditions, these grasses typically allocate a greater proportion of their biomass to root systems to improve water uptake, resulting in a high root-to-shoot ratio (39). The spontaneous herbaceous vegetation growing between vines, subject to sporadic tillage (3–5 events over 20 years of management), accumulates an average of 4.71 ± 1.15 Mg/ha of above-ground biomass. Root biomass averages 5.2 ± 1.7 Mg/ha in the top 10 cm of soil, with an additional 2.7 ± 1.05 Mg/ha in the subsoil layer between 10 and 30 cm depth. Yang et al. (40) studied a chronosequence comprising approximately 2,000 plots from 21 agricultural fields that had been abandoned for periods ranging from 4 to 74 years, in a long-term experiment of grassland recovery in Minnesota (USA) showing that root biomass recovery is related to soil C storage. Mean root biomass across different functional groups in monoculture plots (0–30 cm soil depth) ranged approximately between 2 and 6 Mg/ha. Over the final five years of that experiment, the average root-to-shoot ratio across all plots, measured to a soil depth of 0–30 cm, was 4.3. Although the climatic and soil conditions differ, the observed root biomass values are of similar magnitude.

3.2 Effects on nutrients

As both vineyards are subjected to the same seasonal conditions, instantaneous nutrient pools are strongly influenced by short-term variability—particularly soil moisture—but these effects are superimposed on long-term management-driven differences. In the topsoil layer (0–10 cm), soils under cover cropping (CC) exhibited generally higher nutrient concentrations compared to tilled soils (TILL), as indicated by median values and their respective minimum and maximum ranges shown in Figure 2. For example, ammonium (NH_4^+) concentrations in CC showed a trend toward higher median values compared to TILL; however, high variability prevented statistically significant differences. A similar trend was found for Nitrate (NO_3^-) content under CC. This resulted in a significantly greater mineral nitrogen pool (kg/ha) in CC soils at 0–10 cm depth, as shown by higher median values compared to TILL ($p = 0.0495$). For phosphorus, although differences in extractable P_2O_5 between CC and TILL were not statistically significant, a consistent trend toward higher median values under CC was observed at both depths, suggesting a possible enhancement of phosphorus cycling and biological activity in surface soils.

In the subsurface layer (10–30 cm), differences between management systems were more variable and less pronounced than in the topsoil. Ammonium (NH_4^+) concentrations showed comparable median values between CC and TILL, with overlapping ranges and no significant differences ($p = 0.8273$). In contrast, nitrate (NO_3^-) levels were lower under CC, with a reduced median value compared to TILL, and this difference was statistically significant ($p = 0.0495$), resulting in a trend of lower total mineral nitrogen pool (kg/ha) under CC at this depth. These patterns may reflect increased microbial immobilization or altered nitrification dynamics associated with cover cropping. As biological activity plays a central role in nitrogen cycling—particularly through nitrification and denitrification. The microbial pathways are strongly influenced by soil factors such as nitrogen availability, temperature, soil organic matter, moisture content, and oxygen

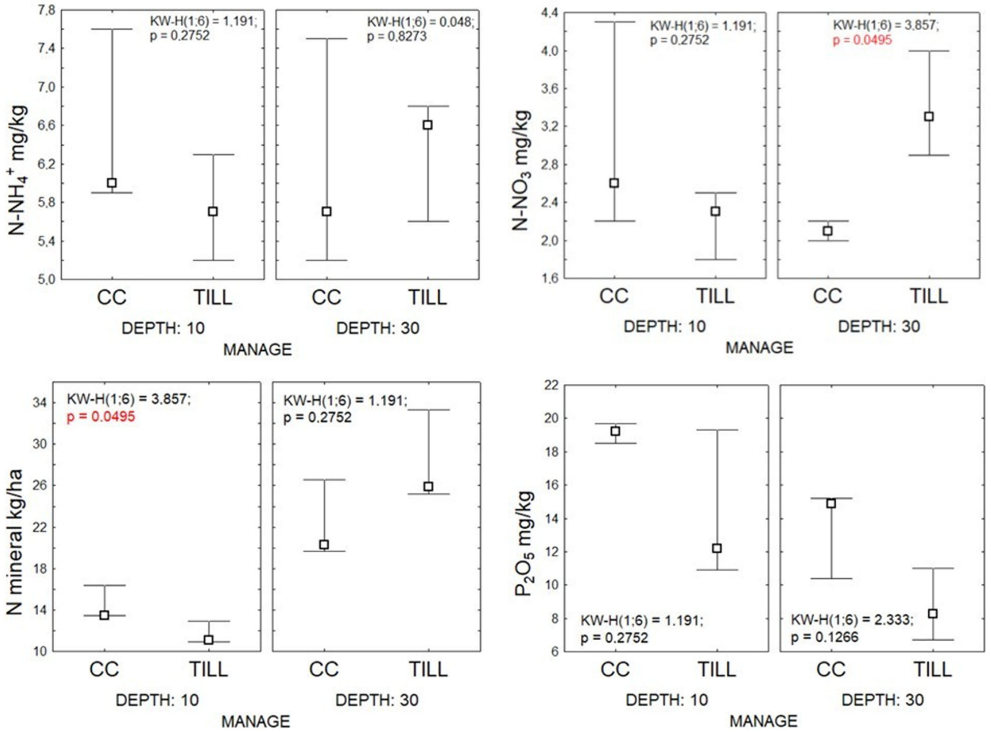

FIGURE 2

Effects of cover cropping in ammonia, nitrates and available phosphorus. (CC, cover cropping; TILL, tillage). Median, minimum and maximum values. Kruskal Wallis test indicates significant differences between treatments.

levels (41). Steenwerth and Belina (42) found that soil inorganic nitrogen levels varied not only with management practices but also with cover crop growth, and thus with the sampling date. In early winter, NO_3^-–N levels declined sharply following rainfall and reduced plant activity, and were consistently lower under cover cropping compared to tillage. Similarly, in this study, where sampling was conducted in early winter, nitrogen levels appeared to be lower under CC management in the deep layers of soils.

At 10–30 cm depth, a trend toward higher phosphorus availability was observed under cover cropping, with median P_2O_5 values exceeding those in tilled soils, although differences were not statistically significant ($p = 0.1266$). This pattern suggests that cover cropping may contribute to deeper phosphorus mobility or retention, potentially linked to greater soil organic carbon content or higher biological activity extending into the subsoil.

When considering the entire 0–30 cm soil profile, available phosphorus (P_2O_5) showed a moderate increase under CC compared to TILL, although the difference was not statistically significant (Kruskal–Wallis H(1, N = 12) = 2.56, $p = 0.1093$). Median P_2O_5 values were higher in cover-cropped soils (16.85 mg/kg) than in tilled soils (10.95 mg/kg), and the interquartile range was slightly broader under CC (14.90–19.20 mg/kg) than under TILL (8.27–12.20 mg/kg). This trend suggests that long-term cover cropping may contribute to enhanced phosphorus availability, potentially through increased soil organic carbon and biological activity; a further stepwise analysis in the following sections will confirm this relationship.

3.3 Enzymatic effects of cover cropping

The increase in soil organic matter observed under cover cropping (Table 1) would provide favorable conditions for microbial activity, as organic carbon serves both as an energy source and structural substrate for soil microorganisms (43). The enzymatic activities measured in this study may reflect the biological response to long-term cover cropping linked to soil health indicators previously described. Five key enzymes analyzed across surface (0–10 cm) and subsurface (10–30 cm) soil layers under the two management systems: cover cropping (CC) and traditional tillage (TILL), are shown in Table 2.

β-glucosidase, an enzyme involved in cellulose degradation and carbon cycling, showed significantly higher activity in soils managed under CC in the topsoil. In the surface layer, activity ranged from 17.4 to 35.9 mU/g under CC (Q25-Q75), approximately doubling the values observed in TILL soils (11.9 to 15.83 mU/g; $p < 0.05$). This difference was not significant in the subsurface layer, where Q25-Q75 values under CC were 7.6 to10.1 mU/g compared with 6.7 to 8.6 mU/g in TILL soils. These results are consistent with higher organic matter content under CC and align with the role of β-glucosidase as a sensitive marker of soil biological response to management. The β-glucosidase activity in Mediterranean arable soils typically ranges from 5 to 35 mU/g in the topsoil (39). The lower values observed in both vineyard treatments—despite the positive effects of cover cropping—clearly indicate the lasting impact of agricultural land use on soil biological functioning.

TABLE 2 **Enzymatic activity analyzed across surface (0–10 cm) and subsurface (10–30 cm) soil layers under the two management systems: cover cropping (CC) and traditional tillage (TILL). Kruskal-Wallis test compares management within the same depth.**

		β glucosidase mU/g				Kruskal-Wallis
Manejo	Profundidad	Mean	SD	Median	Quartiles (25–75%)	
CC	0–10 cm	25.49	9.47	23.14	17.42-35.91	H (1. N = 6) =3.8571 p =0.0495
TILL		13.81	1.97	13.70	11.9-15.83	
CC	10–30 cm	8.59	1.34	8.01	7.63-10.13	H (1. N = 6) = 0.0476 p=0.827
TILL		7.80	0.99	8.10	6.7-8.6	
		Phosphatase mU/g				
		Mean	SD	Median	Quartiles (25–75%)	
CC	0–10 cm	18.82	3.55	20.49	14.75-21.23	H (1. N = 6) =2.333 p =0.1266
TILL		11.87	7.50	9.69	5.71-20.22	
CC	10–30 cm	10.00	6.82	7.48	4.79-17.72	H (1. N = 6) =0.4285 p =0.5127
TILL		10.16	13.02	3.73	1.62-25.14	
		Arylsulfatase mU/g				
		Mean	SD	Median	Quartiles (25–75%)	
CC	0–10 cm	1.70	0.79	1.48	1.03-2.58	H (1. N = 6) =0.42857 p =0.5127
TILL		0.96	0.31	1.14	0.6-1.15	
CC	10–30 cm	0.87	0.18	0.83	0.72-1.07	H (1. N = 6) =0.42857 p =0.5127
TILL		0.89	0.47	0.65	0.6-1.43	
		Dehydrogenase mU/g				
		Mean	SD	Median	Quartiles (25–75%)	
CC	0–10 cm	0.173	0.067	0.202	0.096-0.220	H (1. N = 6) =3.8571 p =0.0495
TILL		0.023	0.004	0.025	0.019-0.026	
CC	10–30 cm	0.028	0.013	0.027	0.016-0.042	H (1. N = 6) =0.4285 p =0.5127
TILL		0.019	0.008	0.017	0.012-0.028	
		Urease mU/g				
		Mean	SD	Median	Quartiles (25–75%)	
CC	0–10 cm	20.45	1.80	20.63	18.56-22.15	H (1. N = 6) =3.8571 p =0.0495
TILL		15.89	0.90	15.53	15.24-16.91	
CC	10–30 cm	11.32	0.93	11.69	10.26-12.02	H (1. N = 6) =2.333 p=0.1266
TILL		18,51	2,64	17,34	16,66-21,53	

Phosphatase activity, associated with organic phosphorus mineralization, was also greater under CC (14.7 to 21.2 mU/g, Q25-Q75) than under TILL (5.7 to 20.2 mU/g) in the surface layer, although the difference was not statistically significant. In the subsurface layer, the difference disappeared. This likely reflects higher root activity in the topsoil, driven by greater organic inputs and biological stimulation. Similarly, phosphatase activity was low in soils of vineyards compared to Mediterranean arable soils where it is ranging from 15 to 85 mU/g (39).

These authors describe urease activity in arable soils, ranging from 2 to 12 mU/g. This enzyme catalyzes the hydrolysis of urea into ammonium, and was also significantly enhanced under CC. In the topsoil, activity was 18.6 to 22.1 mU/g (Q25-Q75) under CC versus 15.2 to 16.9 mU/g under TILL ($p < 0.05$). At 10–30 cm depth, significant differences were no longer observed). Nitrogen exists in various polymeric forms and humic substances, its acquisition from organic matter is more intricate than phosphorus, and may depend on different microbial strategies for acquiring it (16). Changes in microbial activity and nutrient availability can be strongly influenced by management practices, as increased activity of nitrogen-cycling enzymes is often positively correlated with higher concentrations of NH_4^+ and NO_3^- in soil—particularly under conditions that enhance microbial growth and substrate availability (31, 44). The use of cover cropping tends to increase NH_4^+ and NO_3^- concentrations in the topsoil (Figure 2), although only partially significant. Tillage management significantly

TABLE 3 **Summary of multiple linear regression results from the stepwise models evaluating soil functional recovery. Step 1 models tested the influence of vegetation structure—root biomass (Mg/ha), aboveground biomass (Mg/ha), and percent vegetation cover (%)—on two dependent variables: soil organic matter (SOM, %) and soil water availability (% volumetric water content). Step 2 models assessed the effects of SOM, soil water availability, and soil moisture at the time of sampling (g/g) on mineral nitrogen (Mineral Nitrogen kg/ha)) and extractable phosphorus (P_2O_5, mg/kg). For each model, unstandardized coefficients, standard errors, *t*-statistics, and *p*-values are reported, along with R^2 and adjusted R^2 values to assess model fit. Bold values indicate statistically significant predictors ($p < 0.05$).**

Model Fit Statistics

Model	R^2	Adjusted R^2	Model p-value
Step 1 - SOM	0.663	0.536	**0.02717**
Step 1 – Water Available	0.145	-0.175	0.72177
Step 2 - Nitrogen	0.907	0.872	**0.00018**
Step 2 - Phosphorus	0.786	0.706	**0.00469**

Regression Coefficients

Models	Variables	Coef.	Std.Err.	t	P>|t|	[0.025	0.975]
Step 1 - SOM		0.957	0.087	11.022	**<0.001**	0.757	1.157
	root biomass Mg/ha	0.004	0.038	0.097	0.925	-0.083	0.09
	above-ground veg biomass Mg/ha	0.236	0.067	3.498	**0.008**	0.08	0.392
	% area cover living plants	-0.017	0.006	-2.591	**0.032**	-0.031	-0.002
Step 1 – Water Av.		28.805	1.537	18.735	**<0.001**	25.259	32.35
	root biomass Mg/ha	-0.258	0.665	-0.387	0.709	-1.79	1.275
	above-ground veg biomass Mg/ha	-1.314	1.195	-1.1	0.303	-4.07	1.442
	% area cover living plants	0.132	0.114	1.163	0.278	-0.13	0.395
Step 2 – min-N		25.148	12.462	2.018	0.078	-3.59	53.886
	SOM %	-5.325	3.46	-1.539	0.162	-13.303	2.652
	Available water % vol	-0.877	0.31	-2.831	**0.022**	-1.591	-0.163
	moisture sampling day g/g	256.101	37.903	6.757	**<0.001**	168.696	343.505
Step 2 – P_2O_5		11.079	11.897	0.931	0.379	-16.355	38.514
	SOM %	9.025	3.303	2.733	**0.026**	1.41	16.641
	Available water % vol	0.186	0.296	0.629	0.547	-0.496	0.868
	moisture sampling day g/g	-128.7	36.184	-3.559	**0.007**	-212.2	-45.342

increased NO_3^- concentrations at a depth of 10–30 cm, which coincided with higher urease activity at the same depth (Table 2).

Arylsulfatase activity related to sulfur cycling processes was relatively low in both management practices, especially when compared to Mediterranean arable soils (range 6 to 8 mU/g). There were no statistically significant differences observed between CC and TILL management in this study, surface values were low and ranged from 1.0 to 2.6 mU/g in these soils (0 to 30 cm depth). This may indicate that sulfur cycling enzymes are less responsive to cover cropping under these conditions, or that sulfur-related microbial processes maintain a relatively stable baseline regardless of the management system.

Dehydrogenase, a general indicator of microbial respiratory activity, showed a strong response to CC in the topsoil. This activity under CC ranged from 0.096-0.220mU/g (Q25-Q75) compared to only 0.019-0.026 mU/g under TILL ($p < 0.05$), indicating significantly higher microbial metabolic activity. In the 10–30 cm layer, dehydrogenase activity remained low in both systems, reflecting the natural decline of microbial activity with depth and possibly lower oxygen availability.

The observed increase in β-glucosidase activity in the topsoil under long-term cover cropping is particularly relevant in the context of climate change adaptation. This enzyme plays a central role in soil carbon cycling and nutrient release, yet it is highly sensitive to soil moisture. Previous studies have shown that reductions in soil moisture can significantly suppress enzymatic activity, also β-glucosidase depending on depth and severity of drought (45). In our study, β-glucosidase activity remained elevated in cover-cropped soils, coinciding with higher water availability in the topsoil—a likely outcome of improved soil structure, organic matter accumulation, and surface cover. These changes suggest that long-term cover cropping not only supports microbial function under current conditions but may also buffer microbial processes against future drought stress, thereby contributing to soil system resilience.

3.4 Linking vegetation, SOC, and nutrient availability

The stepwise regression analysis allowed us to model a directional sequence from vegetation structure to soil function and nutrient dynamics. This order reflects a management-driven hypothesis: the introduction of cover crops increases significantly plant biomass, particularly in the topsoil layer (Table 1), which could, in turn, modify soil hydrology and organic inputs, eventually influencing nutrient dynamics.

This analysis revealed distinct patterns in the drivers of soil functional recovery under long-term vegetation cover (Table 3). In Step 1, vegetation significantly influenced soil organic matter (SOM), with root biomass, aboveground biomass, and percent vegetation cover jointly explaining 66.3% of the variation in SOM ($R^2 = 0.663$, $p = 0.027$). This highlights the critical role of plant inputs and ground cover in building organic matter in the soil. In contrast, the same vegetation variables had a weak and non-significant relationship with soil water availability ($R^2 = 0.145$, $p = 0.722$), suggesting that water retention may be more influenced by soil physical properties.

In Step 2, the regression model using SOM, water availability, and soil moisture at the time of sampling as predictors showed that extractable phosphorus (P_2O_5) was strongly influenced by these variables ($R^2 = 0.79$, $p = 0.0047$), highlighting the synergistic role of long-term carbon inputs and short-term moisture conditions in promoting phosphorus mobilization. These findings support a conceptual model in which vegetation acts as a first-order driver of SOM accumulation, which in turn enhances phosphorus availability, particularly in semi-arid systems where biological cycling and moisture pulses are critical to nutrient dynamics.

Similar regression model in Step 2 using mineral nitrogen (kg/ha) as the dependent variable revealed a strong and statistically significant relationship with soil organic matter (SOM), water availability, and soil moisture at the time of sampling ($R^2 = 0.907$, $p = 0.00018$). Among these predictors, soil moisture at the time of sampling emerged as the most influential factor, reflecting its critical role in regulating microbial activity, mineralization processes, and nitrogen mobility in the soil profile. Available water also showed a significant negative association with nitrogen content, potentially indicating dilution effects or interactions with soil texture and leaching dynamics. In contrast, SOM exhibited a weaker and non-significant effect, suggesting that while organic matter contributes to long-term nitrogen storage and supply, its short-term influence on mineral nitrogen availability may be less direct than that of immediate soil moisture conditions. These findings highlight the importance of integrating both stable soil properties (like SOM) and short-term soil moisture conditions to understand nitrogen dynamics in semi-arid agricultural systems.

While R^2 reflects the proportion of variance in the dependent variable explained by the independent variables, adjusted R^2 provides a more conservative estimate by penalizing the inclusion of non-informative predictors, making it particularly valuable in studies with limited sample sizes. In the present analysis, the adjusted R^2 for the SOM model was 0.54, indicating a strong and reliable explanatory relationship with vegetation parameters. In contrast, the Step 1 model for available water produced a negative adjusted R^2 (−0.175), indicating that the vegetation variables (root biomass, above-ground biomass, and vegetation cover) failed to explain any meaningful variation in water availability and performed worse than a null model. The Step 2 model predicting mineral nitrogen (kg/ha) yielded an adjusted R^2 of 0.872, indicating that approximately 87% of the variability in mineral nitrogen across samples is explained by the combined effects of SOM, water availability, and soil moisture at the time of sampling, after accounting for the number of predictors and the sample size. In this case, the high adjusted R^2 confirms that the observed model fit is not merely due to overfitting, but reflects a genuinely strong explanatory relationship. The result reinforces the idea that both stable soil characteristics and dynamic moisture conditions jointly govern nitrogen availability at the field scale.

For extractable phosphorus (P_2O_5) in Step 2, the adjusted R^2 was 0.706, indicating a strong association between phosphorus availability, long-term soil organic matter accumulation, and short-term soil moisture dynamics. This study was conducted on calcareous soils with a mean pH of 8.6, classifying them as strongly basic, where phosphorus availability is typically limited by precipitation with calcium and by sorption onto Ca- and Fe-rich mineral surfaces. Under such conditions, phosphate ions ($H_2PO_4^-$ and HPO_4^{2-}), are often rendered poorly available for plant uptake.

Organic matter inputs—either from external inputs such as manure (46) or from *in situ* sources like decomposed cover crops in this study—may modify these constraints. Organic compounds released during the decomposition of organic residues can compete directly with phosphate for sorption sites on soil minerals, effectively blocking or displacing phosphorus from these sites (17). In calcareous or strongly basic soils, where phosphorus commonly precipitates with calcium as poorly soluble calcium phosphate compounds, organic matter may also interfere with calcium activity through weak complexation or surface charge effects, thereby mitigating precipitation reactions. In this context, the higher soil organic matter content observed under cover cropping is consistent with increased phosphorus availability, although the specific mechanisms involved cannot be resolved directly from the present data.

The observed positive effect of SOM on phosphorus may reflect the biological mobilization of P via enzymatic pathways (47), particularly through the action of phosphatases, which catalyze the release of inorganic phosphate from organic compounds. In support of this, phosphatase activity was notably higher in soils with greater root biomass and SOC levels. Although phosphatase showed only moderate correlation with extractable P in the bivariate analysis, its role becomes more apparent in the multivariate context —suggesting that in alkaline soils, enzymatic activity may help partially offset the chemical limitations on P solubility. This underscores the importance of maintaining vegetation cover not only for carbon inputs but also for sustaining biological mechanisms that improve phosphorus bioavailability in calcareous, nutrient-limited systems. Similarly, this study demonstrates that CC in Mediterranean vineyards enhances the biological drivers of nitrogen mineralization through increasing soil organic inputs and stimulating microbial enzyme activity. The CC contributes labile carbon and nitrogen substrates that support microbial growth and influence C and N-cycling enzymes (48, 49), with corresponding gains in biologically mediated nutrient availability.

4 Conclusions

Taken together, these results demonstrate that spontaneous cover cropping can lead to substantial and enduring improvements in soil quality indicators in semi-arid, rainfed vineyards. For example, soil organic matter (SOM) content doubled in both the 0–10 cm (+109%) and 10–30 cm (+96%) layers under cover cropping. Enzymatic activity also showed notable increases, with β-glucosidase activity nearly doubled under cover cropping compared to the tillage treatment in the topsoil. Urease activity was higher in areas with greater soil nitrogen availability, suggesting a strong functional link between N content and urease-mediated processes. Differences in dehydrogenase activity between management systems were detected only in the topsoil, as was the case for phosphatase, although the latter was only marginally significant. No significant differences were observed for arylsulfatase activity.

While short-term trials often fail to capture such effects, this long-term study highlights the cumulative benefits of abandoning tillage in favor of vegetative soil cover. Notably, these outcomes were observed in a real-world, non-experimental vineyard managed by the farmer, where cover cropping was not applied consistently every year. In particularly hot or dry seasons, the farmer occasionally opted for tillage to mitigate weather-related stress. Despite this variability and lack of strict control, clear and significant differences emerged in key soil quality indicators. This underscores the robustness of cover cropping benefits and suggests that policy instruments such as subsidies or agri-environmental schemes should account for the need for adaptive, flexible management — especially under increasingly unpredictable climate conditions. This shift also resulted in a 17.9% increase in available water at 10–30 cm depth and a dramatic rise in biomass production — with above-ground biomass increasing more than 16-fold, and root biomass nearly quadrupling at 0–10 cm. Soil porosity remained stable (44–52%) across treatments, indicating that long-term cover cropping effectively prevents soil compaction, a common issue typically observed during the initial years of implementation.

Furthermore, the absence of pesticide inputs in the studied vineyard enhances the potential of the microbial community to respond positively to vegetation cover, reinforcing biological pathways for nutrient cycling. In this semi-arid Mediterranean setting — where both water scarcity and soil degradation pose serious challenges — the cumulative effects of cover cropping on water availability, microbial functioning, and nutrient turnover indicate a clear potential for nature-based adaptation to global warming at the farm scale, with relevance for similar dryland perennial cropping systems beyond the Mediterranean region.

Phosphorus availability (P_2O_5) showed a trend of increase under cover cropping — especially at 30 cm depth — although not statistically significant, it aligns with higher SOC and phosphatase activity. In contrast, mineral nitrogen (NH_4^+ + NO_3^-) was significantly higher under tillage at 10 cm, possibly due to faster N cycling or reduced immobilization. However**,** nitrogen values were highly variable, likely reflecting its soluble nature and strong dependence on sampling date and short-term weather conditions. Given the increased microbial activity under cover cropping, which may temporarily reduce mineral nitrogen availability, these findings highlight the importance of monitoring soil nitrogen dynamics. Based on these results, we are now initiating further studies on organic nitrogen fractions and potentially mineralizable nitrogen, to better understand the long-term nutrient availability and guide adaptive management practices.

This study is based on a comparison between two adjacent vineyards under long-term farmer management, and results are therefore interpreted at the plot scale. Although soil conditions were homogeneous between plots, the number of sampled sites was limited, and soil sampling was conducted at a single time point; consequently, some variables—particularly mineral nitrogen—may be influenced by short-term seasonal conditions. Nevertheless, the long duration of the management contrast, the use of multiple physical, chemical, and biological indicators, and the real-world farmer-managed context provide robust and ecologically relevant evidence of long-term soil functional responses to cover cropping in semi-arid vineyards.

Ultimately, this study highlights the pivotal role of cover cropping in promoting functional soil recovery through both chemical and enzymatic pathways, particularly in strongly basic, calcareous soils. The results affirm the value of long-term cover cropping as a regenerative practice that enhances soil resilience in dryland vineyard systems. By providing long-term, field-based evidence from a farmer-managed system, these results contribute to the international understanding of sustainable soil management in semi-arid agriculture and offer practical guidance for farmers and policy-makers engaged in sustainable viticulture and agri-environmental planning.

The results of this study provide a strong basis for extending research on cover cropping across semi-arid vineyard systems under different pedoclimatic conditions and management strategies. Ongoing work at additional sites will allow assessment of how soil functional responses vary with soil type, cover crop type, and management intensity, helping to identify robust and transferable mechanisms of soil recovery.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JH-L: Formal analysis, Methodology, Writing – review & editing, Writing – original draft, Investigation. JM-S: Methodology, Writing – review & editing, Investigation. JG-C: Methodology, Writing – review & editing, Data curation, Investigation. MJ-G: Formal analysis, Investigation, Data curation, Writing – review & editing, Methodology. PC: Data curation, Conceptualization, Supervision, Writing – review & editing, Methodology. BS: Writing – original draft, Funding acquisition, Conceptualization, Writing – review & editing, Supervision, Project administration. MM: Investigation, Supervision, Funding acquisition, Writing – review & editing, Conceptualization, Project administration, Writing – original draft, Methodology.

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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.

Generative AI statement

The author(s) declared that generative AI was used in the creation of this manuscript. Was used to improve wording and edit English language and check final versions.

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