

Article

Response of Soil Microbial Biomass and Activity to Cover Crop Incorporation Methods

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Abstract

Cover crop management in vineyards under a semiarid Mediterranean environment needs strategies that enhance soil C and N status and microbial functioning without increasing disturbance. This study compared cover crops biomass incorporation (harrowing, HR; rotary tillage; RT) and non-incorporation (NI, residues left on the topsoil) into the soil in a 12-year Grecanico dorato vineyard. Traditional vineyard soil management (continuously tilled for weeds control) was also used as a control. Soil samples from 0 to 20 and 20 to 40 cm were analyzed for total organic carbon (TOC), total nitrogen (TN), microbial biomass carbon (MBC) and nitrogen (MBN), and enzyme activities. NI and HR raised TOC and TN in the topsoil versus TR, with NI frequently maintaining advantages at depth. NI also maximized MBC/MBN and reduced the metabolic quotient (qCO_2), indicating improved microbial C-use efficiency; RT showed intermediate chemistry but depressed subsoil MBC and altered MBC/MBN. Enzyme profiles reflected contrasting mechanisms: RT boosted β -glucosidase in the topsoil, TR peaked for urease and arylsulfatase but alongside lower biomass and higher specific enzyme activities, while NI supported greater overall functioning via larger biomass and lower per-C enzyme demand. The calculated geometric mean enzyme (GMea) index emphasized transient TR flush versus steadier conservation functioning. Strong vertical stratification occurred for all indices, yet NI transmitted some benefits to 20–40 cm. We conclude that residue retention or moderate incorporation promotes larger, more efficient microbial population and more balanced nutrient cycling, whereas repeated rotary tillage risks subsoil inefficiencies. In semi-arid Mediterranean vineyards, low-disturbance cover-crop incorporation (HR) or, preferably, residue retention at the topsoil (NI) offer a simple, scalable route to sustain soil quality and long-term fertility.

Keywords: soil fertility; microbial biomass; biochemical indicators; Mediterranean environment; vineyard management



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1. Introduction

Soil degradation is a major threat to global food security and ecosystem sustainability. Erosion, organic matter depletion, and loss of soil structure reduce both soil productivity and resilience, particularly in systems of intensive agriculture [1,2]. Tillage, while historically used to control weeds and prepare seedbeds, is one of the main drivers of soil erosion and organic matter decline [3–5]. In Mediterranean environments, where soils are often shallow and vulnerable, unsustainable management has accelerated the decline of fertility and ecosystem services. Given

the importance of these regions for high-value crops such as vineyards and orchards, restoring soil health is a pressing challenge. Conservation agriculture, based on minimal disturbance, permanent soil cover, and crop diversification, has been promoted as a sustainable alternative to conventional management [6]. Within this framework, cover crops play a crucial role by providing multiple agroecological benefits: they reduce erosion, improve infiltration, and contribute organic inputs that enhance soil aggregation and biological activity [7]. Leguminous cover crops are particularly valuable because they simultaneously supply nitrogen (N) through biological fixation and contribute organic matter, thereby improving soil nutrient status and supporting higher yields of cash crops [8]. Mixtures with grasses, in turn, optimize ground cover and residue quality. These attributes make cover crops key tools for restoring soil health and sustaining perennial systems such as vineyards and orchards.

In the more frequent case of temporary cover crops several management options, including mechanical methods such as rolling or mowing, and chemical methods such as herbicide application are available [9,10]. However, the sustainability of cover crops use cannot be fully understood without considering the way how cover crops are buried after their termination. In particular, the impact of cover crops management on soil microorganisms and their functional roles remains less studied compared with more traditional agricultural practices.

Soil microbial biomass are highly sensitive indicators of soil quality, as they regulate nutrient cycling, organic matter decomposition, and soil structure formation [11]. In perennial systems such as vineyards, cover crops can reduce excessive vine vigour while simultaneously supporting soil microbial population [12,13]. Meta-analyses consistently show that cover crops increase microbial biomass, activity, and diversity [14–16]. Indeed, Liu et al. [14] synthesized evidence from 76 studies to evaluate how crop rotation influences soil microbial communities in comparison with monoculture systems. The analysis showed consistent increases in microbial biomass C (MBC) and microbial biomass N (MBN) by 13.4% and 15.8%, respectively. Bacterial diversity, measured by the Shannon index, was also enhanced by 7.7%, while fungal biomass increased substantially by 45.5% when rotations included legumes. In contrast, no significant effects were observed on enzyme activities such as phosphatase and β -glucosidase. Muhammad et al. [15] conducted a meta-analysis of 81 studies to investigate the effects of cover crops compared with bare soil on soil microbial abundance and community composition. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were used as indicators of microbial abundance. Their findings showed that cover crops substantially enhanced microbial abundance, increasing MBC, and MBN by 24%, 40%, and 51%, respectively, relative to no-cover treatments. Kim et al. [16] conducted a comprehensive meta-analysis to evaluate the effects of cover crops on soil microbial communities. The study synthesized data from 60 independent studies and demonstrated that cover cropping significantly enhances soil microbial abundance, activity, and diversity. Specifically, MBC and MBN increased by approximately 27%, indicating a substantial rise in microbial abundance. Soil microbial activity, as measured through enzyme activities, increased by 22%, while microbial diversity, assessed using the Shannon index, showed a 2.5% improvement compared to bare fallow systems.

However, the magnitude and direction of these effects depend on local conditions, crop type, and management practices, including termination strategies. This aspect is particularly critical during the post-incorporation period. At this stage, cover crop residues, including roots and their associated microorganisms, play a central role in regulating nutrient availability for subsequent crops through decomposition processes [17]. Despite the recognized benefits of cover crops, their agronomic and ecological outcomes are strongly influenced by incorporation method. Mechanical incorporation, mowing, and herbicide application differ in how they affect residue placement, decomposition dynamics, and nutrient release [18]. Kichler et al. [18] for example showed that rolling/crimping and mowing left residues on the soil surface, creating a uniform cover that improved soil moisture retention, particularly under dry conditions. In

contrast, mowing followed by incorporation distributed residues more heterogeneously and accelerated residue decomposition, thereby influencing nutrient availability together with a SOC increase of about 10% and N increase on about 30% in the upper 15 cm soil layer.

These findings demonstrate that cover crop management of cover crops residue strongly affect decomposition rates, and nutrient release, with important implications for soil health and crop productivity. Such changes are likely to alter microbial responses. However, comparative studies remain scarce, particularly under Mediterranean conditions, and long-term evidence on cumulative effects is still lacking.

The aim of this study was to evaluate how incorporating and not incorporating cover crops after their termination by mowing affect soil quality indicators in a sloping vineyard of a semiarid Mediterranean environment. By linking management practices to soil chemical and biochemical quality indicators, this study aims to clarify how residue management can enhance soil fertility, support microbial activity, and promote sustainable viticulture in a semiarid Mediterranean environment.

2. Materials and Methods

2.1. Experimental Set-Up

The study was conducted in the countryside of Sambuca di Sicilia (Agrigento, Italy; 37°39'12.35" N; 13°01'27.61" E) on clay soil with an average slope of 10%, classified as a Typic Haploxerept. The main characteristics of the 0–20 cm soil layer, determined at the beginning of the experiment, were as follows: 420 g kg⁻¹ clay, 260 g kg⁻¹ silt, 320 g kg⁻¹ sand (clayey texture), pH 7.8 (1:2.5 H₂O, *w/v*), 157 µS cm⁻¹ electrical conductivity, 21% total carbonates, 10.1 g kg⁻¹ total organic C (TOC) and 0.9 g kg⁻¹ total nitrogen (TN). The climate of the study area is Mediterranean (Köppen climate classification: Csa [19]) characterized by mild and wet winters, and warm to hot and dry summers. Based on the Thornthwaite moisture index ($Im = -29$), the site can also be defined as semiarid.

The twelve-year-old vineyard (cv. Grecanico dorato grafted on 140 Ruggeri rootstock) was northeast-oriented, with vines spaced 2.60 × 0.90 m apart (4273 plants ha⁻¹), trained to a vertical trellised Guyot system, and manually cane-pruned (8 buds per cane and 2 per spur). Since its establishment, the vineyard has been cover-cropped with a mixture of common vetch (*Vicia sativa* L.) and oat (*Avena sativa* L.), sown in late October at 50 kg ha⁻¹ for each species using a power harrow combined with a mechanical seed drill. Over a five-year average, the cover crop produced about 50 t ha⁻¹ of fresh biomass, with a N content of 1.7% on a dry matter basis. Three cover crop incorporation methods after mowing were compared: harrowing (HR); rotary tillage (RT); and no incorporation, (NI). Traditional (TR) inter-row soil management was compared to cover cropped system. Up to six tillage operations were performed each year for inter-row weed and water evaporation control. In the HR and RT treatments, cover crop biomass was incorporated into the soil at approximately 20 cm depth in mid-April using disk arrow. The experimental vineyard was arranged into twelve adjacent inter-rows (2.6 m × 50 m each), with three consecutive inter-rows assigned to each treatment, resulting in an experimental plot area of 390 m² per treatment. In all the treatments, chemical weed control under vine rows (RoundUp Ultra-MAX, Monsanto Company, St. Louis, MI, USA; glyphosate at 3 kg ha⁻¹) was carried out in spring, after cover crop biomass incorporation.

Cover cropped soils received phosphorus fertilization (50 kg ha⁻¹ yr⁻¹ of superphosphate) at the beginning of March, during vine dormancy, each 1–2 years. The vineyard managed under traditional practices was fertilised with an N-P-K 11-22-16 at a rate of 0.5 t ha⁻¹, broadcast on the soil surface.

2.2. Soil Collection and Analyses

Soil sampling was carried out two months after cover crop incorporation. For each treatment and depth (0–20 and 20–40 cm), six soil cores were collected at randomly selected points along the central part of the three inter-rows, avoiding vine rows and wheel tracks. Each core was treated as an independent replicate, resulting in six replicates per treatment and depth ($n = 6$; 48 samples in total). This sampling design ensured that spatial variability within each treatment area was represented while maintaining statistical independence among replicates. Soil samples were air dried, ground, sieved at $\varnothing < 2$ mm and then analysed in laboratory. Soil texture (sand, 2–0.02 mm; silt, 0.02–0.002 mm; clay, <0.002 mm) was determined by pipette method [20]. Reaction (pH) was determined by a pHmeter (FiveEasy, Mettler Toledo Spa, Milan, Italy) in 1:2.5 (w/v) soil/distilled water suspension. Total organic carbon (TOC) and total nitrogen (TN) were determined by the Walkley–Black wet oxidation method [21] and Kjeldahl method [22], respectively.

An aliquot of dried soil samples, moistened up to 50% of their water-holding capacity (WHC), was used for the determination of microbial biomass C and N, and respiration (CO_2 emission). Microbial biomass C (MBC) and N (MBN) were determined by the fumigation-extraction method with 0.5 M K_2SO_4 [23] as described by Paliaga et al. [24]; it corresponded to the difference between organic C extracted by 0.5 M K_2SO_4 from fumigated and not fumigated samples, multiplied by 2.64. The concentration of K_2SO_4 -extractable C from non-fumigated soil was assumed as a proxy of available C [25]. Soil respiration was quantified as CO_2 release ($\mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$) during soil incubation at 25 °C, measured by gas chromatography (TraceGC, Thermo Fisher Scientific Inc., Waltham, MA, USA) with a thermal conductivity detector (TCD). Briefly, 8 g of soil were placed in 50 mL air-tight glass bottles, and CO_2 measurements were performed 1, 3, 6, and 10 days after the start of incubation.

Enzyme activities were determined by incubating soil samples with specific substrates in appropriate buffer solutions. Soil β -glucosidase, arylsulfatase, and phosphatase activities were determined by measuring the *p*-nitrophenol released after incubation of the properly buffered soil with, as substrates, *p*-nitrophenyl glucoside [26], *p*-nitrophenyl sulphate [27], and *p*-nitrophenyl phosphate [28,29], respectively. Used buffers were the modified universal buffer (MUB) at pH 6.0, the acetate buffer at pH 5.8, and the MUB at the soil pH (in water). Urease activity was determined by monitoring the ammonium release from a soil sample treated with urea as a substrate and incubated with borate buffer at pH 10.0 [30]. Triphenyltetrazolium chloride was used as a substrate [31], buffered with TRIS-HCl at pH 7.4 for dehydrogenase determination. Incubated soil without substrate served as control. The specific enzymes activities were calculated by dividing total enzyme activities by MBC [32].

To integrate multiple enzyme activities into a single indicator of soil biochemical functioning, the geometric mean of enzyme activities (GMea) was computed as follows:

$$\text{GMea} = (\text{URE} \times \text{PHOS} \times \text{BG} \times \text{ARY})^{1/4}$$

where URE is urease, PHOS is phosphatase, BG is β -glucosidase, and ARY is arylsulfatase. All activities were expressed on an oven-dry soil basis and in consistent time units prior to calculation. This index is widely used and sensitive to land-use and management effects [33,34].

2.3. Statistical Analysis

Reported data are arithmetic means of six replicated field samples and are expressed on an oven dry basis (105 °C) of soil. Before performing parametric statistical analyses, normal distribution and variance homogeneity of the data were checked by Kolmogorov–Smirnov goodness-of-fit and Levene's tests, respectively.

Within each sampling depth (0–20 cm and 20–40 cm), a one-way ANOVA was conducted using the cover crop termination method (TR, traditional; HR, harrowing; RT, rotary tillage;

NI, no incorporation) as the main factor. Statistically significant differences among cover crop termination methods were identified using Tukey's HSD test at $p < 0.05$. In addition, for each treatment, differences in soil properties between the two depths were assessed using Student's t -test ($p < 0.05$). Pearson's correlation analysis was performed to evaluate the relationships among soil chemical, microbial, and enzymatic variables. Correlation matrices were generated separately for the two sampling depths (0–20 cm and 20–40 cm) to account for depth-related differences in soil properties and biological activity. Results of Pearson's correlation analysis among soil chemical, microbial, and enzymatic variables are reported in Table S1 (0–20 cm) and Table S2 (20–40 cm) in the Supplementary Material. All statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Total Organic C and N Pool

The termination method of the vetch–oat mixture used for cover crop the vineyard soil significantly influenced soil organic carbon (TOC) and total nitrogen (TN) concentrations, particularly in the 0–20 cm layer (Figure 1). Across all treatments, TOC concentrations were significantly higher in the 0–20 cm layer compared to 20–40 cm. Among management practices, NI and HR promoted the highest TOC contents in the surface layer (13.5 g kg^{-1} , on average), while RT showed intermediate values and the traditional system (TR) the lowest (11.2 g kg^{-1}). In the deeper layer, differences among treatments were less pronounced, although NI maintained highest values. These results indicate that conservative practices such as NI and HR favour the accumulation of organic C, likely due to greater retention of plant residues and reduced disturbance of the soil, whereas intensive practices (TR) limit organic carbon storage and increase C mineralization [35].

Similarly to TOC, TN concentrations were greater in the surface horizon than at depth. The highest TN values were observed in NI treatment (1.5 g kg^{-1} at 0–20 cm), followed by HR, while RT and TR showed the lowest contents. At 20–40 cm, the overall trend persisted but with reduced differences among treatments. Notably, all cover crop-based practices (MO, HR, RT) enhanced soil TN on average by 31% compared to TR, reflecting the combined contribution of legume-derived N from vetch and C-rich residues from oat. The greater accumulation of TN under NI and HR is likely linked to a more balanced mineralization process and to the steady incorporation of cover crop biomass, which improve N retention and availability. In contrast, the TR system, characterized by herbicide application and intensive tillage, tends to accelerate organic matter degradation and limits N replenishment, resulting in a reduced soil N pool. The TOC/TN ratio displayed smaller variability across treatments. TR and RT did not differ significantly, both showing lower values than NI and HR (statistically equal to each other), which indicates relatively higher N availability under conservation practices. This balanced C/N ratio is generally favourable for microbial activity and nutrient cycling. Overall, the findings suggest that NI represents the most effective management strategy for enhancing soil organic matter quality and nutrient status, followed by HR. Both practices promote the retention of C and N in the topsoil and contribute to a more sustainable soil fertility management. By contrast, the TR, characterized by herbicide use and intensive tillage, showed the lowest values of TOC and TN and a higher C/N ratio, highlighting its limitations in sustaining soil quality. RT showed intermediate effects, suggesting that repeated intense disturbance may partially offset the benefits of cover crop inputs. At 20–40 cm depth, TOC and TN decreased consistently across all treatments, with the steepest declines observed under NI, where residues remained on the soil surface. This vertical stratification is consistent with findings in Mediterranean vineyards, where conservation practices promote C and N accumulation in the upper layers but have limited impact on subsoil pools [2,4]. Similar increases in TOC and TN with cover crop incorporation have been documented under semiarid conditions [8,36]. These results confirm that minimizing soil disturbance and retaining residues on the surface can improve topsoil fertility, providing a critical buffer against erosion in sloping vineyards [1,37].

The greater TOC and TN concentrations observed under NI and HR likely reflect differences in the biological processes governing organic matter turnover. In both treatments, limited soil disturbance preserves soil aggregation and reduces organic matter mineralization rates, allowing organic C and N to accumulate. The retention of residues at the surface (NI) also enhances root activity and rhizodeposition, sustaining microbial populations that contribute to C and N stabilization. Conversely, shallow incorporation (HR) promotes residue–microbe contact and nutrient recycling without markedly accelerating decomposition. In contrast, the more intensive mixing under RT enhances aeration and microbial respiration, increasing C losses through CO₂ and reducing organic matter stabilization efficiency. These findings are consistent with previous studies showing that residue mulching or shallow tillage enhances C and N storage by fostering microbial-driven stabilization rather than mineralization processes [15,24]. These patterns were further supported by the correlation analysis (Tables S1 and S2), which revealed strong positive relationships among TOC, TN, and microbial biomass (MBC and MBN; $r > 0.80$, $p < 0.01$). This confirms that higher organic matter contents are closely linked to enhanced microbial biomass and overall biological functioning. Along the soil profile, TOC and TN exhibited a clear vertical stratification, with values declining from 0 to 20 cm to 20 to 40 cm across all treatments. However, the magnitude of this decline was smaller under NI, confirming that part of the organic inputs and dissolved C and N compounds moved downward, sustaining subsoil fertility. The preservation of soil structure and macropores under low-disturbance conditions likely enhanced this downward diffusion, while repeated tillage (RT and TR) reduced pore continuity and accelerated C and N mineralization near the surface, limiting transfer to deeper layers. This pattern demonstrates that residue retention improves nutrient storage not only in topsoil but also in deeper horizons, which are crucial for long-term carbon sequestration and resilience in semiarid systems.

3.2. Microbial Biomass C and N

Microbial biomass C (MBC) and N (MBN) exhibited strong responses to termination method (Figure 2). At 0–20 cm, in general all the cover crop management increased the both MBC and MBN. Specifically, NI recorded the highest MBC (372.1 mg C kg^{−1}) and MBN (75.5 mg N kg^{−1}) corresponding to increases of 93% and 40%, respectively, compared to TR. In contrast, HR and RT resulted in markedly reduced MBC compared with NI, despite still exceeding TR. Such reductions highlight how intensive tillage disrupts soil structure, enhances organic matter mineralization, and ultimately limits microbial habitat quality and stability [14,15]. Indeed, these effects can be ascribed to the disruption of soil aggregates caused by tillage, which leaves microorganisms more exposed to environmental stresses and erosion agents [24,38,39]. In the 20–40 cm layer, a clear stratification was observed, with MBC and MBN generally declining with depth across all treatments, reflecting reduced organic matter inputs and more compact soil conditions. Nevertheless, treatment effects remained evident. NI again exhibited the strongest stimulation, with mean MBC and MBN values of 278 mg C kg^{−1} and 59 mg N kg^{−1}, representing increases of 69% and 51% compared with TR. This suggests that residue retention at the soil surface, even without direct incorporation, provides a sustained supply of organic substrates that enhance microbial biomass even in deeper layers. HR maintained intermediate values (193 mg C kg^{−1} and 45 mg N kg^{−1}), exceeding TR but clearly lower than NI. This partial effect is consistent with moderate soil disturbance: while residues are incorporated, aggregate breakdown likely limits microbial habitat quality and organic matter stabilization. By contrast, RT produced the lowest values in the subsoil, with MBC averaging only 110 mg C kg^{−1}, even below TR, while MBN remained comparable to or slightly higher than the control (≈50 mg N kg^{−1}). Interestingly, RT was the only treatment that markedly altered the MBC/MBN ratio. The higher MBC/MBN ratio under NI indicates a shift toward fungal-dominated communities, which typically support more stable C cycling and aggregate formation. While all other treatments, across both

0–20 and 20–40 cm, showed relatively stable values ranging from 3.2 to 5.2, the RT subsoil layer exhibited a much lower ratio (2.2). Such a shift suggests a modification in microbial community structure and function. A reduced MBC/MBN ratio is often associated with a dominance of bacterial over fungal biomass, reflecting a community more reliant on readily available N and less capable of processing recalcitrant C substrates [39]. Such a bacterial-driven system may respond rapidly to labile inputs generated by intensive tillage but lacks the functional stability typically provided by fungal networks, which are more sensitive to aggregate disruption and habitat loss. Indeed, physical stress caused by tillage can lead to the disintegration of fungal hyphae, resulting in a marked decline in fungal biomass [24]. Vertical differentiation of microbial biomass was marked but treatment-dependent. MBC and MBN decreased with depth in all systems, yet NI maintained substantially higher subsoil values, suggesting that surface residue retention supported a more favorable environment for microbial persistence below 20 cm. The smaller decline of MBC/MBN and Q_{micr} under NI, together with a moderate qCO_2 increase, points to efficient carbon use and reduced stress in subsoil microorganisms. These patterns highlight that vertical transmission of microbial benefits results from the combination of soluble organic inputs, biopore continuity, and root-driven C allocation. Similar results were reported by Kabiri et al. [40] and Patra et al. [41], who found that minimal disturbance and root residue turnover enhanced microbial activity and enzymatic potential at depth under semi-arid conditions.

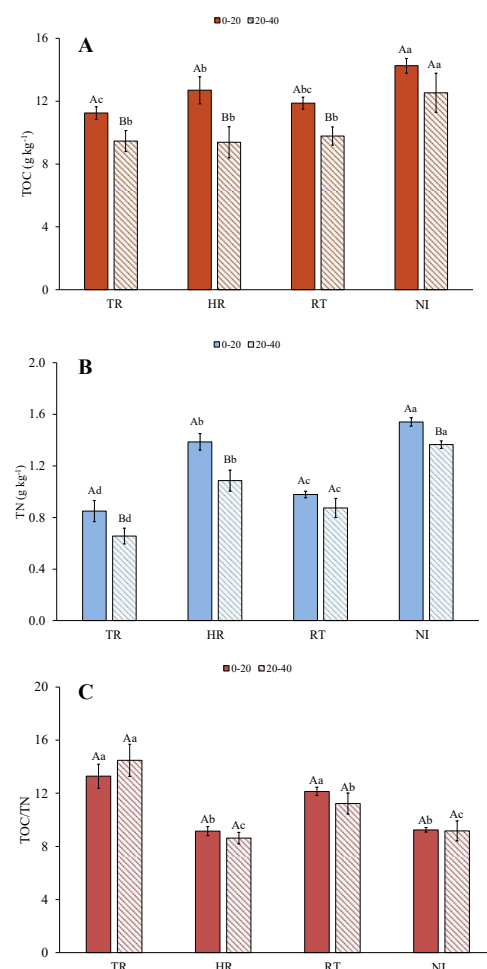


Figure 1. (A) Total organic C (TOC), (B) total N (TN) and (C) their ratio (TOC/TN) of soil subjected to different cover crop termination methods. Different capital letters indicate significant differences ($p < 0.05$) among different soil depth within the same cover crop termination methods; lowercase letters indicate significant differences ($p < 0.05$) among soils within different cover crop termination methods, within the same soil depth.

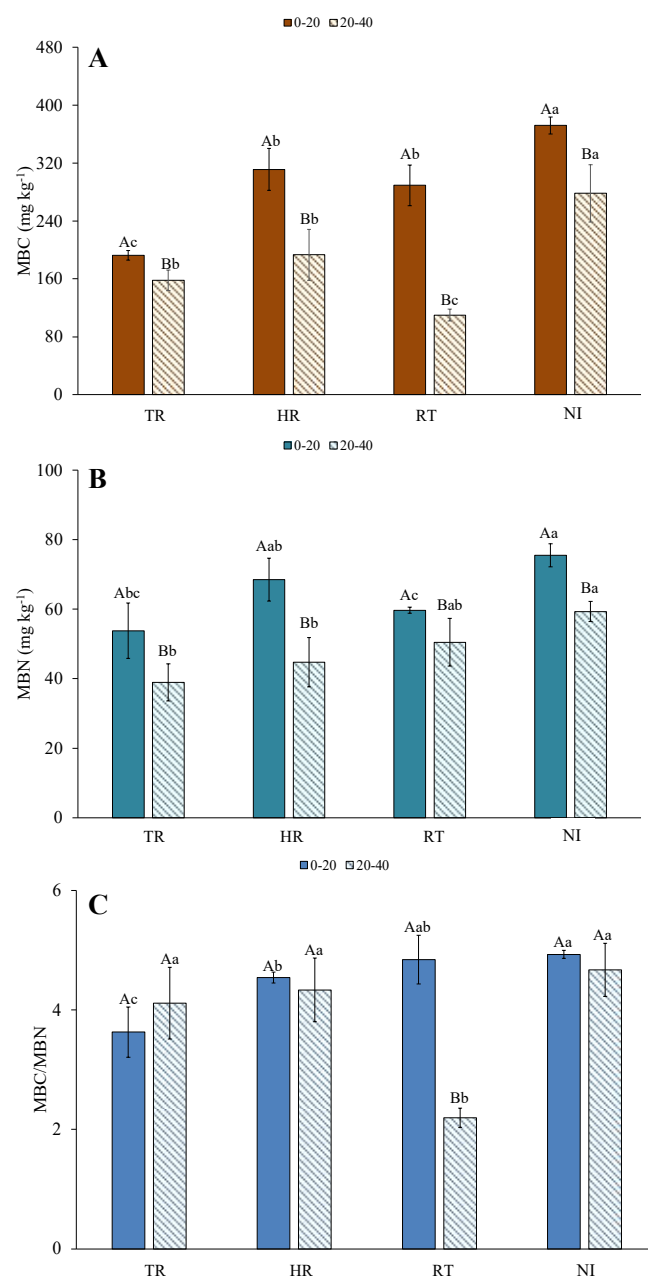


Figure 2. (A) Microbial biomass C (MBC) and (B) N (MBN) and (C) their ratio (MBC/MBN) of soil subjected to different cover crop termination methods. Different capital letters indicate significant differences ($p < 0.05$) among different soil depth within the same cover crop termination methods; lowercase letters indicate significant differences ($p < 0.05$) among soils within different cover crop termination methods, within the same soil depth.

3.3. Microbial Quotients

In both soil layers, the microbial quotient (Q_{micr}) was significantly higher under all cover crop treatments compared with TR (Figure 3), except for RT at 20–40 cm. In this deeper layer, Q_{micr} reached only 1.13%, representing a 50% decrease relative to TR and nearly 90% lower values than in the other cover crop treatments. This drastic reduction can be explained by the greater aeration induced by RT, which may have accelerated organic matter mineralization and limited the labile C, resulting in a reduced microbial biomass at depth. By contrast, in the corresponding 0–20 cm layer no decline was observed, probably due to the input of fresh plant residues, which provide easily available C and N that counterbalance the negative effects of tillage on microbial biomass. Conversely, the metabolic quotient

(qCO_2) was higher under RT in the 20–40 cm layer, suggesting a more stressed and less efficient microbial community. A higher qCO_2 generally indicates that microorganisms are investing more C into respiration than into biomass production, which is often interpreted as a stress signal or a reduced efficiency of carbon use [42]. This pattern aligns with the concomitant decline in MBC and the Q_{micr} at this depth under RT. Such a reduction points to a microbial community unable to effectively incorporate C into biomass, likely because of reduced substrate availability and unfavorable microenvironmental conditions in deeper layers subjected to mechanical disturbance.

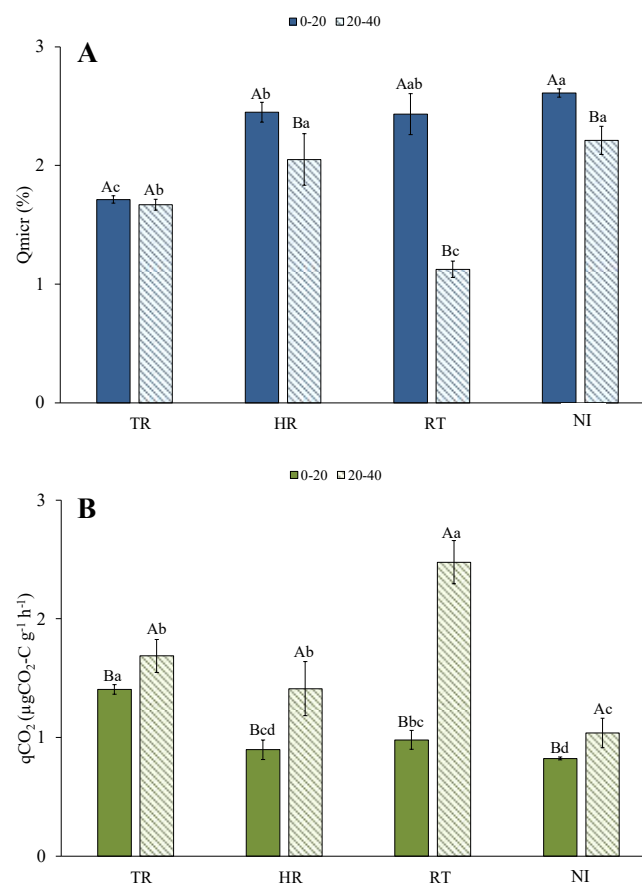


Figure 3. (A) Microbial (Q_{micr}) and (B) metabolic (qCO_2) quotient of soil subjected to different cover crop termination methods. Different capital letters indicate significant differences ($p < 0.05$) among different soil depth within the same cover crop termination methods; lowercase letters indicate significant differences ($p < 0.05$) among soils within different cover crop termination.

In the 0–20 cm layers, under NI and RT, qCO_2 decreased by 53% compared to TR, and was on average $0.9 \mu g CO_2-C g^{-1} h^{-1}$ reflecting a more efficient microbial metabolism [43–45]. Such a reduction points to a microbial community unable to effectively incorporate C into biomass, likely because of reduced substrate availability and the shift in microbial community composition caused by mechanical disturbance. Indeed, higher qCO_2 values in tilled soils have been associated with the disruption of fungal hyphae and the dominance of bacteria less efficient in C use, leading to a stressed microbial community with lower biomass formation efficiency [40,46].

The qCO_2 is a relevant indicator of soil quality, as it integrates information about microbial physiological status and carbon cycling efficiency. Soils exhibiting lower qCO_2 values generally show a more balanced C turnover and a greater capacity to retain organic C, contributing to improved soil structure, nutrient availability, and overall biological functioning [47]. Conversely, elevated soil respiration rates coupled with high qCO_2 values

can signal degradation of soil organic matter and a decline in soil quality, as more C is lost as CO₂ rather than stabilized within microbial biomass or humified forms [48]. The observed variations in qCO₂ across management systems therefore highlight the close linkage between microbial metabolic efficiency, soil respiration, and soil quality, emphasizing the role of reduced tillage and residue retention in maintaining a more sustainable soil C balance. The higher microbial biomass and lower qCO₂ observed under NI indicate a more efficient use of C by soil microorganisms, implying that a greater fraction of assimilated C is stabilized within microbial biomass rather than lost as CO₂, thereby enhancing soil organic matter accumulation and nutrient retention. Pearson's correlation analysis (Table S1) confirmed this pattern, showing significant negative relationships between qCO₂ and Q_{micr} ($r = -0.98$, $p < 0.01$), suggesting that soils with higher microbial biomass exhibited lower respiratory losses and greater C-use efficiency. Over time, these processes can improve soil structure and fertility, supporting long-term vineyard productivity and sustainability under Mediterranean conditions.

3.4. Absolute and Specific Enzyme Activities

Soil enzyme activities are widely recognized as indirect indicators of microbial functioning, as they represent the potential metabolic capacity of soil microorganisms to catalyze nutrient transformations and regulate biogeochemical cycling processes [11]. The soil enzyme activities showed marked differences among the four cover crop management treatments, with clear stratification by depth.

In the 0–20 cm layer, β -glucosidase activity was the highest under the RT treatment and the lowest in HR and NI treatments (Table 1). This enzyme is primarily involved in the hydrolysis of cellulose and the release of glucose, a readily available energy source for soil microorganisms. This pattern may be explained by the intensive mixing and further shredding of cover crop residues by rotary tillage that stimulated a surge in cellulolytic activity by exposing fresh substrate. The elevated values recorded in the RT treatment were consistent with the increase in the specific microbial respiration activity (qCO₂). This likely indicate a short-term pulse of decomposition following intensive residue fragmentation and enhanced aeration. Such transient activation can accelerate organic C mineralization and nutrient release but often at the expense of long-term C stabilization. This reflects a disturbance-driven microbial response in which the community rapidly exploits labile substrates, increasing respiration and reducing C-use efficiency. Similar short-lived boosts in enzyme activity after residue fragmentation have been reported in tilled systems. However, these boosts may not indicate sustainable improvement, as they often coincide with rapid organic matter mineralization [49,50]. In contrast, the tighter coupling between β -glucosidase and microbial biomass observed in NI suggests a more conservative C cycling strategy. The activities of phosphatase, arylsulfatase, and urease responded in yet other ways. Phosphatase was relatively stable across treatments and showed no clear statistical differences. The lack of strong treatment effects on phosphatase aligns with broader findings that cover cropping often yields minor or inconsistent changes in P-cycling enzymes [51].

Table 1. Enzymatic activities of soil subjected to different cover crop termination methods. Value represents mean $\pm$ standard deviation.

Soil Tillage	Depth	Enzyme Activities				
		β -Glucosidase	Dehydrogenase	Phosphatase	Arylsulfatase	Urease
	cm	(mg pNP kg ⁻¹ h ⁻¹)	(mg TPF kg ⁻¹ 24 h ⁻¹)	(mg pNP kg ⁻¹ h ⁻¹)	(mg pNP kg ⁻¹ h ⁻¹)	(mg NH ₄ ⁺ -N h ⁻¹)
TR	0–20	70.2 Aa	2.7 Ab	75.3 Aa	8.3 Aa	3.5 Aa
	20–40	47.2 Ba	2.4 Aa	60.4 Ba	6.7 Ba	1.6 Ba

Table 1. Cont.

Enzyme Activities						
Soil Tillage	Depth	β -Glucosidase	Dehydrogenase	Phosphatase	Arylsulfatase	Urease
	cm	(mg <i>p</i> NP kg ^{−1} h ^{−1})	(mg TPF kg ^{−1} 24 h ^{−1})	(mg <i>p</i> NP kg ^{−1} h ^{−1})	(mg <i>p</i> NP kg ^{−1} h ^{−1})	(mg NH ₄ ⁺ -N h ^{−1})
HR	0–20	54.6 Ab	3.3 Aab	71.1 Ab	7.0 Ab	3.2 Aa
	20–40	29.2 Bb	2.4 Ba	53.5 Ba	5.8 Bab	1.6 Ba
RT	0–20	79.2 Aa	3.3 Aa	61.8 Ac	6.5 Ab	3.2 Aa
	20–40	46.1 Ba	2.2 Ba	54.4 Ba	5.2 Bbc	1.2 Bab
NI	0–20	57.1 Ab	3.5 Aa	71.4 Ab	6.9 Ab	1.8 Ab
	20–40	51.0 Ba	2.4 Ba	54.7 Ba	3.6 Bc	0.7 Bb

SEA						
Soil tillage	Depth	β -Glucosidase/ MBC	Dehydrogenase/ MBC	Phosphatase/ MBC	Arylsulfatase/ MBC	Urease/ MBC
TR	0–20	19.7 Aa	0.014 Aa	1.42 Aa	0.124 Aa	0.34 Aa
	20–40	11.6 Bb	0.015 Ab	1.57 Aa	0.122 Aa	0.18 Ba
HR	0–20	12.0 Ab	0.010 Ab	1.04 Bb	0.089 Ac	0.29 Ab
	20–40	6.8 Bc	0.013 Ab	1.20 Ab	0.087 Ac	0.20 Aa
RT	0–20	16.4 Ba	0.011 Bb	1.04 Ab	0.096 Bb	0.29 Ab
	20–40	21.2 Aa	0.020 Aa	1.09 Ab	0.105 Ab	0.13 Ba
NI	0–20	11.6 Ab	0.009 Ac	0.94 Ab	0.082 Ad	0.15 Ac
	20–40	10.9 Bb	0.009 Ac	0.92 Ac	0.048 Bd	0.07 Bb

Note: Values represent the mean and standard deviation of three samples ($n = 3$). Abbreviations: *p*NP, *p*-nitrophenol; TPF, 3-triphenyl formazan; SEA, Specific Enzyme Activities. Different letters within a column indicate significant differences at $p < 0.05$.

Arylsulfatase activity, involved in sulfur cycling, was highest under TR and markedly lower in all cover-cropped soils suggesting that the disturbance in TR may have unprotected organo-sulfur compounds, whereas the cover crop treatments moderated this response. A similar phenomenon has been noted where conventional plowing induces a flush of certain enzyme activities due to the sudden availability of protected substrates [52]. Over time, however, no-till and cover-cropped soils often surpass tilled soils in arylsulfatase as organic matter accumulates in surface layers [47]. Our short-term results likely capture the initial flush effect in TR, but long-term monitoring would be needed to see if arylsulfatase in NI and HR eventually overtake TR as soil health improves.

Urease activity showed the most pronounced treatment divergence. At 0–20 cm, TR exhibited the highest urease, while NI was the lowest. The HR and RT treatments were intermediate. Urease is largely of plant and microbial origin and tends to be enriched in fresh plant residues [53]. Thus, the low urease under NI can be explained by the lack of residues incorporated into the soil; indeed, being not buried plant-derived urease may remain in the plant biomass in the surface layer rather than mixed into the soil. By contrast, the other treatments, by incorporating plant biomass into soil, released plant urease.

In the NI treatment, where cover crop residues were left on the soil surface without incorporation, dehydrogenase activity (an indicator of overall microbial oxidative activity) showed the highest dehydrogenase, ~30% greater than TR. The two cover crop incorporation treatments, HR and RT, were intermediate, about 20–22% above TR. The elevated dehydrogenase under NI suggests that maintaining surface residues enhanced microbial respiration capacity, likely by improving moisture retention and providing a continuous food source [54]. This result is in line with studies finding higher microbial activity under mulch-based systems and no-till, where soil microhabitats are less disturbed [55]. For example, a meta-analysis reported that conservation tillage practices (especially no-till with residue retention) significantly increase soil enzyme activities globally [55]. Our data support this trend for dehydrogenase, indicating that minimal disturbance (NI) can stimulate oxidative enzymes by preserving microbial biomass and habitat. Notably, Silwana et al. [54] observed in a citrus orchard trial that merely slashing vs. incorporating cover

crops led to no significant differences in enzyme activities after one year. This suggests that the benefits of surface residue retention on enzymes may become more pronounced over longer periods, whereas short-term effects can be subtle [54]. In our case, even within one season NI already enhanced dehydrogenase, highlighting the sensitivity of this enzyme to residue management.

The depth distribution of enzyme activities underscores the strong topsoil focus of biochemical processes. All enzymes declined substantially from 0 to 20 cm to 20 to 40 cm in every treatment ($p < 0.05$). For example, β -glucosidase in TR dropped from 70.2 to 47.2 mg kg⁻¹ h⁻¹ (a 33% decrease) between the two depths. Even larger proportional declines were seen under HR (−46%) and RT (−42%). The NI treatment was distinctive showing only ~11% decrease compared to the topsoil. This anomaly suggests that leaving residues on the topsoil in NI allowed some downward movement of soluble C substrates or root-derived exudates, sustaining microbial activity deeper in the profile. In support of this, we note that the cover crop used (vetch–oat mix) likely developed some roots in the 20–40 cm layer, especially under NI where soil structure was undisturbed; the decay of those deeper roots can directly elevate enzyme activity in situ.

Patra et al. [41] reported that introducing a deep-rooted cover crop increased subsoil carbon inputs and microbial activity, thereby enhancing enzymatic capacity in deeper layers compared to a no-cover control. Such findings align with our observation that the RT treatment (which physically incorporated residues into ~20 cm depth) maintained relatively high subsoil enzyme levels too (e.g., RT and NI had the highest 20–40 cm β -glucosidase of the treatments). Correlation analyses (Table S1) revealed that dehydrogenase activity was positively related to TOC, TN, and microbial biomass ($r = 0.64$ – 0.85 , $p < 0.01$) and negatively to qCO₂ ($r = -0.83$, $p < 0.01$), confirming its role as a sensitive indicator of overall microbial metabolism. Conversely, β -glucosidase and urease showed positive associations with qCO₂ and negative with MBC, indicating that their activity increased under condition of accelerated carbon mineralization.

When enzyme activities were normalized to microbial biomass C (specific enzyme activity, SEA), an interesting inverse pattern emerged (Table 1). At the 0–20 cm depth, TR showed the highest enzyme activities per unit biomass; for instance, β -glucosidase specific activity was ~19.7 mg pNP per mg MBC in TR, compared to 16.4 in RT and only 11.6 in NI (and 12.0 in HR). Likewise, SEA for urease and arylsulfatase in TR were about 17–38% higher than in NI. These differences indicate that in the conventionally managed soil, each unit of microbial biomass was producing more enzymes, whereas in the cover-cropped soils (especially NI) the larger microbial biomass was relatively “less active” on a per-C basis. This can be interpreted as a stress compensation strategy: under frequent disturbance and lower total biomass (TR had the smallest MBC pool in our trial), microbes invest more in enzyme production to scavenge nutrients, resulting in high specific activity. By contrast, in the more organic-rich, less disturbed NI soil, microbes do not need to secrete as many enzymes per cell; they exist in larger numbers (high biomass C) and can meet metabolic needs with lower enzyme expression per unit biomass. Similar observations have been reported by other authors. Wen et al. [55] noted that no-till regimes tend to increase total enzyme activity mainly by expanding microbial biomass, rather than by boosting the activity per microbial cell. In our study, high SEA (for β -glucosidase, phosphatase, etc.) in TR and low SEA in NI reflect this fundamental difference in microbial strategy between an input-limited, disturbance-prone soil and a resource-rich, stabilized soil. At 20–40 cm depth, the trend in SEA shifted somewhat. The RT treatment exhibited an exceptionally high specific β -glucosidase activity (21.2 mg mg⁻¹ MBC), 83% greater than TR at the same depth. This suggests that the microbes that were active in RT subsoil were operating at very high per-biomass capacity, likely because the rotary tiller had placed some fresh residue at depth but the overall microbial biomass there remained low. This combination (few microbes,

abundant substrate) drove per-cell enzyme production up in the RT subsoil. NI, in contrast, had a comparatively low β -glucosidase SEA in the subsoil, consistent with its higher microbial biomass in that layer and less extreme enzyme demand per cell. These SEA patterns reinforce the idea that how cover crop residues are distributed in soil (surface vs. buried) influences not just the total activity, but the metabolic behavior of soil microorganisms. Disturbed soils with low biomass (TR, deep RT) show activated microbes with high enzymatic effort per cell, whereas undisturbed, biomass-rich soils (NI topsoil) have more sedate per-cell activity. Notably, a similar inverse relationship between microbial biomass and specific enzyme activity was reported by Liu et al. [14] under long-term fertilization regimes, implying that it may be a general phenomenon across different management practices.

3.5. Geometric Mean of Enzyme Activities (GMea)

The GMea revealed clear differences among treatments: in the 0–20 cm layer, TR showed the highest values, followed by RT and HR, while NI had the lowest (24% lower than TR, Figure 4). A similar ranking was observed at 20–40 cm, with a general decrease of 33–39% compared to the topsoil, confirming the strong stratification of enzymatic activity. Although TR appeared to promote higher overall enzyme activity, this was largely driven by inflated values of urease and arylsulfatase immediately after tillage. These peaks coincided with reduced microbial biomass and efficiency (low microbial C/N ratio and higher metabolic quotient). Thus, TR soils achieved high enzyme activity through stress-induced microbial turnover and organic matter oxidation, consistent with Raiesi and Salek-Gilani [32]. In contrast, the conservation treatments (NI, HR, RT) displayed slightly lower GMea values but supported larger and more efficient microbial communities (higher microbial C and lower qCO_2). This indicates a more balanced nutrient cycling regime, with less disturbance-driven mineralization. NI and HR, in particular, showed moderate enzyme activity linked to improved nutrient retention and reduced losses, as also reported by Elhaddad et al. [45]. With depth, GMea declined across all treatments, yet NI maintained values comparable to TR at 20–40 cm, suggesting partial transfer of surface-derived benefits into the subsoil. Agronomically, while TR maximized enzyme indices temporarily, it did so at the expense of organic matter stocks, whereas NI and HR promoted microbial efficiency and more sustainable soil functioning. Overall, the GMea proved to be a valuable integrative indicator. In this vineyard system, conventional tillage generated a short-term enzymatic “flush,” whereas conservation practices fostered more stable biochemical functioning, supporting long-term soil fertility.

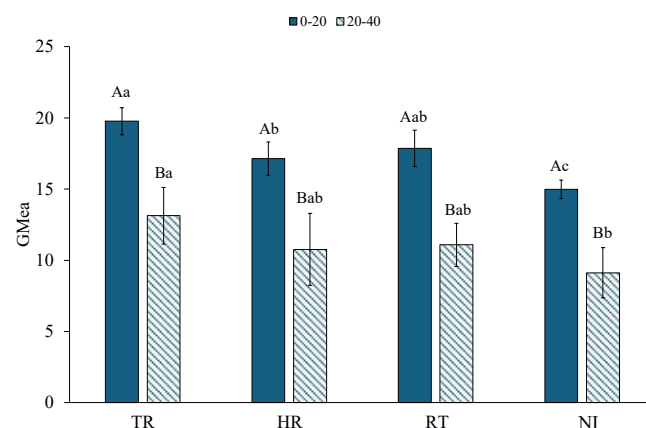


Figure 4. The geometric mean of enzyme activities (GMea) at the 0–20 and 20–40 cm soil depths in soils with different cover crops termination methods. Different capital letters indicate significant differences ($p < 0.05$) among different soil depth within the same cover crop termination methods; lowercase letters indicate significant differences ($p < 0.05$) among soils within different cover crop termination.

The observed differences among treatments highlight how soil disturbance and residue management shape the biological mechanisms that sustain soil fertility. Minimal or shallow disturbance (NI and HR) preserved microbial habitats and favored carbon stabilization processes, resulting in greater microbial efficiency and improved nutrient cycling. In contrast, intensive tillage (RT and TR) enhanced short-term mineralization and enzyme activation but promoted C losses and reduced microbial stability. These findings suggest that, beyond their immediate effects on TOC and TN, conservation practices stimulate a shift toward more efficient and resilient microbial communities, supporting long-term soil health and vineyard sustainability under Mediterranean conditions.

4. Conclusions

Residue handling after cover-crop termination strongly shaped soil C and N stocks and microbial functioning. Leaving residues on the topsoil (NI) consistently increased TOC and TN in 0–20 cm, often retaining advantages at 20–40 cm; harrowing (HR) delivered similar but smaller gains, while rotary tillage (RT) was intermediate yet detrimental at depth. Microbial biomass mirrored these patterns; and, indeed, NI maximized MBC and MBN and lowered qCO_2 , indicating higher C-use efficiency.

Enzyme activities reflected mechanism rather than sustained quality: RT induced a short-term β -glucosidase pulse from residue fragmentation; conventional tillage (TR) showed peaks in urease and arylsulfatase but alongside lower biomass and higher specific enzyme activity, suggesting symptoms of stress-driven turnover. Normalizing enzyme activities by MBC revealed that NI achieved lower per-C enzyme demand yet greater overall functioning due to a larger biomass pool, whereas TR and RT relied on costlier, disturbance-induced processes. Depth stratification was pronounced across treatments, but NI transmitted some benefits into the subsoil.

Overall, retaining cover-crop residues on the surface or incorporating them moderately improved nutrient retention, enlarged and stabilized microbial biomass, and reduced reliance on disturbance-induced mineralization. Repeated rotary tillage should be limited because it stimulates short-lived enzymatic pulses, increases specific respiration, and undermines subsoil microbial efficiency. For semi-arid Mediterranean vineyards, low-disturbance termination offers a pragmatic pathway to conserve soil quality, buffer erosion risk, and support long-term fertility.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy15112504/s1>, Table S1. Pearson correlation coefficients among soil chemical, biochemical, and enzymatic variables in the 0–20 cm soil layer. Level of significance for explained variance: *, $p < 0.05$; **, $p < 0.01$. Table S2. Pearson correlation coefficients among soil chemical, biochemical, and enzymatic variables in the 20–40 cm soil layer. Level of significance for explained variance: *, $p < 0.05$; **, $p < 0.01$.

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