

Article

Soil Quality Responses to Green Undersown Crops in Mediterranean Calcareous Persimmon Orchards

Carmen Orts ^{1,2}, Ángel Marqués-Mateu ³ , Cristina Lull ⁴ , Josep V. Llinares ⁵ , Desamparados Soriano ² and Rafael Boluda ^{6,*} 

- ¹ Department of Agriculture, Water, Livestock and Fisheries, Generalitat Valenciana, 46018 València, Spain; orts_mca@gva.es
 - ² Department of Plant Production, Universitat Politècnica de València, Camí de Vera s/n, 46022 València, Spain; asoriano@prv.upv.es
 - ³ Department of Cartographic Engineering, Geodesy and Photogrammetry, Universitat Politècnica de València, Camí de Vera s/n, 46022 València, Spain; amarques@cgf.upv.es
 - ⁴ Research Group in Forest Science and Technology (Re-ForeST), Research Institute of Water and Environmental Engineering (IIAMA), Universitat Politècnica de València, Camí de Vera s/n, 46022 València, Spain; clull@upvnet.upv.es
 - ⁵ Institut Agroforestal del Mediterrani (IAM), Universitat Politècnica de València, Camí de Vera s/n, 46022 València, Spain; jollipa@qim.upv.es
 - ⁶ Department of Plant Biology, Faculty of Pharmacy, University of València, Avda. Vicent Andrés i Estellés 22, Burjassot, 46100 València, Spain
- * Correspondence: boluda@uv.es

Abstract

Green undersown crops (GUCs) are increasingly promoted as a sustainable management strategy to improve soil quality and ecosystem services in Mediterranean orchards, particularly in the calcareous, low-organic-matter soils typical of Eastern Spain. In this context, soil quality is understood as the soil's overall functional capacity, integrating physical structure, chemical balance, and biological activity, whereas soil fertility refers specifically to the soil's ability to supply nutrients to plants; soil quality therefore encompasses a broader set of ecosystem functions beyond nutrient provision. However, their effects on the calcareous, low-organic-matter soils typical of Eastern Spain remain insufficiently quantified. This study evaluates the effects of seeded undersown (grasses, legumes, and flower mixtures), spontaneous vegetation, and herbicide-managed bare soil on topsoil (0–15 cm) physico-chemical and biological indicators in 54 plots across three irrigated persimmon orchard sites (Granja, Cargol, and Alginet) over 18 months of treatment in the València region (Eastern Spain). Seasonal sampling was conducted at the START (early winter) and END (late spring) of the experiment period. Soil measurements at both sampling times included soil organic matter (SOM), nitrogen (N), C/N ratio, pH, electrical conductivity (EC), soil respiration rate (RR), collembolan abundance, mite abundance, and the QBS-ar index of soil arthropods. Legumes increased SOM by +1.12%, grasses by +0.22%, whereas flower mixtures (−0.44%) and spontaneous vegetation (−1.36%) showed SOM reductions associated with rapid biomass turnover. RR increased under all GUCs (+0.06 to +0.16 g CO₂ m^{−2} h^{−1}), and QBS-ar improved markedly under grasses (+26.6) and spontaneous vegetation (+36.7). EC decreased across all treatments (−16 to −84 μS cm^{−1}). These results were analysed using principal component analysis (PCA). Four PCA components explained 74% of the total variance, revealing functional gradients driven by SOM, N, EC, RR and mesofauna. After 18 months, microbial biomass carbon (MBC) increased by +45–60% under legumes, water-soluble organic carbon (WSOC) by +30–50% under legumes and flower mixtures, and the enzyme activities (EA) by +20–40% under all GUCs. Herbicide-managed soils showed reduced biological activity and detectable residues of glyphosate and oxyfluorfen.



Academic Editors: José L. S. Pereira and Vítor João Pereira Domingues Martinho

Received: 1 June 2026

Revised: 14 July 2026

Accepted: 15 July 2026

Published: 20 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

PCA and linear discriminant analysis (LDA) were used to identify functional gradients and treatment separation. GUCs significantly increased SOM, MBC, EA, and mesofauna abundance compared with herbicide treatments, which showed reduced biological activity and detectable residues of glyphosate and oxyfluorfen. Legumes and flower mixtures produced the strongest improvements in biological functioning due to higher MBC, WSOC, EA and RR. PCA and LDA confirmed clear separation between GUCs and herbicide-managed soils based on multivariate differences in SOM, N, EC, RR, MBC, WSOC and mesofauna indicators. Overall, GUCs modulated soil chemistry and biodiversity and enhanced soil functioning and biological quality, supporting their adoption as a sustainable management strategy in Mediterranean orchards. Legume-based covers are recommended for rapid biological activation, whereas grass-based covers favour longer-term SOM stabilisation. These findings highlight their role as key tools for improving soil resilience in Mediterranean persimmon orchards.

Keywords: legumes; grasses; flowers; spontaneous vegetation; soil organic matter; collembolans; mites; enzyme activities

1. Introduction

The intensive use of fertilisers, pesticides and herbicides, together with high erosion rates, low soil organic matter and biodiversity loss, has led to the progressive degradation of agricultural soils and associated ecosystem services. This process places soil degradation among the major environmental challenges in the region [1,2]. In this context, green undersown crops (GUCs) have emerged as an effective strategy to enhance soil quality by improving soil structure, increasing organic inputs and stimulating biological activity [3,4]. Here, soil quality is understood as the soil's overall functional capacity, integrating physical, chemical and biological attributes, whereas soil fertility refers specifically to the soil's ability to supply nutrients to plants; soil quality therefore encompasses a broader set of ecosystem functions beyond nutrient provision. However, their effects depend strongly on plant functional traits, residue quality and local edaphoclimatic conditions. Recent agricultural policies have promoted soil conservation and sustainable management as part of broader climate-mitigation strategies [5]. In Mediterranean orchards, spontaneous and sown vegetation are increasingly used as GUCs to improve soil functioning, as, when properly managed, they increase soil organic matter, reduce nutrient losses, limit erosion, improve water retention and enhance carbon sequestration, while supporting biological pest control and reducing chemical inputs [6–11].

Despite these benefits, bare-soil management through tillage or herbicides remains widespread in many fruit orchards, despite growing evidence of herbicide-resistant weeds and the weak empirical support for strong water competition in mature trees. Within this framework, the concept of soil quality refers to the capacity of soil to sustain agricultural production, whereas soil health emphasises biological attributes such as biodiversity, microbial activity and ecological functioning [12–14]. Given the multidimensional nature of soil health, its assessment requires the integration of biological, chemical and physical indicators together with crop performance [15,16]. Among these indicators, soil organic matter (SOM) is central due to its sensitivity to management and its influence on aggregation, porosity, nutrient availability and water retention [17–19]. Biological indicators—such as microbial biomass, enzyme activities and soil respiration—provide early signals of changes in soil functioning and respond rapidly to management practices [12–14,20,21]. Microbial biomass carbon (MBC) represents an active fraction of soil organic carbon, whereas water-

soluble organic carbon (WSOC) reflects the most mobile and bioavailable fraction [19,21,22]. In addition, soil mesofauna contributes to organic matter turnover and constitutes a reliable indicator of biological quality [23]. SOM comprises fractions with contrasting biochemical stability, ranging from labile compounds (e.g., soluble carbohydrates, amino acids and microbial by-products) to more recalcitrant constituents such as lignin-rich residues and humified structures. The balance between these fractions determines SOM turnover and its sensitivity to management. Green undersown crops influence this balance through continuous inputs of residues with variable C/N ratios and biochemical composition, thereby modulating both short-term mineralisation and longer-term SOM stabilisation in Mediterranean orchard soils. Recent meta-analyses have shown that residue quality strongly governs SOC responses to management [24–27], supporting the relevance of considering SOM composition and recalcitrance when evaluating soil quality improvements.

Soil organic carbon (SOC) is a key indicator of soil quality because it influences soil structure, nutrient cycling, water retention, and biological activity. Increasing SOC is therefore a major goal of sustainable agricultural management and climate change mitigation. Among the practices shown to be most effective in enhancing SOC are undersown crops, organic amendments, conservation tillage, and diversified crop rotations, which increase carbon inputs and improve soil health and ecosystem functioning [17,24,25,27–29].

Different types of undersown crops exhibit contrasting functional behaviour. Legumes produce biomass with low C/N ratios, favouring rapid decomposition, nitrogen mineralisation and stimulation of microbial processes [30,31]. In contrast, grasses generate lignified residues with high C/N ratios that decompose slowly, promoting carbon stabilisation and aggregate formation [28,29,32]. Spontaneous vegetation often shows intermediate functional behaviour and high adaptability to Mediterranean water stress, thereby contributing to soil protection and biodiversity [33]. Beyond their chemical and structural effects, undersown crops also influence soil fauna, a key indicator of ecological quality. Collembolans and mites respond sensitively to moisture, organic matter quality and disturbance, while the QBS-ar index integrates the functional diversity of soil microarthropods [34,35]. Understanding how undersown crops modulate these biological components is essential for a comprehensive assessment of soil functioning.

The Valencia region (Eastern Spain) produces more than 90% of national persimmon yield, yet only ~15% of orchards currently use undersown crops. Despite numerous studies, the edaphogeochemical and biological responses associated with different GUC types remain poorly integrated in these agroecosystems. Therefore, this study addresses this gap by analysing a broad set of chemical, biological and structural soil variables using principal component analysis (PCA). The objective of this study was to evaluate the effects of seeded and spontaneous undersown crops compared with herbicide-managed bare soil on soil physicochemical and biological indicators in irrigated persimmon orchards. To achieve this, a broad set of variables—including SOM, nitrogen (N), C/N ratio, pH, electrical conductivity (EC), soil respiration rate (RR), collembolan (COLL) and mite abundance, QBS-ar index, enzyme activities (EA), MBC and WSOC—were assessed across 54 plots in three orchard sites using field campaigns, laboratory analyses and multivariate tools (principal component analysis, PCA). This approach allows the dominant functional gradients associated with each GUC type and the mechanisms driving soil quality improvement to be identified.

2. Materials and Methods

2.1. Study Area and Experimental Design

A total of 139 composite samples from different plots across three irrigated persimmon (*Diospyros kaki* L.) orchard sites (Granja, Cargol and Alginet), located near València (Eastern

Spain), were analysed for this study (Figure 1; Table S1). The soils in this area are classified as Calcaric Arenic Fluvisols, according to IUSS-WRB (2022) [36]. This classification is consistent with previous regional soil surveys [37,38], which describe these Fluvisols as having low organic matter content, basic pH, moderate salinity and high carbonate levels. The study was conducted in topsoils (0–15 cm) in two stages. In the first stage, the effect of GUCs on physicochemical and biological soil properties was assessed over an 18-month period. The study lasted 18 months and included two seasonal sampling campaigns: START (early winter) and END (late spring). All physicochemical and biological parameters were measured at both sampling times. Additional indicators (MBC, WSOC, EA and pesticide residues) were determined at the end of the 18-month period. The following variables were measured: SOM, N, C/N, pH, EC, RR, mesofauna (collembolan and mite abundance) and the QBS-ar arthropod index. Two seasonal sampling campaigns were carried out in this phase, at the beginning (START) and at the end of the experimental period, yielding 108 composite samples (54 per sampling time; Table S1, PANEL A). In the second stage, 63 plots were selected according to site and treatment, resulting in 31 composite samples (Table S1, PANEL B). In this stage, selected soil biochemical and biological indicators were analysed, including 19 enzyme activities, MBC, WSOC and the QBS-ar index. The number of samples in PANEL A and PANEL B of Table S1 differs because of the specific objectives and sampling design of each stage.

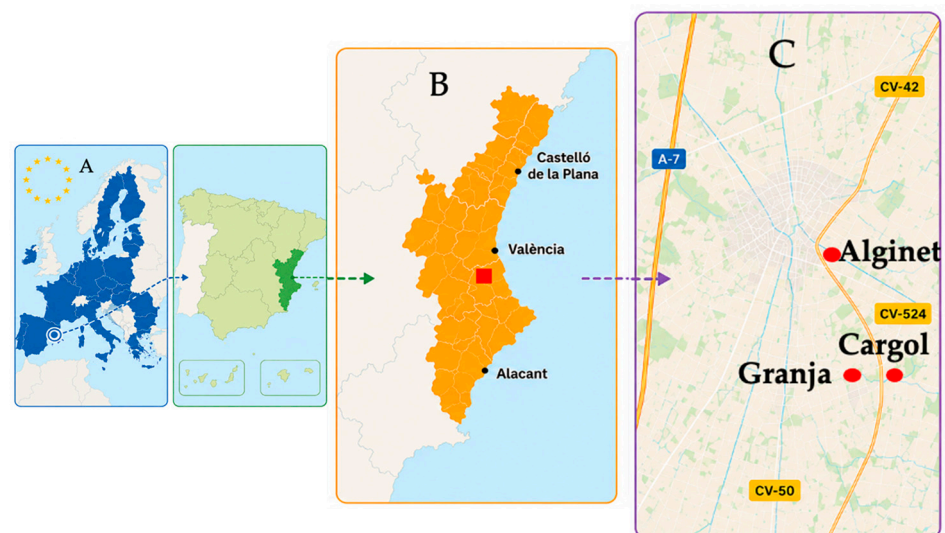


Figure 1. Geographic context of the study area. (A) Location of Spain within the European Union; (B) Valencian Community, highlighting the study region; (C) local map showing the three experimental orchards (Alginet, Granja and Cargol) (red colour). Maps were generated from open-access layers provided by the European Soil Data Centre (ESDAC) and the Valencian Cartographic Institute (yellow symbols indicate regional roads).

Undersown crops differ from conventional cover crops because they remain active during the whole season, continuously providing soil protection, organic inputs and biological stimulation. This management aligns with Mediterranean orchard practices, where permanent green covers are preferred over seasonal cover crops. Three groundcover management systems were compared, using herbicide-managed bare soil as the baseline (control) against which seeded undersown crops and spontaneous vegetation were evaluated.

1. Seeded undersown crops: grasses, legumes and flowering species;
2. Spontaneous vegetation;
3. Herbicide-managed bare soil.

In orchards with spontaneous vegetation, green cover was maintained for longer periods and was either mechanically mown or incorporated into the soil. In herbicide-managed bare soil, weed control was based mainly on applications of glyphosate, oxyfluorfen and pendimethalin.

Seeded GUCs were sown in November–December 2022 using a mechanical seed drill. The grass mixture consisted of *Festuca arundinacea* Schreb. (50%) and *Bromus inermis* Leyss. (50%). Legume species included *Onobrychis viciifolia* Scop., *Vicia sativa* L. and *Trifolium alexandrinum* L. The flower mixture comprised *Brachypodium distachyon* L., *Festuca arundinacea* Schbr, *Bromus inermis* Leyss, *Medicago sativa* L., *Calendula officinalis* L., *Achillea millefolium* L., *Diplotaxis eruroides* L. and *Lobularia maritima* L. Figure S1 shows examples of the undersown treatments.

Vegetation cover was quantified using a 1 × 1 m quadrat subdivided into 25 cells. For each sampling plot, total vegetation cover (%) and the relative abundance of functional groups (grasses, legumes, composites and others) were recorded. Plant classification followed the Braun–Blanquet phytosociological method [39,40].

2.2. Soil Sampling and Laboratory Analyses

Soil samples were collected from the 0–15 cm layer using a graduated soil probe during the sampling campaigns. Plots at each site were systematically established across the orchards and georeferenced for in situ sampling. To optimise analytical costs and processing time, individual samples were composited according to cover crop type and spatial proximity. The composite soil samples were homogenised, air-dried and sieved to 2 mm.

Soil oxidisable organic carbon (COOx) was determined following the Walkley–Black method by oxidation with potassium dichromate in the presence of sulphuric acid and subsequent titration with ammonium ferrous sulphate [41]. SOM was estimated according to Equation (1). The factor 1.29 accounts for the incomplete recovery of oxidisable organic carbon by the dichromate oxidation [41,42]. The factor 1.72 is the classical Van Bemmelen coefficient, used to convert organic carbon to soil organic matter assuming an average SOM composition of 58% C. Total C and N were measured using an elemental analyser (EA 1110 CNHS, CE Instruments, Milan, Italy) by dry combustion at ≥ 900 °C in the presence of oxygen.

$$\text{SOM} = \text{COOx} \times 1.29 \times 1.72 \quad (1)$$

The C/N ratio was calculated as follows (Equation (2)):

$$\text{C/N} = (\text{COOx} \times 1.29) / \text{N} \quad (2)$$

Soil pH was measured in a 1:2.5 soil:water suspension, and EC was measured in a 1:5 soil:water extract using a CRISON pH meter and a CRISON conductivimeter, both calibrated prior to analysis, following the official protocols of the Spanish Ministry of Agriculture, Fisheries and Food [43]. Soil respiration rate (CO₂ efflux) was measured directly in the field using a portable CO₂ analyser (EGM-5, PP Systems International Inc., Amesbury, MA, USA) [44,45]. WSOC was analysed following the method described by Yakovchenko and Sikora [46]. MBC was determined using the chloroform fumigation–extraction method according to Vance et al. [47], as modified by Wu et al. [48]. Soil mesofauna was extracted using Berlese funnels following the Tullgren method [49]. Soil biological quality was assessed using the QBS-ar index, which is based on the presence and degree of morphological adaptation of soil arthropods. This method focuses on major taxonomic groups such as oribatid and gamasid mites and collembolans, without requiring species-level identification [50]. EA was determined using the API ZYM system (BioMérieux, Marcy-l'Étoile,

France). Nineteen soil enzyme activities, including phosphatases, lipases, esterases, β -glucosidases and aminopeptidases, were measured following the protocol described by Boluda et al. [51]. EA was semi-quantitatively assessed according to the manufacturer's scale: no activity (0), low activity (1), moderate activity (2–4) and high activity (5). Values between 1 and 2 correspond to concentrations of 10–20 nmol, whereas values between 3 and 5 correspond to 20–40 nmol. Additionally, 15 pesticides commonly used in fruit orchards were analysed in samples from the herbicide treatment using the QuEChERS extraction method, a widely used dispersive solid-phase extraction (d-SPE) procedure for sample preparation. Quantification of the compounds in the extracts was carried out by UHPLC-MS/MS [52]. The target compounds included three herbicides (oxyfluorfen, pendimethalin and glyphosate), five insecticides (amitraz, acetamiprid, carbofuran-OH, chlorantraniliprole and imidacloprid), six fungicides (azoxystrobin, pyraclostrobin, myclobutanil, trifloxystrobin, imazalil and fenamidone) and one synergist (piperonyl butoxide) in order to determine pesticide residues in herbicide-managed bare-soil samples.

2.3. Statistical Analysis

A principal component analysis (PCA) and a linear discriminant analysis (LDA) were conducted to explore multivariate patterns in soil chemical and biological properties across the different cover-crop treatments using the dataset obtained in the first stage. The analysis included nine variables: SOM, N, C/N ratio, pH, EC, RR, collembolan abundance, mite abundance and the QBS-ar index. Prior to analysis, all variables were examined for normality and homogeneity of variance. Because the variables were measured in different units and scales, the PCA was performed on the covariance matrix after standardisation of the dataset. Sampling adequacy was assessed using the Kaiser–Meyer–Olkin (KMO) statistic, and Bartlett's test of sphericity was used to verify the suitability of the data for factor analysis. Components with eigenvalues greater than one were retained according to the Kaiser criterion. A Varimax orthogonal rotation was applied to improve interpretability of the component structure. Component loadings were used to identify the dominant gradients associated with soil chemical status, organic matter quality, microbial activity and soil mesofauna. LDA was performed to maximise separation among treatments and to assess the classification ability of the variables. The significance of discriminant functions was evaluated using Wilks' lambda and chi-square tests. Prior standardisation of variables was applied to avoid scale-related bias. The combined use of factor analysis and discriminant analysis provides a complementary and statistically rigorous framework for evaluating treatment effects on soil quality. Additionally, a second PCA was performed together with analysis of variance (ANOVA) to explore differences among treatments using the biological indicator dataset obtained during the second stage of the experiment (END). These data were analysed using one-way ANOVA followed by the least significant difference (LSD; DMS) post hoc test ($\alpha = 0.05$) to detect pairwise differences among treatments. Homogeneity of variances was verified using Levene's test, and robust Brown–Forsythe statistics were inspected when necessary. All analyses were performed using standard multivariate procedures in SPSS v31.0 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Soil Physicochemical and Biological Properties in the First Stage of the Experiment

The physicochemical and biological properties of the orchard soils at the beginning (START) and end (END) of the first stage of the experiment are summarised in Table 1, while full plot-level data are provided in Tables S1 and S2. The five groundcover treatments (flowers, legumes, grasses, spontaneous vegetation and herbicide-managed bare soil) exhibited

substantial variability at START, reflecting the heterogeneous baseline conditions typical of Mediterranean calcareous soils historically maintained under bare-soil herbicide regimes.

Table 1. Soil physicochemical and biological indicators at START and END. PANEL (A), grouped by treatment, and PANEL (B), summary statistics of soil indicators by treatment (START + END combined).

PANEL A. Treatment mean										
Treatment	Season	SOM	N	C/N	pH	EC	RR	COLL	Mites	QBS-ar
Flowers	START	2.32	0.17	8.6	7.7	266	0.16	6	2	14
	END	1.88	0.14	8.1	7.8	188	0.25	0	2	11
Legumes	START	0.98	0.09	7.2	7.9	211	0.16	6	1	15
	END	2.10	0.13	9.4	7.8	142	0.22	1	1	17
Grasses	START	1.88	0.10	11.3	7.9	201	0.15	3	1	10
	END	2.10	0.10	12.1	7.8	185	0.31	1	3	37
Spontaneous	START	3.45	0.16	13.1	7.6	269	0.25	0	0	0
	END	2.19	0.12	10.7	7.7	303	0.15	1	1	11
Herbicide	START	1.99	0.10	12.0	7.8	228	0.21	3	0	6
	END	1.50	0.06	14.3	7.9	176	0.16	0	0	0
PANEL B. Summary statistics of soil indicators by treatment (START + END combined)										
Variable	Flowers		Legumes		Grasses		Spontaneous		Herbicides	
SOM	2.10 ± 0.59 (28%)		1.54 ± 0.86 (56%)		1.99 ± 0.45 (23%)		2.82 ± 1.00 (35%)		1.74 ± 0.53 (30%)	
N	0.15 ± 0.04 (27%)		0.11 ± 0.03 (27%)		0.10 ± 0.01 (10%)		0.14 ± 0.04 (29%)		0.08 ± 0.03 (38%)	
C/N	8.4 ± 2.3 (27%)		8.3 ± 3.8 (46%)		11.7 ± 2.7 (23%)		11.9 ± 3.1 (26%)		13.1 ± 4.6 (35%)	
pH	7.8 ± 0.1 (2%)		7.9 ± 0.2 (3%)		7.8 ± 0.2 (3%)		7.7 ± 0.1 (2%)		7.8 ± 0.2 (2%)	
EC	227 ± 68 (30%)		176 ± 73 (41%)		193 ± 161 (83%)		286 ± 97 (34%)		202 ± 50 (25%)	
RR	0.21 ± 0.10 (48%)		0.19 ± 0.10 (53%)		0.23 ± 0.12 (52%)		0.20 ± 0.09 (45%)		0.18 ± 0.04 (22%)	
COLL	3 ± 6 (187%)		3 ± 5 (162%)		2 ± 3 (148%)		0 ± 1 (191%)		1 ± 4 (283%)	
Mites	2 ± 3 (153%)		1 ± 2 (185%)		2 ± 2 (107%)		0 ± 1 (367%)		0 ± 0 (269%)	
QBS-ar	12 ± 13 (105%)		16 ± 18 (111%)		23 ± 18 (75%)		6 ± 10 (185%)		3 ± 8 (283%)	

SOM, soil organic matter (%); N, nitrogen (%); EC, electrical conductivity ($\mu\text{S cm}^{-1}$); RR, soil respiration rate ($\text{g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$); COLL, collembolans, $\text{n}^\circ \text{ m}^{-2}$; QBS-ar, soil microarthropod index. Values in parentheses are the coefficients of variation (CVs).

At START, SOM ranged widely (0.20–4.35%), with spontaneous vegetation showing the highest values (mean 3.45%), followed by flowers (2.32%) and grasses (1.88%). Legume plots displayed the lowest SOM (0.98%), largely due to the severely depleted soils at the Granja-2 site. This heterogeneity is reflected in the coefficients of variation (CVs) in Table 1 (Panel B), where legumes exhibited the greatest dispersion (56%). After 18 months, SOM increased most markedly under legumes and grasses. Legumes rose from 0.98% to 2.10%, with several plots increasing from <0.4% to >3% (Table S2), demonstrating the strong responsiveness of carbon-depleted Mediterranean soils to continuous organic inputs. Grasses also increased moderately (1.88% to 2.10%). Flowers showed variable responses, while spontaneous vegetation remained relatively high but declined slightly. Herbicide-managed soils showed no improvement and remained consistently low (1.50%).

Nitrogen concentrations remained low across treatments (0.06–0.23%), consistent with Mediterranean soils with limited organic inputs. However, the C/N ratio revealed clear functional differences. At START, legumes exhibited very low C/N (1.6–3.0), indicating N-rich residues and rapid mineralisation, whereas spontaneous vegetation showed the highest C/N (12–17). At END, legumes maintained low C/N (4–14), confirming sustained

N availability, while grasses and flowers remained moderate and spontaneous vegetation remained high. These patterns align with established evidence that legume-based cover crops enhance N cycling and accelerate residue turnover.

Soil pH remained remarkably stable across treatments and sampling periods (7.3–8.3; $CV \leq 3\%$), reflecting the strong buffering capacity of calcareous soils. In contrast, EC showed considerable heterogeneity (176–303 $\mu\text{S cm}^{-1}$), with higher variability under grasses due to one plot with exceptionally elevated EC. This chemical heterogeneity is consistent with previous reports for citrus-growing soils in the Valencian region [53].

Biological indicators exhibited the greatest spatial variability. Soil respiration (RR) ranged from 0.02 to 0.48 $\text{g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, with similar means across treatments (0.15–0.25). Collembolans, mites and QBS-ar showed extremely high CVs (105–283%), reflecting strong differences in soil biological quality (Table 1, Panel B). Despite this heterogeneity, clear treatment effects emerged. Grasses produced the highest average QBS-ar values (23) and the lowest CV (75%), indicating consistent biological recovery across plots. Grasses and legumes also improved biological functioning (QBS-ar = 16). In contrast, herbicide-managed soils remained biologically impoverished: at END, no collembolans or mites were detected in any herbicide plot, and QBS-ar was zero.

Overall, the first stage of the experiment demonstrates that GUCs improved soil quality within 18 months, particularly in soils with initially low SOM. Legumes were most effective in restoring soil carbon and nitrogen in degraded soils, while grasses promoted the most consistent improvements in soil biological functioning. These patterns are consistent with the multivariate differentiation observed. Legume-rich mixtures and grasses cluster towards higher SOM, lower C/N and greater biological activity, whereas herbicide-managed soils remain isolated at the low-quality end of the gradient. The complementary information provided by Table 1 (Panels A and B) and Table S2 shows that GUCs enhanced both mean soil properties and reduced response heterogeneity. Legumes improved SOM and nutrient status in degraded soils, while grasses supported stable biological recovery. Together, these results highlight the potential of diversified groundcover systems to restore soil quality in Mediterranean orchard environments.

3.2. Principal Component and Discriminant Analyses of Soil Parameters

PCA and discriminant analysis were performed on the soil parameters measured. The PCA extracted four components with eigenvalues greater than one, together explaining 74.3% of the total variance in soil chemical and biological properties (Table 2). Bartlett's test indicated that the correlation matrix was suitable for data reduction ($\chi^2 = 428.1$, $p < 0.001$), although the KMO value (0.393) suggested limited sampling adequacy.

Table 2. Total variance explained ($n = 108$; n is the number of samples).

Component	Total	Initial Eigenvalues		Rotation Sums of Squared Loadings		
		%	%	Total	%	%
		Variance	Accumulated		Variance	Accumulated
PC1	2.32	25.783	25.783	1.976	21.961	21.961
PC2	1.895	21.053	46.836	1.829	20.328	42.288
PC3	1.278	14.205	61.041	1.518	16.87	59.158
PC4	1.192	13.244	74.285	1.361	15.127	74.285
PC5	0.832	9.244	83.53			
PC6	0.625	6.942	90.471			
PC7	0.588	6.532	97.003			
PC8	0.237	2.636	99.639			
PC9	0.032	0.361	100			

PC1 (21.9%) was strongly associated with mites (0.892) and QBS-ar (0.890), and negatively with electrical conductivity (-0.574) (Table 3). This component represents a biological quality gradient, with higher scores reflecting well-developed soil mesofauna and lower scores corresponding to herbicide-treated soils where fauna was largely absent. PC2 (20.3%) showed high positive loadings for nitrogen (0.883) and soil organic matter (0.654), and a strong negative loading for pH (-0.731). This axis reflects a nutrient and organic matter gradient, distinguishing soils under legumes and spontaneous vegetation from those under herbicide management. PC3 (16.9%) was dominated by the C/N ratio (0.980), with a secondary contribution from SOM (0.626). This component represents organic matter quality, separating high-C/N grasses and spontaneous vegetation from low-C/N legumes. The SOM dynamics observed in this study can be interpreted in light of the biochemical composition and recalcitrance of the organic inputs supplied by GUCs. Legume-rich mixtures contributed residues with low C/N ratios and high proportions of labile compounds, favouring rapid microbial mineralisation and short-term increases in labile SOM fractions. In contrast, grasses supplied lignified, structurally complex residues that decomposed more slowly and contributed to more recalcitrant SOM pools associated with aggregate formation and carbon stabilisation. The flower mixture, dominated by *Medicago sativa* in calcareous soils, generated substantial biomass but with moderate recalcitrance, leading to high turnover rather than sustained SOM accumulation in the 0–10 cm layer. These patterns are consistent with global evidence showing that SOM responses depend strongly on residue quality and biochemical stability [24,26,27]. Overall, the results illustrate how the biochemical traits of GUCs govern SOM trajectories in Mediterranean orchard soils.

Table 3. Coefficients extracted from the rotated component matrix.

Parameter ¹	Principal	Component		
	PC1	PC2	PC3	PC4
SOM	−0.014	0.654	0.626	0.278
N	0.009	0.883	−0.200	0.267
C/N	−0.004	−0.001	0.980	0.080
pH	0.046	−0.731	−0.210	0.225
EC	−0.574	0.221	0.230	−0.389
RR	0.041	0.166	0.031	0.631
COLL	0.234	0.098	−0.158	−0.749

¹ SOM, soil organic matter; N, nitrogen; EC, electrical conductivity; RR, soil respiration rate; COLL, collembolans.

In addition, PC4 (15.1%) accounted for a smaller but ecologically meaningful proportion of the variance. This component was characterised by a positive loading for RR (0.631) and a negative loading for collembolans (-0.749), indicating a microbial activity vs. mesofauna disturbance gradient. High RR values were associated with soils exhibiting greater biological respiration, whereas high collembolan abundance occurred under contrasting conditions. This pattern suggests that PC4 represents a gradient of microbial activity versus mesofauna stability, reflecting differences in microhabitat conditions that differentially favour microbes and soil fauna. High PC4 scores corresponded to soils with elevated microbial respiration but depleted mesofauna, typically under herbicide or disturbed conditions.

The PCA revealed that edaphic and biological variability in the studied systems is primarily structured by two dominant ecological gradients: soil biological quality and organic fertility. These patterns are consistent with previous findings in Mediterranean agroecosystems, where organic matter availability and salt accumulation are recognised as major drivers of soil biological functioning [35,54–56]. Such structuring of below-ground communities along functional gradients aligns with broader evidence linking soil biodi-

versity to ecosystem functioning [57]. The first component (PC1), characterised by strong positive loadings for mites and QBS-ar and a marked negative loading for EC, represents a clear gradient of soil biological quality versus salinity stress. The close alignment between mites and QBS-ar suggests that both indicators respond coherently to environmental conditions, reinforcing the use of QBS-ar as a robust integrative metric of soil ecological status. The strong negative association with EC indicates that salinity acts as a limiting factor for mesofauna communities, a pattern widely documented in Mediterranean and semi-arid soils. This opposition implies that soils with higher concentrations of soluble salts tend to exhibit reduced biodiversity and diminished biological activity. The PC2 component reflects a gradient of organic fertility, with positive loadings for SOM and N, and a negative loading for pH. This configuration is consistent with the tendency of soils richer in organic matter to display slightly lower pH values, often associated with enhanced microbial activity and nutrient mineralisation [58]. The strong correlation between SOM and N further highlights the tight coupling between organic matter dynamics and chemical fertility in these systems. In contrast, the C/N ratio (PC3), which loads predominantly on higher components, appears largely independent of the PC1–PC2 structure. This suggests that the quality of organic matter, expressed as the carbon-to-nitrogen balance, does not substantially contribute to the variability captured by the first two components, although it may influence more specific processes related to decomposition and nutrient turnover. Beyond these dominant gradients, PC4 provides additional insight into the functional organisation of the soil system. The positive loading of RR and the negative loading of collembolans indicate an inverse relationship between microbial metabolic activity and mesofauna abundance. Similar trade-offs have been reported in systems where rapid microbial turnover, reduced moisture or structural instability create conditions that enhance microbial respiration but constrain mesofauna populations [59,60]. Collembolans typically thrive in moist, structurally stable microhabitats with moderate decomposition rates, whereas elevated respiration is often associated with drier, warmer or more disturbed soils, where microbial communities respond rapidly to substrate availability [61]. Such conditions may reduce habitat suitability for collembolans through microclimatic stress, reduced substrate quality or competition for resources. Thus, PC4 captures a secondary but ecologically relevant axis describing the functional tension between microbial metabolism and mesofauna stability.

Overall, the results demonstrate that salinity, organic fertility, and microbial–faunal trade-offs are the principal drivers shaping edaphic and biological variability in the studied soils. These findings have clear implications for agricultural management: practices that enhance organic matter inputs and mitigate salt accumulation—such as the use of undersown crops, reduced tillage or the application of compost—are likely to improve soil biological quality and, consequently, overall ecosystem functioning.

The biplot of PC1 and PC2 (Figure 2) clearly illustrates the multivariate structure of the soil dataset. Variables associated with soil biological quality—mites and QBS-ar—show strong positive loadings on PC1, confirming that this axis represents a gradient of mesofauna abundance and soil biological integrity. In contrast, EC is oriented in the opposite direction, reflecting its negative contribution to PC1. PC2 is dominated by N and SOM, both pointing strongly upwards, indicating that this axis represents a nutrient-richness gradient. Conversely, pH loads negatively on PC2, consistent with its inverse relationship with SOM and N in the dataset. Variables such as C/N, RR and collembolans show short vectors in the PC1–PC2 plane, indicating that their main contribution lies in components beyond the first two (PC3 and PC4), in agreement with the rotated component matrix. Overall, the biplot confirms that PC1 separates soils according to biological quality, while PC2 separates them according to nutrient status, fully consistent with the PCA loadings.

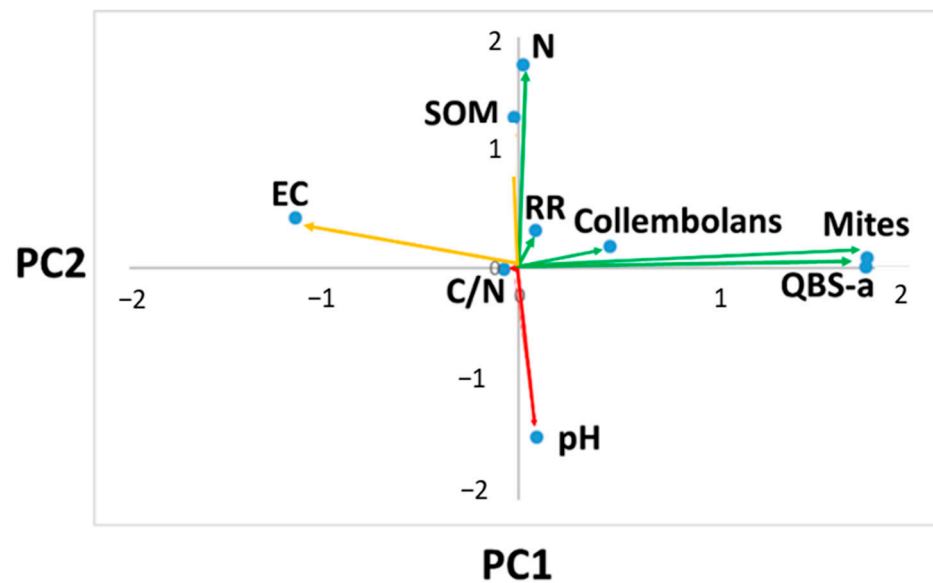


Figure 2. Biplot of the first two principal components (PC1 and PC2) derived from soil chemical and biological variables. Vectors represent the loadings of each variable on the two components, while points correspond to individual soil samples. PC1 is mainly associated with soil fauna (mites and QBS-ar), whereas PC2 reflects gradients in SOM and N content. The length and direction of each vector indicate the strength and sign of the correlation with the components.

Figure 3 shows an infographic illustrating the four functional gradients identified in the PCA of soil variables. Each quadrant represents a functional axis of soil response to cover crop management, integrating chemical, biological and structural dimensions of soil quality. Dry, lignified root residues are associated with reduced detritivore activity, whereas fresh, moist residues favour higher collembolan populations. This pattern highlights the sensitivity of soil fauna to residue quality and moisture, reflecting the functional link between organic matter state and biological activity in Mediterranean calcareous soils.

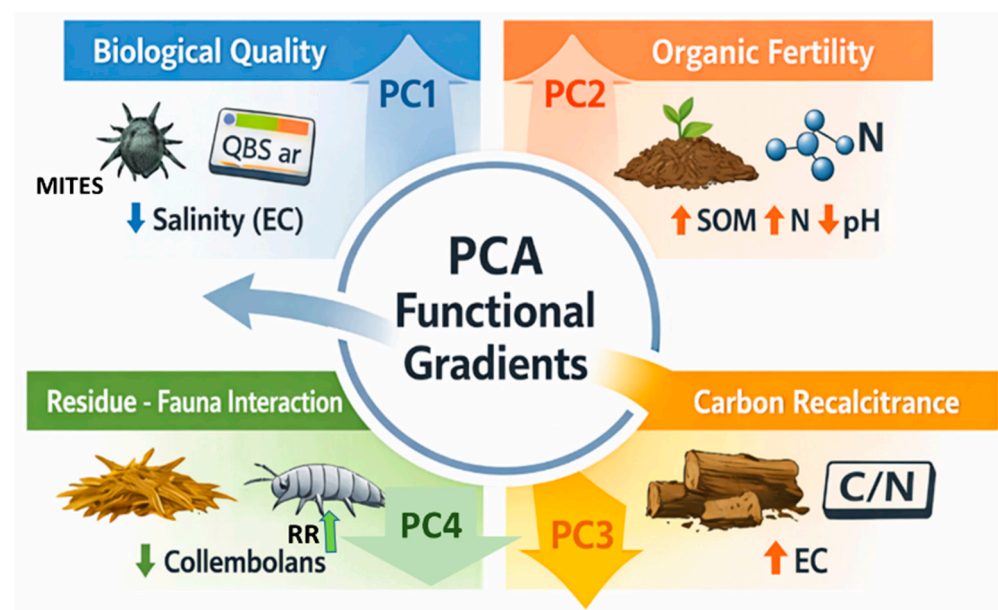


Figure 3. Functional gradients of GUC effects derived from PCA components. PC1—biological quality: mites and QBS-ar associated with lower salinity (↓ EC). PC2—organic fertility: high SOM and N with slightly lower pH (↑ SOM, ↑ N, ↓ pH). PC3—carbon recalcitrance: high C/N and SOM linked to increased EC (↑ C/N, ↑ EC). PC4—residue–fauna interaction: inverse relationship between RR ↑ and collembolan ↓.

Building on the multivariate structure identified through the factor analysis, discriminant analysis was subsequently applied to evaluate whether these same soil quality dimensions were sufficiently robust to distinguish among the experimental treatments. The combined use of factor analysis and discriminant analysis provides a complementary and statistically rigorous framework for evaluating treatment effects on soil quality. Factor analysis was employed to identify the underlying structure of covariation among soil physicochemical and biological indicators, thereby reducing dimensionality and revealing latent ecological gradients. Discriminant analysis, in contrast, explicitly incorporates treatment identity and tests whether these latent dimensions are effective for classifying groups. This sequential approach is widely recommended in multivariate ecology because it allows researchers to first characterise the intrinsic organisation of soil properties and then assess the discriminatory power of those same dimensions [62,63]. By integrating both methods, the analysis ensures that treatment differences are not only statistically significant at the univariate level but also ecologically interpretable and multivariate robust.

The multivariate separation among treatments is illustrated in the canonical discriminant function plot (Figure 4). The chart shows a clear displacement of group centroids along Function 1 and Function 2, consistent with the structure matrix results. Treatments positioned towards the positive side of Function 1 exhibit higher SOM and N and lower pH, whereas those on the negative side are characterised by lower organic inputs. Vertical separation along Function 2 reflects differences in C/N ratio, with treatments showing higher C/N values clustering in the upper part of the plot. The limited overlap among point clouds indicates that treatments produced distinct multivariate soil signatures, confirming the robustness of the discriminant model.

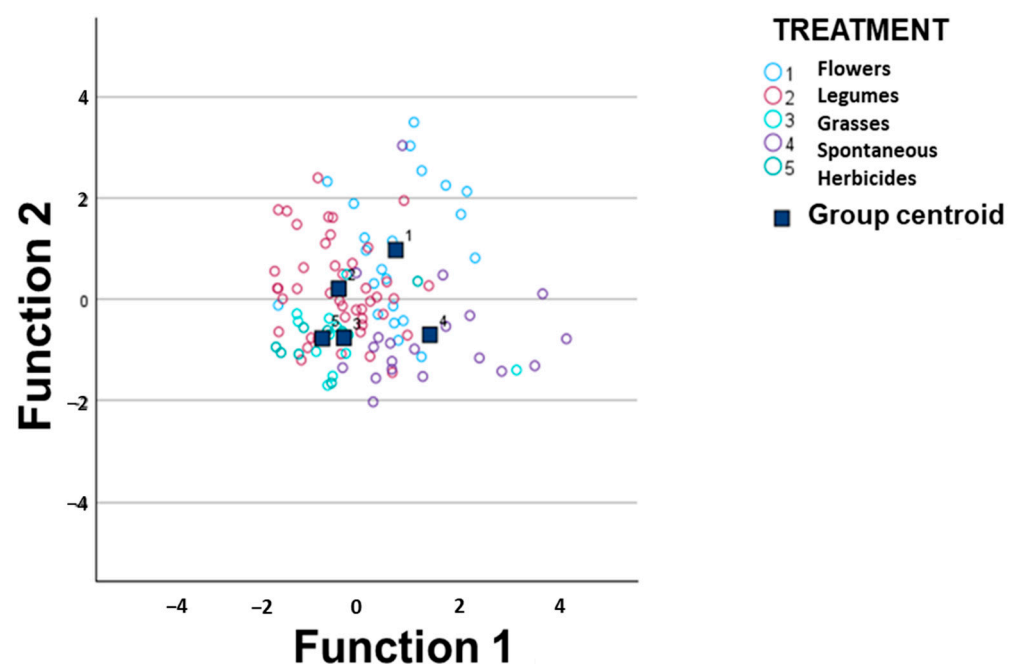


Figure 4. Canonical discriminant function plot showing multivariate separation among the five treatments. Points represent individual soil samples and squares denote group centroids. Function 1 is primarily associated with SOM, N and pH, while Function 2 is dominated by the C/N ratio. The distribution of samples and the displacement of centroids indicate clear differences in soil physicochemical and biological properties among treatments.

Taken together, the univariate and multivariate results demonstrate that the treatments generated clear and ecologically meaningful shifts in soil condition. The ANOVAs identified significant differences in SOM, N, C/N, pH, EC and QBS-ar, indicating that

both physicochemical and biological attributes responded to management. Factor analysis revealed that these variables clustered into coherent ecological dimensions related to organic matter quality, nutrient stoichiometry and soil biological functioning. The discriminant analysis confirmed that these same dimensions were highly effective in separating treatments, with SOM, N and C/N emerging as the strongest discriminators. The distinct positioning of group centroids in canonical space provides strong evidence that each treatment produced a unique multivariate soil signature. Overall, the integrated analysis highlights the multidimensional nature of soil responses and underscores the value of combining physicochemical and biological indicators to detect treatment-induced changes in soil functioning.

The spatial arrangement of treatments in the canonical discriminant plot reinforces the ecological interpretation of the multivariate results. The strong horizontal separation driven by SOM and N aligns with the well-established role of organic matter inputs in shaping microbial activity, nutrient turnover and soil structural development [64,65]. The vertical gradient associated with the C/N ratio further reflects differences in organic matter quality and decomposition pathways, a pattern widely reported in soil ecological studies [66]. The distinct clustering of treatments therefore indicates that management practices influenced not only individual soil properties but also the broader multivariate configuration of soil functioning, supporting the view that soil quality is inherently multidimensional [67]. Overall, the first stage of the experiment revealed that green undersown crops (GUCs) generated measurable improvements in SOM, biological activity and soil quality indicators within a relatively short period (18 months). The strongest responses occurred in soils with initially low SOM, where continuous inputs from roots, rhizodeposition and residues—particularly from legumes—enhanced SOM and biological functioning. These patterns are consistent with the multivariate differentiation observed in Figures 3 and 4, where legume-rich mixtures and grasses cluster towards higher SOM, lower C/N and greater biological activity, while herbicide-managed soils remain isolated at the low-quality end of the gradient.

3.3. Seasonal Dynamics in Soil Physicochemical and Biological Parameters Across Treatments

The analysis of seasonal changes at the start and the end of the first phase of experiment are showed in Table 4. Seasonal changes in soil properties showed clear differences among treatments. SOM increased notably under legumes (+1.12%) and, to a lesser extent, grasses (+0.22%), whereas it declined under flowers (−0.44%), spontaneous vegetation (−1.36%), and herbicides (−0.49%). A similar pattern was observed for N, with small increases in legumes and grasses, and declines in the remaining treatments. The C/N ratio increased strongly under legumes (+2.98) and herbicides (+2.17), indicating an accumulation of carbon-rich material, while it decreased under flowers and spontaneous vegetation. Soil pH remained relatively stable across treatments, highlighting its decrease in legumes and grasses in accordance with the increase in SOM. EC decreased sharply in all treatments, with the strongest reductions in spontaneous vegetation (−84.36 $\mu\text{S cm}^{-1}$) and flowers (−77.55 $\mu\text{S cm}^{-1}$), indicating a general seasonal leaching of soluble salts.

Seasonal changes between the START and END revealed contrasting functional responses among treatments (Table 5). SOM increased markedly under legumes (+1.12%) and moderately under grasses (+0.22%), whereas it decreased under flowers (−0.44%), spontaneous vegetation (−1.36%) and herbicide-managed soil (−0.49%). The reduction in SOM in the flower mixture coincided with increases in RR, mites and QBS-ar, suggesting rapid turnover of organic inputs rather than reduced soil functioning. In spontaneous vegetation, SOM declined despite stable or increased biological indicators, reflecting high initial SOM values and rapid mineralisation under summer conditions. Electrical conductivity decreased across all treatments, indicating seasonal leaching and reduced salt accumula-

tion. Biological indicators showed treatment-specific responses: QBS-ar increased strongly under grasses (+26.57) and spontaneous vegetation (+36.71), while herbicide-managed soils showed declines in most biological metrics.

Table 4. Seasonal changes in soil parameters between the start and end.

Parameter ¹	Flowers	Legumes	Grasses	Spontaneous	Herbicides
SOM	−0.44	+1.12	+0.22	−1.36	−0.49
N	−0.03	+0.04	+0.004	−0.06	−0.04
C/N	−0.56	+2.98	+0.66	−0.98	+2.17
pH	+0.16	−0.18	−0.10	+0.17	+0.13
EC	−77.55	−66.65	−16.59	−84.36	−52.78
RR	+0.10	+0.07	+0.16	+0.06	−0.02
COLL	−6.64	−4.41	−1.29	+1.29	−2.50
Mites	+0.27	−4.41	+2.29	+3.00	−0.25
QBS-ar	−3.36	+3.27	+26.57	+36.71	−5.50

¹ Values represent mean differences between START and END; positive = increase, negative = decrease. SOM, soil organic matter %; N, nitrogen %; EC, electrical conductivity ($\mu\text{S cm}^{-1}$); RR, soil respiration rate ($\text{g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$); COLL, collembolans ($\text{n}^\circ \text{ m}^{-2}$); QBS-ar index, soil microarthropod index.

Table 5. Biological indicators during the second stage of sampling.

Site	Sample	Cover	Enzyme Activity ¹									
			TE	Li-Es	Phos	Gluc	Aminop	MBC	WSOC	QBS-ar	COLL	Mites
Cargol	1	Flowers	11	2	3	2	4	414	394	41	1	0
Granja	2	Flowers	6	2	1	1	2	794	184	76	1	5
Granja	3	Flowers	9	2	2	2	3	504	269	61	1	2
Cargol	4	Legumes	5	2	2	0	1	577	579	0	0	0
Cargol	5	Legumes	3	1	2	0	0	545	1150	0	0	0
Cargol	6	Legumes	12	3	5	1	3	588	786	0	0	0
Cargol	7	Legumes	2	1	1	0	0	547	634	20	0	0
Cargol	8	Legumes	4	1	2	1	0	673	247	0	0	0
Cargol	9	Legumes	2	1	1	0	0	589	376	0	0	0
Cargol	10	Legumes	3	1	2	0	0	639	631	20	0	1
Cargol	11	Legumes	4	2	1	1	0	545	633	155	0	0
Granja	12	Legumes	0	0	0	0	0	889	173	165	0	0
Granja	13	Legumes	6	2	2	1	1	943	163	51	10	7
Granja	14	Legumes	5	2	2	0	1	845	201	40	3	1
Granja	15	Legumes	7	3	2	1	1	902	171	40	4	1
Granja	16	Legumes	0	0	0	0	0	785	186	0	0	0
Granja	17	Legumes	7	2	3	1	1	789	199	50	6	3
Granja	18	Legumes	0	0	0	0	0	829	182	20	2	0
Granja	19	Legumes	8	3	3	1	1	874	244	190	3	4
Cargol	20	Grasses	7	2	3	1	1	516	168	0	0	0
Cargol	21	Grasses	8	2	4	1	1	341	566	60	1	1
Granja	22	Grasses	0	0	0	0	0	921	208	40	1	1
Granja	23	Grasses	8	3	2	2	1	842	190	87	1	5
Alginet	24	Spontaneous	6	2	2	1	1	373	451	17	2	0
Alginet	25	Spontaneous	5	1	2	1	1	724	248	21	1	0
Alginet	26	Spontaneous	5	2	2	0	1	314	75	0	0	0
Alginet	27	Spontaneous	5	1	3	1	0	342	190	65	1	2
Alginet	28	Spontaneous	2	1	1	0	0	218	713	10	0	0
Alginet	29	Herbicide	3	0	2	1	0	335	235	0	1	0
Alginet	30	Herbicide	1	0	1	0	0	77	217	0	0	0
Alginet	31	Herbicide	0	0	0	0	0	233	164	0	0	0

¹ Enzyme Activity was semi-quantitatively assessed according to the manufacturer's scale: no activity (0), low activity (1), moderate activity (2–4), and high activity (5). Values between 1 and 2 correspond to concentrations of 10–20 nmol, while values between 3 and 5 represent concentrations of 20–40 nmol. TE: total enzymes; Li-Es: lipase-esterase; Phos: phosphatase; Gluc: glucosidase; Aminop: aminopeptidase; MBC: microbial biomass carbon (mg kg^{-1}); WSOC: water-soluble carbon (mg kg^{-1}); QBS-ar index, soil microarthropod index; COLL: collembolans.

Biological parameters showed contrasting responses. RR increased in all treatments except herbicides, with the highest rise in grasses ($+0.16 \text{ mg CO}_2 \text{ g}^{-1} \text{ h}^{-1}$). Collembolans declined in most treatments, particularly in flowers (-6.64 individuals per m^{-2}) and legumes (-4.41 individuals per m^{-2}), but increased under spontaneous vegetation ($+1.29$ individuals per m^{-2}). Mites increased under grasses and spontaneous vegetation but declined under legumes and herbicides. The QBS-ar index showed strong improvements under

grasses (+26.57) and spontaneous vegetation (+36.71), while decreasing under flowers and herbicides.

According to these results, seasonal shifts in soil properties revealed distinct functional trajectories across treatments, reflecting differences in vegetation composition, organic inputs and disturbance regimes. The increase in SOM and N under legumes is consistent with the well-known capacity of leguminous species to enhance soil fertility through nitrogen fixation and high-quality litter inputs [57]. In contrast, the decline in SOM under flower and spontaneous vegetation suggests either lower biomass return or faster mineralisation rates, a pattern previously observed in Mediterranean systems with ephemeral vegetation [55]. The strong reduction in EC across all treatments indicates seasonal leaching of soluble salts, a common process in Mediterranean climates characterised by intense autumn–winter rainfall and irrigation farming. The extreme acidification observed under grasses may reflect high root exudation rates or the accumulation of organic acids, processes known to occur in dense grass swards [58].

Biological parameters showed divergent responses. The increase in RR in most treatments suggests enhanced microbial activity during the season, likely driven by improved moisture and substrate availability. However, the simultaneous decline in collembolans in flowers, legumes and herbicides indicates that conditions favouring microbial metabolism did not necessarily support mesofauna stability. This inverse pattern aligns with evidence that rapid microbial turnover, drying–rewetting cycles or structural instability can reduce habitat suitability for collembolans [59,61]. The positive response of collembolans under spontaneous vegetation suggests that unmanaged vegetation may provide more stable microhabitats. The strong increases in QBS-ar under grasses and spontaneous vegetation highlight the sensitivity of soil biological quality to vegetation structure. These treatments likely provided greater litter continuity, root complexity and microhabitat heterogeneity, conditions known to enhance soil fauna diversity and ecological functioning [57,60]. Conversely, the decline in QBS-ar under herbicides reflects the negative impact of vegetation removal on soil biological integrity.

Overall, the seasonal dynamics observed indicate that vegetation type strongly modulates soil physicochemical and biological responses, with spontaneous vegetation and grass cover showing the most favourable trends for soil biological quality. Figure 5 shows a schematic conceptual model of soil treatment effects on soil quality. Arrows illustrate the direction and intensity of physicochemical and biological responses to different management treatments (flowers, legumes, grasses, spontaneous vegetation and herbicide). Green arrows indicate positive changes (increases or improvements), whereas red arrows denote negative changes (decreases or deterioration). The left section summarises physicochemical parameters—SOM, N, C/N ratio, pH and EC—that influence nutrient availability and microhabitat conditions. The right section depicts biological indicators, including RR, collembolans, mites and the QBS-ar index, which collectively reflect soil biological quality. Interconnecting arrows represent functional linkages between nutrient dynamics and faunal responses. Treatments promoting higher SOM, N and QBS-ar values (e.g., grasses and spontaneous vegetation) are associated with improved soil quality, while those reducing these parameters (e.g., herbicides) correspond to degraded soil conditions. The magnitude of SOM increases recorded in this study is consistent with the behaviour of low-organic-matter Mediterranean soils subjected to intensive organic inputs and reduced disturbance. Several plots started with SOM values below 0.4%, a condition typical of long-term herbicide-managed orchards with high erosion and minimal residue return. In such depleted soils, the establishment of GUCs can trigger rapid SOM accumulation, as continuous biomass deposition and absence of tillage enhance carbon incorporation in the upper soil layer. Meta-analyses have shown comparable responses: Crystal-Ornelas

et al. [24] reported significant SOC gains under cover crops and organic amendments; Gross et al. [25] demonstrated consistent SOC increases following manure inputs; He et al. [26] quantified global SOC increases of 4–6% under cover cropping; and Salisu et al. [27] summarised substantial improvements in SOC and soil quality across diverse systems. The convergence of SOM increases with higher MBC, WSOC, enzyme activities and mesofauna abundance further support the interpretation that the observed changes represent genuine improvements in soil functioning rather than short-term artefacts. Overall, the results highlight the capacity of undersown crops to rapidly restore SOM in degraded Mediterranean orchard soils.

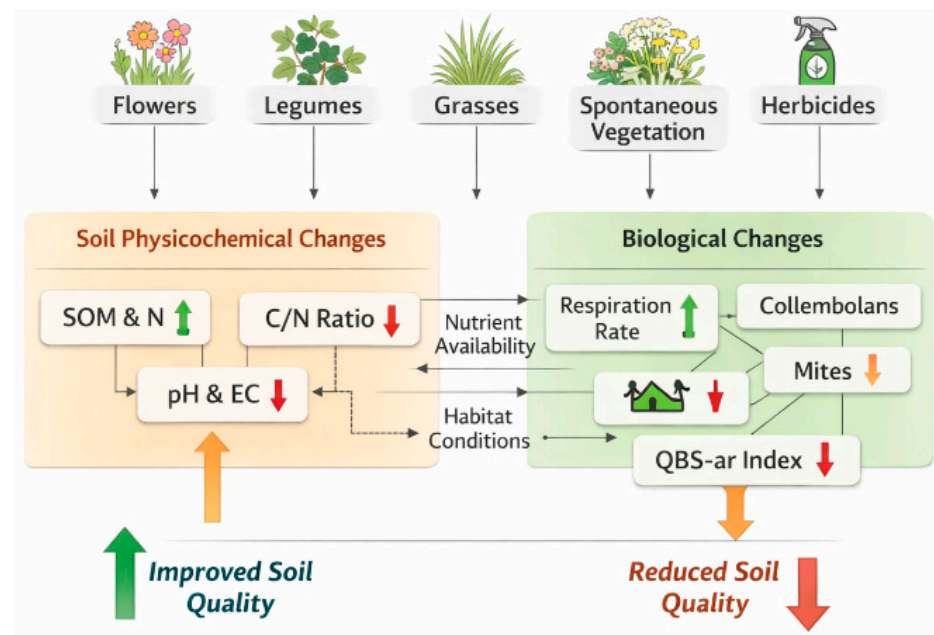


Figure 5. Conceptual model of soil undersown crop treatment effects on soil quality.

The seasonal SOM dynamics observed in Table 5 are consistent with the functional traits of the different covers. The flower mixture showed a small SOM reduction (−0.44%), which can be attributed to the dominance of *Medicago sativa* in calcareous soils. Alfalfa produces residues with moderate C/N ratios and high degradability, promoting rapid microbial mineralisation and carbon turnover. Its deep rooting system also allocates biomass below the sampled 0–10 cm layer, reducing apparent SOM accumulation at the surface. This explains why SOM decreased while biological indicators (RR, mites and QBS-ar) increased, reflecting high turnover rather than loss of soil functioning. This short-term reduction in SOC under the flower mixture is consistent with the low recalcitrance and high degradability of alfalfa residues, which promote rapid microbial turnover rather than long-term carbon stabilisation. In Mediterranean orchard soils, SOC persistence depends strongly on physicochemical protection mechanisms that regulate the stabilisation of plant residues [29], explaining why highly degradable inputs such as alfalfa contribute more to short-term turnover than to SOC accumulation. Spontaneous vegetation exhibited the largest SOM decline (−1.36%), likely due to high initial SOM values and rapid decomposition under Mediterranean summer drought. In contrast, legumes increased SOM substantially (+1.12%), consistent with their low C/N residues and high rhizodeposition. These patterns align with published evidence showing that SOM responses depend strongly on residue quality, rooting depth and turnover rates in Mediterranean orchard soils.

3.4. Effects of Undersown Crops on Selected Soil Biological Indicators at the END Sampling

3.4.1. Enzymatic Activity Responses and Soil Fauna Patterns

At the END sampling (stage 2), cover-crop treatments produced marked differences in soil biological functioning, particularly in extracellular enzyme activities (TE), MBC and soil fauna (Table 5). These contrasts provide a coherent picture of how vegetation structure and management intensity shape microbial and faunal processes.

Biological indicators measured at the END sampling revealed clear differences among cover-crop treatments, particularly in extracellular enzyme activities (Table 6). Flowering species showed the highest enzymatic activity (TE = 6–11), with consistently elevated lipase–esterase, phosphatase, glucosidase and aminopeptidase. This pattern reflects enhanced microbial functional capacity driven by diverse root inputs and rhizodeposition [68–70]. Legume covers displayed greater variability (TE = 0–12), likely linked to differences in establishment and biomass production, but generally supported high MBC (>800 mg kg^{−1}). Grass covers showed intermediate EA (TE = 0–8) and moderate MBC, consistent with slower decomposition of high-C/N residues. Spontaneous vegetation maintained moderate TE but lower MBC than sown covers. These patterns indicate an enhanced microbial functional capacity, likely driven by the greater root diversity and rhizodeposition associated with multispecies flowering covers, which are known to stimulate microbial metabolism and nutrient cycling [71,72].

Table 6. Pesticide concentrations (µg kg^{−1}) found in the herbicide samples at the end of the study.

Pesticide	Mean (n = 3)	Standard Deviation
Acetamiprid	Tr ¹	
Glyphosate	93.08	6.10
Imidacloprid	2.65	3.29
Myclobutanil	3.04	1.47
Oxyfluorfen	53.83	42.59

¹ Tr, traces.

Legume-based covers displayed highly variable enzymatic profiles (TE = 0–12). Some plots showed negligible activity (TE = 0), whereas others exhibited moderate-to-high values (TE = 7–12). This heterogeneity likely reflects differences in legume establishment and biomass production, which strongly influence microbial activity through nitrogen-rich residues and root exudates [73,74]. Despite this variability, legume plots generally supported high microbial biomass carbon (MBC), frequently exceeding 800 mg kg^{−1}, consistent with the well-documented capacity of legumes to enhance microbial growth via N inputs [66].

Grass covers showed intermediate enzymatic activity (TE = 0–8) and moderate MBC values (516–921 mg kg^{−1}). Their WSOC concentrations were also moderate, reflecting the typically high C/N ratio and slower decomposition of grass residues, which promote more gradual microbial responses [28].

Spontaneous vegetation plots exhibited moderate enzyme activities (TE = 2–6) and variable WSOC, with some plots showing high soluble carbon (e.g., 713 mg kg^{−1}). However, MBC values were generally lower than in legume or grass covers, suggesting that unmanaged vegetation maintains microbial substrates but supports less microbial biomass.

In contrast, herbicide-treated plots consistently showed the lowest biological activity, with TE values between 0 and 3, minimal phosphatase and glucosidase activity, and markedly reduced MBC (77–335 mg kg^{−1}). These results align with evidence that herbicide-based weed control reduces root inputs and microbial habitat quality, leading to declines in microbial biomass and enzymatic functioning [75]. Pesticide residues detected in these plots are shown in Table 6.

Soil fauna indicators supported the enzymatic patterns. The QBS-ar index reached its highest values in flower and legume plots (up to 190), reflecting improved soil biological quality under vegetated covers. Collembolan and mite abundance followed a similar trend, with the greatest values in flower and legume treatments and the lowest in herbicide plots. These findings are consistent with the sensitivity of soil mesofauna to vegetation cover, organic inputs and disturbance regimes [76].

3.4.2. ANOVA Results and Interpretation

The ANOVA + LSD test revealed significant treatment effects for TE, Gluc and Aminop, whereas Li-Es, Phos, collembolans and mites showed no significant differences (Figure 6A). TE activity was highest in the flowers treatment and significantly lower in herbicide-managed soils, indicating reduced oxidative capacity under chemical weed control. Glucosidase activity also differed significantly, with flowers outperforming legumes and herbicide, suggesting enhanced β -glucosidase activity under diverse floral cover. Aminopeptidase showed the strongest treatment effect, with flowers significantly exceeding all other treatments and herbicide presenting the lowest values, reflecting a strong positive influence of vegetated cover on N-related enzymatic processes.

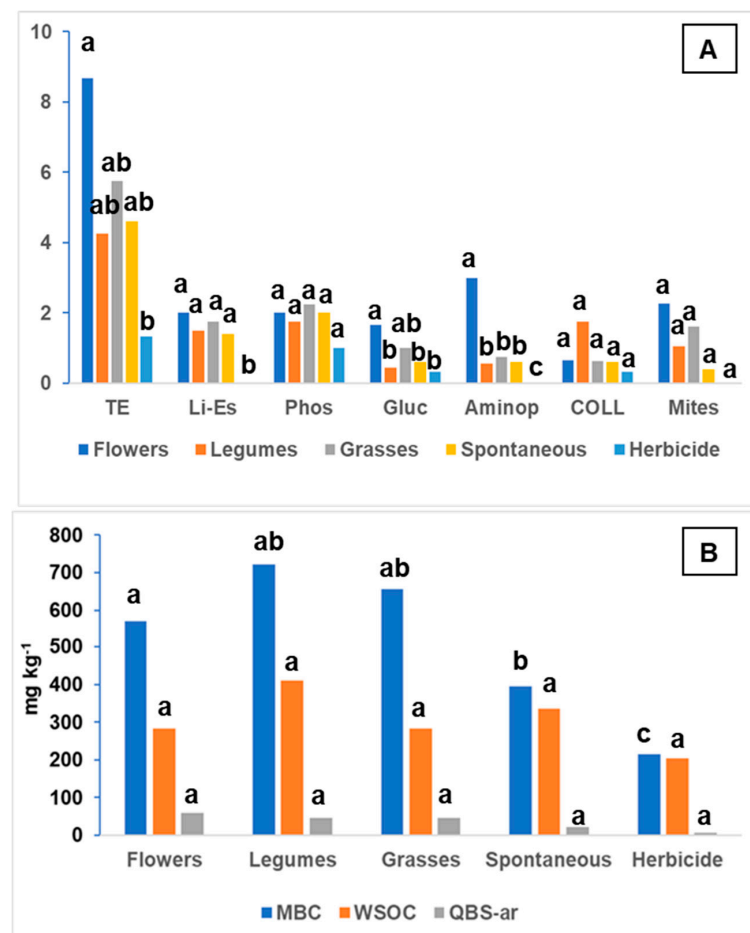


Figure 6. Biological indicators under different groundcover management systems (flowers, legumes, grasses, spontaneous vegetation, and herbicide). (A) Enzymatic soil activities (TE, total enzymes; Li-Es, lipase–esterase; Phos, phosphatase; Gluc, glucosidase; Aminop, aminopeptidase; according to the manufacturer’s scale (see Section 2.2), values between 1 and 2 correspond to concentrations of 10–20 nmol, whereas values between 3 and 5 correspond to 20–40 nmol); COLL, collembolans and mites ($n^{\circ} m^{-2}$). (B) Microbial biomass carbon (MBC), water-soluble organic carbon (WSOC), and soil biological quality (QBS-ar index). Different letters above bars indicate significant differences among treatments according to the LSD post hoc test ($\alpha = 0.05$).

For biological indicators, ANOVA revealed a strong treatment effect for MBC ($F = 7.29$, $p < 0.001$), whereas WSOC ($p = 0.671$) and QBS-ar ($p = 0.631$) showed no significant differences (Figure 6B). Herbicide plots had significantly lower MBC than flowers, legumes and grasses, while spontaneous vegetation showed intermediate values. WSOC exhibited high variability but no treatment effect, suggesting that soluble carbon pools are less sensitive to groundcover type. QBS-ar values were statistically similar across treatments, although numerically higher in flowers, legumes and grasses.

Overall, MBC emerged as the most responsive indicator, with herbicide use consistently suppressing microbial biomass.

The strong response of TE, Gluc and Aminop to groundcover management highlights the sensitivity of soil enzymatic activities to vegetation structure and disturbance. The consistently higher values in the flowers treatment align with studies showing that plant diversity enhances microbial biomass, substrate availability and enzymatic turnover [77,78]. Conversely, reduced enzymatic activity in herbicide-managed soils is consistent with evidence that herbicides can suppress microbial activity, alter community composition and reduce nutrient cycling efficiency [79]. The particularly low Aminop values under herbicide suggest impaired N-mineralisation processes, which are highly sensitive to chemical disturbance [80].

3.4.3. PCA: Multivariate Structure of Biological Indicators

To integrate these responses, a PCA was performed to explore multivariate patterns in biological indicators (Tables 7 and 8). Whereas social sciences often require >70% explained variance, 50–70% is typical in soil ecology [56,66]. The correlation matrix was not positive-definite, indicating multicollinearity, which is expected in datasets comprising enzyme activities and microbial biomass. The model extracted two well-defined and ecologically meaningful components, explaining 65.8% of the total variance. Communalities were high (0.58–0.99), indicating that the model effectively captured the variance of most variables. Component 1 (PC1), biochemical activity axis (39.62% variance), was dominated by all extracellular enzyme activities (TE, Li-Es, Phos, Gluc and Aminop), representing microbial functional capacity. The extremely high loading of TE (0.994) confirms its role as a robust integrator of microbial functioning. Component 2 (PC2), biological community structure (26.20% variance), showed high loadings for MBC (0.760), mites (0.807), collembolans (0.741) and QBS-ar (0.585) and a negative loading for WSOC (−0.588). This axis represents soil biological community structure, integrating microbial biomass and mesofauna. The negative WSOC loading suggests that active biological communities rapidly consume soluble carbon, consistent with the MEMS framework [81].

Table 7. Total variance explained for biological indicators ($n = 31$).

Component	Initial Eigenvalues			Sums of Squared Loadings of the Rotation		
	Total	%	%	Total	%	%
PC1	4.285	42.854	42.854	3.962	39.619	39.619
PC2	2.296	22.964	65.819	2.620	26.200	65.819
PC3	0.924	9.235	75.054			
PC4	0.854	8.539	83.593			
PC5	0.564	5.640	89.233			
PC6	0.369	3.693	92.926			
PC7	0.276	2.759	95.686			
PC8	0.256	2.564	98.249			
PC9	0.175	1.751	100.000			

Table 8. Rotated component matrix for biological indicators.

Parameter ¹	Component	
	PC1	PC2
TE	0.994	0.079
Li-Es	0.851	0.233
Phos	0.870	−0.147
Gluc	0.782	0.267
Aminop	0.831	0.027
MBC	−0.039	0.760
WSOC	0.176	−0.588
QBS-ar	0.135	0.585
COLL	0.213	0.741
Mites	0.309	0.807

¹ TE: total enzymes; Li-Es: lipase–esterase; Phos: phosphatase; Gluc: glucosidase; Aminop: aminopeptidase; MBC: microbial biomass carbon (mg kg^{−1}); WSOC: water-soluble carbon (mg kg^{−1}); QBS-ar index, soil microarthropod index; COLL: collembolans.

3.4.4. PCA Biplot Interpretation and Treatment Positioning

The PCA biplot (Figure 7) illustrates the multivariate relationships among indicators. Enzymes formed a tight cluster of long vectors, indicating strong positive correlations and defining a biochemical functioning gradient. MBC and soil fauna formed a second cluster, defining a biological richness and trophic activity gradient. WSOC pointed in the opposite direction, reflecting its depletion in biologically active systems. Treatment distribution followed clear ecological patterns: flowers and legumes, upper-right quadrant (high enzymes + high fauna + high MBC); herbicide, lower-left quadrant (low enzymes + low fauna + low MBC); grasses and spontaneous vegetation, intermediate positions. This structure is typical of systems where vegetation cover enhances soil biological functioning, whereas herbicide-based management suppresses it [54–57,67–84].

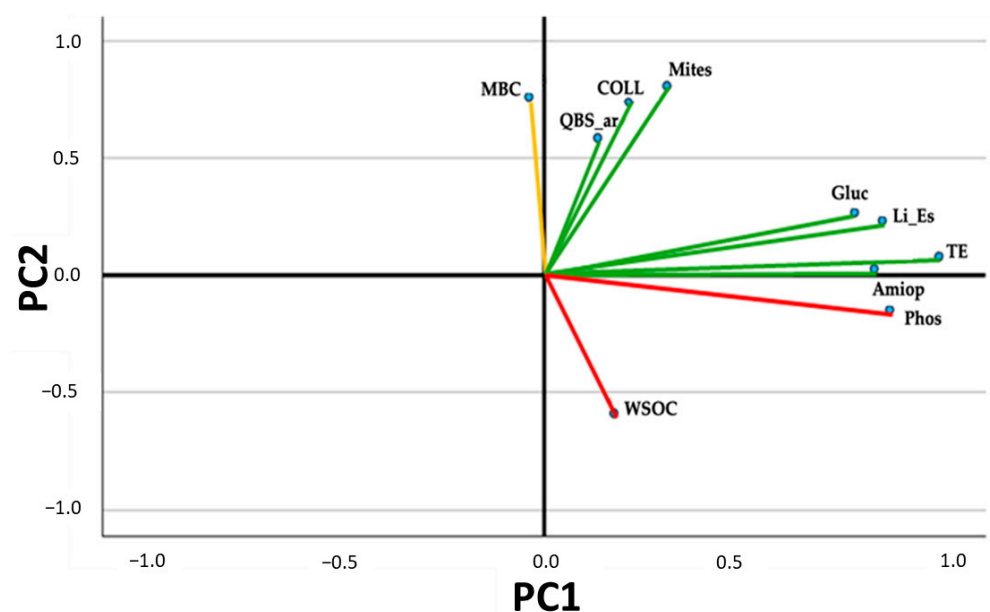


Figure 7. PCA biplot of soil biological indicators under different green undersown crop management systems. The first two principal components (PC1 and PC2) show the multivariate relationships among biological variables. Vectors represent the loadings of microbial biomass carbon (MBC), water-soluble organic carbon (WSOC), soil biological quality (QBS-ar), collembolans (COLL), mites, and enzyme activities (TE, total enzymes; Li-Es, lipase–esterase; Phos, phosphatase; Gluc, glucosidase; Aminop, aminopeptidase). The direction and length of each vector indicate the strength and sign of its contribution to each component, while the angles between vectors reflect correlations among variables. PC1 and PC2 together explain the major gradients of biological functioning across treatments, differentiating soils according to microbial activity, mesofauna abundance and enzymatic performance.

4. Conclusions

This study demonstrates that green GUCs substantially improve soil quality in Mediterranean calcareous persimmon orchards, where low organic matter, high pH and recurrent disturbance typically constrain biological functioning. Across 18 months of management, seeded undersown crops—particularly legumes and flower mixtures—consistently enhanced soil organic matter, microbial biomass carbon, enzyme activities and mesofauna abundance compared with herbicide-managed bare soil. These improvements reflect increased organic inputs, greater rhizodeposition and more favourable microhabitat conditions under vegetated covers.

Multivariate analyses revealed clear functional gradients associated with soil biological quality, organic fertility, organic matter quality and biological–mesofauna interactions. PCA and LDA both showed strong separation between GUC treatments and herbicide-managed soils, confirming that vegetation cover drives coherent shifts in soil functioning. Legumes and flower mixtures produced the most pronounced improvements, whereas herbicide treatments exhibited reduced microbial activity, lower mesofauna abundance and detectable residues of glyphosate and oxyfluorfen.

The second-stage analyses further highlighted the sensitivity of biochemical indicators to groundcover management. Enzyme activities, MBC and WSOC were markedly higher under GUCs, while herbicide-managed soils showed suppressed biochemical functioning. These patterns indicate that GUCs promote both microbial efficiency and trophic activity, reinforcing their role in enhancing soil health.

Overall, GUCs modulated soil chemistry, biological communities and functional processes in complementary ways, improving soil resilience in calcareous Mediterranean orchards. The consistent benefits observed across sites and treatments support the adoption of GUCs as a sustainable management strategy capable of restoring soil quality, reducing reliance on herbicides and strengthening ecosystem functioning in persimmon production systems.

By strengthening soil functioning, biodiversity and long-term resilience, GUCs offer a practical and scalable pathway towards more sustainable, climate-ready Mediterranean orchard systems. Future research should explore the long-term dynamics of these biological indicators under contrasting management regimes, with particular emphasis on multi-year responses, below-ground biodiversity and the interactions between microbial processes, mesofauna and soil physicochemical constraints.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/soilsystems10070082/s1>, Figure S1. Representative images of the different undersown crop treatments. Table S1. Sites, samples, treatments and sampling period (season). Table S2. Sites, samples, treatments, sampling period (season), soil characteristics and mesofauna at the first stage.

Author Contributions: Conceptualisation, C.O., Á.M.-M., D.S. and R.B.; methodology, all authors; validation, formal analysis and investigation, C.O., D.S., J.V.L. and R.B.; resources, D.S., Á.M.-M. and R.B.; data curation, C.O., D.S. and R.B.; writing—original draft, C.O. and R.B.; writing—review and editing, all authors; visualisation, C.O., J.V.L. and R.B.; supervision, Á.M.-M., D.S., C.L., J.V.L. and R.B.; project administration and funding acquisition, Á.M.-M., D.S. and R.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. Internal support was provided, in part, by the Unit of Research in Soils, Waste and Environment (SOREiMA, Prof. Dr. Rafael Boluda), University of València (OTR2016-16953INVES).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in this paper. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors gratefully acknowledge the Laboratory of Plant Production (Universitat Politècnica de València) and the SOREiMA Laboratory of the Plant Biology Department (University of València) for their technical assistance. We also thank David Sales for providing access to the experimental orchards.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

GUCs	Green undersown crops
SOM	Soil organic matter
N	Total nitrogen
C/N	C/N ratio
EC	Electrical conductivity
RR	Respiration rate
COLL	Collembolans
QBS-ar	Soil quality index, arthropods
PCA	Principal component analysis
LDA	Discriminant analysis
MBC	Microbial biomass carbon
WSOC	Water-soluble organic carbon
EA	Enzyme activities
TE	Total enzymes
Li-Es	Lipase–esterase
Phos	Phosphatase
Gluc	Glucosidase
Aminop	Aminopeptidase

References

1. García-Ruiz, J.M.; López-Moreno, J.I.; Vicente-Serrano, S.M.; Lasanta, T.; Beguería, S. Mediterranean water resources in a Global Change scenario. *Earth-Sci. Rev.* **2011**, *105*, 121–139. [\[CrossRef\]](#)
2. Cerdà, A.; González-Pelayo, O.; Giménez-Morera, A.; Jordán, A.; Pereira, P.; Novara, A.; Brevik, E.C.; Prosdocimi, M.; Mahmood-abadi, M.; Keesstra, S.; et al. The use of barley straw residues to avoid high erosion and runoff rates on persimmon plantations in Eastern Spain under low frequency—High magnitude simulated rainfall events. *Soil Res.* **2016**, *54*, 54–165. [\[CrossRef\]](#)
3. Blanco-Canqui, H.; Benjamin, J.G. Impacts of soil organic carbon on soil physical behaviour. In *Quantifying and Modeling Soil Structure Dynamics*; Logsdon, S., Berli, M., Horn, R., Eds.; American Society of Agronomy; Crop Science Society of America; Soil Science Society of America: Madison, WI, USA, 2013; Volume 3, pp. 11–40. [\[CrossRef\]](#)
4. Basche, A.D.; DeLonge, M.S. Comparing infiltration rates in soils managed with conventional and alternative farming methods: A meta-analysis. *PLoS ONE* **2019**, *14*, e0215702. [\[CrossRef\]](#) [\[PubMed\]](#)
5. European Commission. EU Soil Protection Strategy for 2030. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. Available online: <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A52021DC0699> (accessed on 1 May 2026).
6. Nantes, L.S.; Bonini, C.d.S.B.; Santos, M.A.; da Silva Alves, L.; Lourencetti, J.; Bonini Neto, A.; de Souza, J.A.L.; da Silva Lunardelli, G.A.; Silva, M.B.; Alvares, D.V.; et al. Exposed Subsoil Under Recovery for 30 Years: Influence of Management Strategies on Soil Physical Attributes and Organic Matter. *Soil Syst.* **2025**, *9*, 17. [\[CrossRef\]](#)
7. Strauss, S.; Kadyampakeni, D.; Kaniserry, R.; Wade, T.; Diepenbrock, L.; Popenoe, J. Cover Crops for Citrus. Citrus Industry Magazine. 2019. Available online: <https://citrusindustry.net/2019/04/09/cover-crops-for-citrus/> (accessed on 1 May 2026).
8. Altieri, M. *Agroecology: The Scientific Basis of Alternative Agriculture*; Westview Special Studies in Agriculture Science and Policy; Westview Press: Boulder, CO, USA, 1987.

9. Yousefi, M.; Dray, A.; Ghazoul, J. Assessing the effectiveness of cover crops on ecosystem services: A review of the benefits, challenges, and trade-offs. *Int. J. Agric. Sustain.* **2024**, *22*, 2335106. [CrossRef]
10. Vicent, A.; Mira, J.L.; Dalmau, V. Estrategias para la Gestión Integrada de las Enfermedades Causadas por *Phytophthora* en Cítricos. *VIDA Rural*. 2016, pp. 38–43. Available online: https://redivia.gva.es/bitstream/handle/20.500.11939/6552/2016_Vicent_Estrategias.pdf?sequence=4&isAllowed=y (accessed on 29 April 2026).
11. Bello, A.; López-Pérez, J.A.; Díez-Rojo, M.A.; López-Cepero, J.; García-Álvarez, A. *Principios Ecológicos en la Gestión de los Agrosistemas*; Arbor Ciencia, Pensamiento y Cultura CLXXXIV 729; CSIC: Madrid, Spain, 2008. Available online: <https://arbor.revistas.csic.es/index.php/arbor/article/view/158/158> (accessed on 29 April 2026).
12. Alkorta, I.; Aizpurua, A.; Riga, P.; Albizu, I.; Amezcua, I.; Garbisu, C. Soil enzyme activities as biological indicators of soil health. *Rev. Environ. Health* **2003**, *18*, 65–73. [CrossRef] [PubMed]
13. Orts, C. Gestión de la Mejora del Suelo con Cubiertas Vegetales en el Cultivo del Caqui (*Dyospiros kaki* Lf.): Integración de Técnicas Clásicas, Herramientas de Geoprocesamiento, Teledetección y Espectrorradiometría. Ph.D. Thesis, Universitat Politècnica de València, Valencia, Spain, 2025. [CrossRef]
14. Büchi, L.; Wendling, M.; Amossé, C.; Necpalova, M.; Charles, R. Importance of cover crops in alleviating negative effects of reduced soil tillage and promoting soil fertility in a winter wheat cropping system. *Agric. Ecosyst. Environ.* **2018**, *256*, 92–104. [CrossRef]
15. Cottney, P.; Black, L.; White, E.; Williams, P.N. A review of supporting evidence, limitations and challenges of using cover crops in agricultural systems. *Agriculture* **2025**, *15*, 1194. [CrossRef]
16. Porta, J.; López-Acevedo, M.; Poch, R. *Edafología: Uso y Protección de Suelos*; Ediciones Mundi-Prensa: Madrid, Spain, 2014; pp. 211–247.
17. Lal, R. Soil organic matter content and crop yield. *J. Soil Water Conserv.* **2020**, *75*, 27A–32A. [CrossRef]
18. Piotrowska, A.; Wilczewski, E. Effects of catch crops cultivated for green manure and mineral nitrogen fertilization on soil enzyme activities and chemical properties. *Geoderma* **2012**, *189–190*, 72–80. [CrossRef]
19. Tiquia, S.M. Evolution of extracellular enzyme activities during manure composting. *J. Appl. Microbiol.* **2002**, *92*, 764–775. [CrossRef] [PubMed]
20. Xiong, J.; He, Z.; Shi, S.; Kent, A.; Deng, Y.; Wu, L.; Van Nostrand, J.D.; Zhou, J. Elevated CO₂ shifts the functional structure and metabolic potentials of soil microbial communities in a C4 agroecosystem. *Sci. Rep.* **2015**, *5*, 9316. [CrossRef] [PubMed]
21. Naylor, D.; Sadler, N.; Bhattacharjee, A.; Graham, E.B.; Anderton, C.R.; McClure, R.; Lipton, M.; Hofmockel, K.S.; Jansson, J.K. Soil microbiomes under climate change and implications for carbon cycling. *Annu. Rev. Environ. Resour.* **2020**, *45*, 29–59. [CrossRef]
22. Li, S.; Li, D.; Li, J.; Li, G.; Zhang, G. Evaluation of humic substances during co-composting of sewage sludge and corn stalk under different aeration rates. *Bioresour. Technol.* **2017**, *245*, 1299–1302. [CrossRef] [PubMed]
23. Filser, J. The role of collembola in carbon and nitrogen cycling in soil: Proceedings of the Xth international Colloquium on Apterygota, České Budějovice 2000: Apterygota at the Beginning of the Third Millennium. *Pedobiologia* **2002**, *46*, 234–245. [CrossRef]
24. Crystal Ornelas, R.; Thapa, R.; Tully, K.L. Soil organic carbon is affected by organic amendments, conservation tillage, and cover cropping in organic farming systems: A meta-analysis. *Agric. Ecosyst. Environ.* **2021**, *312*, 107356. [CrossRef]
25. Gross, A.; Glase, B. Meta-analysis on how manure application changes soil organic carbon storage. *Sci. Rep.* **2021**, *11*, 5516. [CrossRef] [PubMed]
26. He, Q.; Lu, C.; Cowie, A.; Zhao, S.; Liu, D.L.; Yi, B.; Shi, L.; Zhang, S.; Qiu, T.; Shi, Y.; et al. Optimizing cover cropping application for sustainable crop production. *npj Sustain. Agric.* **2025**, *3*, 10. [CrossRef]
27. Salisu, M.A.; Ampim, P.A.Y.; Oyebamiji, Y.O.; Kotochi, A.B.A.; Imoro, M.M. Cover Crops Enhance Soil Organic Carbon and Soil Quality for Sustainable Crop Yield: A Systematic Review. *Agronomy* **2025**, *15*, 2865. [CrossRef]
28. Poelplau, C.; Don, A. Carbon sequestration in agricultural soils via cultivation of cover crops—A meta-analysis. *Agric. Ecosyst. Environ.* **2015**, *200*, 33–41. [CrossRef]
29. Gerke, J. The central role of soil organic matter in soil fertility and carbon storage. *Soil Sys.* **2022**, *6*, 33. [CrossRef]
30. Sanz-Cobena, A.; Lassaletta, L.; Aguilera, E.; del Prado, A.; Garnier, J.; Billen, G.; Iglesias, A.; Sánchez, B.; Guardia, G.; Abalos, D.; et al. Strategies for greenhouse gas emissions mitigation in Mediterranean agriculture: A review. *Agric. Ecosyst. Environ.* **2017**, *238*, 5–24. [CrossRef]
31. Plaza-Bonilla, D.; Nogué-Serra, I.; Raffaillac, D.; Cantero-Martínez, C.; Justes, É. Carbon footprint of cropping systems with grain legumes and cover crops: A case-study in SW France. *Agric. Syst.* **2018**, *167*, 92–102. [CrossRef]
32. Kaye, J.P.; Quemada, M. Using cover crops to mitigate and adapt to climate change. A Review. *Agron. Sustain. Dev.* **2017**, *37*, 4. [CrossRef]
33. Novara, A.; Minacapilli, M.; Santoro, A.; Rodrigo-Comino, J.; Carrubba, A.; Sarno, M.; Venezia, G.; Gristina, L. Real cover crops contribution to soil organic carbon sequestration in sloping vineyard. *Sci. Total Environ.* **2019**, *652*, 300–306. [CrossRef] [PubMed]
34. Parisi, V. La qualità biologica del suolo. Un metodo basato sui microartropodi. *Acta Nat. L'Ateneo Parm.* **2001**, *37*, 105–114.

35. Menta, C.; Conti, F.; Pinto, S.; Bodini, A. Soil Biological Quality index (QBS): 15 years of application at global scale. *Ecol. Indic.* **2018**, *85*, 773–780. [CrossRef]
36. IUSS-WRB (IUSS Working Group WRB). *World Reference Base for Soil Resources 2022: International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*, 4th ed.; International Union of Soil Sciences (IUSS): Vienna, Austria, 2022.
37. MAPA (Ministerio de Agricultura, Pesca y Alimentación). *Mapa de Suelos de España. Escala 1:1.000.000*; Ministerio de Agricultura, Pesca y Alimentación: Madrid, Spain, 1994.
38. ESDAC (European Soil Data Centre). *Soil Map of Europe (1:1,000,000)*; European Commission, Joint Research Centre (JRC): Ispra, Italy, 2020; Available online: <https://esdac.jrc.ec.europa.eu> (accessed on 3 July 2026).
39. Ivanova, N. Global overview of the application of the Braun-Blanquet approach in research. *Forests* **2024**, *15*, 937. [CrossRef]
40. Alcaraz, F.J. *El Método Fitosociológico. Vegetación y Tipos de Hábitats de Interés en la Unión Europea. Tema 3*; Universidad de Murcia: Murcia, Spain, 2011.
41. Walkley, A.; Black, J.A. An examination of the Detjareff method for determining soil organic matter and a proposed modification on the chromic acid titration methods. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
42. Nelson, D.W.; Sommers, L.E. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis, Part 3*; SSSA Book Series; Wiley: Hoboken, NJ, USA, 1996.
43. Ministerio de Agricultura, Pesca y Alimentación (MAPA). *Métodos Oficiales de Análisis. Tomo III: Suelos*; Gobierno de España: Madrid, Spain, 1994.
44. PP Systems. *EGM-5 Portable CO₂ Gas Analyzer: Operation Manual*; Version 1.03; PP Systems: Amesbury, MA, USA, 2018. Available online: https://ppsystems.com/download/technical_manuals/80109-1-EGM-5_Operation_V103.pdf (accessed on 4 July 2026).
45. Norman, J.M.; Kucharik, C.J.; Gower, S.T.; Baldocchi, D.D.; Crill, P.M.; Rayment, M.; Savage, K.; Striegl, R.G. A comparison of six methods for measuring soil-surface carbon dioxide fluxes. *J. Geophys. Res.* **1997**, *102*, 28771–28777. [CrossRef]
46. Yakovchenko, V.P.; Sikora, L.J. Modified dichromate method for determining low concentrations of extractable organic carbon in soil. *Commun. Soil Sci. Plant Anal.* **1998**, *29*, 421–433. [CrossRef]
47. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]
48. Wu, J.; Joergensen, R.G.; Pommerening, B.; Chaussod, R.; Brookes, P.C. Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. *Soil Biol. Biochem.* **1990**, *22*, 1167–1169. [CrossRef]
49. Tullgren, A. Ein sehr einfacher Ausleseapparat für terricole Tierfaunen. *Z. Angew. Entomol.* **1918**, *4*, 149–150. [CrossRef]
50. Parisi, V.; Menta, C.; Gardi, C.; Jacomini, C.; Mozzanica, E. Microarthropod communities as a tool to assess soil quality and biodiversity: A new approach in Italy. *Agric. Ecosyst. Environ.* **2005**, *105*, 323–333. [CrossRef]
51. Boluda, R.; Roca-Pérez, L.; Iranzo, M.; Gil, C.; Mormeneo, S. Determination of enzymatic activities using a miniaturized system as a rapid method to assess soil quality. *Eur. J. Soil Sci.* **2014**, *65*, 286–294. [CrossRef]
52. Boluda, R.; Alejos-Campo, A.; Fernández-Gómez, E.; Gamón, M.; Roca-Pérez, L.; Andreu-Sánchez, O. A comparison of the QUECHERSER mega method for pesticide determination in loamy-clayed soil and the effect of organic amendments on pendimethalin, oxyfluorfen, and trifloxystrobin soil persistence. *J. Xenobiot.* **2025**, *15*, 98. [CrossRef] [PubMed]
53. Visconti, F.; Peiró, E.; Baixauli, C.; de Paz, J.M. Spontaneous plants improve the inter-row soil fertility in a citrus orchard but nitrogen lacks to boost organic carbon. *Environments* **2022**, *9*, 151. [CrossRef]
54. FAO (Food and Agriculture Organization of the United Nations). *Voluntary Guidelines for Sustainable Soil Management*; FAO: Rome, Italy, 2017.
55. García, C.; Hernández, T.; Costa, F. Soil microbial activity under different environmental conditions in Mediterranean ecosystems. *Soil Biol. Biochem.* **1997**, *29*, 17–25. [CrossRef]
56. Morales-Salmeron, L.; Fernández-Boy, E.; León, R.; Navarro-Fernández, C.M.; Dominguez, M.T. Influence of the quality of organic amendments on Mediterranean agricultural soils following a drought episode. *J. Environ. Manag.* **2025**, *380*, 125093. [CrossRef] [PubMed]
57. Bardgett, R.D.; van der Putten, W.H. Belowground biodiversity and ecosystem functioning. *Nature* **2014**, *515*, 505–511. [CrossRef] [PubMed]
58. Manzoni, S.; Taylor, P.; Richter, A.; Porporato, A.; Ågren, G.I. Environmental and stoichiometric controls on microbial carbon-use efficiency. *New Phytol.* **2012**, *196*, 79–91. [CrossRef] [PubMed]
59. Chagnon, M.; Paré, D.; Hébert, C.; Camiré, C. Effects of experimental liming on collembolan communities and soil microbial biomass in a southern Quebec sugar maple (*Acer saccharum* Marsh.). *Appl. Soil Ecol.* **2001**, *17*, 81–90. [CrossRef]
60. Nielsen, U.N.; Ayres, E.; Wall, D.H.; Bardgett, R.D. Soil biodiversity and carbon cycling: A review and synthesis of studies examining diversity–function relationships. *Eur. J. Soil Sci.* **2010**, *62*, 105–116. [CrossRef]
61. Hopkin, S.P. *Biology of the Springtails (Collembola)*; Oxford University Press: Oxford, UK, 1997. [CrossRef]
62. Legendre, P.; Legendre, L. *Numerical Ecology*, 3rd ed.; Elsevier: Amsterdam, The Netherlands, 2012; 1007p.
63. McLachlan, G.; Peel, D. *Finite Mixture Models*; Wiley: New York, NY, USA, 2004; 456p.

64. Six, J.; Conant, R.T.; Paul, E.A.; Paustian, K. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant Soil* **2002**, *241*, 155–176. [\[CrossRef\]](#)
65. Lehmann, J.; Kleber, M. The contentious nature of soil organic matter. *Nature* **2015**, *528*, 60–68. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Manzoni, S.; Trofymow, J.A.; Jackson, R.B.; Porporato, A. Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. *Ecol. Monogr.* **2010**, *80*, 89–106. [\[CrossRef\]](#)
67. Bünemann, E.K.; Bongiorno, G.; Bai, Z.; Creamer, R.E.; De Deyn, G.; de Goede, R.; Fleskens, L.; Geissen, V.; Kuyper, T.W.; Mäder, P. Soil quality—A critical review. *Soil Biol. Biochem.* **2018**, *120*, 105–125. [\[CrossRef\]](#)
68. Nannipieri, P.; Kandeler, E.; Ruggiero, P. Enzyme activities and microbiological and biochemical processes in soil. In *Enzymes in the Environment: Activity, Ecology, and Applications*; Burns, R.G., Dick, R.P., Eds.; Marcel Dekker: New York, NY, USA, 2002; pp. 1–33.
69. García Sánchez, I.S.; García Orenes, F.; Morugán Coronado, A. Actividades Enzimáticas Determinadas en Suelos Bajo Manejo Agrícola Sostenible. Bachelor's Thesis, Universidad Miguel Hernández de Elche, Elche, Spain, 2017. Available online: <https://hdl.handle.net/11000/4299> (accessed on 29 April 2026).
70. Ochoa, V.; Hinojosa, B.; Gómez-Muñoz, B.; García-Ruiz, R. *Actividades Enzimáticas como Indicadores de Calidad del Suelo en Agroecosistemas Ecológicos*; Universidad de Jaén: Jaén, Spain, 2007. Available online: <https://revistaselectronicas.ujaen.es/index.php/ininv/article/view/251> (accessed on 29 April 2026).
71. Finney, D.M.; White, C.M.; Kaye, J.P. Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. *Agron. J.* **2016**, *108*, 39–52. [\[CrossRef\]](#)
72. Tiemann, L.K.; Grandy, A.S.; Atkinson, E.E.; Marin-Spiotta, E.; McDaniel, M.D. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecol. Lett.* **2015**, *18*, 761–771. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Drinkwater, L.E.; Wagoner, P.; Sarrantonio, M. Legume-based cropping systems have reduced carbon and nitrogen losses. *Nature* **1998**, *396*, 262–265. [\[CrossRef\]](#)
74. Kaye, J.P.; Hart, S.C. Competition for nitrogen between plants and soil microorganisms. *Trends Ecol. Evol.* **1997**, *12*, 139–143. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Zaller, J.G.; Heigl, F.; Ruess, L.; Grabmaier, A. Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. *Sci. Rep.* **2014**, *4*, 5634. [\[CrossRef\]](#) [\[PubMed\]](#)
76. Ponge, J.F. Plant–soil feedbacks mediated by humus forms: A review. *Soil Biol. Biochem.* **2013**, *57*, 1048–1060. [\[CrossRef\]](#)
77. Allison, S.D.; Martiny, J.B.H. Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11512–11519. [\[CrossRef\]](#) [\[PubMed\]](#)
78. Eisenhauer, N.; Beßler, H.; Engels, C.; Gleixner, G.; Habekost, M.; Milcu, A.; Partsch, S.; Sabais, A.C.W.; Scherber, C.; Steinbeiss, S.; et al. Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* **2010**, *91*, 485–496. [\[CrossRef\]](#) [\[PubMed\]](#)
79. Rose, M.T.; Cavagnaro, T.R.; Scanlan, C.A.; Rose, T.J.; Vancov, T.; Kimber, S.; Kennedy, I.R.; Kookana, R.S.; Van Zwieten, L. Impact of herbicides on soil biology and function. *Adv. Agron.* **2016**, *136*, 133–220. [\[CrossRef\]](#)
80. Burns, R.G.; DeForest, J.L.; Marxsen, J.; Sinsabaugh, R.L.; Stromberger, M.E.; Wallenstein, M.D.; Weintraub, M.N.; Zoppini, A. Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biol. Biochem.* **2013**, *58*, 216–234. [\[CrossRef\]](#)
81. Cotrufo, M.F.; Wallenstein, M.D.; Boot, C.M.; Deneff, K.; Paul, E. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Glob. Change Biol.* **2012**, *19*, 988–995. [\[CrossRef\]](#) [\[PubMed\]](#)
82. Gamarra, C.; Díaz, M.; Vera de Ortiz, M.; Galeano, M.P.; Cabrera, A. Relación carbono-nitrógeno en suelos de sistemas silvopastoriles del Chaco paraguayo. *Rev. Mex. Cienc. For.* **2018**, *9*, 4–26. [\[CrossRef\]](#)
83. Wang, H.; Zhong, L.; Liu, J.; Liu, X.; Xue, W.; Liu, X.; Yang, H.; Shen, Y.; Li, J.; Sun, Z. Systematic analysis of the effects of different green manure crop rotations on soil nutrient dynamics and bacterial community structure in the Taihu Lake Region, Jiangsu. *Agriculture* **2024**, *14*, 1017. [\[CrossRef\]](#)
84. Aronsson, H.; Hansen, E.; Thomsen, I.K.; Liu, J.; Øgaard, A.; Kankanen, H.; Ulén, B. The ability of cover crops to reduce nitrogen and phosphorus losses from arable land in southern Scandinavia and Finland. *Soil Water Conserv.* **2016**, *71*, 41–55. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.