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Physical, Chemical, and Biological Indicators of Soil Quality in Mediterranean Vineyards under Contrasting Farming Schemes

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Abstract: The soil of most Spanish vineyards is strongly eroded and carbon depleted and is very poor in biodiversity. Growing evidence of the negative impacts of soil degradation on climate change mitigation, water quality, and plant production is pushing a shift from intensive viticulture to more sustainable management strategies of the vineyards. Among them, minimum impact and regenerative viticulture are gaining ground. However, field data are still necessary to assess the real effect of these new farming schemes on soil carbon stocks and soil functional biodiversity. We compared soil quality at three vineyards managed under intensive, regenerative, and minimum impact strategies using physical, chemical, and biological indicators. Soil carbon stocks were 2.3 and 3.4 times greater in the regenerative and the minimal impact vineyards than in the intensive vineyard, respectively. Soil biota was particularly favored by regenerative viticulture, with 26.2 times more protists, 3.1 times more nematodes, and 29.4 more microarthropods in the regenerative than in the intensive vineyard. Our results indicate that the ecological intensification of agricultural practices is highly promising to restore degraded agricultural soils under Mediterranean conditions. We also propose cost-effective soil bioindicators sensitive to agricultural management for their possible inclusion in soil monitoring programs.

Keywords: regenerative farming; minimum impact farming; tillage; cover crops; soil carbon stocks; soil biodiversity

1. Introduction

Soil contains the largest terrestrial organic carbon pool (2400 Pg C to 2 m depth), which is three times the amount of CO_2 currently in the atmosphere [1]. It also provides most of the ecosystem services we rely upon, with soil biodiversity being the regulating agent behind this [2].

Soil ecological degradation can be defined as the disruption of the functions that support the net productivity on the ecosystem [3]. Across Europe, intensive agriculture is among the main drivers of soil physical and chemical degradation, erosion, and organic matter loss [4]. In turn, the decay of soil organic carbon (SOC), together with the use of agrochemicals, is at the root of the decline in the biomass and functional diversity of the soil biota [5]. However, belowground biodiversity is crucial for agricultural soils since it correlates with soil multifunctionality and with most soil functions that underpin plant production [6]. Intensive farming also lessens the resilience of the soil food web to climate change and, in particular, its ability to withstand drought [7]. It had been posited that the negative effects of intensive management on the soil biota are restricted to the largest belowground animals [8], but recent molecular analyses show that soil bacteria, fungi, and protists are even more affected than metazoans [9].

Vineyards are the form of agricultural use that causes the greatest rates of soil loss under Mediterranean climate, with measured values of 2.4 to 9.3 Mg ha^{-1} year^{-1} [10].

These high rates are attributable to frequent cultivation on steep slopes, tillage, mineral fertilization, and the absence of soil plant cover in inter-row spaces [11]. Tillage alters the soil carbon balance through negative effects on the methanotrophic microorganisms of the soil, thus lessening its CH_4- oxidizing capacity [12] and its potential as a carbon sink. Due to erosion, limited organic inputs, and high mineralization rates, intensive vineyards often behave as net CO_2 emitters [13]. However, when properly managed, they offer great potential for carbon sequestration in trees and soil [14]. In the latter, sequestration not only helps remove atmospheric CO_2, but also improves the environment (e.g., soil structure, aggregate stability, pore space water retention capacity, etc.) on which the living soil community relies upon [4].

Several SOC-centered agricultural practices have been proposed to recarbonize agricultural soils, such as tillage cessation or reduction, mulching, cover and catch crop seeding, and organic fertilization [15,16]. No-tillage and reduced tillage can increase SOC in croplands, with sequestration potentials of 0.1 to 1.0 t C ha^{-1} year^{-1} [17], due to the larger amount of SOC physically protected inside the microaggregates of no-tilled soils, which reduces SOC turnover [18,19]. However, neutral or even negative effects of tillage cessation on SOC stocks have been described under specific environmental conditions [20–22]. With a few reported exceptions [23,24], cover crops enhance SOC content, due to increasing plant inputs from above and below ground. The size of this effect varies with soil properties and climate [16] and is sensitive to crop type and agricultural management (e.g., plant cover composition and diversity) [25,26]. Organic fertilization also increases SOC stocks in woody crops [27,28], with different results depending on the supplied biomass. Green compost is the best option, while manure might have little or no effect unless combined with cover crops [29,30].

Besides enhancing SOC content, the above-mentioned practices are meant to increase soil biodiversity [31], with varying success for different groups of soil inhabitants [32]. Tillage cessation and reduction favor belowground invertebrates and mycorrhiza [33]. When plant residues are left to decompose on the ground, they benefit generalist predators and contribute to pest control [34]. Organically managed soils with high SOC content show high levels of microbial biomass and diversity [35], two key drivers of the resistance of soil microbial communities to disturbance [36].

When applied alone, many of these agricultural practices regarded as "sustainable" often generate little benefit compared with intensive methods. In fact, restoring the multifunctionality of degraded agricultural soils requires implementing integrated management schemes that wisely combine complementary actions [27,37]. Minimum impact (also known as "low-input") and regenerative farming are among these alternative schemes. "Minimum impact" farming refers to those systems managed with limited use of external inputs (including fossil fuels, agrochemicals, or mineral fertilizers), and that generally rely on the improved management of on-farm resources. "Regenerative agriculture" is a farming approach that seeks to close nutrient cycles and to increase farm biodiversity and resilience, which applies locally tailored combinations of practices that contribute to sequestering carbon, to building soil fertility and health, and to improving the hydrological cycle [38]. A combination of tillage cessation (or significant reduction), cover and catch crops, enhanced plant diversity, and integration between animal raising and cropping are the main principles of this strategy [39]. There is abundant information about the effects of each of the main sustainable agricultural practices in isolation on soil carbon stocks. However, the effect of their joint application under integrated agricultural schemes on carbon sequestration and soil biodiversity are much less studied [40,41]. More field data are necessary to inform agricultural planning at the landscape scale.

Physical and chemical indicators that provide information about soil fertility are commonplace [42] but, despite increasing appeals for action [43–45], there is a striking absence of indicators addressing the effects of agricultural management on the soil biota [46]. An important drawback for implementing bioindicator systems useful for policy making

is the scarcity of soil biodiversity data at the management scale, which impedes setting reference values and defining desired trajectories and rates of change [47,48].

The aim of this work is (a) to provide indicators of soil carbon stocks and soil biodiversity that are appropriate to evaluate farming schemes in Mediterranean vineyards, and (b) to compare vineyards managed under intensive and sustainable schemes based on these indicators. We hypothesize that (i) the effects of management on soil biodiversity can be measured by sensitive bioindicators that are affordable in terms of cost and work effort and that (ii) both minimum impact and regenerative viticulture contribute to improve soil biodiversity by increasing soil C stocks and by reversing soil physical and chemical deterioration.

2. Materials and Methods

2.1. Study Site

This study was conducted in an agricultural zone of the Girona province, in Catalonia, Spain. The topography is smooth, and the climate is typically Mediterranean, with 15.2 °C of mean annual temperature, 703 mm of annual precipitation, and dry summers characterized by soil water deficit.

We selected three adjacent vineyards that occupy a homogeneous spot of well drained Haplic Regosols developed on silty, clay, and sandy sediments from the Pliocene. The three fields are totally comparable in terms of climate, topography, geological substrate, and soil type. After a long-lasting shared history of intensive viticulture, the three fields currently undergo contrasting management strategies: intensive management (INT), regenerative management (REG), and minimum impact management (MIN).

In the intensive vineyard (42°19′06.6″ N, 3°04′42.2″ E, 27 m.a.s.l., 7100 m^2 area) (Figure 1a), the vine trees are 30 years old, and the management has always been intensive: soil is tilled at 20 cm five times a year. Soil is fertilized with 150 kg ha^{-1} of NPK. Weeds are eliminated with glyphosate, and plant pests are controlled with chemical pesticides and systemic fungicides. In the regenerative vineyard (42°19′09.0″ N 3°04′41.8″ E, 27 m.a.s.l., 8300 m^2 area) (Figure 1b), the vine trees are 30 years old. The management had been intensive until 2015. Since then, under regenerative principles, cultivation entails no tillage, cation regulation, and permanent cover crops made of a mixture of *Lolium rigidum*, *Dactylis glomerata*, *Festuca arundinacea*, *Onobrychis viciifolia*, *Vicia sativa*, *Trifolium alexandrinum,* and *Sinapis alba*. The soil is fertilized with calf manure (6 t ha^{-1}). In spring and summer, the herbs growing in the inter-rows are smashed with a crop roller, and the space between vines in the same row is cleared up to minimize competition. The soil is decompacted in winter with air injectors, and humic acids are added. Microbial teas that are locally produced [49] are applied. Fungal attacks are prevented with copper and sulfur, and insect pests are controlled with pheromones. In the minimum impact vineyard (42°19′01.5″ N 3°04′36.5″ E, 27 m.a.s.l, 6700 m^2 area) (Figure 1c), the vines are 50 years old. The field was intensively managed until 2007, when tillage was suppressed and the vegetation was allowed to grow wild between vines. The herbaceous cover is mown yearly with a lightweight mower, and plant residues are allowed to decompose in situ. Pruning remains are removed from the field and burnt. Bordeaux broth and sulfur are regularly applied to prevent fungal attacks.

All three vineyards are rainfed, and the vines are planted 1.5 m away from each other in rows separated by 2.5 m wide inter-rows. The vine trees are trellised in the intensive and regenerative vineyards but are allowed to develop their natural shape in the minimum management vineyard.
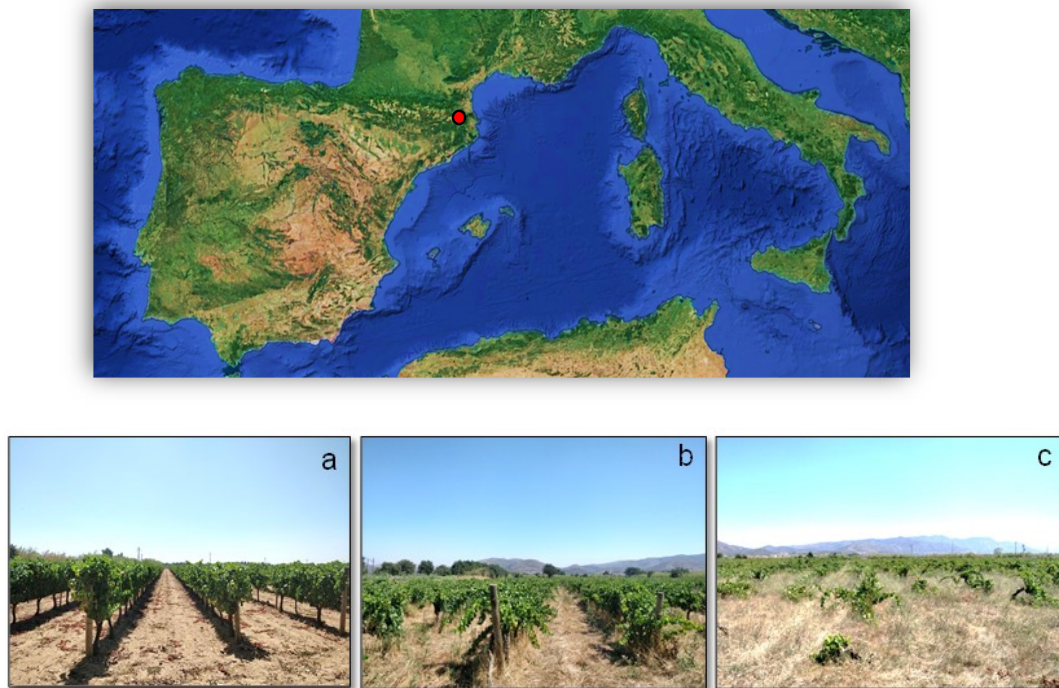
Figure 1. Geographical localization of the study area in the Girona province (Catalonia, Spain), and view of the three vineyards under (**a**) intensive management, (**b**) regenerative management, and (**c**) minimum impact management, respectively.

2.2. Sampling Design

Real replication is often impossible in farming and grazing field studies. Due to the spatial soil variability of the region, to the nature of the farming activity, and to the predominance of intensive agriculture, we were not able to replicate our three vineyards. Although the samples collected within each field were, strictly speaking, pseudo-replicates, the inter-sample distance was large enough to consider them independent from each other [50,51].

We used a systematic line transect sampling design, with transects placed in a zigzag fashion. At the center of each vineyard, we delimited a 30 × 30 m area containing 10 vine rows. At each area, following a diagonal direction, we established 16 pairs of sampling points, with all points placed in the space between two vine trees (Figure 2). The diagonal distribution of the sampling points and their location beneath the trees was intended to hedge the spatial variability at each field while avoiding uncontrollable disturbances due to the passing of machinery between trellises.
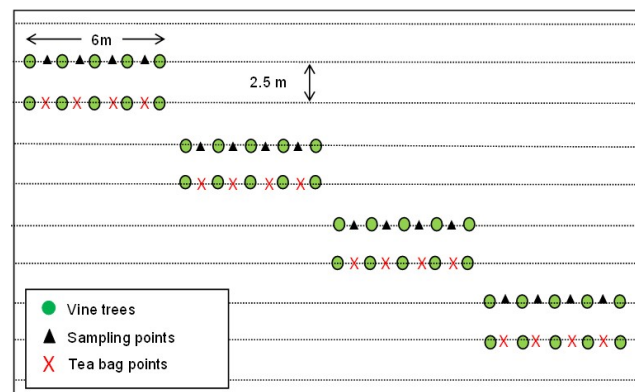

Figure 2. Sampling design at each vineyard.

In May 2019, we visited the vineyards and buried one pair of tea bags (one rooibos and one green tea bag (16 pairs of bags per vineyard) at the "tea bag" sampling points (Figure 2). All bags were buried at a depth of 8 to 10 cm and allowed to decompose belowground for 85 days.

In July 2019, we revisited the fields for sampling. We took three soil cores (25 cm^2 section and 15 cm long) at each of the 16 sampling points of each vineyard (Figure 2). The first sample was allocated to soil physical and chemical analyses; the second to soil microarthropods analyses; and the third to soil nematode analyses, soil incubation, extraction of soil protists, and soil microbial community profiling. A fourth core (5 cm Ø and 5 cm long cylindrical probes) was taken for a bulk density measurement. All samples were transported to the CREAF labs in coolers for immediate processing.

2.3. Lab Analyses

Dry soil samples sieved at 2 mm were analyzed for water content, texture (gravimetric method), pH (water, 1:2.5), electric conductivity (extract 1:5), carbonates, nitrogen (Kjeldahl), available phosphorus (Olsen), and cations (Ca, Na, K and Mg) (ICP-OES Plasma-Mass inductively coupled plasma-atomic emission spectrometry). Aggregate stability was measured by the Mean Weight Diameter (MWD), obtained by the wet sieving method [52,53]. Total organic carbon (TOC) content was assessed in a Thermo Scientific FLASH 2000 CHNS analyzer after removing the carbonates by acidification. The size of the labile and recalcitrant C pools was obtained by incubation of 50 g soil samples (sieved at 2 mm) in sealed Mason jars (25 °C, 60% WHC, in the dark) for 275 days. The CO_2 evolved was measured in the atmosphere of the headspace of the jars at increasing time intervals by infrared absorption spectrometry. We estimated the size of the active C pool by fitting the curve of the CO_2-C evolved per unit time to a two-pool first-order equation. The size of the recalcitrant C pool was estimated from the difference between TOC and labile C.

A community-level physiological profiling of the soil microbes was done with MicroResp™ [54] using the following substrates: two simple sugars (D-glucose, D-fructose), one disaccharide (sucrose), one polysaccharide (cellulose), three amino acids (γ-aminobutyric acid, L-proline, L-arginine), three carboxylic acids (α-ketoglutarate, citric acid, L-malic acid), one aromatic carboxylic acid (protocatechuic acid), one polymer (a-cyclodextrin), one chiral (mannitol), one polyol (glycerol), and a sugar alcohol (meso-erythritol). We used original MicroResp™ plates (https://www.microresp.com/), and the substrates were purchased from Sigma Aldrich. Plate reading was performed with a microplate reader spectrophotometer at 570 nm. Due to budgetary limitations, the microbial physiological profiling was only performed in half of the 16 soil samples taken at each vineyard. We estimated the abundance of soil bacteria (bacterial cells/m^2) and fungi (mm^3 hyphae/m^2) by direct counting in slides under an epifluorescence microscope and divided the resulting fungal biomass by 10 to assess only its live fraction, since the epifluorescence method estimates not only living bacteria, but also both the dead and living fungi [55].

We estimated the abundance of the three main groups of protists (ciliates, amoebas, and flagellates) by the most probable number method [56]. We extracted the nematodes from the soil samples with Baermann funnels for 72 h and sorted them into feeding groups. We extracted the microarthropods in Berlese funnels for 7 days and sorted them into functional groups based on common trophic positions and life traits. We calculated the carbon abundance of every group of the soil biota by multiplying its abundance by one-half of the individual body weight attributed to the group (see Table A1 in Appendix A), assuming that 50% of the dry weight of the living biomass is made of carbon.

We used the "Tea Bag" method to compare our vineyards for the effectiveness of their soil in decomposing plant residues. The method is based on the differential in decomposition between the fast-decomposing leaves of *Camellia sinensis* (green tea) and the slow-decomposing leaves of *Aspalathus linearis* (rooibos tea) for about three months. Following the original protocol of the test [57], we used Lipton rooibos tea (EAN: 87 22700 18843 8) and Lipton green tea (EAN: 87 22700 05552 5) as proxies of low- and high-quality litter,

respectively, based on differences in their chemical quality and recalcitrance to biological decomposition. We oven-dried a set of 20 bags of each type for five days at 40 °C and weighed their dry tea content. After 85 days of incubation in the soil of the vineyards, we retrieved the bags, let them dry in the oven, and weighed the tea they contained. From these data, we calculated the decomposition rate constant of the labile fraction of the bags' content as follows [58]:

$$K_r = -\text{LN}\left(\frac{X_r - (1 - a_r)}{a_r}\right)/t \tag{1}$$

where X_r is the fraction of rooibos tea remaining after incubation, a_r is the predicted labile fraction of rooibos tea, and t is the incubation time expressed in days.

2.4. Data Treatment

Soil microbial functional diversity (H') and evenness (E') were calculated based on the relative utilization of all substrates used in the MicroResp test, as follows [59]:

$$H' = -\sum_{i=1}^{S} p_i \, \text{LN}(p_i) \tag{2}$$

$$E' = \frac{1}{\sum_{i=1}^{S} p_i^2} \tag{3}$$

where p_i is the relative respiration induced by the ith substrate relative to the sum of all respiration rates.

We compared the three vineyards for differences in soil physical, chemical, and biological properties with a univariate linear model (ANOVA, SPSS Statistics, v23.0) after log transformation when data did not meet the normality conditions. Differences in soil community and microbial physiological profile between vineyards were tested with non-parametric multivariate analyses of variance (PERMANOVA). When differences were significant, we calculated the contribution to dissimilarity of each trophic group or MicroResp substrate by means of similarity percentage analyses (SIMPER).

We used distance-based linear models (DistLMs) [60] to identify soil properties influencing the configuration of the soil community and of its microbial physiological profile. Soil properties included SOC, electrical conductivity, clay content, pH, P, Na K, Ca, Mg, and the decomposition constant Kr. To visualize the models in the multivariate space, we drew dbRDA plots using only the variables selected by the best model (PERMANOVA + for PRIMER package, v.7).

We used the V Index [61] to graphically explain the effect of regenerative and minimum impact management on soil properties relative to intensive management. We calculated the index as follows:

$$M_{REG} = \frac{2M_{\text{REG}}}{M_{\text{REG}} + M_{\text{INT}}} - 1 \text{ and } M_{MIN} = \frac{2M_{\text{MIN}}}{M_{\text{MIN}} + M_{\text{INT}}} - 1 \tag{4}$$

with M_{INT} representing the value of a given soil property under intensive management, and M_{REG} and M_{MIN} representing its value under regenerative and minimum impact management, respectively.

3. Results

3.1. Physical and Chemical Soil Properties under Contrasting Management

We found significant differences between vineyards for all physical and chemical properties except for Mg content (Table 1).

Table 1. Physical, chemical, and biological properties of the soils of the three vineyards studied in this work under contrasting management. Mean ± standard error, with n = 16 samples, except for microbial substrate utilization, microbial functional diversity, and microbial functional evenness, with n = 8. The abundance of the soil functional groups is expressed in g C m^{-2}. All data correspond to the upper 15 cm of the soil. MWD: Mean Weight Diameter; For each line, different letters (a, b) indicate significant differences between treatments after ANOVA; ns: non-significant difference.

	Unit	Intensive Management (INT)	Regenerative Management (REG)	Minimal Management (MIN)	*p*
Physical and chemical properties					
Silt + clay	%	24.3 ± 2.3 [b]	53.7 ± 6.7 [a]	45.6 ± 4.9 [a]	0.002
Bulk density	g cm^{-3}	1.64 ± 0.05 [a]	1.36 ± 0.10 [b]	1.15 ± 0.02 [b]	<0.0001
Aggregate stability (MWD)	mm	0.87 ± 0.14 [ab]	0.54 ± 0.06 [b]	1.16 ± 0.13 [a]	0.005
pH		5.97 ± 0.25 [b]	7.1 ± 0.09 [a]	5.97 ± 0.08 [b]	0.002
EC	dS m^{-1}	0.06 ± 0.01 [b]	0.14 ± 0.05 [a]	0.08 ± 0.01 [ab]	0.014
Total SOC	%	0.54 ± 0.08 [b]	1.27 ± 0.20 [a]	1.84 ± 0.32 [a]	0.002
Labile SOC	%	0.007 ± 0.001 [b]	0.043 ± 0.007 [a]	0.052 ± 0.052 [a]	<0.0001
Nitrogen	%	0.056 ± 0.006 [b]	0.121 ± 0.017 [a]	0.121 ± 0.009 [a]	0.004
Available P	mg kg^{-1}	7.27 ± 0.75 [b]	39.07 ± 11.19 [a]	19.85 ± 3.81 [ab]	0.003
Ca	mg kg^{-1}	739.5 ± 86.4 [b]	1498.5 ± 174.9 [a]	904.7 ± 162.1 [ab]	0.016
K	mg kg^{-1}	69.5 ± 4.4 [b]	251.2 ± 27.6 [a]	202.7 ± 18.7 [a]	<0.0001
Mg	mg kg^{-1}	130.5 ± 14.9	228.2 ± 30.8	167.5 ± 24.4	ns
Na	mg kg^{-1}	30.5 ± 1.9 [b]	42.5 ± 2.5 [a]	39.0 ± 2.1 [a]	0.008
Microbial properties					
Microbial substrate utilization	µg CO_2-C g^{-1} h^{-1}	0.382 ± 0.067 [b]	2.032 ± 0.718 [a]	1.82 ± 0.406 [a]	0.001
Microbial functional diversity (*H'*)		2.772 ± 0.0002 [a]	2.764 ± 0.002 [b]	2.766 ± 0.0024 [ab]	0.019
Microbial functional evenness (*E'*)		0.999 ± 0.0002 [a]	0.991 ± 0.002 [b]	0.994 ± 0.0024 [b]	0.014
Decomposition rate (*K$_r$*)	−year	0.010 ± 0.001	0.011 ± 0.002	0.014 ± 0.003	ns
Soil functional groups' properties					
Bacterial biomass C	g C m^{-2}	7.31 ± 2.68	12.78 ± 3.98	12.73 ± 2.63	ns
Fungal biomass C	g C m^{-2}	8.33 ± 1.07	13.90 ± 2.42	16.65 ± 2.61	ns
Total microbial biomass C	g C m^{-2}	15.64 ± 4.16 [b]	26.68 ± 5.68 [ab]	29.38 ± 7.59 [a]	0.01
Fungal-to-bacterial biomass C	unitless	0.595 ± 0.057	0.593 ± 0.064	0.564 ± 0.056	ns
Flagellates	g C m^{-2}	0.0006 ± 0.0002 [b]	0.009 ± 0.002 [a]	0.001 ± 0.0004 [b]	<0.0001
Amoeba	g C m^{-2}	0.016 ± 0.007 [b]	0.423 ± 0.193 [a]	0.057 ± 0.027 [b]	0.004
Ciliates	g C m^{-2}	0.0006 ± 0.0002	0.013 ± 0.009	0.0003 ± 0.0001	ns
All protists	g C m^{-2}	0.017 ± 0.007 [b]	0.445 ± 0.034 [a]	0.059 ± 0.027 [b]	<0.0001
Bacteriophagous nematodes	g C m^{-2}	2.703 ± 0.699 [b]	8.243 ± 2.028 [a]	9.439 ± 2.632 [a]	0.037
Fungivorous nematodes	g C m^{-2}	0.82 ± 0.14 [b]	2.70 ± 0.83 [b]	3.87 ± 0.98 [a]	0.004
Plant associated nematodes	g C m^{-2}	0.90 ± 0.23 [b]	3.24 ± 0.24 [a]	4.48 ± 1.18 [a]	0.012
Omnivorous nematodes	g C m^{-2}	1.148 ± 0.483	3.102 ± 1.052	7.945 ± 2.10	ns
All nematodes	g C m^{-2}	5.57 ± 1.32 [b]	17.29 ± 4.03 [a]	26.22 ± 5.95 [a]	0.011
Poduromorpha (collembola)	g C m^{-2}	2.19 ± 1.13	11.62 ± 3.53	7.37 ± 2.12	ns
Entomobryomorpha (collembola)	g C m^{-2}	0.0007 ± 0.0002 [b]	0.006 ± 0.001 [a]	0.0002 ± 0.0001 [b]	<0.0001
All collembola	g C m^{-2}	0.002 ± 0.001 [b]	0.015 ± 0.002 [a]	0.005 ± 0.001 [b]	<0.0001
Predatory mites	g C m^{-2}	0.0001 ± 0.0001 [b]	0.0006 ± 0.0004 [a]	0.0004 ± 0.545 [b]	<0.0001
Fungivorous oribatid mites	g C m^{-2}	0.001 ± 0.0003 [c]	0.02 ± 0.003 [a]	0.003 ± 0.001 [b]	<0.0001
All microarthropods	g C m^{-2}	0.023 ± 0.005 [b]	0.676 ± 0.183 [a]	0.122 ± 0.082 [b]	<0.0001

The proportion of fine particles (silt and clay) was significantly lower under intensive than under any alternative management (r^2 = 0.744, df = 2, F = 13.089, *p* = 0.02; REG > INT, *p* = 0.002; MIN > INT, *p* = 0.01). Soil bulk density was very high under intensive management and significantly higher than under the two alternative management types (r^2 = 0.639; df = 2; F = 13.271; *p* < 0.0001; INT > REG, *p* = 0.033; INT > MIN, *p* < 0.0001). Soil aggregate stability was the lowest under regenerative management and the highest under minimum impact management, with the intensively managed vineyard showing intermediate values (r^2 = 0.397, df = 2; F = 6.917, *p* = 0.005; MIN > REG, *p* = 0.004).

Electrical conductivity was lower under intensive than under alternative managements (r^2 = 0.613, df = 2, F = 7.119, *p* = 0.014; REG > INT, *p* = 0.018). Soil pH was significantly higher

under regenerative management than under intensive or minimum impact management ($r^2 = 0.757$, df = 2, F = 14.043, *p* = 0.002; REG > INT, *p* = 0.004; REG > MIN, *p* = 0.004). The vineyards with regenerative and minimum impact management showed higher levels of soil N ($r^2 = 0.711$, df = 2, F = 11.083, *p* = 0.004; REG > INT, *p* = 0.007; MIN > INT, *p* = 0.007), available *p* ($r^2 = 0.723$, df = 2, F = 11.738, *p* = 0.003; ECO > INT, *p* = 0.002; MIN > INT, *p* = 0.043), and cations (for Ca: $r^2 = 0.599$, df = 2, F = 6.711, *p* = 0.016; REG > INT, *p* = 0.02; for K: $r^2 = 0.922$, df = 2, F = 53.217, *p* < 0.0001; REG > INT, *p* < 0.001; MIN > INT, *p* < 0.0001; for Na: $r^2 = 0.658$, df = 2, F = 8.672, *p* = 0.008; REG > INT, *p* = 0.008; MIN > INT, *p* = 0.037) than the intensively managed vineyard. Total SOC content ($r^2 = 0.736$, df = 2, F = 12.56, *p* = 0.002; REG > INT, *p* = 0.028; MIN > INT, *p* = 0.002) and the proportion of labile organic carbon relative to total SOC ($r^2 = 0.845$, df = 2, F = 22.554, *p* < 0.0001; REG > INT, *p* = 0.001; MIN > INT, *p* < 0.0001) were significantly lower under intensive management than under any of the alternative management regimes.

3.2. Abundance of Soil Groups under Diverse Management Strategies

We did not find differences between vineyards in the C abundance of fungi and bacteria or in the fungal-to-bacterial ratio (Table 1), but total C microbial abundance was significantly higher under minimum impact management than under intensive management ($r^2 = 0.192$, df = 2, F = 5.113, *p* = 0.01; REG > INT, *p* = 0.008; MIN > INT, *p* = 0.008), with intermediate values in the regenerative vineyard. Contrastingly, vineyards significantly differed in the C abundance of all soil invertebrates, except in the cases of the order Poduromorpha (collembolans) and omnivorous nematodes.

The C abundance of flagellates ($r^2 = 0.488$, df = 2, F = 21.454, *p* < 0.0001; REG > INT, *p* < 0.0001; REG > MIN, *p* < 0.0001), amoeba ($r^2 = 0.225$, df = 2, F = 6.338, *p* = 0.004; REG > INT, *p* < 0.004; REG > MIN, *p* < 0.004), total protists ($r^2 = 0.575$, df = 2, F = 30.419, *p* < 0.0001; REG > INT, *p* < 0.0001; REG > MIN, *p* < 0.0001), total collembolans ($r^2 = 0.470$, df = 2, F = 16.399, *p* < 0.0001; REG > INT, *p* < 0.0001; REG > MIN, *p* = 0.001), Entomobryomorpha (collembolans) ($r^2 = 0.631$, df = 2, F = 18.785, *p* < 0.0001; REG > INT, *p* < 0.0001; REG > MIN, *p* = 0.003), predatory mites ($r^2 = 0.503$, df = 2, F = 20.221, *p* < 0.0001; REG > INT, *p* < 0.0001; REG > MIN, *p* < 0.0001), fungivorous oribatid mites ($r^2 = 0.691$, df = 2, F = 42.479, *p* < 0.0001; REG > INT, *p* < 0.0001; REG > MIN, *p* < 0.0001), and total arthropods ($r^2 = 0.539$, df = 2, F = 25.757, *p* < 0.0001; REG > INT, *p* < 0.0001; REG > MIN, *p* < 0.0001) was significantly higher in the regenerative vineyard than in the two other vineyards.

The C abundance of the bacteriophagous ($r^2 = 0.141$, df = 2, F = 3.570, *p* = 0.037; REG > INT, *p* = 0.053), plant-associated nematodes ($r^2 = 0.193$, df = 2, F = 4.905, *p* = 0.012; MIN > INT, *p* = 0.010), and total nematodes ($r^2 = 0.185$, df = 2, F = 5.008, *p* = 0.011; REG > INT, *p* = 0.028; MIN > INT, *p* = 0.023) was similar in the regenerative and minimum impact vineyards, and significantly higher in both of them than in the intensively managed vineyard. The C abundance of the fungivorous nematodes was significantly higher in the vineyard under minimum impact management than in the intensive and regenerative vineyards ($r^2 = 0.224$, df = 2, F = 6.21, *p* = 0.004; MIN > INT, *p* = 0.003). All groups were significantly less abundant under intensive management than under any alternative type of management (Table 1). PERMANOVA did not reveal significant differences between vineyards in the functional structure of the soil community.

3.3. Soil Microbial Properties under Contrasting Management

Total microbial substrate utilization (Table 1) was the lowest under intensive management ($r^2 = 0.480$, df = 2, F = 9.709, *p* = 0.001; REG > INT, *p* = 0.002; MIN > INT, *p* = 0.005). Microbial functional diversity (*H'*) was the highest in the regenerative vineyard and the lowest in the intensive vineyard ($r^2 = 0.316$, df = 2, F = 4.846, *p* = 0.019; REG > INT, *p* = 0.019), and evenness (*E'*) was higher under intensive than under regenerative management ($r^2 = 0.329$, df = 2, F = 5.154, *p* = 0.015; INT > REG, *p* = 0.014).

The most exploited carbon substrate in all plots was L-malic acid. In terms of the contribution of each substrate to total induced microbial respiration, we only found

significant differences between the three vineyards for D(+) glucose (Figure 3), which was relatively more exploited in the vineyard under minimum impact management than in the other two (r^2 = 0.384, df = 2, F = 6.546, *p* = 0.006; MIN > REG, *p* = 0.007; INT > REG, *p* = 0.049). The regenerative and the intensive vineyards also differed significantly in the exploitation of citric acid, L-arginine, cellulose, L-malic acid, α-ketoglutarate, Υ-aminobutyric acid, and fructose. The vineyards under regenerative and minimum management only differed in the exploitation of D(+) glucose and L-arginine.
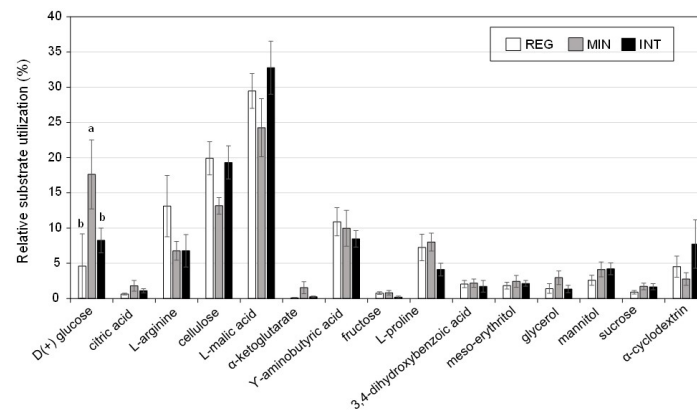

Figure 3. Respiration profiles of the soil microbial communities in the three vineyards studied in this work under intensive management (INT), regenerative management (REG), and minimum impact management (MIN). Mean ± standard error for n = 8. Different letters (a, b) indicate significant differences between treatments after ANOVA.

PERMANOVA indicated significant differences between the microbial physiological profiles of the three vineyards (*p* = 0.0002). Specifically, the intensively managed vineyard differed from the regenerative (*p* = 0.002) and from the minimally managed vineyards (*p* = 0.0003). The SIMPER analysis showed that D(+) glucose (13.4%) and L-malic acid (12.04%) were the main contributors to the difference between the intensive and minimum impact vineyards (the two of them were more exploited in the minimum impact plot), and L-malic acid (15.2%), cellulose (11.1%), g-aminobutyric acid (11.1%), and L-proline (10.2%) were the main contributors to the difference between the intensive and the regenerative vineyards.

According to the DistLM analysis, when considered alone, none of the variables have a significant relationship with the multivariate data cloud defined by the exploitation of the MicroResp substrates. The soil microbial physiological profile is best explained by combining the concentration of calcium and the concentration of magnesium (Figure 4).
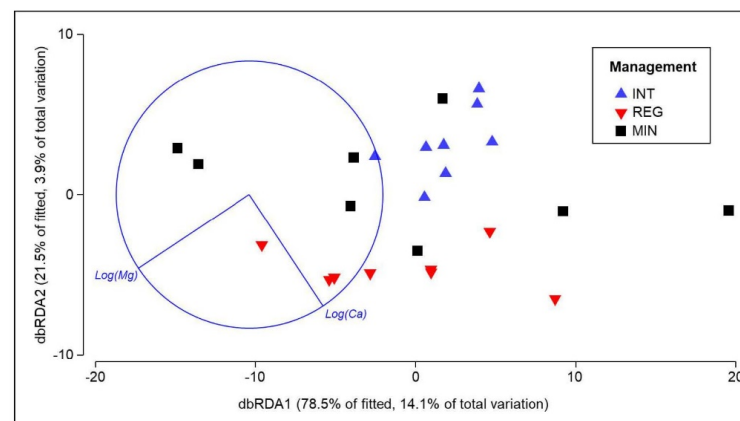

Figure 4. Distance-based redundancy analysis (dbRDA) plot of the DistLM using the two explanatory variables included in the best model. Vectors indicate the direction of the parameter effect in the ordination plot.

3.4. Response of Soil Properties to Agricultural Management

The V Index showed that the soil characteristics more responsive to our three types of management are the abundance of amoeba ($V_{REG} = 0.935$, $V_{MIN} = 0.577$), total protists ($V_{REG} = 0.905$, $V_{MIN} = 0.414$), oribatid mites ($V_{REG} = 0.898$, $V_{MIN} = 0.532$), and flagellates ($V_{REG} = 0.881$, $V_{MIN} = 0.313$), followed by the abundance of entomobryid collembola ($V_{REG} = 0.801$, $V_{MIN} = -0.524$), total arthropods ($V_{REG} = 0.776$, $V_{MIN} = 0.331$), total collembolans ($V_{REG} = 0.753$, $V_{MIN} = 0.424$), and predatory mites ($V_{REG} = 0.732$, $V_{MIN} = 0.189$), as well as the proportion of labile carbon relative to total soil organic carbon ($V_{REG} = 0.711$, $V_{MIN} = 0.752$) (Figure 5). The percent difference between pairs of treatments for the mean value of each indicator is shown in Table 2.
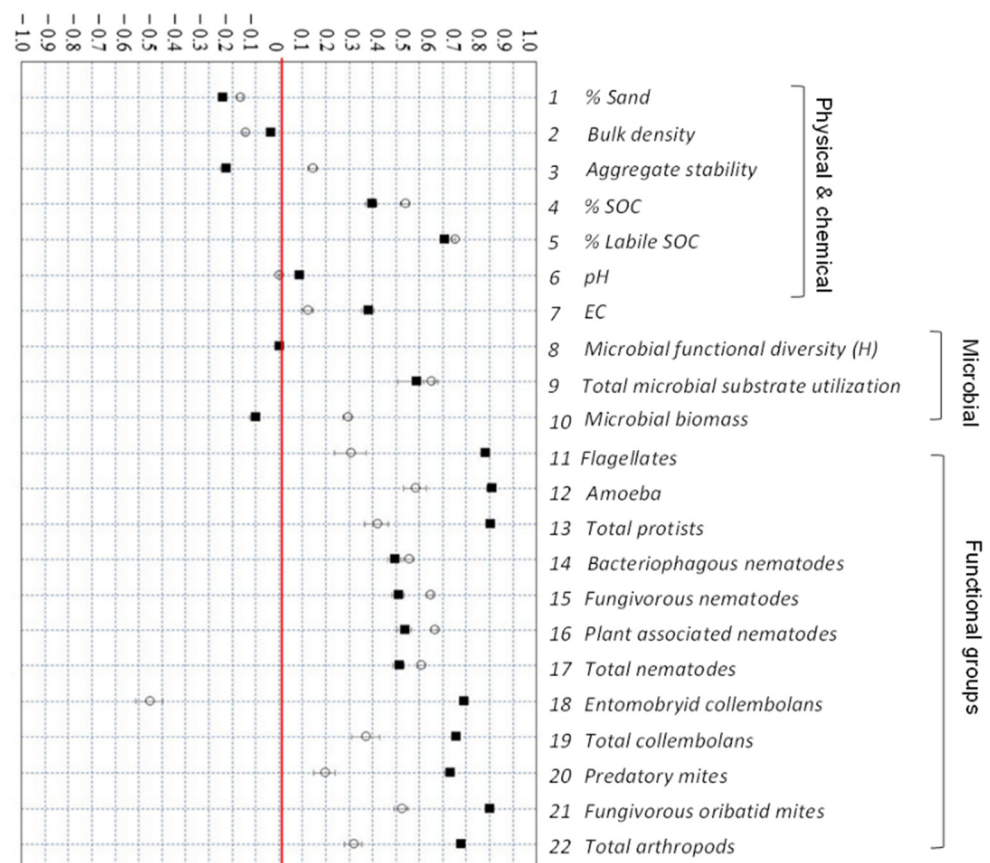

Figure 5. Value of the V Index (mean ± standard error) for differences between the regenerative and intensive vineyards (black squares) and between the minimum impact and intensive vineyards (empty circles). The greater the distance to the reference red line, the greater the difference between treatments. The red line represents the reference value of each soil property in the intensive vineyard.

Therefore, the soil response to management was more apparent when using biological properties than when using physical and chemical characteristics.

The regenerative vineyard got the best marks for all biological properties, while the minimum impact vineyard showed the best values for physical properties and soil organic carbon content. The soil of the intensive vineyard showed the lowest quality for all biological indicators, carbon content, and all physical properties, except soil aggregate stability (Figure 6).

Table 2. Percentage difference (% Δ) between pairs of vineyards for all the selected indicators. INT: intensive vineyard; REG: regenerative vineyard; MIN: minimum impact vineyard. .

|	REG-INT % Δ	MIN-INT % Δ	MIN-REG % Δ
% Sand	−63.5	−39.2	14.9
Bulk density	−20.6	−42.6	−18.3
Aggregate stability	−61.1	25.0	53.4
% SOC	57.5	70.7	31.0
% Labile SOC	83.7	86.5	17.3
pH	15.9	0.0	−18.9
EC	57.1	25.0	−75.0
Microbial functional diversity (*H′*)	−0.3	−0.2	0.1
Total microbial substrate utilization	81.2	79.0	−11.6
Microbial biomass	41.4	46.8	9.2
Flagellates	93.7	47.7	−728.2
Amoeba	96.7	73.1	−703.1
Total protists	95.0	58.6	−727.4
Bacteriophagous nematodes	67.2	71.4	12.7
Fungivorous nematodes	69.7	78.8	30.2
Plant associated nematodes	72.1	79.9	27.8
Total nematodes	68.8	75.6	21.6
Entomobryid collembolans	89.0	−222.6	−2822.6
Total collembolans	85.7	59.5	−183.1
Predatory mites	84.6	31.9	−341.9
Fungivorous oribatid mites	94.6	69.6	−465.3
Total arthropods	87.4	49.7	−297.9
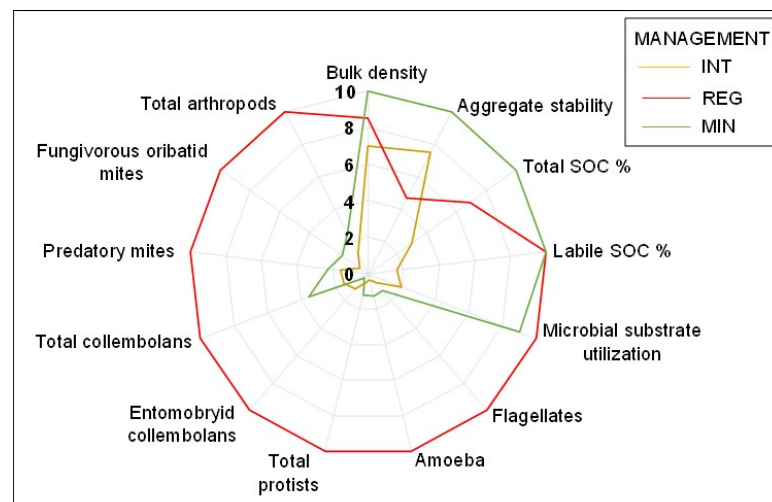
Figure 6. Spider graph showing the performance of the three vineyards for the most discriminating indicators. The value of each indicator is standardized relative to its maximal value in the three vineyards. For bulk density that is inversely related to soil quality, standardization was done based on the lowest value in the three vineyards. (INT) intensive vineyard; (REG) regenerative vineyard; (MIN) minimum impact vineyard.

4. Discussion

Among the studied soil physical and chemical properties, texture, bulk density, aggregate stability, pH, electric conductivity, cation content, and total and labile organic carbon content were responsive to changes in agricultural management.

Texture is often considered an inherent and immutable soil characteristic, but it can be altered by practices causing erosion, due to preferential removal of fine particles by runoff [62]. In agreement with previous works [63], we found the proportion of fine particles about twice as high in the soil of the regenerative and minimal impact vineyards than in the soil of the intensive vineyard. By protecting soil clay, which is fundamental in stabilizing soil organic matter, regenerative and minimum impact management would thus contribute to increase the capacity of soil to stock carbon [64].

With some exceptions [25], most studies suggest that cover crops combined with no-tillage lessen soil bulk density [65], with positive effects on soil water holding capacity, water dynamics, and root development. This effect has also been found in the present work, with bulk densities significantly lower in soils under regenerative and minimum impact management than in intensively managed soils. Soil decompaction in the absence of tillage can be achieved by fostering the development of a natural or introduced herbaceous cover with a dense root system and can be helped by injecting air below ground. The situation of our intensive vineyard, with bulk density above the degradation threshold for sandy loam soils ($1.58 \, \text{g cm}^{-3}$) [66], is representative of the condition of many Mediterranean vineyards, and regenerative and minimum impact management appear as promising strategies to reverse soil physical deterioration in the region.

Cover crops are also believed to benefit aggregate stability in Mediterranean woody crops [67,68] by increasing carbon content and microbial and plant root activity [69]. In the present work, we expected to find the stability of soil aggregates improved under both management strategies including cover crops relative to intensive management. Surprisingly, we found the lowest aggregate stability in the regenerative vineyard, and we posit that this effect is due to the higher soil pH in this field, since soil aggregate stability and pH correlate inversely [70]. The relatively high pH found in the regenerative vineyard as compared to the two other studied fields can be explained by the soil cation balance, that has been managed in the regenerative vineyard to approach a 65%:15%:4%:1–3% Ca:Mg:K:Na ratio, following the "Base Cation Saturation Ratio" (or "soil balancing"), which is assumed to provide the best soil structure and oxygenation, while guaranteeing nutrient availability to plants. This concept is widely accepted by organic farmers despite being generally disregarded by the scientific community [71].

As is often the case in Mediterranean woody crops, the soil of our intensive vineyard was carbon depleted, while the SOC content in the regenerative and minimum management vineyards was about two and three times higher, respectively. Assuming a constant rate of carbon sequestration in soil over time, the efficiency of the regenerative strategy in increasing soil C stocks has been particularly remarkable compared to the minimum impact strategy, since the abandonment of the intensive management occurred 4 years before our sampling campaign in the regenerative vineyard and 12 years before in the minimum impact vineyard. In both cases, this high effectiveness reinforces the idea that integrated management strategies are more efficient in restoring agricultural soils than isolated practices such as tillage suppression, which has been found to have zero effects during the two first years of implementation [72]. However, our results must be interpreted with caution since, due to economic constraints, we only sampled the top 15 cm of the soil profile, instead of the whole soil layer occupied by roots, as would be advisable, since the estimates of soil carbon stocks are deeply influenced by sampling depth [73].

An important proportion of the soil biological properties considered in this work were sensitive to agricultural management. With the aim to propose soil analyses that are affordable for farmers, we addressed the effects of agricultural management on soil microbial functions by means of two low-cost and user-friendly methods: the Tea Bag test and the MicroResp test. The Tea Bag test [59] measures the ability of soil to decompose

plant debris and was promising a priori for its use in soil surveys involving farmers. Unfortunately, as anticipated in previous works [74–76], this test was not sensitive to the management types considered in this study.

The MicroResp test provides information about the ability of soil microbes to exploit carbon sources of varied chemistry and recalcitrance. As expected, our data confirmed that the regenerative and minimum impact strategies contribute to restoring the catabolic capacity of the microbial community in soils previously managed intensively. Surprisingly [77], we found the highest catabolic diversity and evenness in the soil of the intensive vineyard. This might be explained by the fact that the microorganisms living in oligotrophic soil environments are adapted to exploit a wider spectrum of carbon sources than those living in more fertile soils, as those promoted by regenerative and minimum impact management [78].

In line with previous works, our results indicate that agricultural management affects the dietary preferences of soil microbes [79]. In particular, amino acids, sugars, and L-malic acid are relatively more exploited in our regenerative and minimum impact vineyards than in the intensive vineyard, which can be attributed to the presence of microbes adapted to utilize the labile carbon provided by the roots of the cover crops [80]. Regenerative and minimum impact management also contributed to the recovery of soil microbial biomass, as was expected based on previous data [81]. However, contrary to what some authors sustain [82], we could not find any effect of management on the soil fungi-to-bacteria ratio. The insensitivity of this indicator might be due to conflicting effects of diverse practices combined under our integrated agricultural strategies on the soil microbiota. On the one side, tillage cessation might have favored fungi by reducing physical disturbance to soil hyphal networks while, on the other hand, cover crops might have fostered soil bacteria [83,84].

Our results add to the growing evidence that regenerative viticulture and low-impact management improve soil biodiversity in vineyards that have been previously managed intensively, and that this improvement affects all elements of the soil food web [85]. Overall, and as we expected based on the available literature, we found that both the regenerative and the minimum impact options have positive effects on soil invertebrates relative to the intensive option, although this effect is dramatically higher for the regenerative management option. Organic management and cover crops are known to favor soil protists and nematodes at different trophic levels of the soil trophic web [86]. The great abundance of nematodes in the regenerative and minimum impact vineyards can be explained by the rich offering of bacterial and plant root resources in their soils.

There is little information about the response of soil microarthropods to agricultural management, and the available data are often conflicting. The abundance and species richness of soil collembolans and mites are known to be much higher in organic than in intensive vineyards [87,88]. Negative effects of tillage, as a component of the intensive management, on collembolans and oribatid and mesostigmatic mites have been frequently reported [89,90]. Consistent with these authors, we found the regenerative management especially favorable to microarthropods and, in particular, to predatory and fungivorous mites and entomobryid collembola. Since high abundances of these groups are indicative of carbon-rich and productive soils [91,92], we were expecting to find the highest abundance of microarthropods associated with the minimal impact management, but instead we found it in the regenerative vineyard, which is less rich in soil carbon. This fact can be explained by a favorable stoichiometry in the soil of the regenerative vineyard, with the lowest C:Ca, C:N, and C:P ratios. Calcium is a crucial element in the construction and hardening of the exoskeleton of soil arthropods. In the same sense, high nitrogen content has been found to favor soil predators, since high C:N ratios in the body tissues of their preys can be limiting for assimilation. Finally, the C:P ratio is a key regulator of the density of the soil food web at all trophic levels [93]. In this sense, it is worth mentioning that, in the studied vineyards, the soil microbial catabolism is greatly influenced by cations and particularly by Ca and Mg concentrations. From these results, it appears that soil stoichiometry and soil cations are key drivers of soil biodiversity in agricultural soils.

Protists and nematodes are important regulators of the abundance of soil bacteria and key actors in the availability of nitrogen to plants in agricultural soils [94]. Directly (by bioturbation, comminution of plant residues, and production of feces) or indirectly (as top-down controllers of soil microbes), soil microarthropods regulate the soil carbon cycle [95]. Therefore, any management strategy promoting these elements of the soil food web should be considered valuable in restoring the environmental services provided by degraded soils in winegrowing regions.

From the above points, it can be seen that, to evaluate the effect of agricultural management on soil environmental quality, biological indicators should be used together with soil physical and chemical properties. To be useful at the farm scale, soil indicators must be easy to measure, understandable and meaningful, and cost-effective in terms of monetary costs and workload [96]. Bioindicators have often been considered inappropriate for this purpose due to high costs in terms of expertise, equipment, and workload. When describing the composition and structure of the soil microbial community, molecular analyses are still too expensive [97] and, in practice, total soil microbial biomass is the most frequently used bioindicator in monitoring programs [98]. Earthworms and collembolans are the most widely studied elements of the soil biota, together with nematodes [99,100] and microarthropods [101]. With all this in mind and based on our results, we propose soil protists, collembolans, and mites as highly appropriate indicators of the effects of agricultural management on soil biodiversity in vineyards. Extracting and counting soil microarthropods and protists demand basic laboratory equipment, and technicians may be easily trained to classify soil microfauna into functional groups. In very degraded soils, microarthropods are often very scarce and, in these cases, the use of the more pervasive protists may be more appropriate. To approach soil microbial functionality, community-level microbial physiological profiling is advisable as a low-cost tool. SOC content is a key indicator of the fertility and environmental quality of agricultural soils, and soil labile carbon is a small but very dynamic fraction of total SOC. Its value, in combination with total SOC and aggregate stability can provide important clues about the ability of soil to stabilize organic carbon under diverse management types.

5. Conclusions

Regenerative and minimum impact management of Mediterranean vineyards are promising strategies to restore the environmental quality of degraded agricultural soils. In this work, we found that vineyards managed under regenerative and minimum impact strategies for 5 and 12 years, respectively, after a long history of intensive farming, contain significantly more organic carbon and support significantly more soil invertebrates than a comparable vineyard that is managed intensively. Regenerative viticulture particularly benefits functional groups of the soil food web that are key players in regulating soil microbial populations and in stabilizing organic carbon in the soil.

These strategies should be soundly evaluated for their possible incorporation into European environmental policies. Moreover, by contributing to increase soil carbon stocks and to foster soil biological diversity, regenerative and minimum impact viticulture will help to attain the UN development goals number 13 (Climate Action) and 15 (Protecting Life on Land) while contributing to achieve food security (Zero Hunger objective). Biological indicators must be included, together with basic physical and chemical indicators, in any soil monitoring program intended to evaluate effects of management on agricultural sustainability

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

Appendix A

Table A1. Individual body weight of the trophic groups found in the soil of the three vineyards.

	Individual Body Weight (g, d.w.)
Chilopoda (Geophilomorpha)	2.59×10^{-3}
Pseudoescorpionida	0.00×10^{-5}
Predaceous diplurans	3.40×10^{-5}
Predaceous mites	7.70×10^{-6}
Nematophagous mites	1.00×10^{-6}
Predaceous nematodes	1.04×10^{-6}
Omnivorous nematodes	9.00×10^{-7}
Phytophagous nematodes	6.00×10^{-8}
Bacteriophagous nematodes	7.40×10^{-8}
Fungivorous nematodes	1.10×10^{-7}
Ciliates	1.00×10^{-9}
Amoeba	1.20×10^{-9}
Flagellates	1.90×10^{-11}
Fungivorous Cryptostigmata	2.70×10^{-6}
Fungivorous Prostigmata	1.00×10^{-6}
Symphyla	6.60×10^{-6}
Collembolans	2.70×10^{-6}
Bacteria	6.65×10^{-13}
Fungi	2.30×10^{-6}

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