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Biofertilization with Liquid Vermicompost-Activated Biochar Enhances Microbial Activity and Soil Properties

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Abstract: Biochar (Bc) and liquid vermicompost extracts (LVEs) are increasingly being used as biofertilizers in agriculture to promote soil-microbe-crop interactions. However, although both these products can potentially act synergistically due to their complementary characteristics, their co-application in different soils has not yet been investigated. Therefore, firstly, an LVE-activated biochar (B_{LVE}) was experimentally formulated and the persistence of LVE bacteria over a 60-day storage period was determined. The total number of LVE bacteria increased by 10-fold after 7 days and was stable throughout the entire biochar storage period. In addition, changes in the composition of the bacterial community were observed after 30 days of storage, indicating that taxa less represented in pure LVE may be advantaged upon biochar colonization. Secondly, a microcosm experiment was performed to evaluate whether the biological fertility and enzyme activities of two soils, differing in organic matter content, could be enhanced by the addition of LVE-activated biochar. In this experiment, three different doses of Bc, LVE, and B_{LVE} against the carbon-related biological fertility index (i.e., biological fertility index, BFI) and three enzyme activities over a 21-day incubation period were tested. The B_{LVE} treatment yielded the best results (i.e., BFI +32%, enzyme activities +38%). This indicates that Bc and LVEs can act synergistically to promote soil fertility, quality, and microbial activity. By integrating LVE-activated biochar into their soil management practices, farmers could achieve higher crop yields and healthier products.

Keywords: bacteria; organic fertilizers; soil amendments; soil enzyme activities; soil quality; tea vermicompost

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1. Introduction

Animal and plant biomass, and particularly products derived from its biological and thermochemical conversion (e.g., vermicompost and biochar, respectively), are attracting increasing attention in agriculture for their ability to improve soil quality and crop yield [1,2].

Vermicompost is the product of the decomposition of organic waste by microorganisms through the digestive tract of earthworms [3]. Both water-soluble nutrients and microorganisms can be further extracted during steeping of vermicompost in water and formulated into nutrient-rich and microbiologically active liquid vermicompost extracts

(LVEs) [4]. Bacteria are essential components of LVEs, as they fulfill multiple functions that promote both soil and plant health, including nutrient solubilization, biocontrol, or the active release of plant growth stimulators [5,6]. On the other hand, the appeal of biochar, the solid byproduct of biomass pyrolysis, stems primarily from its capacity to improve the soil structure and quality in a wide array of ways; for instance, biochar can improve soil carbon sequestration, soil water- and nutrient-holding capacity, and soil porosity and thus aeration [7–9]. Interestingly, biochar has recently emerged as a promising carrier material for the delivery of beneficial microorganisms in the soil, as it provides a stable environment for bacterial survival and activity, potentially enhancing their longevity and effectiveness in the soil [10,11].

Although LVEs and biochar are both considered useful and sustainable soil organic amendments, their broad-scale application has not yet been widely adopted as contrasting findings have been reported on their effects on soil health. As an example, depending on the soil type, the sole application of LVEs can be prone to runoff and leaching under excessive rainfall/irrigation conditions [12], while variable biochar dosages can increase soil salinization in the long term, decrease soil fertility, and/or adversely affect soil microbial activity [13,14]. In this scenario, there is a growing consensus that these potentially adverse effects of LVEs and biochar could be mitigated by their co-application, as both amendments have been shown to act in synergy given their different, yet potentially complementary attributes [15–17]. The co-application of LVEs and biochar has usually involved a prior soil amendment with biochar followed by the application of LVEs by fertigation [18,19]. While this strategy has been shown to provide benefits in terms of both plant growth and protection [20–23], recent studies have investigated the formulation of "pre-conditioned" or "activated" biochars [24]. The process of biochar activation offers a more targeted approach, allowing more controlled integration of both organic amendments and facilitating the contact of LVE-associated microorganisms and biochar particles without the interference of the soil matrix [25]. In addition, effective biochar formulations could also increase its ease of application, transport, and commercialization, while minimizing the potential water wastage that may occur in the dual application process. However, analyses of the bacteria present in LVEs are scarce [4,26], and no study exists on their persistence in biochar particles after activation. Moreover, previous studies have focused on the use of vermicompost-activated biochars in the bioremediation context, showing the synergistic effects of microorganisms and biochar in metal-metribuzin- and cadmium-contaminated soils [27–29]. However, the functional consequences of amendment with LVE-activated biochar for soil quality have not yet been investigated.

In the first part of this study, both in-plate isolation methods and 16SrRNA-based sequencing were used to monitor bacterial composition and persistence in LVE-activated biochar. In the second part, the efficacy of LVE-activated biochar was evaluated in two soils with very low and moderate organic matter content. We hypothesized that (1) the biochar can be a suitable environment for the growth and persistence of the inoculated bacteria, and (2) the soil quality and biological fertility could be improved by the addition of LVE-activated biochar.

2. Materials and Methods

2.1. Biochar and LVE Characteristics

Biochar was produced from pruning residues' woodchips (G30–G50) of a consortium of selected tree species (*Abies* sp., *Alnus* sp., *Castanea sativa*, *Fraxinus* sp., *Quercus* sp., and *Robinia pseudoacacia*) by a pyrolysis process (Bio-Esperia S.r.l., Arezzo, Italy). Biochar production was characterized by a 10 h process duration with an average heating rate of 75–80 °C min^{-1} before reaching a peak of 1100 °C. The parameters for the characterization of biochar were analyzed through certified methods approved by Italian regulations (Law Decree 75/2015): 2–5 mm particle diameter, 400% water-holding capacity (WHC), 9.8 pH, 69% total organic carbon (TOC), <0.1% total carbonate (total $CaCO_3$), <0.4% total nitrogen (TN), 0.034% available phosphorus (P), and 115 $cmol_{(+)}$ kg^{-1} cation-exchange capacity

(CEC). The TOC content was Class 1 following the Guidelines for Certification of the International Biochar Initiative [30].

The liquid vermicompost extract (LVE) was derived from cattle and horse manure vermicompost produced by Centro di Lombricoltura Toscano (CLT S.r.l, Pisa, Italy). Manure was converted into vermicompost in an open-air litter with two earthworm species (i.e., *Eisenia fetida* and *Eisenia andrei*) in a 6-month composting process; after the vermicompost was fully matured, a portion was cold water-extracted using air insufflation over a 48 h period to produce the LVE. The chemical properties of the LVE were as follows: 4.8 pH, 4.05 mS cm^{-1} electrical conductivity (EC), 0.1% TOC, 101 ppm TN, 100 ppm total organic nitrogen (TON), and 10 C/N ratio.

2.2. Formulation of LVE-Activated Biochar and Microbiological Analysis

2.2.1. Biochar Sterilization and Contamination Check

Biochar was sterilized to remove the presence of inherent microbial populations. Briefly, 1 g of biochar was transferred in triplicate (n = 3) to 100 mL flasks and autoclaved at 121 °C for 20 min. Flasks were closed tightly and stored for five days at 25 °C in the dark. At each storage day, flasks were filled with 20 mL of Luria Bertani (LB) medium, composed as follows (g L^{-1}): 10 tryptone, 5 yeast extract, 10 sodium chloride (NaCl) at 7.0 pH, and incubated overnight in a rotary shaker (120 rpm). Subsequently, a 50 µL aliquot from these flasks was serially diluted up to the 10^5-fold, and each dilution spread on LB agar (1.5%, w/w) plates, which were incubated for five days at 25 °C. Finally, the number of colony-forming units (CFUs) was counted and recorded. The same experiment was repeated using non-autoclaved biochar as the control. Both the sterilization process and the activation of biochar with the LVE were performed at the Biology Department of the University of Florence (Italy).

2.2.2. Preparation of Water- and LVE-Activated Biochars

An amount of 1 g of autoclaved biochar was mixed either with sterile distilled water (B_C = biochar as control) or with LVE (B_{LVE} = LVE-activated biochar) at 1:20 (w:v) ratio in 100 mL flasks, and incubated statically at 25 °C for 24 h. Flasks were then drained, closed tightly, and stored for 1, 7, 15, 30, and 60 days at 25 °C in the dark for subsequent analyses (Figure 1).

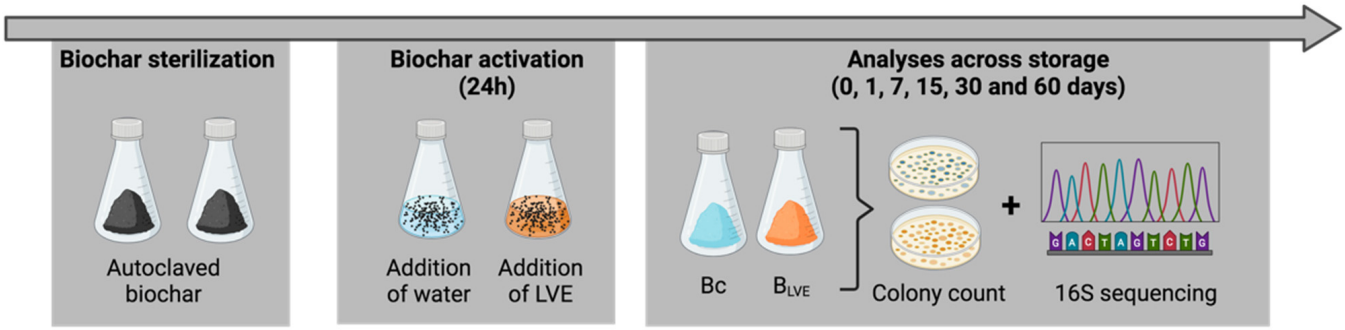

Figure 1. Flowchart illustrating the different tasks carried out throughout the formulation of water- and LVE-activated biochars (B_C and B_{LVE}, respectively).

2.2.3. Bacterial Quantification

At each storage day, CFUs were quantified through the drop-plate method as follows: both B_C and B_{LVE} were mixed with a sterile 0.8% NaCl solution at 1:20 (w:v) ratio and incubated in a rotary shaker at 25 °C and 150 rpm for 2 h. A 20 µL aliquot of each mixture was serially diluted in a 0.8% NaCl solution up to the 10^8-fold, and 10 µL of each dilution dropped on LB agar and incubated overnight at 25 °C. Dilutions containing between 30 and 300 CFUs were counted, and their concentration expressed as CFU g^{-1} of biochar. The number of CFUs was also determined in the pure LVE as described for B_C and B_{LVE}, even

if, in this case, their concentration was expressed as CFU mL^{-1}. All experiments in each storage day were performed in triplicate.

2.2.4. Bacterial Isolation and Molecular Identification

Isolation of bacteria from pure LVE, Bc, and B_{LVE} was carried out from the above-described serial dilutions performed. Hence, 50 µL from the dilutions, containing between 30 and 300 CFUs, was plated on LB agar and incubated for one week at 25 °C. Plates were thoroughly observed under a stereomicroscope and unique morphotypes were isolated and re-plated as many times as necessary until pure colonies were obtained. The assay was performed in triplicate.

Molecular identification of bacteria was carried out through the amplification and sequencing of the partial 16S recombinant DNA region using universal forward primers 104F 5′-GCACGGGTGAGTAACACGTG-3′ [31] and reverse 1392R 5′-ACGGGCGGTGTGTRC-3′ [32]. A single colony was resuspended in sterile water and the DNA extracted with thermal shock (96 °C for 10 min and cooled at −20 °C for 30 min). Samples were then centrifuged, and the supernatant used as the template for amplification. Each PCR reaction was performed in 25 µL; the mixture contained the following: 5 µL of 5X Colorless GoTaq® Reaction Buffer (Promega, Madison, WI, USA) (1X final concentration), 0.5 µL of forward primer (final concentration 0.5 µM), 0.5 µL of reverse primer (final concentration 0.5 µM), 0.5 µL of dNTPs mix (10 mM), 0.5 µL magnesium chloride (25 mM), 0.2 µL of Taq polymerase (1 U/sample), 1 µL of DNA, and sterile deionized water to 25 µL. Amplification protocol: initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s; 56 °C for 40 s and 72 °C for 1 min; and a final elongation step at 72 °C for 5 min. The PCR products were purified using NucleoFast® 96 PCR Plate, 96-well ultrafiltration plate for PCR clean-up (MACHEREY-NAGEL, Düren, Germany), and sequenced with the Sanger method. DNA sequences were deposited into the GenBank repository. The taxonomic affiliations and respective accession numbers of the isolated bacteria are listed in Table S1.

2.3. pH and EC Determination in LVE-Activated Biochar

Both B_C and B_{LVE} were mixed with distilled water at 1:20 (*w*:*v*) ratio, and mixtures were left to stand for 30 min. Subsequently, both the pH and EC were measured using a pH meter (Edge® HI2002, HANNA Instruments Inc., Woonsocket, RI, USA) and an EC meter (BASIC 30, Crison Strumenti SpA, Carpi, Italy), respectively [33]. These measurements were carried out in triplicate.

2.4. Validation of the Biochar-Activation Protocol on Two Different Soils

2.4.1. Soil Experimental Setup

The soil experiment was conducted twice to increase the amount of data and improve the robustness of the statistical analysis. To differentiate between the effects of the B_C, LVE, and B_{LVE} doses, each soil experiment was conducted in triplicate with nine treatments for both soil types, using 250 mL microcosms (a total of 60 microcosms). This study applied four treatments to evaluate the soil parameter changes: a control (Cnt), and low, medium, and high doses of Bc, LVE, and B_{LVE} at 0.5%, 1%, and 2% (*w*/*w*), respectively. The treatments (B_C, LVE, and B_{LVE}) were combined with the acronyms L- (low), M- (medium), and H- (high) based on the indicated dose (0.5%, 1%, and 2%). The Cnt, B_C, and B_{LVE} samples were moistened with deionized water at 60% of WHC, which is considered an ideal moisture value for soil biological activity. The LVE samples were moistened with LVE solutions diluted in deionized water to achieve 0.5%, 1%, and 2% (*w*/*w*) doses, reaching 60% of WHC. The microcosms used in the experiment were made of glass containers sealed with rubber stoppers. The soil inside the containers was moistened daily to maintain the water content at the initial WHC. The jars were opened daily to aerate the samples and measure soil respiration. The samples were kept in the dark for an incubation period of 21 days at 25 ± 1 °C. At the end of this period, the samples were stored at 4 °C for chemical and enzyme analyses.

2.4.2. Soil Recovering

The surface (0–15 cm) of two agricultural sandy soils was collected from two locations. The first one was derived from a field owned by the agricultural research center "Enrico Avanzi" of the University of Pisa (Pisa, Italy; Lat. 43°39′38.96″ N; Long. 10°18′22.17″ E; 1 m above sea level) and it was given the name "Weak Soil" (W-S), due to its very low (1.0%) soil organic matter (SOM) content. The second one was collected in a countryside field at Colle di Compito (Lucca, Italy; Lat. 43°46′21.34″ N; Long. 10°36′24.86″ E; 42 m above sea level) and, for its moderate (2.2%) SOM content, was named "Middle Soil" (M-S). The soil samples were collected in September 2023 by drilling 20 cores, each measuring 5 cm in diameter and 15 cm in depth. The soils were air-dried and passed through a 2 mm sieve to remove larger residues.

2.4.3. Soil Analyses

The main physical and chemical parameters of both soils (i.e., W-S and M-S) are listed in Table 1. The parameters, such as texture, WHC, pH in water, and CEC, were measured according to standard methods (n = 3) [34].

Table 1. Main soil physical and chemical parameters (mean ± SD; n = 3) of "Weak Soil" (W-S) and "Middle Soil" (M-S).

Parameter	Unit	W-S	M-S
Sand	%	86	74
Silt	%	8	16
Clay	%	6	10
WHC	%	21	25
pH	-	8.2 ± 0.1	7.6 ± 0.1
TC	%	0.7 ± 0.1	1.6 ± 0.1
Total $CaCO_3$	%	0.9 ± 0.1	2.5 ± 0.2
TOC	%	0.6 ± 0.1	1.3 ± 0.1
SOM	%	1.0 ± 0.1	2.2 ± 0.1
CEC	$cmol_{(+)}\ kg^{-1}$	4.93 ± 0.05	8.12 ± 0.03

WHC: water-holding capacity; TC: total carbon; TOC: total organic carbon; SOM: soil organic matter; CEC: cation-exchange capacity.

Total organic carbon was determined by subtracting the inorganic carbon (total $CaCO_3$) amount from the total carbon (TC) content. A vario Micro Element Analyzer (Elementar Italia Srl, Lomazzo, Como, Italy) was used to conduct the TC analysis. Soil organic matter content was derived using the van Bemmelen coefficient (1.724). The total $CaCO_3$ was measured using a Scheibler apparatus (Gabrielli Technology, Calenzano, Firenze, Italy) (n = 6).

To determine the soil's microbial biomass carbon (MB-C), a method was used where organic C was extracted from both fumigated and non-fumigated soils using 1 N K_2SO_4 [35]. The extracted organic C was then measured by QBD1200 Laboratory TOC Analyzer (Hach Co., Loveland, CO, USA). The difference in soluble C between the fumigated and non-fumigated soils was then converted into microbial biomass carbon (MB-C) using an extraction efficiency coefficient (Kc) of 0.45 (n = 6).

During the experimental period, a 21-day aerobic incubation was carried out to determine the sample's potential to mineralize organic C. The evolution of carbon dioxide (CO_2) was monitored daily from day 1 to day 21. An amount of 100 g of soil was placed in 250 mL glass containers, which were sealed with rubber stoppers. The soil was moistened to 60% of WHC and incubated at 25 ± 1 °C. The CO_2 that evolved was trapped in NaOH solution, and the excess alkali was titrated with HCl [36]. The results were normalized with respect to time and expressed as mg of mineralized C 100 g^{-1} of dry soil (n = 6).

The soil biological fertility index (BFI) [37] is based on six variables as follows:

$$BFI = Res\_Bas + Res\_Cum + MB - C + SO + qCO_2 + qM$$

where Res_Bas (ppm) is the basal respiration, Res_Cum (ppm) is the cumulative respiration, MB-C (ppm C-CO_2) is the microbial biomass carbon, SO (%) is the organic matter, qCO_2 (mg C-$CO_2 \cdot 10^{-2} \cdot h^{-1} \cdot$mg C mic^{-1}) is the specific respiration of biomass, with an increase in CO_2 being related to microbial stress [38] or to changes in the bacterial-to-fungal ratio [39,40], and, finally, qM (%) is the mineralization quotient expressing the ratio of cumulative respiration to organic C content (Res_Cum$\cdot TOC^{-1} \cdot 100$). The values for each variable that contribute to the BFI were grouped into five levels [41], based on previous studies [42,43]. These levels were then added together to create a BFI score that ranges from 1 to 30. The BFI levels indicate the different levels of soil fertility as follows: (I) BFI < 9 represents stressed soils with very low fertility, (II) 9 < BFI < 12 represents pre-stress soils, (III) 13 < BFI < 18 represents soils with intermediate fertility, (IV) 19 < BFI < 24 represents good fertility soils, and (V) BFI > 24 represents soils with very high fertility.

All the enzyme activities were analyzed by spectrophotometric methods (n = 3). Alkaline phosphatase activity (APA) was measured using p-nitrophenyl phosphate incubated with soil samples at pH 11 and 37 °C for 60 min. The reaction product (p-nitrophenol) was extracted by dilute alkali (0.5 M $CaCl_2$ and 0.5 M NaOH) and determined at 410 nm [44]. β-glucosidase activity (βGA) was determined using a substrate of 4-nitrophenyl-β-D-glucopyranoside. After incubation at 37 °C for 60 min, the production of p-nitrophenol was measured at 410 nm [45] as for APA analysis. Both APA and βGA activities were expressed as μmol p-nitrophenol$\cdot g^{-1}$ dry soil$\cdot h^{-1}$. Urease activity (UA) was spectrophotometrically (690 nm) measured according to Kandeler and Gerber [46] by analyzing the ammonia production after a 2 h incubation of soil samples with urea substrate at 37 °C; UA activity was expressed as μg of NH_4^+-N g^{-1} soil 2 h^{-1} [46].

The effect of the treatments on the soil quality was assessed by determining the soil alteration index 3 (SAI3), as defined by Puglisi et al. [47]. This index was calculated by processing the enzyme data according to the following formula:

$$SAI3 = (7.87 \times \beta - glucosidase) - (8.22 \times phosphatase) - (0.49 \times urease)$$

where the enzyme activities were expressed in μmol of p-nitrophenol g^{-1} of dry soil h^{-1} (for phosphatase and β-glucosidase), and in μg of urea g^{-1} of dry soil 2 h^{-1} (urease).

2.5. Statistical Analyses

All results are presented as mean ± SD and the Levene's test was performed to assess the equality of variances among the samples. The data regarding both biochars' pH and EC approached a normal distribution (Shapiro-Wilk test, $p < 0.05$), and hence a Student's t-test for independent samples was used to check for statistically significant differences in these parameters between B_C and B_{LVE} at each storage day.

The statistical analysis of the soil chemical and biochemical results was conducted using JMP software v. 17.2 (SAS Institute, Inc., Cary, NC, USA). The soil data were calculated on a dry weight basis. One-way ANOVA was used to check for differences among treatments. The Tukey-Kramer post-hoc test was performed to identify significantly different means at $p < 0.05$.

3. Results

3.1. Biochar Sterilization Test

Biochar sterilization through autoclaving was effective in removing the biochar's inherent bacterial populations, as shown in Table 2. On the other hand, 2.5 and 9.3 × 10^3 CFU g^{-1} were recovered from non-autoclaved biochar after 24 and 48 h, respectively, where the CFU number stabilized after 72 h (20 × 10^3 CFU g^{-1}) (Table 2).

Table 2. Number of CFU (mean CFU $g^{-1} \times 10^3 \pm$ SE), grown in LB plates from non-autoclaved and autoclaved biochar after 24, 48, 72, and 96 h.

Biochar	Storage Time (h)			
	24	**48**	**72**	**96**
Non-autoclaved	2.5 ± 0.5	9.3 ± 0.8	20.0 ± 2.0	20.0 ± 1.0
Autoclaved	0	0	0	0

3.2. Quantitative and Qualitative Analyses of Bacteria across B_C and B_{LVE} Storage

Regardless of the storage time examined, no CFUs were recovered in the Bc. On the other hand, nearly 3×10^6 CFUs g^{-1} were recovered from the B_{LVE} one day after storage, more than double compared to those present in the pure LVE (~1.3×10^6 CFUs mL^{-1}). The bacterial population in the B_{LVE} reached its maximum at 7 days after storage (~4×10^7 CFUs g^{-1}) and remained around this order of magnitude until 60 days after storage (Figure 2a).

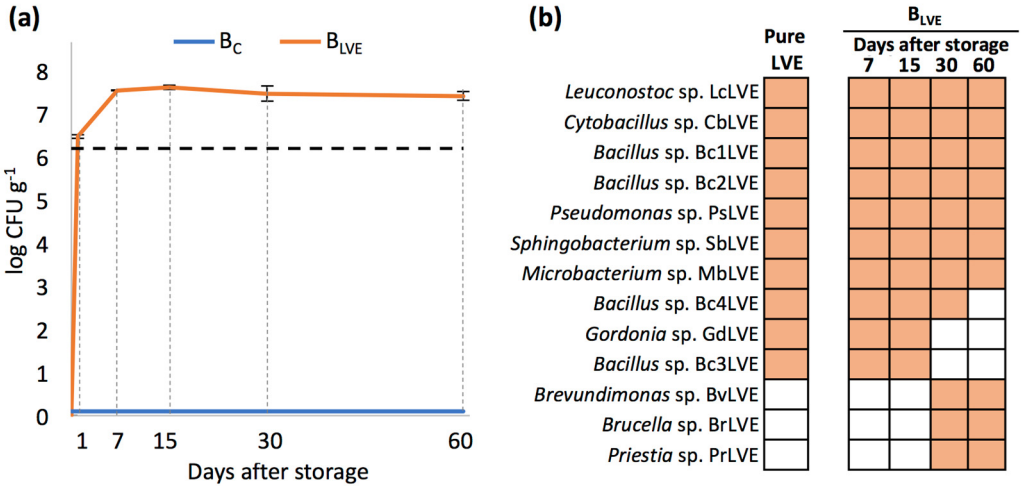

Figure 2. (**a**) Total number of CFUs (mean CFU $g^{-1} \pm$ SD) isolated from B_C and B_{LVE} across the different days after storage. The dashed line represents the number of CFUs counted in the initial pure LVE; (**b**) presence of each bacterium in the pure LVE and in B_{LVE} across the different days after storage.

As for qualitative analyses, ten bacterial morphotypes were isolated from the pure LVE, which were all recovered from the B_{LVE} both 7 and 15 days after storage. Most of these bacteria belonged to the phyla Bacillota (i.e., *Leuconostoc* sp. LcLVE, *Cytobacillus* sp. CbLVE, *Bacillus* sp. Bc1LVE, *Bacillus* sp. Bc2LVE, *Bacillus* sp. Bc3LVE, and *Bacillus* sp. Bc4LVE) followed by Actinomycetota (i.e., *Microbacterium* sp. MbLVE and *Gordonia* sp. GdLVE), Pseudomonadota (i.e., *Pseudomonas* sp. PsLVE), and Bacteroidota (i.e., *Sphingobacterium* sp. SbLVE). On the other hand, two bacterial genera, including *Gordonia* sp. GdLVE and *Bacillus* sp. Bc3LVE, were not detected in the B_{LVE} 30 days after storage, while three previously undetected genera, including *Bervundimonas* sp. BvLVE, *Brucella* sp. BrLVE, and *Priestia* sp. PrLVE, were recovered for the first time at this stage. Bacterial members recovered 30 days after storage, except for *Bacillus* sp. Bc4LVE, were also recovered from the B_{LVE} 60 days after storage (Figure 2b).

3.3. pH and EC Analyses of B_C and B_{LVE}

The activation of the biochar with the LVE led to significant changes in both the pH and EC (Figure 3a,b). Compared to the B_C, activation with the LVE decreased the biochar's pH from 9.3 to 7.6, while it increased the EC from 665 to 806 µS cm^{-1} on the first day of storage. The extent of these changes was maintained throughout the entire 60-day storage period.

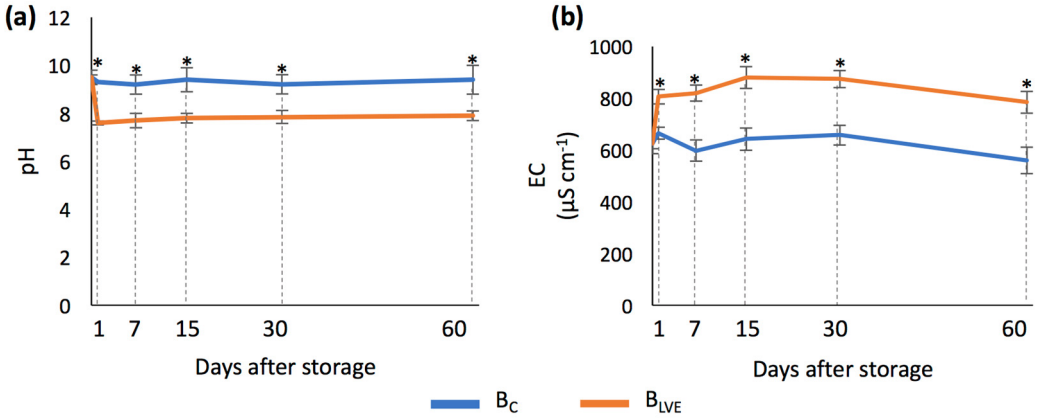

Figure 3. (**a**) pH (mean ± SD) of B_C and B_{LVE} across the different days after storage; (**b**) electrical conductivity (EC) (mean ± SD) of B_C and B_{LVE} across the different days after storage. Asterisks indicate significant differences between B_C and B_{LVE} each day after storage.

3.4. Testing the Effect of Increasing Doses on Soil Quality

3.4.1. Carbon Fractions and BFI

In both soils, the M-S and W-S, the application of the B_C and B_{LVE} treatments resulted in an increase in the SOM. Regardless of the concentration tested, the average increase recorded in the M-S was 69% for both the B_C and B_{LVE} treatments (Table 3). The W-S showed an average increase of 128% for the B_C treatments and 134% for the B_{LVE} treatments (Table 4).

The different soil types had varying effects on the microbial biomass carbon (MB-C) based on the treatment used. In the M-S, the B_C alone treatment increased the MB-C by an average of 18%, while the LVE alone treatment showed positive effects only at the M and H dose with an average increase of 12%. On the other hand, the B_{LVE} treatment showed a general increase of 32% (Table 3). In the W-S, only the highest dose showed statistically significant effects, with the B_C increasing by 18%, LVE by 19%, and B_{LVE} by 30% (Table 4).

Table 3. Soil fertility parameters comprising the soil biological fertility index (BFI) and its level (BFI lv.) of M-S (mean ± SD; $n = 6$). Means with the same letters within the column are not significantly different from each other at $p < 0.05$ according to Tukey-Kramer test.

Treatment	Res_Bas	Res_Cum	MB-C	M-S SOM	qCO_2	qM	BFI	BFI lv.
Cnt	21.6 ± 0.4 ^{d}	1302 ± 14 ^{cd}	849 ± 33 ^{e}	2.2 ± 0.1 ^{d}	1.06 ± 0.04 ^{a}	10.0 ± 0.1 ^{a}	14.0 ± <0.1 ^{e}	III
L-B_C	21.2 ± 0.7 ^{d}	1285 ± 49 ^{cd}	963 ± 23 ^{bc}	3.1 ± 0.1 ^{c}	0.92 ± 0.02 ^{cd}	7.1 ± 0.3 ^{d}	17.0 ± <0.1 ^{cd}	III
M-B_C	21.3 ± 0.4 ^{d}	1299 ± 2 ^{d}	1025 ± 85 ^{b}	3.4 ± 0.2 ^{b}	0.87 ± 0.07 ^{d}	6.4 ± <0.1 ^{ef}	18.3 ± 0.6 ^{c}	III
H-B_C	23.6 ± 0.8 ^{bcd}	1405 ± 49 ^{bc}	1025 ± 10 ^{b}	4.8 ± 0.2 ^{a}	0.92 ± 0.03 ^{cd}	5.1 ± 0.2 ^{g}	20.7 ± 0.6 ^{b}	IV
L-LVE	23.9 ± 1.0 ^{abcd}	1388 ± 67 ^{bcd}	886 ± 22 ^{de}	2.4 ± 0.2 ^{d}	1.12 ± 0.03 ^{a}	9.8 ± 0.5 ^{ab}	14.7 ± 1.2 ^{e}	III
M-LVE	22.5 ± 1.1 ^{d}	1360 ± 7 ^{bcd}	939 ± 19 ^{cd}	2.4 ± 0.2 ^{d}	1.00 ± 0.02 ^{b}	9.8 ± 0.1 ^{a}	14.3 ± 0.6 ^{e}	III
H-LVE	22.8 ± 0.4 ^{cd}	1315 ± 26 ^{cd}	967 ± 16 ^{bc}	2.4 ± 0.2 ^{d}	0.98 ± 0.02 ^{b}	9.2 ± 0.2 ^{b}	15.3 ± 0.6 ^{de}	III
L-B_{LVE}	26.6 ± 2.1 ^{a}	1406 ± 13 ^{bc}	1110 ± 29 ^{a}	3.1 ± 0.2 ^{c}	1.00 ± 0.03 ^{b}	8.0 ± 0.1 ^{c}	18.3 ± 1.5 ^{c}	III
M-B_{LVE}	25.5 ± 0.5 ^{abc}	1447 ± 20 ^{b}	1109 ± 35 ^{a}	3.6 ± 0.2 ^{b}	0.96 ± 0.01 ^{bc}	7.0 ± 0.1 ^{de}	20.3 ± 0.6 ^{b}	IV
H-B_{LVE}	26.3 ± 1.5 ^{ab}	1753 ± 54 ^{a}	1136 ± 12 ^{a}	4.8 ± 0.2 ^{a}	0.96 ± 0.01 ^{bc}	6.3 ± 0.2 ^{f}	22.7 ± 0.6 ^{a}	IV

Cnt: control; L-Bc: low Bc dose; M-Bc: medium Bc dose; H-Bc: high Bc dose; L-LVE: low LVE dose; M-LVE: medium LVE dose; H-LVE: high LVE dose; L-B_{LVE}: low B_{LVE} dose; M-B_{LVE}: medium B_{LVE} dose; H- B_{LVE}: high B_{LVE} dose; Res_Bas: basal respiration (ppm CO_2-C); Res_Cum: cumulative respiration (ppm CO_2-C); MB-C: microbial biomass carbon (ppm C); SOM: soil organic matter (%); qCO_2: specific microbial biomass respiration (mg CO_2-C·10^{-2}·h^{-1}·mg C mic^{-1}); qM: mineralization quotient (%).

Table 4. Soil fertility parameters comprising the soil biological fertility index (BFI) and its level (BFI lv.) of W-S (mean ± SD; n = 6). Means with the same letters within the column are not significantly different from each other at $p < 0.05$ according to Tukey-Kramer test.

Treatment	Res_Bas	Res_Cum	MB-C	W-S SOM	qCO_2	qM	BFI	BFI lv.
Cnt	13.1 ± 1.0 ^{c}	896 ± 81 ^{b}	807 ± 71 ^{c}	1.0 ± 0.1 ^{d}	0.68 ± 0.06 ^{ab}	14.7 ± 1.3 ^{bc}	10.7 ± 0.6 ^{f}	II
L-B_C	13.5 ± 2.0 ^{bc}	959 ± 87 ^{b}	840 ± 37 ^{c}	1.5 ± 0.2 ^{c}	0.67 ± 0.03 ^{ab}	10.4 ± 0.9 ^{de}	11.3 ± 0.6 ^{ef}	II
M-B_C	13.4 ± 1.0 ^{bc}	1032 ± 94 ^{b}	946 ± 33 ^{b}	2.2 ± 0.1 ^{b}	0.64 ± 0.02 ^{bc}	8.1 ± 0.7 ^{ef}	13.0 ± 0.6 ^{cde}	III
H-B_C	13.4 ± 0.5 ^{bc}	1103 ± 100 ^{ab}	869 ± 43 ^{bc}	3.4 ± 0.1 ^{a}	0.59 ± 0.02 ^{c}	5.6 ± 0.5 ^{f}	16.3 ± 0.6 ^{ab}	III
L-LVE	14.4 ± 0.4 ^{bc}	948 ± 86 ^{b}	894 ± 97 ^{bc}	1.0 ± 0.1 ^{d}	0.68 ± 0.08 ^{ab}	15.4 ± 1.4 ^{ab}	11.0 ± 1.0 ^{f}	II
M-LVE	14.5 ± 1.0 ^{bc}	992 ± 90 ^{b}	901 ± 34 ^{bc}	1.0 ± 0.1 ^{d}	0.67 ± 0.03 ^{ab}	16.6 ± 1.5 ^{ab}	11.7 ± 1.2 ^{ef}	II
H-LVE	14.6 ± 1.0 ^{abc}	1098 ± 100 ^{ab}	957 ± 50 ^{ab}	1.0 ± 0.1 ^{d}	0.64 ± 0.03 ^{bc}	18.2 ± 1.7 ^{a}	12.3 ± 0.6 ^{def}	II
L-B_{LVE}	15.4 ± 0.3 ^{abc}	1103 ± 100 ^{ab}	892 ± 26 ^{bc}	1.7 ± 0.2 ^{c}	0.72 ± 0.02 ^{a}	11.5 ± 1.0 ^{cd}	13.7 ± 0.6 ^{cd}	III
M-B_{LVE}	15.8 ± 0.4 ^{ab}	1153 ± 105 ^{ab}	902 ± 14 ^{bc}	2.2 ± 0.2 ^{b}	0.73 ± 0.01 ^{a}	8.8 ± 0.8 ^{def}	14.7 ± 0.6 ^{bc}	III
H-B_{LVE}	17.2 ± 0.2 ^{a}	1318 ± 120 ^{a}	1052 ± 45 ^{a}	3.4 ± 0.1 ^{a}	0.68 ± 0.03 ^{ab}	6.6 ± 0.6 ^{f}	17.3 ± 0.6 ^{a}	III

Cnt: control; L-Bc: low Bc dose; M-Bc: medium Bc dose; H-Bc: high Bc dose; L-LVE: low LVE dose; M-LVE: medium LVE dose; H-LVE: high LVE dose; L-B_{LVE}: low B_{LVE} dose; M- B_{LVE}: medium B_{LVE} dose; H-B_{LVE}: high B_{LVE} dose; Res_Bas: basal respiration (ppm CO_2-C); Res_Cum: cumulative respiration (ppm CO_2-C); MB-C: microbial biomass carbon (ppm C); SOM: soil organic matter (%); qCO_2: specific microbial biomass respiration (mg CO_2-C·10^{-2}·h^{-1}·mg C mic^{-1}); qM: mineralization quotient (%).

No statistical differences were found between individual treatments in the soil Res_Bas. However, a significant increase in the parameter was observed in the B_{LVE} treatment for both the M-S and W-S. The average increase was 21% in the M-S (Table 3) and 26% in the W-S, but only for the M and H doses (Table 4). As for the Res_Bas, the soil Res_Cum followed a similar trend for both analyzed soils. It was an increase in the M-S at the highest dose of B_C (+8%), and for the M and H doses of B_{LVE} (+23% on average, Table 3). In the W-S, this increase is only observed for the H-B_{LVE}, with an increase of 47% (Table 4).

The trend of the qCO_2 varied depending on the soil type and treatments. In the case of the M-S, there was a general decrease in the B_C-based treatments with a reduction of 15% for the B_C and 8% for the B_{LVE}, and a decrease of 7% for the M and H doses of the LVE (Table 3). As for the W-S, only the B_C-based treatments showed a significant difference and had opposite trends compared to the Cnt, with a decrease of 13% for the H-B_C, and an increase of 6% for the L and M doses of B_{LVE} (Table 4). The trend of the qM is also dose-dependent, unlike qCO_2. As reported in Table 3 for the M-S and in Table 4 for the W-S, in both soils, there was a decrease observed for the M-B_C and H-B_C doses (−20% in M-S and −19% in W-S) and LVE-based treatments (−35% in M-S and −52% in W-S). On the other hand, for the B_{LVE} treatments, negative results were obtained for the L-B_{LVE} dose (−47% in M-S and −60% in W-S), while an increase of 37% and 41% for each soil was observed for the M and H doses.

The final calculation of the BFI and the corresponding levels indicated that treatments based on the B_C and B_{LVE} positively influenced soil fertility. For the M-S, the B_C and B_{LVE} treatments increased the BFI by 17% and 33%, respectively (Table 3), while for the W-S, the H-B_C dose increased the BFI by 25% and an average of 31% for the M and H doses of the B_{LVE} (Table 4). A potential synergistic effect may occur between the B_{LVE} treatments at the M and H doses in both soils.

3.4.2. Enzyme Activities and SAI3

The enzyme activities showed an overall positive response to both the individual and combined treatments (Table 5). Compared to the Cnt, the phosphatase activity increased in both soils with the application of increasing doses of B_C (+32%), LVE (+24%), and B_{LVE} (+33%). In the M-S, the activity of urease was increased by 18% for the B_C and 17% for the LVE. However, when amended (B_{LVE}), the increase became more significant (+22%). In the W-S, only the B_C- and B_{LVE}-based treatments showed a substantial increase, with 44% for the B_C and 129% for the B_{LVE}. Out of all the enzymes analyzed, β-glucosidase showed a different trend. This enzyme activity decreased by 16% and 17% in both the M-S

and W-S when treated with the B_C-based doses, and by 18% and 12% when treated with the B_{LVE}. Only in the M-S was there a decrease of 22% in enzyme activity when treated with the LVE. Despite the decreasing trend of β-glucosidase, the SAI3 calculation showed an overall improvement in soil quality as the doses increased. Regarding any synergistic effect between the materials in the B_{LVE}, it was only observed in the M-S at the highest dose (H). However, in the W-S, differences were visible at the M dose compared to the other treatments.

Table 5. Soil quality parameters comprising the soil alteration index 3 (SAI3) of M-S and W-S (mean ± SD; n = 6). Means with the same letters within the column are not significantly different from each other at $p < 0.05$ according to Tukey-Kramer test.

	M-S				W-S			
Treatment	**APA**	**βGA**	**UA**	**SAI3**	**APA**	**βGA**	**UA**	**SAI3**
Cnt	80 ± 2^{d}	152 ± 6^{a}	156 ± 6^{e}	1.1 ± 0.2^{e}	166 ± 9^{f}	60 ± 4^{a}	13 ± 4^{c}	-6.6 ± 0.7^{f}
L-B_C	101 ± 3^{b}	139 ± 5^{ab}	163 ± 4^{e}	-1.0 ± 0.1^{d}	187 ± 7^{de}	56 ± 4^{a}	18 ± 5^{bc}	-8.2 ± 0.1^{de}
M-B_C	105 ± 5^{ab}	125 ± 9^{cd}	187 ± 5^{bcd}	-2.4 ± 0.7^{c}	197 ± 7^{cd}	52 ± 5^{ab}	19 ± 4^{bc}	-9.1 ± 0.6^{cd}
H-B_C	111 ± 3^{a}	118 ± 13^{cde}	201 ± 7^{a}	-3.4 ± 0.8^{ab}	197 ± 2^{cd}	43 ± 7^{b}	21 ± 3^{b}	-9.6 ± 0.4^{bc}
L-LVE	87 ± 2^{c}	121 ± 5^{cd}	182 ± 3^{cd}	-1.5 ± 0.2^{d}	178 ± 9^{e}	59 ± 3^{a}	13 ± 3^{c}	-7.4 ± 0.6^{ef}
M-LVE	106 ± 4^{ab}	115 ± 6^{de}	187 ± 2^{bcd}	-3.0 ± 0.2^{bc}	184 ± 6^{e}	57 ± 5^{a}	17 ± 5^{bc}	-8.0 ± 0.2^{e}
H-LVE	105 ± 6^{ab}	119 ± 9^{cd}	180 ± 3^{d}	-2.6 ± 0.4^{bc}	185 ± 5^{e}	56 ± 4^{a}	18 ± 3^{bc}	-8.1 ± 0.1^{e}
L-B_{LVE}	101 ± 3^{b}	139 ± 5^{ab}	190 ± 4^{bc}	-1.5 ± 0.1^{d}	205 ± 2^{c}	55 ± 6^{a}	29 ± 4^{a}	-9.5 ± 0.4^{bc}
M-B_{LVE}	108 ± 4^{ab}	130 ± 4^{bc}	187 ± 5^{bcd}	-2.3 ± 0.1^{c}	218 ± 6^{b}	53 ± 8^{ab}	31 ± 3^{a}	-10.4 ± 0.8^{b}
H-B_{LVE}	109 ± 4^{a}	104 ± 5^{c}	195 ± 7^{ab}	-3.9 ± 0.3^{a}	278 ± 4^{a}	51 ± 6^{ab}	32 ± 3^{a}	-14.1 ± 0.1^{a}

Cnt: control; L-Bc: low Bc dose; M-Bc: medium Bc dose; H-Bc: high Bc dose; L-LVE: low LVE dose; M-LVE: medium LVE dose; H-LVE: high LVE dose; L-B_{LVE}: low B_{LVE} dose; M-B_{LVE}: medium B_{LVE} dose; H-B_{LVE}: high B_{LVE} dose; APA: alkaline phosphatase activity (μmol p-nitrophenol·g^{-1} dry soil·h^{-1}); βGA: β-glucosidase activity (μmol p-nitrophenol·g^{-1} dry soil·h^{-1}); UA: urease activity (μg NH_4^+-N g^{-1} soil 2 h^{-1}).

4. Discussion

4.1. Potential Properties of LVE-Activated Biochar

The biochar activation method used allowed the efficient colonization and active multiplication of the LVE bacteria in biochar particles. This effect was clearly depicted by the 10-fold increase in bacterial abundance in the B_{LVE} during the first week of storage and by the maintenance of these population levels for at least 60 days of storage. On the other hand, the observed changes in community composition across biochar storage indicated that taxa lying outside detectability thresholds in pure LVE can later prove to be advantaged upon biochar colonization, as observed for *Bervundimonas* sp. BvLVE, *Brucella* sp. BrLVE, and *Priestia* sp. PrLVE. The colonization of biochar by these taxa could contribute to increasing the microbial diversity in the soil upon amendment with LVE-activated biochar, which could further enhance nutrient availability through nutrient mineralization and organic matter decomposition [48]. Nevertheless, it is important to consider that the specific consequences of amending soils with activated biochar can vary depending on factors such as the type of biochar, the composition of the soil, and the specific microorganisms involved. In addition, although the 16S rRNA gene can provide poor discriminatory power at the species level, the top 100 blast hits for the first two genera showed a high correspondence to the species *Brevundimonas bullata* and *Priestia aryabbhatai*, respectively. Studies correlating the presence of *B. bullata* with improved soil quality are missing. However, Li et al. [49] characterized the antagonistic activity of this species against plant pathogenic nematodes. Furthermore, *P. aryabhattai* has been shown to significantly promote plant growth and drought tolerance in different plant species, including *Arabidopsis*, tobacco, and maize [50,51] and to have phosphate-solubilizing and N-fixing roles, which may potentially contribute to nutrient availability and uptake by plants [52]. Conversely, other bacteria present in LVE may gradually decrease their abundance in biochar particles, as observed for *Gordonia* sp. GdLVE, *Bacillus* sp. Bc3LVE, and *Bacillus* sp. Bc4LVE. A possible reason for the decrease in these taxa may be linked to functional redundancy with other

phylogenetically close members and/or faster-growing microorganisms present in LVE, or even to antagonistic interactions among specific taxa.

Another explanation for the observed changes in community composition over time could lie in the varying ability of the isolated bacteria to persist in biochar particles. In this regard, the top 100 blast hits for *Leuconostoc* sp. LcLVE, *Bacillus* sp. Bc1LVE, and *Bacillus* sp. Bc2LVE showed a high correspondence to the species *Leuconostoc mesenteroides*, *Bacillus subtilis,* and *Bacillus cereus*, which are well known for their capability to secrete extracellular polymeric substances that attach to surfaces and promote biofilm formation [53–55]. However, although biochar can potentially serve as a substrate for biofilms, further research is needed on the role of biofilm formation in enhancing bacterial survival in biochar. In addition, it is reasonable to assume that microbial communities may experience some degree of fluctuation in structure due to their high metabolic activity; this can occur particularly in OM-rich environments, which can induce a more active production of microbial metabolites that can ultimately affect the overall community structure [56,57].

Finally, activation with the LVE led to a significant decrease in the pH of the biochar and an increase in its EC. As for the pH, this effect may have positive consequences on plant growth, as the alkalinity of biochar can lead to the unavailability of assimilable forms of nutrients to plants [58]. On the other hand, the increase in EC could indicate LVE as an additional source of soluble salts, which could compete in uptake with the availability of important nutrients, leading the plant to a nutritional and metabolic imbalance and decreased or increased cell osmotic potential. However, the potential impacts of increased EC on the measured soil quality parameters remain to be elucidated.

4.2. Validation of LVE-Activated Biochar on Soil

4.2.1. Effects on Linked C Parameters and Biological Fertility of Soil

As expected, the addition of Bc and B_{LVE} to the soil increased the SOM content in both soil types (i.e., M-S and W-S), with a greater dose leading to a higher increase. This agrees with other studies [59,60], which have shown that the use of organic fertilizers such as biochar and composted materials (i.e., vermicompost) can enhance the SOM. This is primarily due to biochar contributing stable organic carbon to the soil [61].

Most studies suggest that biochar applications increase the MB-C, due to its ability to create a favorable habitat for microorganisms [62]. In this study, the addition of B_C and B_{LVE} modified the biological activity in the soils tested and it was observed that the positive impact of Bc was more evident at higher doses and in the M-S. This could be attributed to the fact that the M-S had a better initial condition of biological fertility in terms of the SOM and MB-C, with respect to the W-S. Other research also supported the ability of high doses of Bc to enhance the MB-C in soils that share similar characteristics to the M-S [63,64]. The higher MB-C values in the B_{LVE}-treated soils, than Bc-treated ones, suggest that the LVE and/or soil microorganisms could be increased by the biochar. On the other hand, several studies demonstrated the ability of vermicompost to increase the MB-C [65–67] and this would also explain the positive effect of the high dose of LVE treatment in both soils.

The addition of the B_{LVE} increased the soil respiration parameters (i.e., Res_Cum and Res_Bas) in both soils and at higher application doses, indicating a potential role of LVE bacteria in the promotion of a higher and more efficient soil respiration process. Some studies show that biochar does not increase soil respiration [68,69], while LVE can improve it [65,70]. The increased effect of the B_{LVE} on soil respiration could be attributed to (1) the activity of inoculated microorganisms on the native SOM and (2) the ability of the biochar to promote the activity of both inoculated and native microorganisms.

The qCO_2 measures the respiration rate of CO_2-C per unit of MB-C [71]. It is widely used as an indicator of ecosystem succession [72], during which it is expected to decline, and maturity [73]. It has been seen that a low qCO_2 value indicates that soil conditions were improved by the addition of organic matter to soil [74]. Overall, here, the treatments contributed to a reduction in the qCO_2 in both soils. However, the reduction was more significant in the M-S than in the W-S. This study confirms that the use of biochar, vermi-

compost, or a combination of both can reduce this parameter and this observation is in line with other similar studies [64,75,76]. The different trend observed between the two soils could be attributed to the lower amount of native SOM in the W-S compared to the abundance of microorganisms.

The qM represents the TOC fraction that has been mineralized throughout an incubation period [77]. Biochar-based treatments substantially reduced this parameter in both soils, confirming the results of previous studies [78,79] and qualifying it as an effective tool for increasing C storage in the soil.

The BFI is a robust multi-domain indicator of fertility developed for Italian soils [37]. In the B_{LVE} treatments, the biochar and LVE showed a mutual enhancement of their properties, resulting in an increase in the BFI level in both soils, hence reflecting an improved biological activity and soil quality. Although the effect was observable in the M-S, it was more pronounced in the W-S. Indeed, even with the lowest dose of B_{LVE}, there was a significant increase in the BFI in the W-S.

4.2.2. Effects on Enzyme Activities and Quality of Soils

In general, the different treatments positively influenced the APA and UA at various doses, but a negative trend for the βGA was observed. Although in other studies [80–83] the biochar had positive effects on the UA and APA, in our case, the Bc treatments led to a decrease in the βGA. This is in line with Gunal et al. [84], who found that the addition of biochar reduced the βGA and the decline was higher in sandy loam soils compared to loamy soils. Foster et al. [85] suggest that the surface area and pore size distribution of biochar are key indicators of potential biochar-enzyme interactions. They assume that the decline in the βGA is mainly a result of direct absorption. At the same time, adsorption of a small amount of substrate can also occur, since the polar glucose substrate can easily adsorb onto negatively charged solid phases. Other factors may also have influenced the βGA, including the presence in the biochar of inhibiting compounds such as phenols and polyphenols.

In general, vermicompost-based treatments enhance soil enzyme activities and our results align with those of other researchers [19,86,87]. However, it is possible that the decrease in the βGA, as a result of the LVE treatment in the M-S, was due to a reduction in the enzyme present in the vermicompost used to produce the extract. Previous studies have shown that the βGA in the vermicompost decreases as it matures [88,89]. This decrease can be attributed to the presence of more humified organic substances and a reduction in cellobiose and other disaccharides. It is possible that LVE-based treatments have altered the composition of the SOM, thereby reducing the need to produce this enzyme by microorganisms.

Although the βGA showed a negative trend, the SAI3 revealed that the soil quality increased with increasing doses of the B_{LVE}-based treatments in both soils. It is interesting to note that even the lowest dose showed a clear improvement, indicating the magnification of the interaction between the biochar and inoculated microorganisms.

Moreover, the observed changes in the soil enzyme activities may partly lie on the identity and metabolic attributes of the bacteria present in the activated biochar. Microbial APA has often been recognized as a plant- and soil-promoting attribute due to its role in making P more accessible from the SOM into more available forms [90]. On the other hand, microbial UA is a key component of soil biological activity, since it promotes nutrient cycling and the enhancement of N in the soil, and thus contributes to the maintenance of soil health and fertility [91]. In this regard, several members of the phylum Bacillota, including *B. subtilis*, *B. cereus*, and *P. aryabhattai*, are known for their high APA and UA [92–95]. Moreover, other identified bacterial species from the phylum Pseudomonadota, including *B. bullata* as well as several *Brucella* and *Pseudomonas* species, can produce diverse types of phosphatases [93,96–100] and ureases [101,102].

5. Conclusions

The present study showed that biochar can be successfully activated with LVE and that LVE-associated bacteria can undergo compositional shifts across biochar storage. However, while the presence of LVE bacteria could be correlated to the observed beneficial effects on soil quality, further characterization of these isolates is necessary to allow their safe application in agriculture, including the evaluation of potential ecological impacts, the assessment of potential risks of horizontal gene transfer, and the examination of potential side-effects on non-target organisms resulting from the introduction of non-native bacteria into the environment. Moreover, to better understand the role of vermicompost bacteria in soil improvement, further genetic analysis, metabolic profiling, and functional assessments are needed. These additional investigations could provide insights into the genetic composition and metabolic capabilities of the bacteria, as well as their specific functions in enhancing soil health and nutrient cycling. It is important to conduct these studies to gain a comprehensive understanding of the mechanisms underlying the beneficial properties of vermicompost bacteria and to address potential ecological impacts, risks of horizontal gene transfer, and effects on non-target organisms.

In addition, the use of LVE-activated biochar significantly enhanced the soil parameters of C-related soil biological fertility and enzyme-linked soil quality. This study found that activated biochar can significantly improve soil quality and fertility, particularly in less fertile soil (i.e., W-S). However, the increase was also substantial in medium-fertile soils (i.e., M-S), making activated biochar a highly valuable tool for enhancing soil quality and fertility. By incorporating LVE-activated biochar into soil management practices, farmers could achieve higher yields and healthier crops.

However, it is necessary to conduct more comprehensive research to confirm the effectiveness of this approach, considering different types of soils with different physical, chemical, and biological properties. Furthermore, it would be essential to conduct long-term respiration tests and a B_{LVE} storage period in various environmental conditions on other biochars and LVEs produced using different matrices and methods.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/soilsystems8020054/s1, Table S1: list of bacteria isolated from the liquid vermicompost extract (LVE) (genus and assigned strain name), primers used for amplification, and respective accession numbers.

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