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Article

Eco-Sustainability of Soils in Baby-Leaf Crop Systems under Tunnel through the Application of C-Rich Inputs: Towards Combating Soil Degradation

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Abstract: Fresh-cut leafy vegetables are produced in Southern Italy in very intensive crop systems under tunnel greenhouses in which continuous cropping has triggered soil organic carbon (SOC) depletion and the risk of degradation of soil fertility. A two-year trial of soil organic amendment was carried out on a private farm producing baby-leaf crops on a very poor OC soil (<1%). Biowaste compost, two types of olive pomace composts and buffalo manure were compared to evaluate their ability to recover a positive SOC balance and sustain crop growth and yield. The effects on soil health and crop system were studied by measuring different aspects such as SOC stock change and SOC sequestration rate, soil microbial biomass and nine enzyme activities, yields of rocket and concentration of nitrates in leaves. Soil amendments were distributed once a year at doses of 15 and 30 Mg ha^{-1} as fresh matter without integration of mineral fertilizers. In our study, the SOC stock improved in the amended soils in a range of 4–6 Mg ha^{-1}, except for dose 30 of buffalo manure, with the highest values where biowaste compost was applied. Our data showed an increase in biological parameters in all the amended soils with respect to Control. In soil amended with olive pomace, however, compost mineralization rates likely did not match crops' nutrient needs so the yields of rocket were lower than with the biowaste compost and buffalo manure. Biowaste compost showed the best results as it balanced the best C conversion efficiency, the higher increment of SOC and yields of rocket.

Keywords: buffalo manure; olive pomace compost; biowaste compost; soil organic carbon; soil enzymes; soil microbial biomass; rocket; nitrates

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1. Introduction

Fresh-cut leafy vegetables are produced in specialized farms that are equipped with plastic tunnels and suitable mechanical equipment. Compared to the conventional cropping systems in greenhouses, based on 2–3 crop cycles per year, the production of leafy vegetables increased the crop intensification in terms of number of crop cycles (up to 5–7 per year), number of harvests/cuts (around 10 cuts per year), number of soil tilling per year [1], high use of synthetic fertilizers and agrochemicals (including soil fumigants) and systematic removal of crop residues. All these practices, applied under the favorable microclimatic conditions realized under tunnels, have favored soil organic matter mineralization and depletion [2]. The phenomenon is even more pronounced in Italy, characterized by a Mediterranean climate; here, the area cultivated with fresh-cut leafy vegetables has been growing continuously starting from the early 2000s [3,4]. The main Italian area involved in the cultivation of leafy vegetables for the ready-to-eat sector (IV gamma) is the Sele River Valley, south of Salerno, where about 5.400 ha of plastic tunnel-greenhouses are dedicated to this purpose [5]. The adoption of intensive agriculture systems in tunnel-greenhouses for a long period may lead to a deterioration in the physical, chemical and biological quality of soil, which in turn, may negatively affect crop yield and quality [6,7].

Nowadays, the need to ensure the food supply must be coupled with the need to preserve soil health. According to the Intergovernmental Technical Panel on Soils of the FAO Global Soil Partnership [8], soil health is "the ability of the soil to sustain the productivity, diversity and environmental services of terrestrial ecosystems". Regarding the issue of improving the sustainability of leafy vegetable crop systems, in the last few years, some authors have oriented their efforts toward studying the effects of some biostimulants based on *Trichoderma* or protein hydrolysates. The main target of this approach has been to increase the nitrogen fertilization efficiency, yield and quality of perennial-wall rocket by improving its adaptation in low-fertility soils [9–11].

In the last decade, solutions put in practice by farmers to improve the amount of SOC are the introduction during summer months of green manure crops of *Sorghum bicolor* or *Setaria italica*, which are also able to recover N, P and K nutrients from soil [12]. In addition, recently, different soil organic amendments have been tested with different aims: as an alternative to mineral fertilization for Swiss chard cropped in a pot experiment and amended with biochar, olive pomace compost or cattle digestate compost [13]; to examine and characterize the effects of the combined application of bio-based fertilizers (composts, digestate, manure) on lettuce yield and quality and soil properties in a trial conducted under open field conditions [14]; and to explore the possibility of soil fertility recovery and promotion by the application of biochar, buffalo manure compost or alfa-alfa straw in a two-year-long mesocosm experiment [15].

In recent years, the trend observed in the assessment of soil quality has been to integrate the chemical-physical parameters with the biological ones. Soil microbial parameters can be used as indicators to assess soil quality [16]. Since enzyme activities respond rapidly to changes in soil management, they are often used as indicators of soil quality [17]. In addition, soil enzymes can indicate the potential of the soil to support the biochemical processes, essential for the maintenance of its fertility [18].

In the present study, we addressed the problem of soil organic carbon depletion in a baby leaf crop system managed under the tunnel-greenhouses of a private farm, using three kinds of C-rich inputs: (1) Olive pomace compost obtained from the fibrous part of the olive fruit and kernel fragments; (2) Biowaste compost produced by organic fraction of municipal solid wastes separately collected; and (3) Buffalo manure, which is readily available in the Sele River Plain, to be considered as a possible source of organic biomass by farmers devoted to baby leaf crops. Olive pomace compost, due to its high content of organic matter, exchangeable cations and high C/N ratio, is considered to be a valuable resource for soil fertility in olive orchards [19,20]; however, some doubts are posed by Altieri and Esposito [21] and Morra et al. [22] toward the viability of its use in the amendment of more intensive agricultural systems. Meanwhile, nowadays, in Italy, biowaste compost represents the most abundant organic soil amendment: over 2 million tons per year are produced by recycling 7 million tons of municipal organic wastes from source-separated collection [23]. Thus, the use of compost in agriculture gives a double answer to two relevant problems: reduces the quantity and environmental impact of wastes sent to landfills [24] and improves the soil properties with the addition of stabilized organic matter and valuable nutrients (e.g., nitrogen and phosphorus) [25,26].

In light of these considerations, we aimed to evaluate, in the short term, if growing amounts of C input supplied through the above-listed soil organic amendments can reverse the ongoing degradation of soil fertility and sustain crop productivity without the input of chemical fertilizers under plastic tunnels. To verify the achievement of the objective, we studied some modifications occurring to soil and crop:

(a) After a two-year period, the SOC change and conversion efficiency of C input were assessed in relation to the organic C sources and two levels of supply.
(b) In the second year of organic amendment, the repeated measurement (three times) of soil microbial biomass and some enzymatic activities linked to C, N, P and S biogeochemical cycles were carried out.
(c) In the second year, the yield of rocket (fresh and dry matter) as a response to the repeated amendment was evaluated.
(d) In the second year, as already measured in the first year, the possible influence of total N applied by organic amendments on the uptake of nitrate in leaves of rocket, which, as is known, is a nitrate hyper-accumulating species, was verified [9].

2. Materials and Methods

2.1. Experimental Site

The trial was carried out on a farm associated with the Terramore Cooperative in the Sele River Plain (Lat 40°52′, Lon 14°96′), Eboli (south to Salerno), largely devoted to the production of leafy vegetables harvested as baby leaf (rocket and basil) or as mature heads (lettuce, endive), both kinds of vegetables intended for the production of fresh ready-to-eat, packed vegetables. Leafy vegetables are produced under multiple tunnels with a cubic capacity higher than 3 m^3 m^{-2}, covered with plastic film, and not heated. The soil under tunnel had a sandy-clay texture with clay 360 g kg^{-1}, sand 520 g kg^{-1}, silt 120 g kg^{-1}, pH 8.2, electrical conductivity 1.38 dS m^{-1}, soil OC content 5.4 g kg^{-1} (0.9% as organic matter), total N 0.62 g kg^{-1}, C/N 8.7, P_2O_5 19 mg kg^{-1} and K_2O 210 mg kg^{-1}. C content is <1% (10 g kg^{-1}), the threshold value for considering a soil degraded [27].

2.2. Experimental Design and Crop Management

Four soil organic amendments were applied: (1) Olive pomace compost with low C/N ratio containing three-phase-olive-oil-mill waste mixed with wooden chips and waste from the production of sheep wool as supplementary N source (OPCmix); (2) Olive pomace compost with high C/N ratio produced with three-phase-olive-oil-mill waste mixed to wooden chips (OPC); (3) Biowaste compost produced with the organic fraction separately collected from municipal solid wastes (BWCom); and (4) Buffalo manure aged 5 months and supplied from a livestock farm near the research area (BMan). The first two composts were supplied by the composting plant CESCO in Laurino (SA) managed by the National Park of Cilento and Vallo di Diano, while the biowaste compost was supplied by the composting plant of the city of Salerno. Table 1 shows the chemical characteristics of the selected composts and the manure distributed in 2013 and 2014. The two olive pomace composts differ in that OPC had a higher total organic carbon (TOC) and lower total N contents than OPCmix; C/N ratio was 30 in OPC and ranged from 14 to 16.8 in the OPCmix. The buffalo manure showed high annual variation in dry matter, total N and C/N ratio, while the biowaste compost showed steady chemical characteristics in the two years.

Table 1. Concentrations of total organic Carbon and total Nitrogen, C/N ratio and dry matter content of the four soil organic amendments used in 2013 and 2014.

Amendments	Total Organic C g kg^{-1} d.m.	Total N g kg^{-1} d.m.	C/N	Dry Matter g kg^{-1} Fresh Matter
	2013			
BMan	31.5	2.50	12.6	400
OPCmix	36.7	2.18	16.8	760
OPC	38.3	1.25	31	740
BWCom	26.4	1.65	16	890

Table 1. *Cont.*

Amendments	Total Organic C g kg^{-1} d.m.	Total N g kg^{-1} d.m.	C/N	Dry Matter g kg^{-1} Fresh Matter
	2014			
BMan	33.8	1.54	21.9	212
OPCmix	24.7	1.75	14.1	840
OPC	38.3	1.26	30.4	835
BWCom	21.2	1.57	13.5	863

Legend: BWCom = biowaste compost; OPC = Olive pomace compost; BMan = Buffalo manure; OPCmix = Olive pomace compost with wool residues.

The organic amendments were distributed in the 0–30 cm soil layer on 28 April 2013, and on 19 June 2014; the amounts applied for each organic amendment were 15 and 30 Mg ha^{-1} as fresh matter (f.m.). Therefore, the combination of 4 kinds of soil amendments for 2 rates of fertilization gave 8 treatments arranged according to a split-plot design with three replicates. Main plots hosted composts or manure, while in sub-plots were set the two doses. Each soil organic amendments were hand-distributed on the experimental units constituted by the raised beds where rocket was mechanically seeded. Each plot measured 1.6 × 15 m. Mean dry matter supplied annually with all composts was quite similar, ranging from 12 to 13 Mg ha^{-1}, corresponding to a level of 15 Mg ha^{-1} f.m., and ranging from 24 to 26 Mg ha^{-1}, corresponding to a level of 30 Mg ha^{-1} f.m. (Table 2). Conversely, buffalo manure supplied an amount of dry matter reduced by about 1/3 (in both levels 15 and 30 f.m.) compared to the composts. In absolute terms, buffalo manure supplied the lowest annual amounts of TOC and total N, while among composts, OPC supplied the highest annual amount of TOC (4.5–9 Mg ha^{-1}) and the lowest of total N (148–297 kg ha^{-1}); OPCmix and BwCom added more comparable amounts of TOC and N.

Table 2. Average annual amounts of dry matter, total organic C (TOC) and total N supplied with 15 or 30 Mg ha^{-1} as fresh matter of different organic soil amendments in the two-year trial.

Soil Organic Amendment	Dry Matter (Mg ha^{-1})	TOC (Mg ha^{-1})	N Total (kg ha^{-1})
BMan 15	4.4	1.5	100
BMan 30	8.8	3	199
BWCom 15	13	3.1	211
BWCom 30	26	6.2	423
OPCmix 15	12	3.6	234
OPCmix 30	24	7.3	469
OPC 15	12	4.5	148
OPC 30	24	9	297

Legend: BWCom = biowaste compost; OPC = Olive pomace compost; Bman = Buffalo manure; OPCmix = Olive pomace compost with wool residues.

Table 3 summarizes the main information about intensive tillage, organic fertilization and crop cycles carried out in the whole trial, which started in May 2013 and concluded in May 2015. After the first amendment, in 2013/2014, the sequence rocket–rocket–basil–rocket was followed while in 2014/2015, after the second amendment, a rocket–rocket sequence was pursued. Productive and qualitative results related to the first year of the experiment were presented in Morra et al. [28]. In this paper, which considers the second year, we focus on the last, major rocket cycle, which occurred in the cycle from November 2014 to April 2015.

Table 3. Tillage, soil organic amendments distribution, crop sequence, date of sowing, number of cuts (harvests) per cycle and last harvest for the whole trial between 2013 and 2015.

	Soil Tillage	Organic Fertilization	Crop Sequence	Seeding	Number of Cuts and Last Harvest Time
2013–2014	Rotavator to bury amendments along the 0–30 cm soil layer	Compost and manure distribution on 28 April 2013	Rocket (*Diplotaxis tenuifolia*), cv. Reset	05/02/13	(3) 06/17/13
	Before each short cycle: Rotavator cultivation at 20 cm + raised seedbed preparation		Rocket (not monitored)	July	
			Basil (*Ocimum basilicum*), cv. Compatto	08/28/13	(2) 10/10/13
	Before each long cycle: Chisel cultivation at 50 cm depth + rotavator cultivation at 20 cm + raised seedbed preparation		Rocket (*D. tenuifolia*), cv. Winter	11/7/13	(7) 05/08/14
2014–2015		Compost and manure distribution on 19 June 2014	Rocket (*D. tenuifolia*), cv. Reset	07/31/14	(2) 08/27/14
	Tillage as above		Rocket (*D. tenuifolia*), cv. Winter	11/04/14	(5) 04/01/15

2.3. Soil C Balance

Before the start of the trial (April 2013), samples of soil were collected in the 0–30 cm soil layer of the experimental area to define the baseline SOC content. On 19 May 2015, in each experimental plot, four soil samples were collected and pooled to determine the changes that had occurred in soil C contents. Bulk soil samples were sieved at 2 mm, then finely ground to <500 µm and analyzed for SOC. The content of organic C was determined by the Walkley–Black procedure [29]. Soil bulk density (bd) at 15 cm depth was determined at the start of the trial according to the 'Intact core method' for soils without coarse fragments [30]. The bulk density measured at the end of the trial did not change. To estimate the C change across the two-year period, we took into account as inputs of C in soil, the total amounts supplied in two years by different soil organic amendments, and the crop residues of all the crop cycles carried out in the two-year period (Table 4). Dry matter in crop residues was estimated by collecting in one quadrat of 0.25 m^2 per plot, the residual crowns of rocket or basil, including the roots of the first 5 cm soil layer. The crop residues were dried in an oven at 60 °C and weighed. C content of crop residues was estimated to be 45% of dry matter according to FAO [30]. The SOC pool (Mg ha^{-1}) for the 0–30 cm depth layer was calculated using the equation: SOC pool = SOC (g kg^{-1}) × bd (kg cm^{-3}) × d (cm) × 0.1 [30], where, Bd is bulk density 1.33, d is the soil depth and 0.1 is a factor for converting g C cm^{-2} to Mg C ha^{-1}. Based on these elements referred to each fertilization treatment, we calculated SOC changes, OC losses and C conversion efficiency of C supplied to soil by organic amendments + crop residues as follows: SOC changes = Final SOC amount − Initial SOC amount; C losses = Final SOC amount − (Initial SOC amount + total C input); C conversion efficiency = SOC changes/Total C input by organic amendments and crop residues.

Table 4. Initial mean amount of soil organic Carbon (SOC) in the experimental area, Total C input supplied by composts, buffalo manure and crop residues in the two-year period and final SOC amounts measured in the experimental units differently fertilized.

Treatments	Initial SOC Amount	Total C Input by Compost/Manure	Total C Input by Crop Residues	Final SOC Amount
	(Mg ha^{-1})	(Mg ha^{-1})	(Mg ha^{-1})	(Mg ha^{-1})
BWCom 15	21.6	6.2	5.1	28.1 (±1.11) a
BWCom 30	21.6	12.5	5.1	27.8 (±0.35) a
OPC 15	21.6	9.1	5.2	26.8 (±1.30) a
OPC 30	21.6	18.1	5.1	27.4 (±0.21) a
OPCmix 15	21.6	7.3	4.9	25.7 (±0.12) ab
OPCmix 30	21.6	14.6	4.9	27.1 (±0.62) a
BMan 15	21.6	3	5.2	26.4 (±0.13) a
BMan 30	21.6	5.9	5.1	22.8 (±0.58) b

Legend: means followed by different letters are significantly different to Tukey's Test ($p \leq 0.05$) while numbers in brackets represent the standard error of means with n = 3. BWCom = biowaste compost; OPC = Olive pomace compost; BMan = Buffalo manure; OPCmix = Olive pomace compost with wool residues.

2.4. Microbial Biomass and Soil Enzyme Activities

Soil samplings were carried out in the layer 0–20 cm to analyze microbial biomass and some enzymatic activities in the second year of the trial, after the second distribution of organic amendments (19 June). To better appreciate changes due to organic fertilization, samplings were extended to a Control (CNT) area strictly surrounding the experimental plots, cultivated with the same crop sequence but fertilized by chemical fertilizers according to the farm schedule. Soil in this area had similar chemical-physical characteristics to that in the experimental area. The samplings occurred on 17 October 2014, before rocket seeding, 12 February 2015, during the harvest cycle of rocket, and 8 April 2015, at the end of the rocket cycle. Each sampling was executed by taking five independent soil cores per plot with a steel gauger, 2.5 cm in diameter; a total of 120 soil samples were collected in the experimental units. All samples were stored at −20 °C until further processing as described hereafter. Double-strand DNA (dsDNA) was used as a proxy of soil microbial biomass and was determined according to Fornasier et al. and Bragato et al. [31,32]. The enzymatic activities were determined by applying an extraction–desorption procedure [33] and using fluorescent analogs of each enzyme's substrate on microplates. After centrifugation, supernatants containing desorbed enzymes were dispensed in 384-well microplates with appropriate buffer to determine enzymatic activities using fluorescent 4-methyl-umbelliferyl based substrates. We determined the following enzymatic activities: β-glucosidase (betaG) involved in C cycle; arylsulfatase (ArylS) involved in S cycle; chitinase (chit) and leucine aminopeptidase (leu) involved in N cycle; acid- and alkaline-phosphomonoesterase (acP and alkP), phosphodiesterase (bisP), pyrophosphate-phosphodiesterase (piroP) and phytase (inosit), involved in P cycle.

2.5. Yield Measurements and Concentration of Nitrate in Leaves

Crop yields of the autumn–winter cycle carried out in 2014–2015 were estimated by harvesting in two sampling areas per plot. Specifically, we cut the baby leaves (10–15 cm high) into a 0.25 m^2 square to determine fresh biomass and, successively, the dry matter biomass after drying in an oven at 70 °C. The calculation of yield per hectare took into account the sum of rocket collected in each cut and the area sown, corrected for roadways (factor of correction= 0.84). The influence of the organic amendments on the accumulation of nitrates in the leaves of rocket was evaluated by measuring their contents in samples coming from four out of five cuts. The limits in concentration fixed by EU Reg. n. 1258/2011 [34] are 6000 mg kg^{-1} f.m. for rocket harvested from April to September and 7000 mg kg^{-1} f.m. from October to March. Samples of leaves were harvested always in the same hours (10–12 a.m.) and successively frozen. We sampled on 9 January, 12 February, 13 March and 2 April 2015. According to European Commission Regulation (2006), after unfreezing, 50 g of leaves were homogenized, then centrifuged for 5 min at 4500 rpm, the supernatant removed, and the solution diluted before being injected in an HPLC/UV Shimadzu, SCL-10 AVP.

2.6. Statistical Analysis

SOC stock variations as well as yields of rocket were analyzed using two-way ANOVA for a split plot, having as fixed factors, Organic amendment and Dose, and their interaction. When a factor's effect was significant, means were separated by a Tukey HSD Test at $p = 0.05$. Data about nitrate in leaves need a log transformation; they were measures repeated in time, so a mixed model was adopted with Organic amendment, Dose, Time of cut and all their interaction as fixed effects and replicates nested in Time of cut as a random effect. Statistical analyses were conducted by software JMP v. 16 (SAS Institute Inc., Cary, NC, USA).

Regarding the biological parameters, data matrices were square-root transformed and normalized prior to the analyses in order to down-weight the importance of dominant biological parameters, and the Euclidian index of dissimilarity was calculated to measure ecological distance. To evaluate the multivariate separation of the groups defined by soil treatments, non-metric multidimensional scaling (NMDS) with superimposition of the confidence ellipses (for $\alpha = 0.05$) for the type of Organic amendment was applied. The permutational analysis of variance (PERMANOVA) [35] was used to test the effect of Organic amendment and sampling time on the enzymatic activity and dsDNA. *p*-values were calculated using the Monte-Carlo test [36]. A PAIR-WISE TEST was finally applied to test statistically whether there is a significant difference between the groups (types of Organic amendment).

The significance of the differences in enzymatic activity among treatments was then evaluated by two-way RM ANOVAs for the factors Organic amendment and sampling time and their interaction, followed by Tukey post hoc tests (for $\alpha = 0.05$). The statistical analyses were performed using the "tidyverse", "nlme", "multcomp", "emmeans", and "vegan" packages in the R 4.1.2 programming environment [37].

3. Results

3.1. Soil C Stock Changes

The initial SOC concentration measured in the experimental area was determined to be 5.4 g Kg^{-1} (=0.54%), below the threshold of 1%, indicating severe degradation of the soil that [38], in addition, was characterized by a high clay content of 36%. The corresponding amount in the soil layer 0–30 cm was 21.6 Mg ha^{-1} (±0.37 standard error of the mean) (Table 4). Table 4 shows that BMan supplied in both doses an amount of OC ranging from half of that given with BWCom and OPCmix to a third of that given with OPC. Conversely, C input from crop residues did not vary among treatments, ranging around 5 Mg C ha^{-1} in the two years. Overall, at the end of the trial, soil organic C amounts improved from 5 to 6.5 Mg ha^{-1} with respect to the beginning for all treatments except Bman 30. Based on the data shown in Table 4, C stock change, C loss, C conversion efficiency and C sequestration rate per year were calculated and are shown in Table 5.

The average (not significant) effect of organic amendments on the SOC change highlighted the highest increases with BWCom, OPC and OPCmix and the lowest with BMan. SOC change was not influenced by the dose of amendment applied while the interaction Organic Amendment × Dose was highly significant and it is shown in Figure 1. BMan at dose 15 gave an increase in SOC of nearly 5 Mg ha^{-1}, but on doubling the dose, a low and negligible increase was recorded.

Table 5. SOC change, SOC losses, SOC change/OC input ratio and C sequestration rate derived from the SOC balance equation and analyzed according to a split-plot model with the factors Organic amendment and Dose and their Interaction.

Source of Variation		**SOC Change**	**SOC Losses**	**C Conversion Efficiency**	**C Sequestr. Rate**
		(Mg C ha^{-1})	**(Mg C ha^{-1})**	**(%)**	**(Mg C ha^{-1} y^{-1})**
Organic Amendment (OA)					
BWCom		6.3	−8.0 a	47	3.2
OPC		5.5	−13.2 b	35	2.7
OPCmix		4.8	−11.0 b	27	2.4
BMan		3.0	−6.5 a	35	1.5
	p	n.s.	***	n.s.	n.s.
Dose (D)					
15		5.1	−6.2	47	2.6
30		4.7	−13.0	25	2.3
	p	n.s.	***	***	n.s.
OA × D	*p*	**	n.s.	***	*

Legend: p = level of probability: *, **, *** are the levels of statistical significance of F test in the two-way ANOVA for $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively; n.s.: not significant effect. Means followed by different letters are significantly different by Tukey's Test ($p \leq 0.05$). SOC change = Final SOC amount − Initial SOC amount; SOC losses = Final SOC amount − (Initial SOC amount + total C input); C conversion efficiency = (SOC change/Total C input by compost + crop residues) × 100.

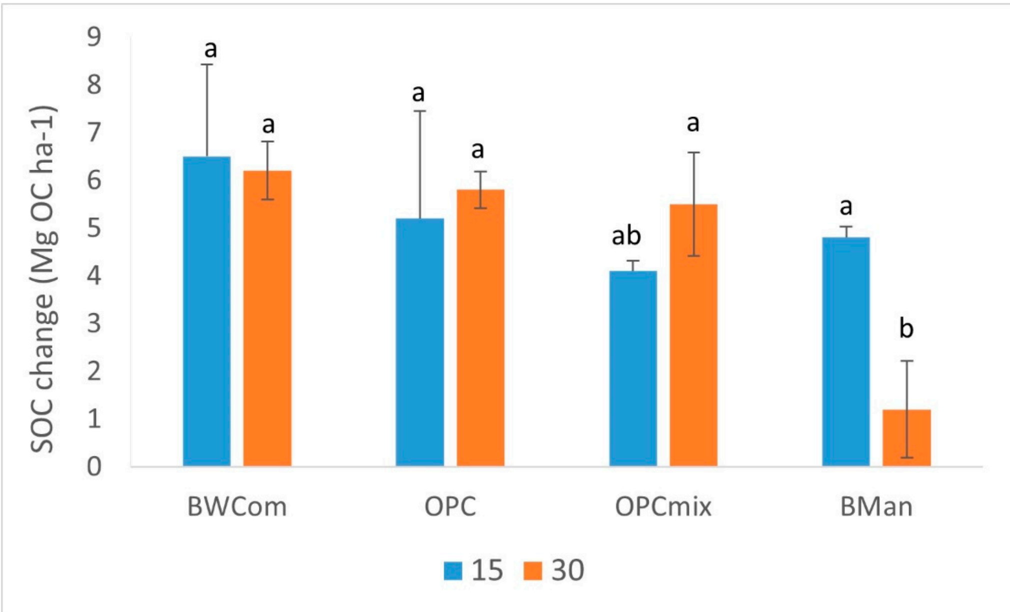

Figure 1. Interaction Organic amendment × Dose on SOC change (p = 0.01) at the end of the two-year trial. SOC changes = Final SOC amount − Initial SOC amount. Bars represent standard deviation (n = 3). Means followed by different letters are significantly different by Tukey's Test ($p \leq 0.05$).

The two OPC composts gave the highest SOC changes with dose 30 but the difference with dose 15 was not significant; BWCom effects were substantially similar at both doses, which promoted a SOC change above 6 Mg ha^{-1}. Coming back to the examination of Table 5, SOC losses after a two-year period highlighted that: (a) on average the highest and most significant losses were recorded by distributing OPC composts (11–13 Mg ha^{-1}) and the lowest losses with BMan and BWCom. (b) When doubling the dose of amendment and OC supplied, we detected a significant doubling of the losses. The C conversion efficiency, which represents normalized data derived from the SOC change/C input ratio, helps us to appreciate which combinations among the different organic soil amendments and doses gave the best results in terms of soil OC enrichment coupled with minimum OC mineralization as CO_2 (Table 5). On average, the conversion efficiency of OC obtained with the four organic amendments was not significantly different. In particular, 47% of the OC introduced by BWCom plus crop residues were stored in the soil and this was higher than the figure for OPCmix (27%), while OPC and BMan showed the same value of 35%. On average, at dose 15, 47% of the OC added was stored, against 25% at dose 30 ($p < 0.0001$). The significant interaction Organic amendment × Dose is shown in Figure 2. The conversion efficiency of OC input supplied with all the organic amendments was higher at dose 15 than at dose 30. The highest values of around 60% were recorded with BWCom 15 and Bman 15; overall, doubling the dose of the amendment halved the conversion efficiency in BWCom, while it was reduced to 10% in BMan. In the case of OPC and OPCmix, the conversion efficiency ranged between 25 and 40%, but the difference between the applied doses tended to decrease to zero in the case of OPCmix. The C sequestration rate showed significant differences for the interaction Organic amendment × Dose ($p < 0.05$), with the highest values shown by BWCom 15 (3.2), BWCom 30 (3.1), OPC 30 (2.9) and OPCmix 30 (2.8) compared to BMan (0.6).

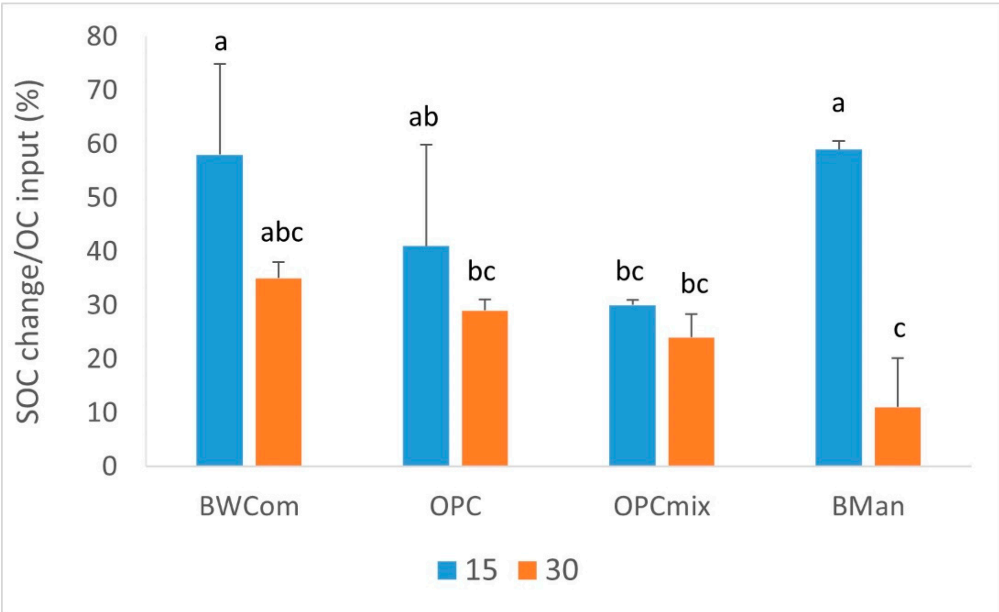

Figure 2. Interaction Organic amendment × Dose on SOC change/SOC input ratio ($p = 0.008$) at the end of the two-year trial. C conversion efficiency = SOC changes/Total C input by organic amendments and crop residues. Bars represent standard deviation (n = 3). Means followed by different letters are significantly different by Tukey's Test ($p \leq 0.05$).

3.2. Microbial Biomass and Soil Enzyme Activities

The temporal dynamic of each biological parameter (Figure S1) showed that dsDNA decreased from October 2104 to April 2015; BetaG, Chit, AcP, BisP and Leu decreased from October to February, then increased again; AryS reached the highest value in February, while PiroP and AlkP showed a growing trend from October to April.

The NMDS with the superimposition of confidence ellipses clearly grouped and separated treatments CNT and BMan with respect to the other treatments in October 2014 (Figure 3); all the biological parameters were higher in the soil amended with the composts. In February 2015 (Figure 4), soil biological activities decreased and no separation was visible among all treatments. In April 2015 (Figure 5), CNT soil was again clearly separated from all the other treatments; in this month, CNT showed biological parameters that were lower than those of the amended soils. The PERMANOVA highlighted significant differences among the combinations Organic amendment × Dose in October 2014 (F = 4.39, $p < 0.001$)

and April 2015 (F = 11.01, $p < 0.001$). We proceeded with the PAIR-WISE TEST for Organic amendment factor, and in Table S1, are reported the results for the pairs of treatments with significant differences. In October 2014, CNT treatment was significantly different from all treatments; in addition, on average BWCom was significantly different from Bman and BMan was significantly different from OPC and OPCmix. In April 2015, CNT soil was significantly different from all treatments, except for BMan30.

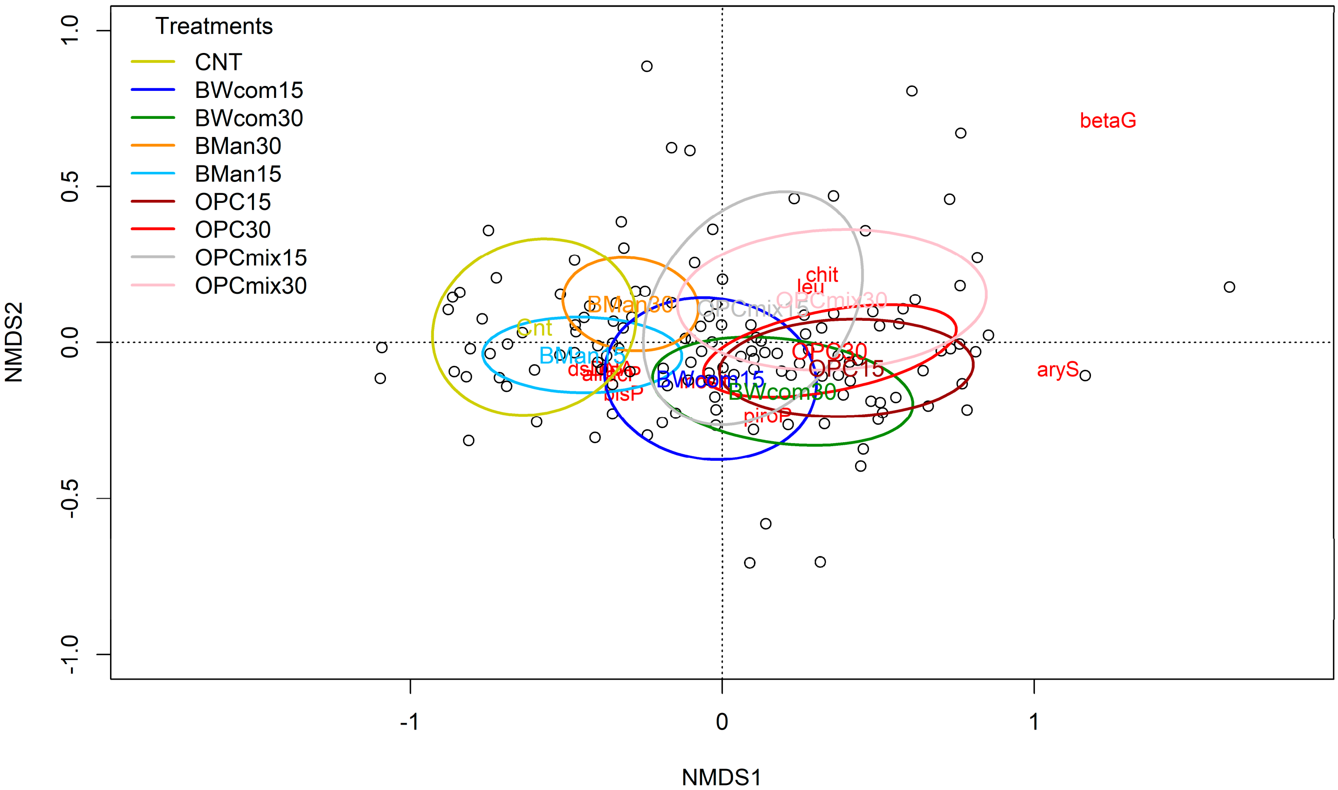

Figure 3. Non-metric multidimensional scaling (NMDS) biplot showing the differentiation among kinds of amendment in relation to biological parameters measured, with superimposition of confidence ellipses (for $\alpha = 0.05$) in October 2014. The biological parameters represented are: dsDNA = Double strand DNA, ArylS = arylsulfatase activity, betaG = β-glucosidase activity, chit = chitinase activity, acP and alkP = acid- and alkaline-phosphomonoesterase, bisP = phosphodiesterase activity, piroP = pyrophosphate-phosphodiesterase, inosit = phytase activity and leu = leucine aminopeptidase activity. The treatments are CNT = Control, BWCom 15 and BWCom 30 = biowaste compost 15 Mg ha^{-1} and 30 Mg ha^{-1}, OPCmix 15 and OPCmix 30 = mixed olive pomace compost 15 Mg ha^{-1} and 30 Mg ha^{-1}, OPC 15 and OPC 30 = Olive pomace compost 15 Mg ha^{-1} and 30 Mg ha^{-1} and BMan 15 and BMan 30 = buffalo manure 15 Mg ha^{-1} and 30 Mg ha^{-1}.

Since the PERMANOVA showed significant differences among the Organic amendment × Dose combinations, we executed RM-ANOVA to deepen the differences in the biological parameters. In October 2014, dsDNA (Table S2) was significantly lower in CNT (12.5 µg dsDNA g soil^{-1}) than in all soils with doses of composts but not than in those with BMan15 and 30 doses (14 µg dsDNA g soil^{-1}); in February 2015, levels of dsDNA flattened in all treatments, while in April 2015, we again detected the same ranking as in October. BetaG activity was higher in OPCmix30 (2.2 nmol of 4-MUF g soil^{-1} h^{-1}), OPC15 (1.7 nmol of 4-MUF g soil^{-1} h^{-1}) and 30 (1.6 nmol of 4-MUF g soil^{-1} h^{-1}) than in CNT (1.2 nmol of 4-MUF g soil^{-1} h^{-1}) in October 2014, while in February and April 2015, betaG activity was only higher in OPCmix30 with respect to CNT and BMan 15 and 30. In October 2014, PiroP and AlkP activities showed values higher in all compost-treated soils than in CNT (2.1 nmol of 4-MUF g soil^{-1} h^{-1}), while BMan treatments had intermediate levels (2.4 nmol of 4-MUF g soil^{-1} h^{-1} on average). All enzymatic activities leveled out among treatments in the sampling of February. In April, conversely, Chit, BisP, AryS and PiroP were higher in all amended treatments than in CNT, and AcP was higher (5.3 nmol of 4-MUF g soil^{-1} h^{-1}) in compost-amended treatments than in CNT.

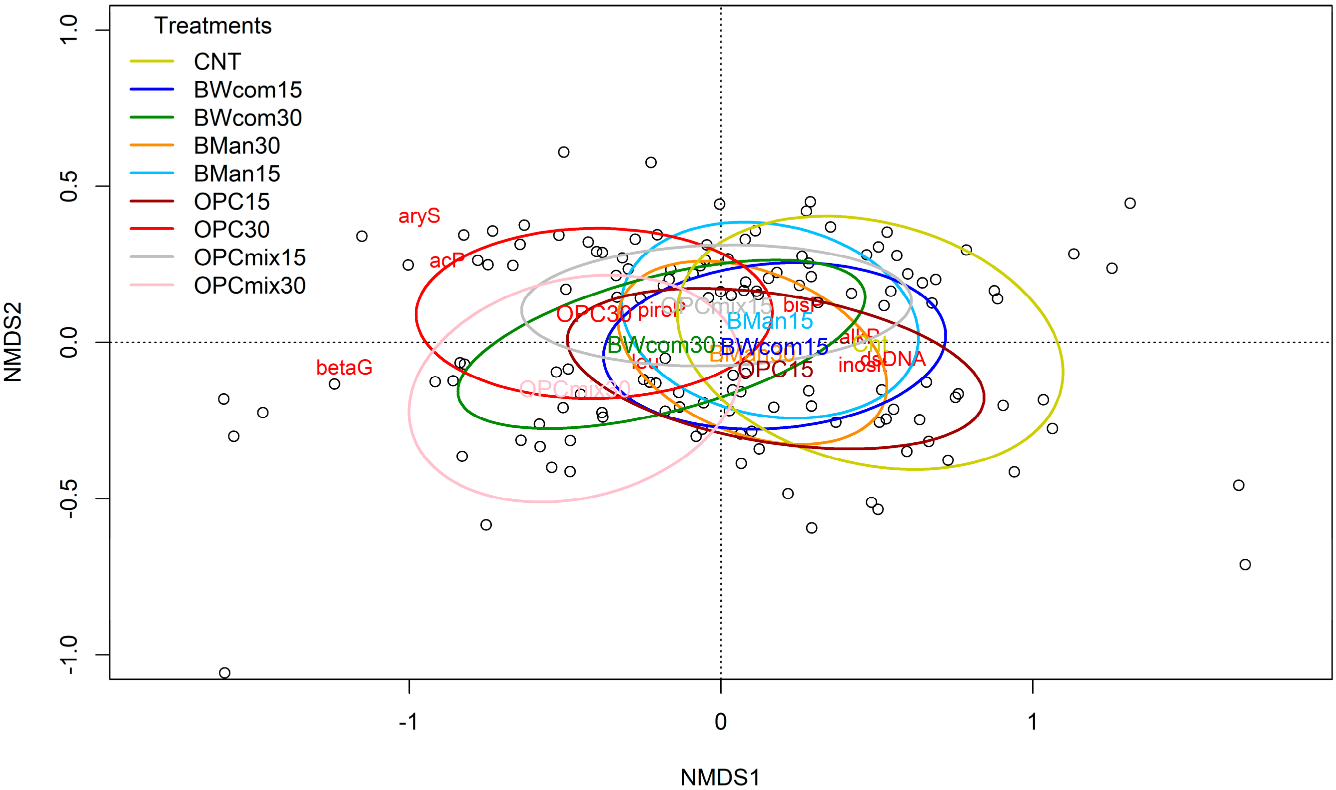

Figure 4. Non−metric multidimensional scaling (NMDS) biplot showing the differentiation among kinds of amendment in relation to biological parameters measured, with superimposition of confidence ellipses (for α = 0.05) in February 2015. The biological parameters represented are: dsDNA = Double strand DNA, ArysS = arylsulfatase activity, betaG = β−glucosidase activity, chit = chitinase activity, acP and alkP = acid− and alkaline-phosphomonoesterase, bisP = phosphodiesterase activity, piroP = pyrophosphate-phosphodiesterase, inosit = phytase activity and leu = leucine aminopeptidase activity. The treatments are CNT = Control, BWCom 15 and BWCom 30 = biowaste compost 15 Mg ha^{-1} and 30 Mg ha^{-1}, OPCmix 15 and OPCmix 30 = mixed olive pomace compost 15 Mg ha^{-1} and 30 Mg ha^{-1}, OPC 15 and OPC 30 = Olive pomace compost 15 Mg ha^{-1} and 30 Mg ha^{-1} and BMan 15 and BMan 30 = buffalo manure 15 Mg ha^{-1} and 30 Mg ha^{-1}.

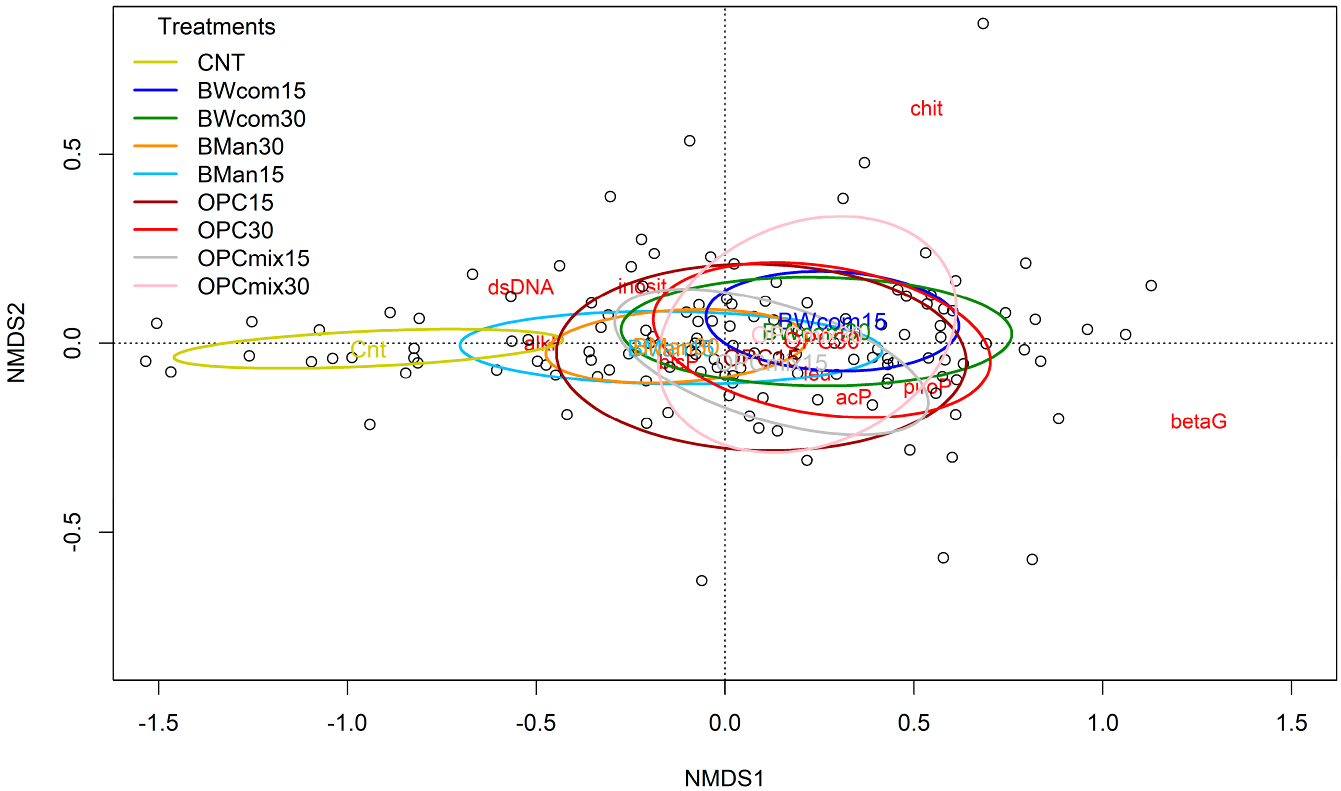

Figure 5. Non-metric multidimensional scaling (NMDS) biplot showing the differentiation among kinds of amendment in relation to biological parameters measured, with superimposition of confidence ellipses (for α = 0.05) in April 2015. The biological parameters represented are: dsDNA = Double strand DNA, AryS = arylsulfatase activity, betaG = β-glucosidase activity, chit = chitinase activity, acP and alkP = acid- and alkaline-phosphomonoesterase, bisP = phosphodiesterase activity, piroP = pyrophosphate-phosphodiesterase, inosit = phytase activity and leu = leucine aminopeptidase activity. The treatments are CNT = Control, BWCom 15 and BWCom 30 = biowaste compost 15 Mg ha^{-1} and 30 Mg ha^{-1}, OPCmix 15 and OPCmix 30 = mixed olive pomace compost 15 Mg ha^{-1} and 30 Mg ha^{-1}, OPC 15 and OPC 30 = Olive pomace compost 15 Mg ha^{-1} and 30 Mg ha^{-1} and BMan 15 and BMan 30 = buffalo manure 15 Mg ha^{-1} and 30 Mg ha^{-1}.

3.3. Yield Measurements and Concentration of Nitrate in Leaves

Table 6 shows the fresh marketable yields of rocket and the corresponding dry biomass calculated as the sum of the produce harvested in the five cuts executed during the last crop cycle, 2014–2015. Split-plot analysis of variance showed the significant effect of the organic amendment on both variables. Significantly higher fresh marketable yields were obtained with BWCom and BMan amendment, which, on average, gave 20% more than the mean yield of the OPC composts. Likewise, the dry biomass was on average 13% higher with BWCom and BMan than with the OPC composts. From the productive point of view, the effect of the dose of the amendment was not significant. The interaction between the two factors was not significant for fresh yield, but significant for dry biomass. Lastly, in Table 6, the contents of nitrates in fresh leaves of rocket are shown as mean effects of each organic amendment, of the dose applied and of the time of cut; the two orders of interactions and their levels of probability are also shown but we chose to not use graphics to describe significant interactions that do not add interesting knowledge.

Table 6. Mean effects of Organic amendment, Dose of amendment and Time of cut and their interactions on fresh and dry biomass yields of rocket, cv Winter in 2014-2015 cycle, and nitrate content in fresh leaves of rocket.

Source	Fresh Marketable Yield	Dry Biomass Yield	Nitrate Content
	(Mg ha^{-1})		(mg kg^{-1} f. m.)
Organic amendment (OA)			
BWCom	44.6 a	4.3 a	4408 a
OPC	35.3 b	3.7 b	2681 bc
OPCmix	37.9 b	3.7 b	2251 c
BMan	43.4 a	4.1 ab	3156 b
p	***	***	***
Dose			
15	40.0	4.35	3466
30	40.6	4.28	2643
p	n.s.	n.s.	***
Time of cut			
9 January 2015			4211 a
2 February 2015			4532 a
13 March 2015			3293 a
2 April 2015			1335 b
p			***
OA × Dose	n.s.	*	n.s.
OA × Time of cut			***
Dose × Time of cut			*
OA × Dose × Time of cut			n.s.

Legend: p = level of probability: *, *** are the levels of statistical significance of F test in the two-way ANOVA for $p \leq 0.05$ and $p \leq 0.001$, respectively; n.s.: not significant effect. Means followed by different letters are significantly different by Tukey's Test ($p \leq 0.05$); BWCom = biowaste compost; OPC = Olive pomace compost; OPCmix = Olive pomace compost with wool residues; BMan = Buffalo manure.

4. Discussion

4.1. Soil C Stock Changes

In our on-farm case study, interesting SOC increases were obtained with all combinations of organic amendments and doses except BMan 30.

SOC contents grew to 6.5–7 g C kg^{-1} vs. the initial status of 5.4 g C kg^{-1} (+20–29%, respectively). In terms of SOC amounts, we determined an increase of 4–6.5 Mg C ha^{-1} after two years, corresponding to a C sequestration rate ranging from 2 Mg C ha^{-1} year^{-1} with OPCmix 15 to 3.2 Mg C ha^{-1} year^{-1} with BWCom 15 and 30 (Table 5). Bonanomi et al. [15], through applying a holistic approach to an intensive crop system of rocket, demonstrated the possibility of achieving soil fertility recovery, high crop yield, good plant health and leaf quality by the application of organic amendments such as composted buffalo manure and biochar. The study was carried out in a mesocosm experiment whose main limit, however, was represented by the lack of the aggressive tillage usually practiced under real cultivation conditions. That is why a very high increment of OC in soil treated by compost vs. the initial status (26.4 vs. 15.4 g kg^{-1}) was measured. Aguilera et al. [39], in a review of the effects on SOC levels of recommended management practices studied in long-term trials in Mediterranean agroecosystems, reported that compost applied in agronomic rates under 10 Mg C ha^{-1} year^{-1} gave a C sequestration rate of 1.32 Mg C ha^{-1} year^{-1}, while manure application gave a poorer increase in C sequestration rate over conventional plots. This could be due to the more stabilized forms of C present in composts than in raw manures. Tiefenbacher et al. [40] confirmed that compost application, in a set of different agricultural practices tested over at least 20 years, had the highest C sequestration rate of 0.7 ± 0.4 Mg C ha^{-1} year^{-1}, while farmyard manure amounted to 0.29 ± 0.13 Mg C ha^{-1} year^{-1}. The high C storage observed in our experiment can be explained taking in mind the short time of observation and the severe C depletion of a soil with high content of mineral size fraction, namely 36% clay and 12% fine silt; as is known, this fraction is responsible for stabilizing OC in soils [38] and determining the potential for increasing the stock of SOC with long residence time [41]. However, the capacity of soils to preserve organic C could be further increased with respect to the C saturation capacity of fine soil particles by practices that favor the formation and stabilization of soil aggregates. Soil aggregates, in fact, are effective in protecting organic matter against microbial decomposition, as reported in several studies [42]. However, we experienced that, under plastic tunnels devoted to leafy vegetable crops, repeated tillage used to prepare the soil for continuous crop cycles cannot be easily modified. This frequent disturbance can block physicochemical processes that stabilize and protect organic compounds, disrupting aggregate and mixing soil particles; this favors the contact of microbial degraders with organic compounds in favorable microclimatic conditions for their activities, such as those under tunnels (T and water content of soil) [43]. Therefore, the SOC stock increases we have measured are explainable principally, as the initial accumulation in a depleted soil, far from saturation [44,45].

The analysis of SOC changes, obtained by the different combinations of organic amendment and dose, clearly highlighted the lowest increase with BMan treatment at the highest dose (Figure 1). Buffalo manure was not composted and likely, the addition of a major amount of more labile OC could have activated a priming effect with a near-complete microbial mineralization. In general, the doubling of the dose of amendment from 15 to 30 Mg ha^{-1} as fresh matter, was associated with a doubling of OC losses, as shown in Table 5. In this case, however, it was possible to distinguish between the organic soil amendments. Indeed, OPC and OPCmix showed mean OC losses that were significantly higher than those of BWCom and BMan. A key factor in this result, obtained with olive pomace composts irrespective of low or high C/N ratio, comes from the shift of paradigm that has occurred in the last 10 years indicating that biotic and abiotic environment is more important than the molecular structure of organic matter (considering C/N ratio as a proxy) in determining organic matter stability in soil [40,46]. It would seem that a decreasing efficiency in C stabilization in soil occurred with an increasing amount of C supplied from organic residues and/or organic fertilizers as reported by Campbell et al. (2002), Shahbaz et al. (2017) and Morra et al. [47–49]. For this reason, the SOC change/OC input ratio (=conversion efficiency of C) was always better with all combinations of organic amendments at the lowest dose (Figure 2). Nevertheless, it must be noted that the best ratios were observed with BWCom 15 and BMan 15, corresponding to total OC inputs of 5.5 and 4.1 Mg C ha^{-1} y^{-1} on average, respectively.

4.2. Microbial Biomass and Soil Enzyme Activities

The increase in SOM in all treatments except BMan 30 stimulated an increase in microbial biomass and activity, indicating that organic amendment represented a new energy source for soil microorganisms. However, this stimulation did not seem to involve a particular group of activities but involved all the activities regardless of the treatment (Figures 3–5, Table S1). This can be explained as being the response of the microbial community to the addition of new organic matter in a degraded soil. Indeed, soils with low organic matter or degraded soils are more susceptible to increases in microbial biomass and activity [50,51].

In the monitoring period of microbial biomass and enzymatic activities, all the biological parameters in October and April showed significant differences between soils treated with composts or manure vs. CNT soil. Based on a pair-wise test, the enzymatic activities in October separated the OPC and OPCmix treatments from BMan. On the basis of RM ANOVA results, the trend in dsDNA in October was significantly higher in all compost-amended plots than in the CNT, but in the manure-amended plots, it was not different from in the CNT and showed values lower than those in soil treated with composts (Table S2). dsDNA was relatively higher in the organic-amended soils as a consequence of microbial growth stimulation through greater availability of resources, as well as changes in the microbial community composition [52]. However, amendment quality is of major importance in the regulation of microbial activities and biomass [53]. Thus, the lower values of dsDNA in soil amended with BMan could be attributable to the rapid decomposition rate of C input from manure. Our monitoring, in fact, took place 4 months after the distribution of amendments (June 2014), when a portion of the most easily biodegradable macromolecules were probably already degraded, according to degradation rates that varied with the kind of organic amendment [53,54], and this determined an increase in SOC accumulated, probably as mineral-associated organic matter. Instead, OPCmix 15 and 30, OPC 30 showed the highest microbial biomass, indicating that the high C input of olive pomace composts was degraded slower than that of BWCom and BMan and, hence, was still subjected to microbial decomposition at the date of the sampling; however, it showed the same increase in SOC as BMan 15. These results agree with the concept that C input characterized by rapid decomposition rates should lead to faster and more efficient accumulation of mineral-associated organic matter than C input characterized by slow decomposition rates [55]. The differences in dsDNA among organic treatments progressively flattened in February and April. The OPC treatments (in February and April) and BWCom (in April) continued to show a significant difference with respect to CNT, but the absolute values of dsDNA decreased due to the probable depletion of the more labile organic fraction added to the soil (Figure S1).

BetaG, bisP, piroP, alkP and leu (Table S2) showed similar values among treatments in October and February, but they reached higher values in compost- or manure-amended treatments than in CNT in April. Seasonality influenced this behavior regardless of the treatments (Figure S1), showing that the temporal factor influenced enzymatic activities. The decrease in February was linked to the lower temperature of the season, which slowed the microbial metabolism and flattened differences between the treatments. Conversely, in April, the increase in soil temperature and the stimulating action of root exudates of rocket supplied new resources to the microbial biomass in the amended plots. These results are consistent with those of other studies [56,57]. In concordance with results reported by López-Piñeiro et al. and Innangi et al. [17,58], we found that treatments with olive pomace compost improved the enzyme activities. Notwithstanding, we can suppose that olive pomace compost mineralization rates did not match crop nutrient needs. In fact, as will be discussed in the next section, yields of rocket, as well as nitrate contents in leaves, were lower than those obtained with biowaste compost and buffalo manure treatments, despite the increases in soil biological parameters measured at the end of the crop cycle.

4.3. Yield Measurements and Concentration of Nitrate in Leaves

The fresh marketable yields tell us how much the variations in soil fertility induced by organic amendments with different materials benefited the soil ecosystem, expressed as food production. In the last cycle of rocket cultivation, the different kinds of organic amendments influenced fresh and dry yields; indeed, BMan and BWCom gave significantly higher production than both the OPC composts. Conversely, the two doses of amendments gave similar fresh and dry yields, so it can be inferred that dose 15 is already sufficient from the productive point of view (Table 6). This behavior was observed also in the first year of the trial [28] and it is consistent with the findings discussed by Morra et al. [19] with regard to the effects of olive pomace compost and biowaste compost applied to open-field vegetable crops at doses of 10 and 20 t ha^{-1} d.w. The olive pomace compost stressed competition for nitrogen between soil microorganisms and roots, causing a decrease in yields in the first crops after compost distribution. In particular, the olive pomace compost, regardless of its C/N ratio, was confirmed to immobilize nitrogen during its decomposition in the short term (3–12 months), as also stated by Roberto et al. [59]. Cardarelli et al. [7] demonstrated that the application of organic fertilizers such as poultry manure, insect frass and vinasse-based fertilizer in greenhouse lettuce production was a good strategy for reducing stressful conditions arising from soil salinization and soil organic matter depletion due to intensive vegetable crop production. As concerns the concentration of nitrates in leaves, as is known, perennial wall rocket can accumulate large amounts of nitrates [15,60]. Nitrate accumulation depends not only on nitrogen availability but also on specific environmental conditions, such as low radiation, which reduces the nitrate reductase activity [61]. The issue of nitrates in vegetables, encompassing the aspects of toxicity, content, intake and cation exchange regulation, was reviewed by Santamaria [62]. The content of nitrates represents on one the hand, a crucial parameter because the marketing of rocket must comply with European food safety legislation fixing limits of concentration [34], and on the other, an indicator of the availability in the soil of mineral N provided from microbial mineralization of the organic N pool. BWCom was, on average, the amendment that demonstrated a major uptake of mineral N with significantly higher nitrate concentrations (4408 ppm) in leaves than all other organic amendments; significantly lower concentrations were measured in leaves grown on soil amended with OPC and OPCmix. However, all the detected concentrations of nitrate did not ever exceed the limit of 7000 mg kg^{-1} f.m. fixed by EU Reg. The mean effect of the dose of the amendment showed a significantly higher concentration of nitrate with dose 15. The significantly lower concentration of nitrates measured, on average, with dose 15 with respect to dose 30, is explainable considering that, in soil initially poor in C and N, the higher amount of stabilized OC added with dose 30 stimulated an intense microbial activity in competition for N with plant roots. Morra et al. [28] in the first year of this trial, found concentrations of nitrates in BWCom 15 and BMan 15 slightly over the legal limits in one out of the three cuttings analyzed per cycle carried out in the spring–summer cycle and autumn–winter cycle. Conversely, the time of cut showed a decreasing trend in nitrates, always under the legal limit; the concentrations significantly decreased in the cuttings in the spring season compared to those in the winter. The decreasing trend in nitrate concentration measured in the cuttings from winter to spring in all treatments is attributable to the known effects of growing temperature, light intensity and photoperiod on the nitrate reductase enzyme responsible for reducing the nitrates to nitrites for their assimilation in organic molecules [61]. Overall, our data showed that different organic amendments did not cause nitrate concentrations over EU legal limits [34] despite the high inputs of total N distributed in some cases in the two years (Table 2). The release of nitrogen in composts is slower than in inorganic fertilizers, since organic fertilization typically does not provide nitrogen in a readily accessible form [63]. For this reason, nitrate accumulation in the edible parts of crops is usually lower in organically grown crops than in conventionally grown crops [61].

5. Conclusions

In our study, the SOC content improved in the amended soils, with the highest values seen where biowaste compost was applied. It is plausible that the increment in OC stored in the soil was favored by the severe SOC depletion and the high potential capacity to store C due to the high silt-clay mineral fraction of the soil. The use of biowaste compost at dose 15 appeared to be the best combination as it couples the lower losses in organic C and the best conversion efficiency with the highest increment in SOC. In addition, biological activities and rocket yield and quality were also improved. Our data showed an increase in biological parameters in all the amended soils with respect to Control. In soil amended with olive pomace, however, compost mineralization rates likely did not match crops nutrient needs. In fact, yields of rocket were lower in this case than with biowaste compost and buffalo manure despite the increases in soil biological parameters. Buffalo manure supplied a good result in terms of C stock change at the dose with the lowest input of C among all treatments, while the higher amount was nearly completely mineralized. It is likely that composting this organic source would represent a way to stabilize organic matter and reduce its ready degradability. A key factor in reversing the trend in soil degradation is undoubtedly a reduction in tillage intensification. While a complete transition from intensive tillage to no-tilling may not be readily practical for the considered vegetable cropping systems, the frequency, depth and timing of tillage operations must be modified to minimize negative effects on soil health. Further research is necessary to implement and test combinations of agroecological practices with the aim of obtaining a transition of these intensive crop systems to preserve soil health and crop productivity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10050476/s1, Figure S1: dsDNA and enzymatic activities (mean ± s.d.), arylsulfatase (ArylS), β-glucosidase (betaG), chitinase (chit), leucine aminopeptidase (leu), acid- and alkaline-phosphomonoesterase (acP and alkP), phosphodiesterase (bisP), pyrophosphate-phosphodiesterase (piroP) and phytase (inositP) showed as temporal dynamics in CNT = Control, BWcom15 and BWcom30 = biowaste compost 15 Mg ha^{-1} and 30 Mg ha^{-1}, OPCmix15 and OPCmix30 = mixed olive pomace compost 15 Mg ha^{-1} and 30 Mg ha^{-1}, OPC15 and OPC30 = Olive pomace compost 15 Mg ha^{-1} and 30 Mg ha^{-1} and BMan15 and BMan30 = buffalo manure 15 Mg ha^{-1} and 30 Mg ha^{-1}; Table S1: Sum of Square, F model and p adjusted values of pairwise tests. Asterisks indicate significant differences (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$). Table S2: Result of RM-ANOVA on biological parameters for "Sampling time" and the combination "Organic amendment × Dose". Different letters indicate significant differences ($\alpha = 0.05$) among the treatments in each sampling time.

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

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