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ORIGINAL PAPER

Agricultural Waste Recycling in an Organic Zucchini-Lettuce Rotation: Soil Microbial Parameters Under Laboratory and Field Conditions, and Crop Production Parameters Assessment

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Abstract

Agricultural Wastes, Co-products, and By-products (AWCB) can be recycled to produce profitable added-value products, such as organic fertilizers. Newly produced AWCB-based fertilizers were thus tested both under laboratory and field conditions in a two-year organic zucchini-lettuce rotation. In a split plot experimental design, the main-plot factor being the green manure (GM) presence or absence, the following fertilizing treatments were compared: (i) co-composted cattle manure anaerobic digestate; (ii) re-composted olive waste compost; (iii) a commercial dried manure organic fertilizer, and iv) a municipal solid waste compost. The aims were to assess: the potential C and N mineralization, changes on soil microbial and chemical properties and the crop yields. Moreover, the residual effect of the fertilization applied before zucchini transplanting on lettuce yield was evaluated. The composts and the commercial organic fertilizer did not significantly change the soil microbial and chemical properties, and crops yield. The GM was the most effective treatment, as highlighted by the highest microbial biomass carbon and dehydrogenase activity, the highest C and N input and the increase of soil TOC. Weather conditions may have contributed to a 55% higher zucchini yield in the second cropping cycle, despite the lowest soil mineral N in GM, and reduced the lettuce yield by 59%. The residual effect of the fertilization applied before zucchini did not affect the subsequent lettuce yield in the first year. In conclusion, AWCB-based fertilization can enhance the soil biochemical dynamics in organic vegetable systems, particularly combined with other agroecological practices, such as GM.

Statement of Novelty

Agricultural Wastes, Co-products, and By-products (AWCB), generated in huge amounts along the agrifood systems, should be recycled to meet the circular economy principles, thus producing profitable added-value products in agriculture, such as organic fertilizers. Although the possible utilization of many AWCB in agriculture was already evaluated by many authors, there is a need of further knowledge on the main effects on the soil of newly produced AWCB-based fertilizers under Mediterranean conditions. This is also linked to the unpredictable behaviour of AWBC due to a wide variability in physicochemical and biological properties, depending on the treatment process and raw materials used. The lack of knowledge may lead to improper application to soil, resulting in low agronomic efficiency and environmental pollution. To allow efficient and environmentally sound waste recycling, laboratory or pot experiments were set up in other studies not combining both types of experimentation. By contrast, we combined laboratory incubation with field experiments in

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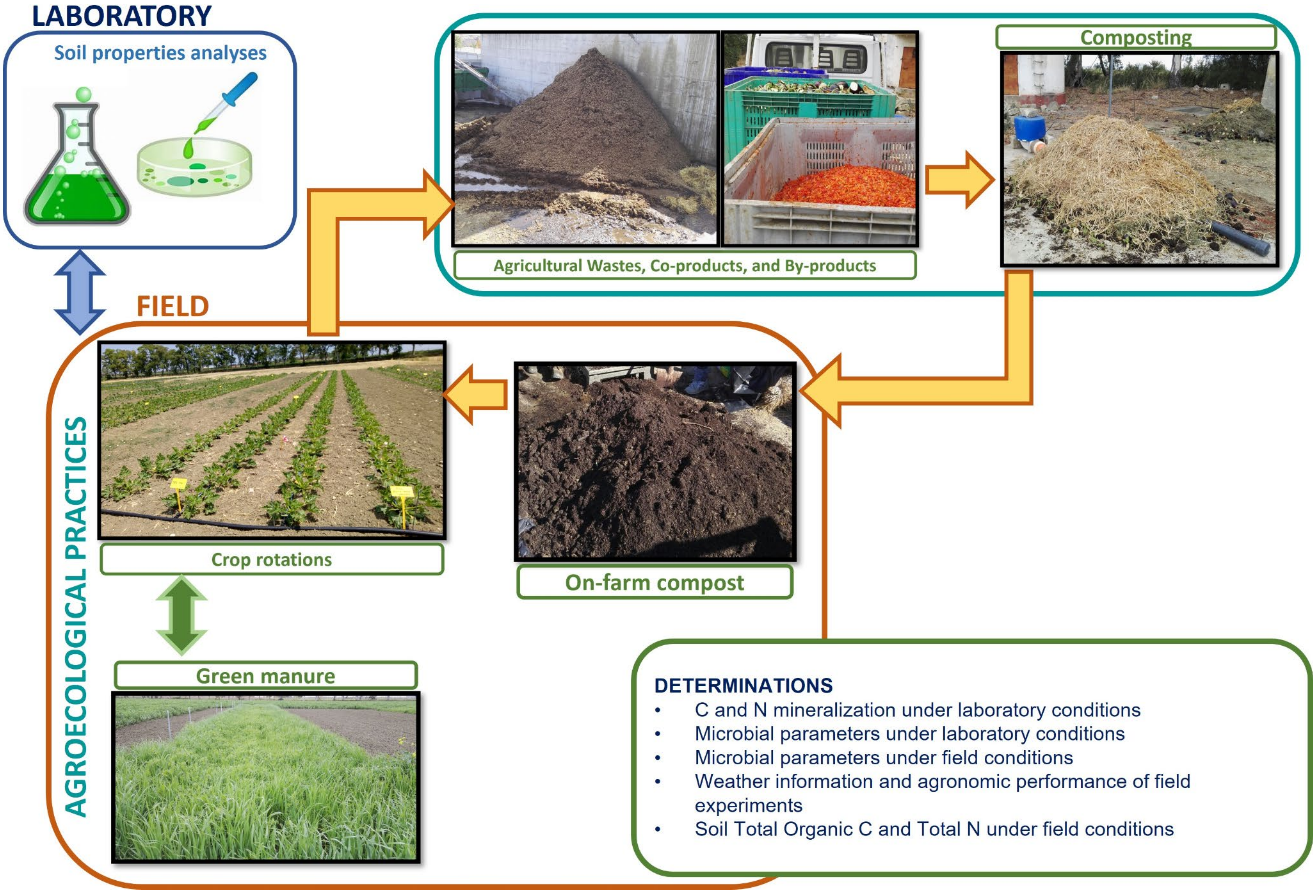
organic crop rotations, testing newly produced AWCB-based fertilizers directly related to farm reality and site-specific context.

Highlights

- Fertilizers can be produced also on-farm by recycling agricultural wastes.
- Combined incubation experiments and field trials on AWCB fertilizers were performed.
- AWCB and GM are effective agroecological practices in Mediterranean area.

Graphical Abstract



DETERMINATIONS

- C and N mineralization under laboratory conditions
- Microbial parameters under laboratory conditions
- Microbial parameters under field conditions
- Weather information and agronomic performance of field experiments
- Soil Total Organic C and Total N under field conditions

Keywords Agricultural residues · Co-products · By-products · Microbial biomass carbon · Dehydrogenases activity · Soil mineral nitrogen · Soil organic carbon · Green manure · Crop rotation

Introduction

Boosting circularity can be crucial in European policy response towards a sustainable agriculture, to address systemic crisis such as climate change and the recent COVID-19 pandemic, which put in evidence all weakness of food systems from production to consumption and wastes generation [1, 2]. The organic fraction of these wastes is generated along all the supply chain, ranging between about 160 and 300 kg per capita^{-1} year^{-1} at the European level [3]. In particular, in the context of world population increase, closed agrifood systems could allow to reduce the disposal of Agricultural Wastes, Co-products, and By-products (AWCB) [1, 2].

The environmental impacts caused by incorrect disposal of AWCB extend from groundwater contamination to soil pollution and GHG emissions [4]. Therefore, it becomes mandatory to achieve high levels of recycling AWCB on-farm and sustainable valorisation, making use of landfilling only for non-recyclable wastes [5, 6]. Circular economy aims at closing the loops, reducing consumption and environmental discharges [7]. Valuable components like proteins, sugars, and lipids from AWCB can be recovered using a circular chain model, which can generate profitable, added-value products in agriculture through controlled microbial/enzymatic transformation processes [8–10].

AWCB from farm livestock (cattle manure), olive mill (wet olive pomace and olive pruning) and vegetable processing residues can be considered the most relevant wastes of the production chains in Southern Italy [11] (and similar areas of the Mediterranean basin), which could be processed and valorised with innovative treatment methods, such as anaerobic digestion and on-farm composting. The biogas supply chain for methane production in Italy is currently in second place in Europe after Germany and is expected to grow considerably in the future [12, 13]. The anaerobic digestion (AD) allows to valorise many organic wastes, and a solid–liquid phase separation is usually carried out prior to further post-treatments. Wastewater treatment plants allow treating the liquid phase, whereas a treatment before the return to the soil of the solid fraction of digestate is required, to enable closing the organic materials loop. In fact, although such fraction can be valorised as a bio-fertilizer [14], the direct application on agricultural soils is limited by its phytotoxicity, viscosity and odour emissions, thus requiring to better stabilize biodegradable matter. The solid fraction of digestate is characterised by valuable contents of organic matter and phosphorus (P; 60–80%) compared to the original material), as well as of nitrogen (N; 20–25%) [15], which can be recycled by biological degradation under aerobic conditions (i.e., co-composting), with an activating inoculum of organic vegetable residues and bulking agents to support digestate stabilization, obtaining a co-composted anaerobic digestate [16].

Olive pomace (OP), the semisolid fraction obtained after the extraction of olive oil, is another organic AWCB that is widespread mainly in the Mediterranean area. Due to phytotoxic and antimicrobial effects of phenolic compounds and lipid fraction, its incorrect disposal may determine environmental impact [17]. By contrast, OP is composed by about 90% of organic matter, which can be profitably recycled through composting [18, 19]. During the composting process, the easily degradable organic matter of OP decreases steadily, while the concentrations of humic substances increase.

A re-composting process could thus be necessary to further decompose potentially phytotoxic compounds and promotethe formation of stable organic matter, thus reducing the risk of harmful effects when the organic material is applied to soil. This enhances the stability and maturity of OP compost, making it suitable for use in agriculture, as reported in Diacono et al. [20].

Co-composting and re-composting of the pre-processed waste materials are aimed to provide organic amendments, allowed in organic farming according to the European Regulation (Commission Regulation N° 889/2008 - EU Council Regulation N° 834/2007), with a higher degree of stability and maturity. Their use can improve soil fertility by fostering microbial activity, changing organic C dynamics and enhancing nutrient availability particularly in the medium to long run. Since soil microbial communities are much more responsive to changes in soil management than physico-chemical properties, understanding soil biological processes after AWCB application is crucial. Both microbial biomass and activity are closely linked with soil fertility, playing a key role in the mineralization of organic matter and nutrient turnover [21–23]. Microbial biomass carbon (MBC) and soil enzymatic activity, such as dehydrogenase activity (DHA), are relevant biological indicators, being related to the availability of carbon (C) as energy source for microbial growth. Moreover, laboratory incubations can be used to estimate C and N mineralization dynamics in amended soils, to better synchronize the mineralization rate with the plant growth, thus reducing the risk of leaching of excess N.

Although many authors have already evaluated the possible utilization of various AWCB in agriculture, there is still the need for understanding the main effects of newly produced AWCB-based fertilizers on soil under Mediterranean conditions. The lack of knowledge could lead to improper application resulting in low agronomic efficiency and environmental pollution. To allow efficient and environmentally sound waste recycling, laboratory or pot experiments were setup in other studies [24]. By contrast, we opted to combine laboratory incubation with field experiments in organic crop rotations, aiming for results more directly related to farm reality and site-specific context.

Therefore, the objective of the research was to promote the agricultural waste recycling in an organic zucchini-lettuce rotation in Mediterranean environment, by testing different AWCB-based fertilizers both under laboratory and field conditions. In particular the aims were: (i) to assess the potential C and N mineralization and changes on soil microbial properties both under laboratory and field conditions; (ii) to evaluate the agronomic performance following the application of biobased fertilizers produced from available AWCB, on a zucchini-lettuce rotation with organic farming management (e.g., green manuring) under Mediterranean conditions; (iii) to find agronomic practices to increase the overall environmental sustainability of organic horticultural systems.

Materials and Methods

Experimental Site

The study has been conducted in 2016–2018 on a two-year organic zucchini (*Cucurbita pepo* L. cv President) – lettuce (*Lactuca sativa* L. var. Iceberg) rotation, at the experimental farm "Campo 7" (CREA-AA), in Metaponto (MT) - South Italy (40° 24' N, 16° 48' E; 8 m a.s.l.).

At the experimental site, the climate is accentuated thermo-mediterranean, according to the UNESCO-FAO Bioclimatic map [25]. The soil, classified as Typic Epiaquerts [26], is poorly drained, consisting mostly of swelling clays, with the clay (42%) and silt (39%) contents increasing with depth, and a soil bulk density of 1350 kg m^{-3}.

Field Experimental Design and Treatments

In this study, composted organic waste materials were tested, which were obtained from selected AWCB derived by local production systems and residues from farm activities [20]. In particular, the anaerobic digestate from cattle manure (80% of total dry weight) was co-composted with an activating inoculum of highly degradable organic vegetable wastes (10%) and straws (10%), producing an anaerobic digestate-activated compost (Acti_AD). Moreover, an OP compost (75% of the final product), which was obtained by olive pomace plus olive pruning, was re-composted by processing with a municipal waste compost (5% of total dry weight) and an on-farm compost (20%), obtaining an olive waste-based compost (OWC). Composting process details are reported in Diacono et al. [20].

The field experiment had a split plot design (each subplot was 4×5 m) with two factors and three replications (blocks), the main-plot factor being the green manure presence (GM+) or absence (GM-), and the subplot factor the fertilizers. The following fertilizing treatments were compared among them and to an unfertilized control (CTR): (i) anaerobic digestate-activated compost (Acti_AD); (ii) re-composted olive waste compost (OWC); (iii) a commercial organic fertilizer (COF), consisting of dried cattle, horse, and poultry manures from non-industrial farms (Ca' verde - ED & F Man Liquid Products Italia srl); iv) a municipal solid waste compost (MWC), obtained with organic waste collected separately from households and mixed to biodegradable wastes from management of parks and gardens (Tersan S.p.A., Bari). All these tested fertilizers were sampled before distribution in triplicate, dried for 48 h at 70° to determine dry content and stored for further analysis. The physicochemical characteristics of the organic amendments and the application rate are reported in Supplementary Information 1 (SI 1).The fertilizers were applied to soil at 150 kg N ha^{-1}, which is equal to the sum of the marketable yield N uptake of both crops in the site-specific environmental conditions. Therefore, different amounts of organic materials (according to their N content) were applied. The fertilization was split in two amounts each year, as follows: (1) before green manure crops sowing (70% of the total quantity) and (2) before zucchini transplanting (the remaining 30%), without considering N input by GM, to reduce potential N immobilization phenomena during the cash crop cycle. Conversely, no fertilization was applied before lettuce, to exploit a residual effect of the fertilization of the previous crop.

The green manure treatment was a mixture of common vetch (*Vicia sativa* L.) and oat (*Avena sativa* L.) sown each year in late autumn at the rate of 80 and 220 kg ha^{-1} for vetch and oat, respectively [20]. At termination, the green manured crops and weed aboveground biomass were sampled by placing two randomly selected 1.0×1.0 square meter within each sub-plot. Samples were dried for 48 h at 70° to determine dry content and stored for further analysis. The mixture of vetch and oat was then chopped and incorporated into the soil (about at 20 cm depth) by plowing in spring in both years.

Zucchini seedlings were hand-transplanted at an inter-row× in-row distance of 1.0×1.0 m (1.0 plant m^{-2}) at 3rd and 10th May, in 2017 and 2018, respectively, and the harvest occurred weekly from the end of June till 25th and 23rd of July, respectively. The zucchini cropping cycle lasted 83 and 79 days, in 2017 and 2018, respectively. Lettuce transplanting was done after a rotary tillage on 24th August and 5th September in 2017 and 2018, respectively, and harvested at once on the 23rd October and 19th November corresponding to a cropping cycle of 59 and 75 days in 2017 and 2018, respectively.

Soil Sampling for Microbial and Chemical Analyses

At the beginning and at the end of the crop rotation, composite soil samples were randomly collected using an auger at 0–30 cm depth from 10 to 12 points in each subplot. Part of the composite soil sample was sent to Ghent University, Belgium, under cool conditions for microbial analysis. The remaining soil was air-dried, sieved through 2 mm, and then analyzed for total organic carbon (TOC) and total nitrogen (TN) by dry combustion methods.

The N availability to plants was monitored at different plant phenological phases during each crop cultivation. Soil samples were collected to 0–30 cm depth at transplanting time, first flowering, the start of harvesting, and the end of the crop cycle for zucchini, and at transplanting, middle of the cycle, and harvest for lettuce. Moist soil samples were then extracted with 2 M KCl (1:10 w/v). Soil mineral N

(SMN) was calculated as the sum of nitrate N (NO_3^-−N) and ammonium N (NH_4^+−N) and determined according to Henriksen and Selmer-Olsen [27] and Krom [28], respectively.

Laboratory Incubation Experimental Design and Measurements

The soil samples collected from the field experiment were used to separately set up two incubation experiments to determine potential C and N mineralization under laboratory conditions. The same four fertilizer treatments of the field experiment (Acti_AD, MWC, OWC, COF) were applied considering the rate of application to soil, also using a control without fertilizer (CTR). Each experiment was designed with five treatments and 3 replicates.

For C mineralization, a total of 15 (5 treatments*3 replicates) PVC tubes (r = 3.75 cm, h = 7.5 cm) were filled with moist soil equivalent to 263 g dry soil, after mixing with the corresponding amount of each fertilizer. No fertilizer was added to the CTR. Each of the tubes filled with soil was placed inside a glass jar (1 L), which was sealed airtight together with a vial containing 15 ml of 1 M NaOH and placed in an incubation chamber at 16 °C for 118 days. The C mineralization was monitored 16 times (days: 1, 4, 6, 13, 20, 27, 34, 46, 48, 55, 62, 69, 76, 90,104, 118) by measuring the CO_2 trapped in the vial containing 1 M NaOH, and by titrating the excess NaOH with 0.5 M HCl in the presence of $BaCl_2$, as explained in Gebremikael et al. [29].

The percentage of net cumulative C mineralized from the AWCB was calculated at each time as follows:

$$Cmin_{net}\,(\%) = \frac{Cmin\_amended - Cmin\_unamended}{Total\,C\,added} x100 \tag{1}$$

Where:
$Cmin_{net}$ = percentage of net cumulative CO_2-C mineralized from the specific AWCB;
Cmin_amended = cumulative total CO_2-C measured in samples treated with AWCB;
Cmin_unamended = C from the control samples without AWBC.

The impact of fertilizers application on soil organic C stock was assessed by deriving the humification coefficient after fitting the C mineralization data with a second-order kinetics model (Eq. 2) [30]. A mean soil temperature of 10 °C was considered since the temperature varies from experiment to experiment and soil organic matter decomposition occurs after the cropping season as well [30]. Using a temperature dependence model of C mineralization [31], we calculated that an incubation period of 175 days at 16 °C (which is the average temperature of the experimental site) under controlled conditions is equivalent to one year mineralization under field conditions.

$$C\,(t) = CA - \frac{CA}{1 + k2a(1-a)CAt} \tag{2}$$

Where:
C (t) = the cumulative net C mineralized at time t,
CA = amount of mineralizable C,
k2 = second-order mineralization rate constant,
α = fraction of C incorporated into microbial biomass C.

The parameters k2 and α cannot be estimated separately by fitting this model to observed data and, therefore, only a 'lumped value', k2 α (1- α) was estimated.

To determine the net N released from the organic fertilizers and the impacts on microbial community and activity dynamics, a total of 105 PVC cores (5 treatments x 3 replicates x 7 sampling times) (r = 2.3 cm, h = 18 cm) were filled with 225 g dry soil equivalent of the moist soil, mixed with the corresponding amount of fertilizer at the rate of 150 kg N ha^{-1} soil. Three replicates of each treatment and the control soil were destructively sampled at 10, 24, 38, 59, 87, 108 and 140 days. Mineral N (sum of NH_4^+ and NO_3^-) was then measured in the suspension extracted from 30 g of soil, with a continuous flow analyzer (Chem-lab 4, Skalar 223 Analytical, Breda, The Netherlands). The net N release was calculated as the difference between total mineral N in the amended and in the unamended control samples, according to De Neve and Hofman [32], and expressed as a percentage of the total N added with fertilizers.

$$Nmin_{net}\,(\%) = \frac{Nmin\_amended - Nmin\_unamended}{Total\,N\,added} x100 \tag{3}$$

Where:
$Nmin_{net}$ = percentage of total mineral N mineralized from the specific AWBC;
Nmin_amended = total mineral N measured in samples treated with AWCB;
Nmin_unamended = the control samples without AWBC.

Microbial Biomass and Enzyme Analysis

The soil microbial biomass carbon (MBC) and dehydrogenase enzyme activities (DHA) were determined at the 7 sampling points. The MBC was determined by fumigation extraction method, according to Vance et al. [33], by using fresh soil and 0.5 M K_2SO_4 (1:2 w: v ratio). The extracts were filtered using Whatman no 5-filter paper and stored in a freezer (-18 °C) until analysis by TOC/TN analyzer (Skalar Analytical B.V). The extraction efficiency coefficient used for microbial biomass carbon was 0.45 [34].

The DHA was analyzed with the procedure explained in a previous study [35], by using five grams of moist soil and triphenyl tetrazolium chloride (TTC) as a substrate. The color intensity of the filtrates was measured at 485 nm with Cary 50 UV-Visible spectrophotometer (Varian Inc.). All measurements were carried out in duplicate with one blank.

Weather Information and Agronomic Performance of Field Experiment

Weather Conditions

During the field trial, the mean monthly temperatures and the rainfall for each cropping season were continuously monitored by collecting data from a nearby weather station and were compared to the long-term average (about 40 years, from 1981 to 2018; Fig. 1).

The mean monthly temperatures in the second cultivation period for both zucchini and lettuce were quite comparable to the long-term averages. On the contrary, in the first cultivation period for zucchini, the mean monthly temperatures were higher compared to the long-term averages. The lowest temperature was found in January 2017, that can be considered an extreme weather event, showing 4.5 °C compared to the long-term average of 8.2 °C. In that period, the green manure crops were cultivated.

The cumulative rainfall during the period of investigation was 87.5 mm from October to December 2016, and 401 and 760 mm in 2017 and 2018, respectively. The rainfall values over the first and the second zucchini cycles were lower by 40% and higher by 11.6%, respectively, than the long-term values of the same seasons. The rainfall values over the first and the second lettuce cycles were lower by 54% and higher by about 90%, respectively, than the long-term values of the same seasons. In particular, in the second cycle of lettuce the average monthly rainfall was higher by 535 and 397%, in August and October than the long-term mean rainfall, respectively.

Crop Yields, Total C and N Input to Soil

Yield and residues were sampled from a 1.0 m^2 area in the middle of each sub-plot at zucchini and lettuce harvest. Biomasses were then dried for 48 h at 70 °C for their dry content determination and stored at room temperature until further analysis. All the collected aboveground biomasses (except for crop marketable yield that was removed from the systems) and fertilizers were analyzed for C and N content to calculate the total C and N input to soil in each treatment. The C content was determined by a LECO analyzer (LECO RC-612; LECO Corporation, St. Joseph, MI) using a dry combustion method. The N content was analyzed by Dumas's method, using the elemental analyzer LECO FP 528.

Statistical Analysis

Incubation Experiment

The incubation experiment under laboratory conditions was set up with two fixed factors: treatments and incubation time (7 sampling dates). A two-way analysis of variance model

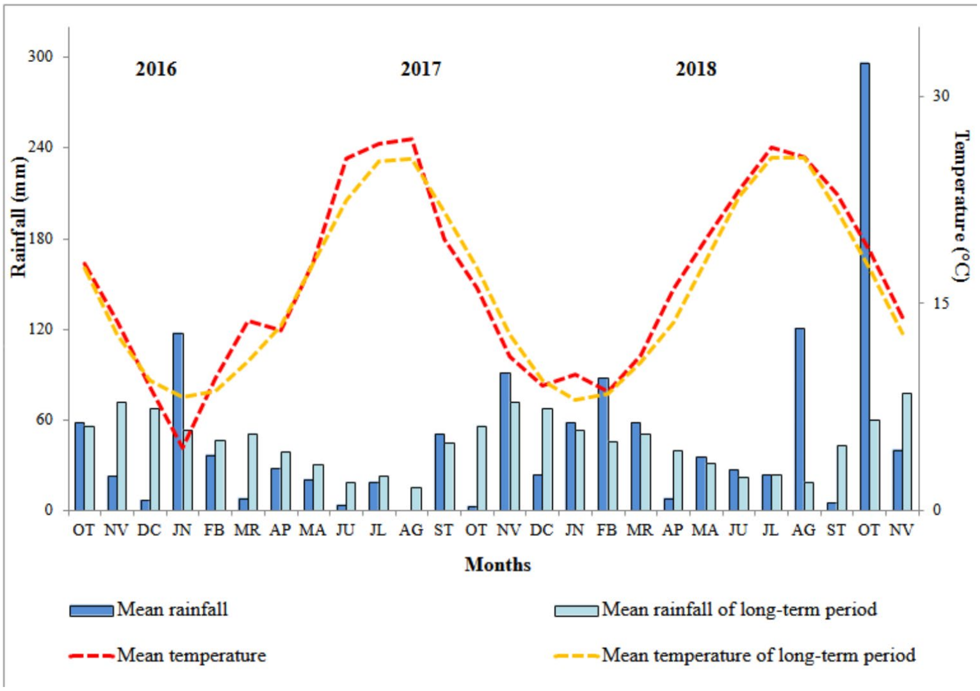


Fig. 1 Mean monthly rainfall and temperature during the experiment (October 2016 –November 2018), in comparison with the long-term averages (1981–2018)

with two factors was fitted on the parameters determined during and at the end of the incubation experiment using a parametric one-way ANOVA (aov) function in R studio version 3.5.3 [36]. The assumptions for ANOVA were checked based on the residuals using the combination of generic functions *plot, shapiro-test and levene Test*. The data were further split based on the incubation time following the significant interactions between time and treatments. Analysis of variance was then conducted for each sampling time using aov model with the treatments as a factor. The post hoc function Tukey, HSD multiple comparisons of means, was applied for ANOVA with $p < 0.05$. When the assumptions of ANOVA were not fulfilled, the data was transformed (log and sqrt values) for the specific variables that did not fulfil the assumptions. Parameters with heteroscedastic data were fit to the conservative Welch's heteroscedastic F-test function (oneway.test) and a pairwise comparison using the conservative Games-Howell test. Differences in mean values are reported in letters for selected parameters in supporting information (SI 2.1-3).

Field Experiment

Univariate analysis of variance (ANOVA) for the combined dataset of 2 years was performed considering Year as a random factor and Green manure (GM + and GM-) and fertilizer treatments (the same as for the incubation experiments) as fixed one. ANOVA model was fitted to the data collected from the field experiments using SPSS for windows, version 16.0. Before analysis, the Levene test was performed to assess the homogeneity of error variances, whereas the Kolmogorov–Smirnov and Shapiro–Wilk tests were computed to check the normality.

ANOVA on mean SMN, C and N inputs (calculated as the sum of C and N amount derived from the fertilizer, the GM, weeds, and crop residues in each treatment), and soil content of TOC and TN, was carried out considering time at sampling phase instead of year, i.e., PHASE: beginning of the rotation (T0), end of first year rotation (Tm), and the end of second-year rotation (Tf).

Mean comparison was carried out according to the Least Square Difference (LSD) statistics and the Duncan Multiple Range Test (DMRT), respectively, for two and more than two comparisons, testing for significance at $p \leq 0.05$.

Results and Discussion

Carbon and Nitrogen Mineralization Under Laboratory Conditions

Laboratory incubations can be used to estimate C mineralization dynamics in amended soils. The data obtained are generally fitted to kinetic models to extract complementary information, such as the C-mineralization rates and the potentially mineralizable C [30, 37]. In our experiment, at the end of the incubation period, all the organic fertilizing treatments significantly (df = 4, F = 137.7, $p < 0.0001$) increased the cumulative CO_2 evolved from organic C (CO_2-C) compared to the control (CTR) (Fig. 2a), showing substantial differences among them. The significantly highest increase in cumulative CO_2-C, compared to the CTR, was recorded in MWC (+ 567%), while the lowest one was found in Acti_AD (+ 62%). Conversely, COF and OWC amended soils showed comparable and intermediate trends in their cumulative CO_2-C mineralization. These different C mineralization patterns of fertilizers could be due mainly to differences in their composition (C/N ratio varying from 10.5 to 25 in COF and MWC, respectively), and the total C application rates [24, 38]. The total C applied in MWC treatment was nearly twice as high as in the Acti_AD (SI 1). It is also likely that MWC contains higher amounts of rapidly mineralizable components coming from the organic household raw waste materials used for compost preparation [39]. On the contrary, labile C compounds in Acti_AD is present in lower proportion, since most of them have been converted to biogas during the anaerobic digestion process and stabilized in the subsequent co-composting. During the digestion process, in fact, the degradation of the more labile fractions (e.g., carbohydrate-like molecules) of the feedstocks leaves a residue (digestate) that is rich in recalcitrant molecules, such as lignin and non-hydrolysable lipids [24, 40]. Moreover, according to Torres-Climent et al. [14], water-soluble C and N generally decrease at the end of the co-composting of digestate from cattle manure, indicating a reduction of its biodegradability and potential environmental pollution. By composting, the stability and maturity of organic matter of digestate can indeed be improved, thereby enhancing the nutrient retention capacity in the soil and reducing the risk of nutrient leaching and N_2O volatilization, which conversely could occur applying not-composted digestate [16, 41, 42].

The net cumulative CO_2-C, expressed as percentage of organic C added with each fertilizer, varied significantly among treatments (Fig. 2b). In particular, the percentage of net cumulative CO_2-C mineralized as percent of added C followed the trend COF (35%) > MWC (25%) > OWC (21%) > Acti_AD (5%). Thus, considering the different

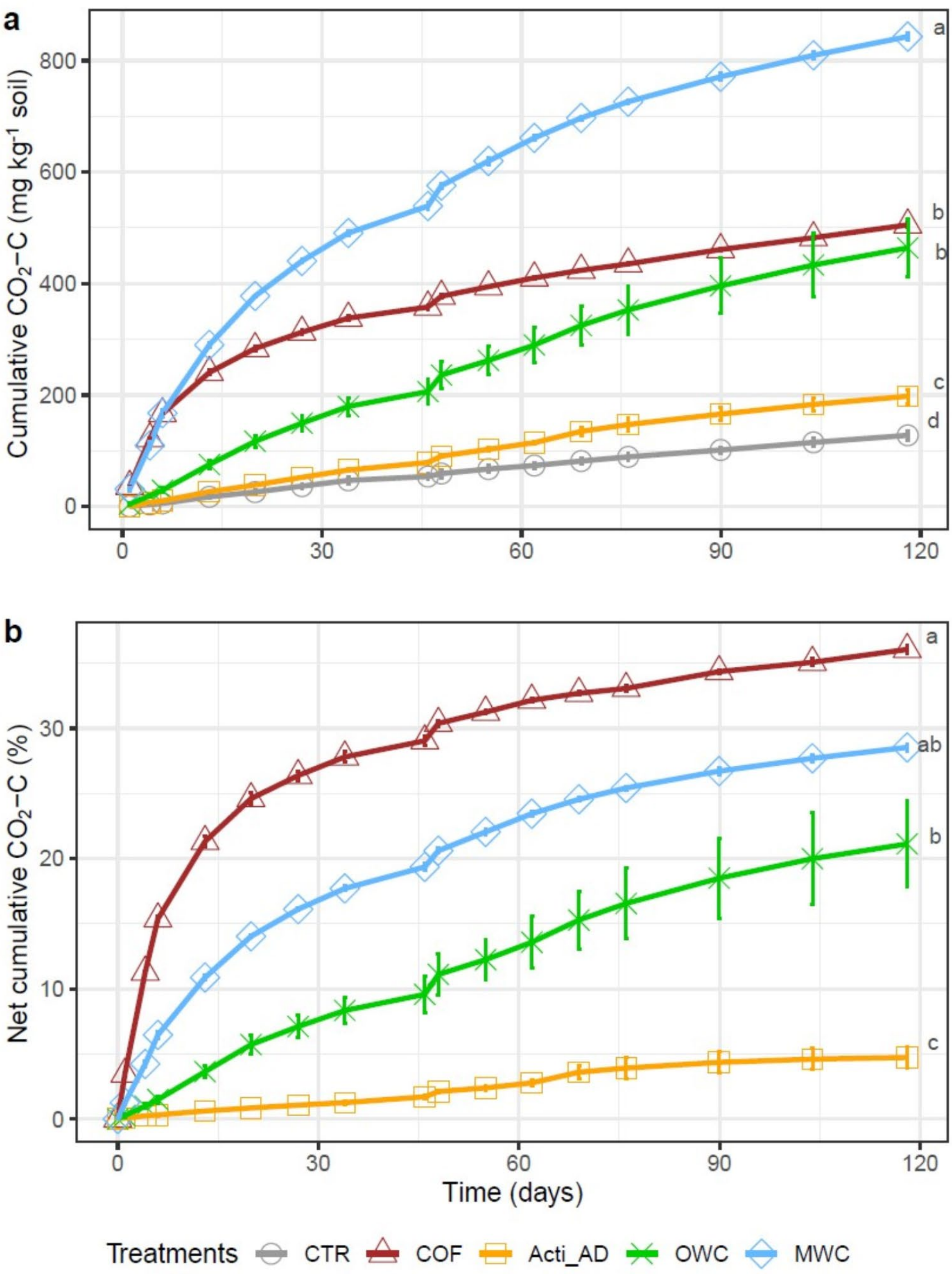


Fig. 2 The dynamics of cumulative CO_2-C mineralized from native and added organic fertilizers **(a)** and percentage of net C mineralized from the added organic fertilizers **(b)** (CTR = unfertilized control; COF = commercial organic fertilizer; Acti_AD = co-composted anaerobic digestate; OWC = re-composted olive waste compost; MWC = municipal solid waste compost)

amounts of C added with the organic materials (SI 1), the highest percentage of cumulative C evolved as CO_2 at the end of the incubation period mineralized from COF and MWC, highlighting the content of more easily decomposing compounds of these organic materials. As indicated in previous studies [37, 43], a higher percentage of net cumulative C-mineralization (as for COF) may not exclusively be attributed to the presence of more labile C fractions in the amendment. It could also be due to a positive priming effect [44], likely induced by higher N input per unit of applied C. Because of the low total C added, N was not a limitation in COF treatment. Therefore, the microbes utilized N from the COF and likely decomposed more native SOM that contributed to the highest percentage (35%) of CO_2-C.

The humification coefficient represents the fraction of the organic material added to the soil converted into humus, and it can vary greatly depending on the input material [45]. In fact, in line with the cumulative net C mineralized from the tested amendments, a significantly higher humification coefficient (0.93) was estimated in Acti_AD than in the other organic fertilizers, which were not different among them as reported in Table 1. The coefficient provides an estimation of the stable organic matter in the soil as SOC, thus representing an indicator of C sequestration potential [46].

Table 1 The mean humification coefficient estimated based on the 2nd order kinetics model. (CTR = unfertilized control; COF = commercial organic fertilizer; Acti_AD = co-composted anaerobic digestate; OWC = re-composted olive waste compost; MWC = municipal solid waste compost). Different letters indicate significant differences (df = 3, F = 83.9, $p = 0.001$) among the treatments

Treatments	Humification Coefficient (mean ± se)
MWC	0.73 ± 0.03b
OWC	0.73 ± 0.05b
COF	0.66 ± 0.01b
Acti_AD	0.93 ± 0.01a

Therefore, the organic C provided by Acti_AD highlights its potential to preserve/restore SOC.

Despite the possible higher concentration of labile organic matter in COF, MWC and OWC, the C sequestration potential has been only partially affected resulting in lower humification coefficients in comparison with Acti_AD, and remaining potentially suitable to preserve/restore C in the soil. This result would confirm the evidence of higher organic C sequestration rate due to composted wastes compared to mineral and other organic commercial fertilizers, particularly over longer periods [47].

As regards N mineralization, the highest value of total mineral N was recorded in soil samples treated with COF, followed by Acti_AD (Fig. 3a). In both cases, the total mineral N was significantly (df = 4, F = 467.4, $p < 0.0001$) higher than the CTR, OWC and MWC throughout the incubation period (SI 2.1). Similarly, the highest percent of net total mineral N released from the fertilizers was also recorded in COF (40% of total N released within 59 days), followed by Acti_AD (Fig. 3b). No net N release from OWC and MWC was observed within the incubation period, likely due to their higher C: N ratio, which might have resulted in N immobilization into soil microbial biomass [21]. The N immobilization trend observed after the application of OWC and MWC may be considered as a drawback in terms of N availability for plant uptake and the subsequent yield reduction. Anyway, a system approach combining agroecological practices should be followed and the application time for OWC and MWC needs to be adjusted accordingly, to reduce competition for N between the microbes and plant roots and synchronize N availability with the crop need [48, 49].

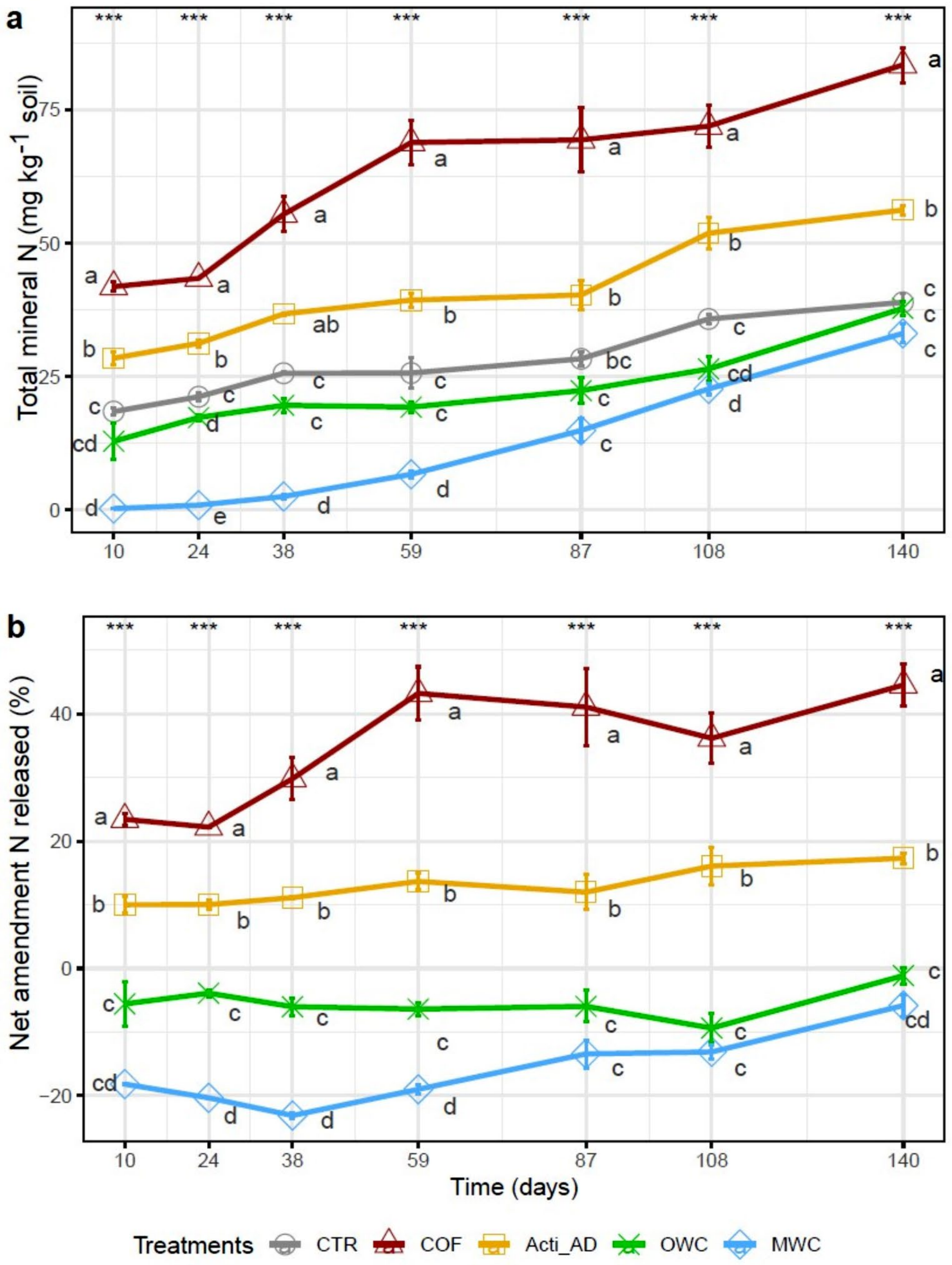
Microbial Parameters Under Laboratory Conditions

The parameter microbial biomass carbon (MBC), which is more sensitive to changes in soil quality than total C or N, can be used as an early indicator of improvement of soil fertility in the medium- short-term [22]. Many studies reported the MBC dynamics following the addition of different organic amendments [21, 24, 50], indicating that both the quality and quantities of organic amendments applied can differently affect the microbial biomass in soils [51, 52]. The present study confirmed these findings, since all the organic fertilizers, except Acti_AD, resulted in significantly (df = 4, F = 101.7, $p < 0.05$) higher MBC compared to the CTR (Fig. 4a), during most of the incubation period (SI 2.2). In particular, MWC treatment showed the significantly highest MBC value compared to all the other treatments, at least until day 38 after the start of the incubation, correlating with the highest N immobilization level in MWC-amended soils achieved on day 38 (Fig. 3b). The gradual increase in the accumulation of mineral N after day 38 was probably due to the microbial turnover that likely resulted in the release of N bound in the microbial biomass. The described increase of MBC is generally due to the presence of a labile C fraction in the organic amendments, which stimulate the growth of microbial communities [53]. The decline of MBC over time is thus likely related to a decline of such labile C.

Dehydrogenase activity (DHA) measurement is usually related to the presence of viable microorganisms and their oxidative capability, playing a significant role in the biological oxidation of soil organic matter [54]. Like the MBC, all organic fertilizers, again except Acti_AD, resulted in significantly (df = 4, F = 112.7, $p < 0.0001$) higher DHA compared to the CTR during the first two months of incubation period (day 59) (Fig. 4b, SI 2.3). In particular, the highest DHA was recorded throughout the experiment in the samples treated with MWC, which seems related to the higher total C applied. The DHA decreased in all the treatments and the CTR until two months after the start of the incubation and became stable during the rest of the incubation period, except for the MWC. The DHA also showed a significant positive correlation ($r = 0.78$, $p < 0.001$) with MBC, indicating that the microbial community in the soil actively utilized carbon sources for their metabolic activities.

As reported by several short and long-term experiments, the dynamics of MBC and DHA are primarily related to the composition and availability of C input, since the available C in the amended soils is used as an energy source for microbial growth [21, 51], and microbial activities in soils are limited by C availability [53]. Therefore, the slow microbial growth recorded in Acti_AD could be primarily related to a large proportion of stable C not readily available to soil microorganisms. Conversely, ammonium and other substances, which are normally present in the digestate [55], cannot be detected in co-composed digestate in a concentration sufficient to cause a toxic effect that can subsequently slow the microbial growth [24].

Fig. 3 The total mineral N dynamics in the control and each treatment **(a)** and percentage of net N released from the added organic fertilizers **(b)**. The values refer to mean($n=3$) and standard error of the mean (CTR = unfertilized control; COF = commercial organic fertilizer; Acti_AD = co-composted anaerobic digestate; OWC = re-composted olive waste compost; MWC = municipal solid waste compost)



Microbial Parameters Under Field Conditions

There was no significant interaction effect of green manure and fertilizer application both on MBC (df = 4, F = 0.29, $p = 0.88$) and DHA (df = 4, F = 0.99, $p = 0.42$) under field conditions (SI 2.4). The MBC did not show significant differences among the fertilizer treatments at both samplings within GM treatments (Fig. 5). However, green manuring resulted in a significant increase (+ 19%, df = 1, F = 11.35, $p = 0.003$ and + 35%, df = 1, F = 25.8, $p < 0.0001$) in MBC compared to the soil without GM at the end of the first- and second-year crop rotation, respectively. This result could be due to increased organic matter input from green manure that likely stimulated the possibility of microorganisms to grow. In fact, several studies reported a significant increase of MBC in soil by using cover crops, due to the input of easily decomposable organic residues [56–58].

Like MBC, DHA did not show significant differences among fertilizers within GM treatments. However, DHA was significantly higher (83%, df = 1, F = 11.35, $p = 0.003$ and + 53%, df = 1, F = 7.96, $p < 0.011$) in GM + plots than GM- at the end of the first and second-year crop rotation, respectively (Fig. 6). It also significantly increased by nearly two-fold (df = 1, F = 181.69, $p < 0.0001$) at the 2nd

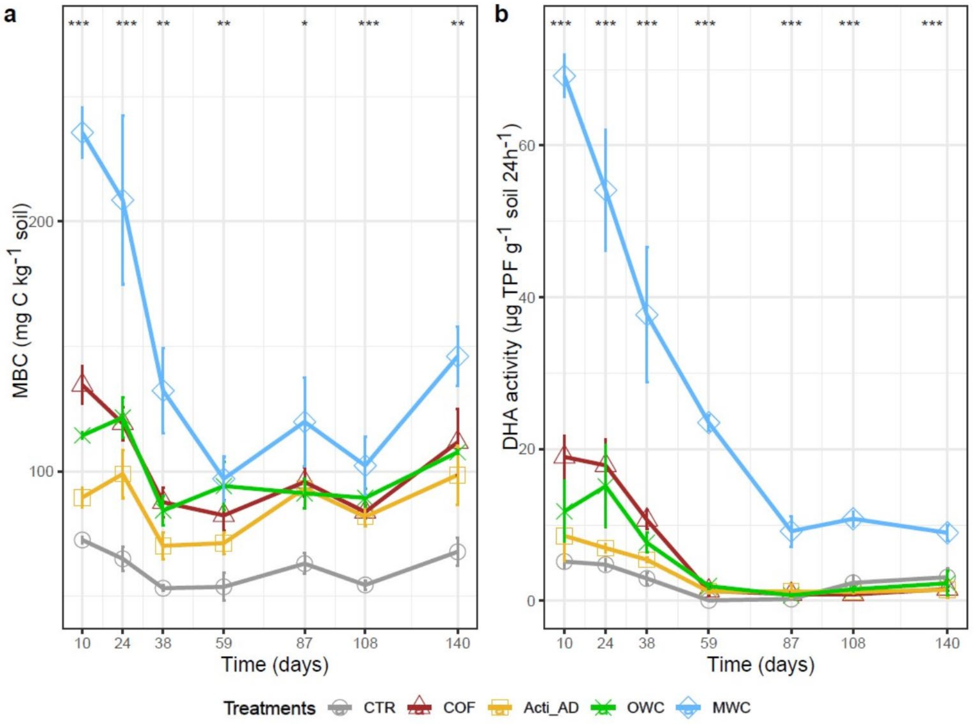


Fig. 4 The dynamics of microbial biomass carbon (MBC) **(a)** and Dehydrogenase activity (DHA) **(b)** over time in the unamended control and treatments. The values refer to the means (*n* = 3) and their standard error (CTR = unfertilized control; COF = commercial organic fertilizer; Acti_AD = co-composted anaerobic digestate; OWC = re-composted olive waste compost; MWC = municipal solid waste compost)

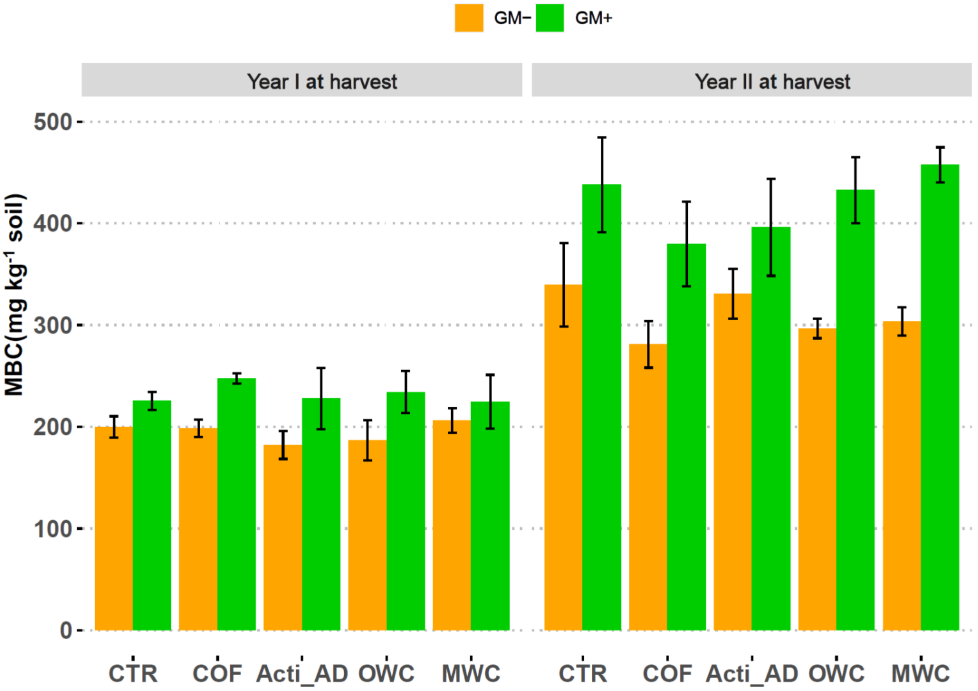


Fig. 5 The dynamics of MBC over time in the unamended control and treatments. The values refer to the means (*n* = 3) and their standard error. MBC did not significantly (df = 4, F = 0.30, *p* = 0.87) respond to fertilizer application, but significantly (df = 1, F = 36.09, *p* = 0.87, $p < 0.0001$) responded to GM addition at harvest of lettuce at the end of first- and second-year rotation (CTR = unfertilized control; COF = commercial organic fertilizer; Acti_AD = co-composted anaerobic digestate; OWC = re-composted olive waste compost; MWC = municipal solid waste compost)

year harvest, as compared to the 1st year, indicating a cumulative effect of the green manuring and fertilizer treatments on micro-organisms activity. The significantly higher DHA in GM + soils suggest the presence of more biodegradable substrates than in GM-, which stimulate and sustain the microbial activities at least until the harvest, thus affecting soil health and likely crop production The DHA also showed a significant correlation ($r = 0.62$, $p < 0.001$) with MBC, in line with previous studies that found DHA increase strictly linked with the increase of soil microbial biomass and soil respiration [59]. The observed differences between laboratory and field results for the two parameters (MCB and DHA) was likely due to a relevant influence of the environmental biotic and abiotic variables, which determined in the

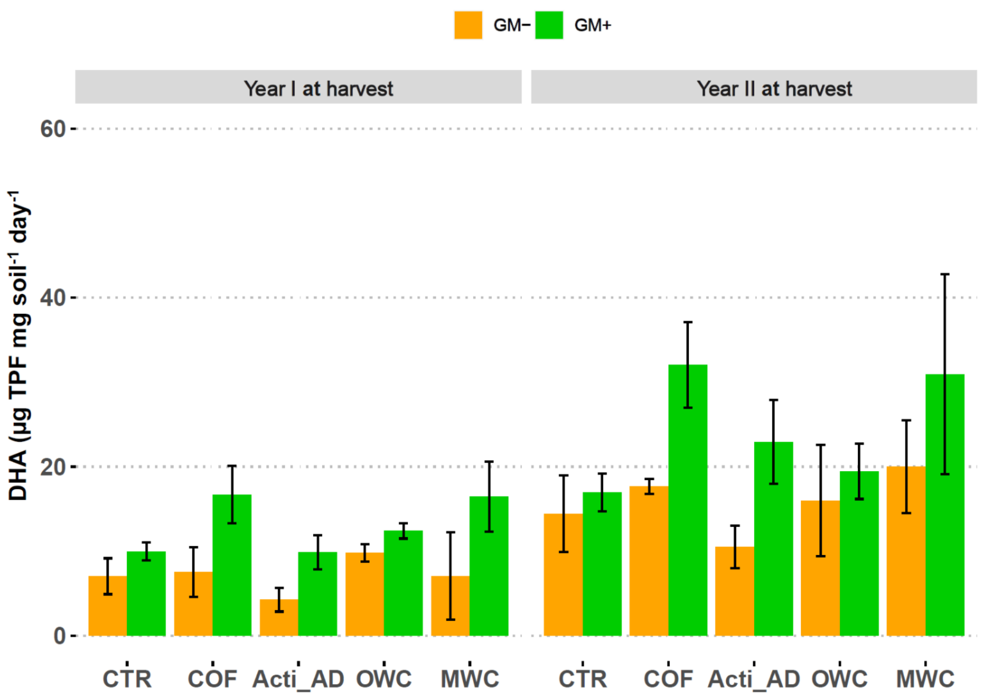


Fig. 6 The dynamics of DHA over time in the unamended control and treatments with and without GM. The values refer to the mean ($n = 3$) and their standard error. DHA did not significantly (df = 4, F = 2.14, $p = 0.09$) respond to fertilizer application, but significantly (df = 1, F = 19.53, $p < 0.0001$) responded to GM addition at harvest of lettuce at the end of first- and second-year rotation (CTR = unfertilized control; COF = commercial organic fertilizer; Acti_AD = co-composted anaerobic digestate; OWC = re-composted olive waste compost; MWC = municipal solid waste compost)

Table 2 Analysis of variance (ANOVA) of the zucchini and lettuce marketable yield, Mean soil mineral nitrogen (SMN) during zucchini and lettuce cycle (0–30 cm) C input and N input during the whole rotation

	df	Zucchini marketable yield (Mg ha^{-1})	Mean SMN zucchini (mg kg^{-1})	Lettuce marketable yield (Mg ha^{-1})	Mean SMN lettuce (mg kg^{-1})	C input (Mg ha^{-1})	*N* input (Mg ha^{-1})
YEAR	1	***	n.s.	***	n.s.	***	**
GM	1	n.s.	n.s.	***	**	***	***
FERT	4	n.s.	**	n.s.	**	***	***
YEAR*GM	1	n.s.	***	**	n.s.	**	*
YEAR*FERT	4	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
GM*FERT	4	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
YEAR*GM*FERT	4	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Adjusted R^2		23.33%	61.06%	57.04	24.74%	78.86%	80.29%

Note n.s., not significant. *, **, *** significant differences at $p < 0.05$, 0.01 and 0.001, respectively. GM, green manure; FERT, fertilizer

field a controversial behaviour of the (although stabilized) organic materials than that under controlled conditions. Moreover, according to Jian et al. (2020) [60], parameters estimated from short- versus long-term datasets may differ by at least an order of magnitude, suggesting that it could be necessary to further analyse microbially driven processes in laboratory to support projection of field results.

Agronomic Performance of Field Experiments

The analysis of variance revealed a significant effect of YEAR on zucchini and lettuce marketable yields, as well as for C and N inputs, while the GM significantly affected lettuce marketable yields, mean SMN during lettuce cycle, and C and N inputs (Table 2). The fertilizer treatments (FERT) affected the mean SMN during zucchini and lettuce cycle, C and N input. Finally, a significant interaction between YEAR x GM was also found for all the parameters, except for zucchini marketable yield and mean SMN during lettuce cycle.

The YEAR effect was likely due to different weather conditions recorded in the experimental period, confirming the results of a previous study in the same area, in which the climatic variability more than other factors influenced the lettuce responses to organic fertilization [20]. Moreover, GM and FERT effects show that integrating these agroecological practices could be important for enhancing C and N input and thus the resilience of the agroecosystems [61].

As reported in Table 3, the highest zucchini yield was found in the second-year trial (+55% than in the first one), while SMN was higher in GM+ in the first year compared to GM-, and the contrary was found in the second year.

Table 3 Zucchini and lettuce marketable yield, Mean soil mineral nitrogen (SMN) during zucchini and lettuce cycles, C input and N input during the whole rotation (CTR = unfertilized control; COF = commercial organic fertilizer; Acti_AD = co-composted anaerobic digestate; OWC = re-composted olive waste compost; MWC = municipal solid waste compost)

		Zucchini marketable yield (Mg ha^{-1})		Mean SMN zucchini (mg kg^{-1})		Lettuce marketable yield (Mg ha^{-1})		Mean SMN lettuce (mg kg^{-1})		C input (Mg ha^{-1})		*N* input (Mg ha^{-1})	
Year	I	10.24	b	15.76	a	30.19	a	10.64	a	3.29	a	0.20	a
	II	15.91	a	16.38	a	12.38	b	9.41	a	2.47	b	0.17	b
GM	GM+	12.23	a	16.53	a	24.15	a	11.50	a	3.71	a	0.23	a
	GM-	13.92	a	15.61	a	18.42	b	8.55	b	2.04	b	0.14	b
FERT	OWC	11.13	a	13.11	b	21.41	a	9.05	b	4.05	a	0.21	a
	Acti_AD	14.89	a	16.46	ab	17.73	a	9.49	b	2.93	bc	0.22	a
	MWC	11.21	a	15.26	ab	18.49	a	8.58	b	3.84	ab	0.24	a
	COF	14.99	a	19.40	a	25.00	a	14.27	a	2.64	c	0.22	a
	Ctr	13.16	a	16.11	ab	23.80	a	8.73	b	0.93	d	0.05	b
YEAR x GM	I GM+	10.33	a	19.79	a	36.30	a	12.35	a	4.47	a	0.26	a
	I GM-	10.15	a	11.72	b	24.08	b	8.93	a	2.11	b	0.15	b
	II GM+	14.13	a	13.26	b	12.00	c	10.65	a	2.96	b	0.19	b
	II GM-	17.69	a	19.51	a	12.77	c	8.16	a	1.98	b	0.14	b

Notes Mean values in each column, followed by different letters, are significantly different according to LSD and DMRT (two and more than two comparisons, respectively). GM, green manure; FERT, fertilizer

Maybe this higher yield in the second year, despite the lowest N supply by GM+, could be explained by the more favourable weather conditions for plant development in the second cultivation period (like temperatures consistent with long-term period and higher rainfall, although the crop was irrigated in both years). The zucchini yield was 21% lower and 23% higher, respectively, than the mean yield of Matera province, which is about 13 Mg ha^{-1} on the average of the last ten years [62]. On the average, although fertilizers did not show any significant difference in zucchini yield, the N availability for zucchini crop was the highest in absolute value in COF and the lowest in OWC, confirming the laboratory results that recorded the highest release of mineral N in soil treated with COF (Fig. 3). The N immobilization trend observed in the incubation experiment for the OWC and MWC treatments was comparable with the immobilization tendency recorded during zucchini production where the SMN in the plots treated with OWC and MWC was slightly lower than in the CTR.

The yield of lettuce crop was significantly higher in the first year (+ 144%), with the highest value recorded in GM + treatment. This was due to the high N supply with GM, since the GM + treatment recorded the highest mean SMN value (by + 35% than in GM-), confirming that the introduction of GM increased SMN and lettuce yield [63]. Even though a higher N availability with COF was detected, the fertilizers used did not show any significant difference in lettuce yield. In this regard, it is worthy to remark that no fertilization was applied before lettuce transplanting, thus the N availability for lettuce was due to a residual effect of the fertilization applied before zucchini transplanting. This fertilization strategy did not affect the first-year yield, which was higher than the mean of Matera province, which is about 23 Mg ha^{-1} on the average of the last ten years [62]. On the contrary, the extreme rainfall values during the second cycle of lettuce reduced the yield by 59%, probably due to a temporary flooding of the soil that overcame the potential beneficial effect of the fertilization strategies adopted, like stated by De Benedetto et al. [64].

Finally, the total C and N inputs to the soil during the whole rotation were significantly higher in GM + than in GM-, confirming the results found in other studies in Mediterranean environment [65, 66]. This result was clear particularly in the first year, which had the highest input values probably due to the different biomass (GM, weeds and crop residues) produced each season. According to the C/N ratio of the fertilizers applied, the C input was significantly higher in OWC and MWC treatments. This result was particularly true considering COF and CTR, the latter receiving the lowest C input (-77% than in OWC treatment). Similarly, the N input in the fertilized plots was significantly higher than in CTR (about − 80% less), particularly in the first year

Table 4 Analysis of variance (ANOVA) of soil total Organic C (TOC) and total N (TN)

	df	TOC (Mg ha^{-1})	TN (Mg ha-^{1})
PHASE	2	***	***
GM	1	*	n.s.
FERT	4	n.s.	n.s.
PHASE*GM	2	n.s.	*
PHASE*FERT	8	n.s.	n.s.
GM*FERT	4	n.s.	n.s.
PHASE*GM*FERT	8	n.s.	n.s.
Adjusted R^2		33.55%	49.36%

Note n.s., not significant. *, **, *** significant differences at $p < 0.05$, 0.01 and 0.001, respectively. PHASE, phase of sampling during the two-year rotation; GM, green manure; FERT, fertilizer

with GM. In accordance with our results, Tittarelli et al. [67] found that the C and N input were higher in the case of agro-ecological systems that included both green manuring and organic amendments, compared to the application of the sole organic amendment.

Soil Total Organic C and Total N under Field Conditions

The analysis of variance revealed highly significant effect of the PHASE (referring to subsection 2.7.2) on both soil TOC and TN, and significant effect of GM on TOC (Table 4). A significant interaction between PHASE and GM was found for soil TN parameter, while no other interactions were recorded both for the second and third order. The different fertilization strategies did not significantly affect either soil TOC or TN during the field trials.

Soil TOC significantly decreased over time (from 1.37 to 1.21%) at the end of the two-year rotation (Fig. 7a), indicating how SOC declines rapidly even under organic vegetable cropping system. However, the mean TOC values were significantly higher in GM + than GM- (Fig. 7b) resulting in lower decline over time with GM application. This result highlights the importance of the introduction of cover crops for the sustainability of organic agricultural systems, since it can increase soil microbial biomass [68] and reduce soil loss [64, 69], confirming the findings of increased microbial biomass C and microbial activities under field condition (Fig. 5). A different trend was recorded for TN, which significantly increased from T0 to Tm in both GM treatments (about + 30% for both), whereas a significant decrease from Tm to Tf for the GM- treatment was found (Fig. 7c).

The different organic amendments did not significantly affect both TOC and TN over the two-year rotation and both in GM + and GM-, except for Acti_AD, which showed a significant higher TOC value by 13% at Tf as compared to CTR in GM- plot (data not reported). The significant increase in TOC in Acti_AD treatment is in line with the humification coefficient estimated under laboratory experiment conditions (Table 1). However, this period may be not enough to draw general conclusions, thus, assessing the possible long-term effects of the tested fertilizers under the study conditions is required. On the contrary, short-term changes in soil organic carbon are mainly linked to the dynamics of the labile fraction of TOC (i.e., microbial activity) [70]. The decrease of TOC was probably due to the mineralization of the easily degradable organic C. On the other hand, the significant increase of TN recorded up to the end of first-year rotation (Tm) can be attributed to the N released by the mineralization of fertilizers particularly in GM+. The rapid decomposition of GM added at the beginning of the second-year rotation extended this trend up to Tf, while in GM- the crop N uptake caused the decrease of TN and likely influenced the yields. This decrease can also be attributed to the high monthly rainfall in the second part of the rotation, which could have determined N leaching in absence of cover crops, as highlighted in other studies conducted in Mediterranean climate [71, 72].

Conclusions

Closed agrifood systems aim to recycle Agricultural Wastes, Co-products, and By-products, thus improving the sustainability of crop systems also integrating other agroecology

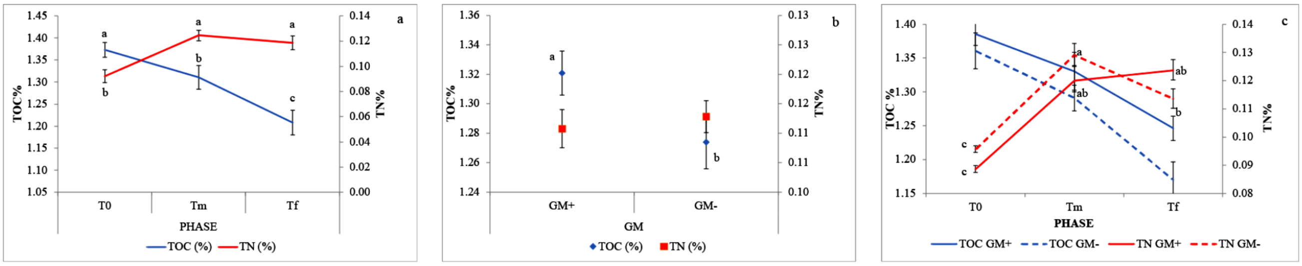


Fig. 7 Dynamics of soil total organic carbon (TOC) and total nitrogen (TN): **(a)** PHASE effect; GM effect **(b)** and PHASE x GM interaction **(c)**. Different letters mean significantly differences according to LSD and DMRT (two and more than two comparisons, respectively). T0, trial starting; Tm, end of the first-year rotation, Tf, end of second year rotation

practices (such as crop rotation, timing of fertilizer application, and green manuring), and considering a system approach. The combination of laboratory and field experiments can be crucial to evaluate the effectiveness of using different recycled wastes, in the circular economy framework. In our study, laboratory incubation experiment highlighted the presence of easily decomposing compounds in COF. This is due to the animal manure origin of the amendment that determined a high percentage of C-mineralization of its more labile C fractions, and N release. The field experiment confirmed these results, showing higher mineral N available in the soil compared to the other treatments for both crops of the rotation. Moreover, the N availability for zucchini crop was the highest in absolute value in COF and the lowest in OWC, although fertilizers did not show any significant difference in zucchini yield, confirming the laboratory results showing the highest release of mineral N in soil treated with COF. Under laboratory conditions, the highest MBC and DHA were achieved in MWC, whereas under field conditions, these parameters were significantly higher in plots with GM, regardless of the fertilizer treatments. This last result suggests the effectiveness of green manuring in boosting the microbial activity more than the fertilizers, as also confirmed by the higher C and N input detected in GM+, which are required for microbial growth. The importance of combined agroecological practices, which include cover crops for the sustainability of the organic vegetable systems, is further confirmed by the increase of soil TOC in GM+than GM-, as well as by the findings of the determination of microbial parameters under field conditions. However, our study highlights that weather conditions could affect the yield more than treatments. Even though a higher N availability with COF was detected, the N availability for lettuce was due only to a residual effect of the fertilization and GM before zucchini transplanting, which did not affect the first-year yield, thus highlighting that it could be possible to use this sustainable agronomic strategy. Anyway, the application of such strategy based on residual fertilizer effect, should consider the impact of weather conditions, since the extreme rainfall values during the second cycle of lettuce reduced the yield probably due to N leaching. The effects of application of newly produced AWCB-based fertilizers, which were supposed to be all stabilized with a potential known behaviour, indicate that the influence on the results of the environmental factors was more relevant than expected. Overall, it is crucial to consider a system analysis integrating fertilizer treatments and cover crop management in the rotation, instead of focusing on a single factor, assessing the potential synergistic effects of different factors also in the long-term.

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Author Contributions Mariangela Diacono, Mesfin T. Gebremikael, Francesco Montemurro and Stefaan De Neve contributed to the study conception and design. Material preparation and data collection were performed by Alessandro Persiani and Angelo Fiore. Data analysis was performed by Mesfin T. Gebremikael, Elena Testani and Alessandro Persiani. The first draft of the manuscript was written by Mariangela Diacono, Mesfin T. Gebremikael, Corrado Ciaccia and Vincenzo Alfano. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets generated and analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors have no financial interests to disclose.

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