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Improving the Sustainability of Tomato Production With Biochar and Biofertilizers in Emilia-Romagna, Italy

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Keywords: biochar | microbial consortia | sustainable agriculture rhizospheric microbes | tomato yield

ABSTRACT

Conventional agriculture is mainly dependent on chemistry and energy to promote growth and yield and on pesticides to protect crops from pests or pathogens. Biofertilizers as plant growth-promoting microbes (PGPM) and novel soil amendments, as biochar, are considered sustainable alternatives to diminish the excessive use of chemicals in agriculture. The effects of biochar, whether used alone or in combination with biofertilizers, are still not fully understood, and its potential benefits have yet to be thoroughly investigated. Additional field studies across various agronomic conditions are needed to confirm the impact of biochar-microbial consortia before they can be widely adopted in sustainable agricultural practices. Industrial tomato is a paradigmatic example of a culture requiring resources as well as fertilisers and energy. Results from field trials performed in two different locations in Emilia-Romagna (Italy): Parma and Ferrara (two different fields), with both conventional and organic practices, are here reported. Biochar, PGPM and arbuscular mycorrhizal fungi (AMF) were applied in field trials for two to three consecutive years. Soil characteristics, both chemical and biological, revealed that the fields had features comparable to the average European arable soils; specifically, the rhizospheric biological diversity indexes were similar in the three locations. The most interesting results were obtained in the Parma field location, where it was observed that biochar and PGPM treatment determined a reduction in the number of not ripe fruits (average 65%). When biochar was combined with newly developed PGPM in the two conventional fields, the value of commercial production and the Brix index of the harvest increased. The biofertilizers, when combined with biochar, remarkably permitted saving 20% of chemical fertilisers with a consequent saving in greenhouse gas (GHG) emissions. These results were also considered from an economic perspective, which revealed that PGPM and biochar increase the gross margins while reducing the environmental impact of the tomato cultivation.

S. Graziano and M. Caldara contributed equally to this work.

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1 | Introduction

Tomato (*Solanum lycopersicum* L.) is a globally important cash crop (Ronga, Laviano, et al. 2020). There are two types of tomato cultivars used, those for fresh consumption, usually grown in greenhouses, and those used for industrial transformation (processing), which are usually grown only under field conditions (Cammarano et al. 2020). According to the FAO database (www.fao.org/faostat; updated in December 2024), the total world tomato production, for both processing and fresh consumption, amounted to over 189.1 million metric tonnes (MmT) in 2021, with an increase of 2% with respect to the previous year and of 4% to the average (182.7MmT) of the previous 3 years (2018–2020). Italy is the top European producer, representing 51.7% of European production, with approximately 3.2% of the global production, and it is the third largest processed tomato producer in the world (behind the United States and China) (www.fao.org/faostat, accessed on 16/05/2024; www.ec.europa.eu/eurostat/data/database, accessed on 16/05/2024). Almost half of the Italian production is in the tomato cluster in northern Italy, and most of the cultivated areas are concentrated in Emilia-Romagna (Donati et al. 2019). Over the centuries, tomato was grown in areas that optimise yield and quality. Italy has an optimal climate for this crop with long, hot summers and abundant sunshine. In addition to its economic importance as a cash crop, tomato also plays a relevant role in the human Mediterranean diet (Nasuelli et al. 2023). Tomatoes are a great source of vitamin C, potassium (K), folate, vitamin K and secondary metabolites such as lycopene used in pharmaceutical, nutraceutical, healthcare and cosmetic industries (Pattani et al. 2023). Tomato production and consumption increased in parallel to the population (Ingrao et al. 2019). Like most vegetables, tomato requires a significant amount of agronomic inputs, such as irrigation (between 400 and 600 mm year⁻¹ of water), nitrogen (N) and phosphorus (P) fertilisation during the growing season (Cammarano et al. 2020; Ronga, Caradonia, et al. 2019; Ronga, Francia, et al. 2019). Tomato cultivation gives great results in terms of yield, but can cause negative effects on ecosystems, including poor soil fertility, salinization and contamination of aquifers, and the generation of waste derived from the intensive use of fertilisers and phytosanitary treatments (Ortega Pérez et al. 2023). Therefore, to meet the sustainable development goals, it is necessary to minimise the environmental impact. Relevance can be given to the supply of silicon during irrigation due to its role in plant water stress tolerance. However, this may result in uptake and translocation of silicon ions into the fruits and in the equal proportion of arsenic, when present in the soil (Marmioli et al. 2017; Marmioli et al. 2014).

Biofertilizers, such as plant growth-promoting microbes (PGPM) or arbuscular mycorrhizal fungi (AMF), can facilitate the growth and development of crops (Caldara et al. 2024; Cesaro et al. 2020) and, therefore, represent a sustainable alternative to chemical fertilisers. Several studies (Mhlongo et al. 2020; Wei et al. 2019; Zuluaga et al. 2021) reported that PGPM can increase crop yields and reduce the inputs of fertilisers and pesticides in tomato plants. Very often the positive action of PGPM and AMF occurs when they are inoculated together, creating a microbial consortium (Caldara et al. 2024; Graziano et al. 2022). These PGPM populate the rhizosphere and colonise the root tissues of the host plant, producing and releasing several compounds,

such as phytohormones and organic volatiles (VOCs), to facilitate nutrient uptake by the plant, lower the level of ethylene in the plant, and stimulate systemic resistance (Fusco et al. 2023). In tomato, the effects of these consortia have been correlated with an increase in the number and biomass of fruits (Bona et al. 2017, 2018). Ortega Pérez et al. (2023) reported that the treatments with PGPM determine larger fruits, which resulted in a better sale price, while others showed that biofertilizers can mitigate tomato drought stress by stimulating stress responsive genes, improve soil health and yield (Krishna et al. 2022). These results indicate that the application of microorganisms may present advantages from environmental and economic points of view. Furthermore, a mixed inoculant of fungi and phosphate-solubilising bacteria, combined with a P-rich substrate, improves plant growth and yield, while allowing for a reduction in fertiliser and cost (El Maaloum et al. 2020).

It is possible to improve biofertilizers' performance by combining them with soil amendments, such as biochar. The latter is an inorganic carbon-rich matrix obtained from biomass gasification or pyrolysis in the total or partial absence of oxygen at a temperature below 700°C (Marmioli et al. 2018). Biochar applied to soil causes changes in pH, organic material content, cation exchange capacity and nutrient retention, thus positively affecting soil fertility (Graziano et al. 2022; Vassura et al. 2023). Furthermore, its structural porosity provides niches in which microorganisms can better cope with environmental stress, thus stimulating microbial growth and survival (Marmioli et al. 2022). The application of biochar to the soil can cause, in some cases, significant and rapid changes in the microbial community; these can then affect nutrient cycling or soil structure and subsequently affect plant growth directly and indirectly (Kuryntseva et al. 2023; Wang et al. 2022). In tomato cultivation, it was observed that biochar promotes N transformation by regulating N-related microorganisms; hence, it increases the inorganic N in the roots, reduces N lost by leaching, and promotes N absorption (Guo, Bornø, et al. 2021). Furthermore, under low fertilisers, the combination of biochar and PGPM significantly improves the diversity of the microbial community and enzyme activity in rhizospheric soil, improves the availability of N in the soil and the uptake of N, and increases the accumulation of dry matter (Wang et al. 2022). The effects of adding biochar to commodity crops for improving yield and plant health were also investigated in major crops like wheat and maize (Caldara et al. 2024; Gulli et al. 2025; Pavlicevic et al. 2022, 2023; Vassura et al. 2023). The functionalization of this supplement with the supply of PGPMs across different agronomic conditions has confirmed that in part or totally, chemical fertilisers, pesticides and insecticides can be replaced, thus increasing sustainability.

This study investigated the effects of the combination of biochar (as a carrier and soil improver) and biofertilizers based on microbial consortia on industrial tomato. In field experiments, tomatoes were grown using both organic and conventional agricultural practices. The latter represents the most common cultivation practice adopted in the Emilia-Romagna region and is based on the principles of a holistic pest management approach known as Integrated Pest Management (IPM), which is regulated by the European Union under Directive 2009/128/EC on the sustainable use of pesticides (European Parliament 2009). These regulations promote best practices for the use of the

most sustainable and least risky agrochemicals for human and environmental health, providing valuable guidelines for minimising their impact on the ecosystem. Integrated production is recognised as one of the most advanced agricultural methods for achieving sustainability, offering several benefits such as increased productivity, improved resource management and reduced environmental footprint (OI Pomodoro Nord Italia 2024).

The effect of biochar, either individually or in combination with biofertilizers, has not been fully explored yet. Further field trials combining different agronomic settings were necessary to confirm the effects of microbial consortia and biochar before any widespread implementation in sustainable agricultural practices. The working hypothesis was that the use of biofertilizers combined with biochar could give excellent yield while, in parallel, reducing the environmental impact but maintaining an economic value to the production. To support this, the study involved: (i) a soil chemical analysis; (ii) a 16S rDNA and ITS profiling of the soil microbial communities to evaluate the main taxa and the changes in their relative composition; (iii) the evaluation of the effects of the different treatments on plant growth, physiology and production and (iv) a socioeconomic and environmental evaluation of these procedures in the route of transition to a more sustainable tomato cropping.

2 | Materials and Methods

2.1 | Plant Materials and Experimental Field Trials

The experiments were carried out using *Solanum lycopersicum* L. cv Heinz 1648, an industrial tomato cultivar provided by Furia Seed (Monticelli Terme, Parma, Italy). Tomato field trials were carried out over 3 years (2020–2021–2022) in three locations in Italy: (i) Mezzani (Parma) (44.921 N; 10.420 E, 27 m above sea level) conventional (integrated) farmer Ganazzoli, (ii) Codigoro (Ferrara) (44.805 N; 12.106 E, 3 m above sea level) organic farmer Contarini and (iii) Codigoro (Ferrara) (44.80536 N; 12.10669 E, 3 m above sea level) organic farmer Trombini (Figure S1). Six different fields were considered for trials, in 3 years, to follow the traditional crop rotation used by farmers.

The seedlings were transplanted in the Ganazzoli field on 10/05/2020, 23/05/2021 and 19/04/2022, in the Trombini field on 18/05/2020 and 06/05/2021 and in the Contarini field on 21/05/2020 and 20/05/2021. A randomised complete block design with three replications was adopted in the three locations, in plots of 30 m² (3.4 plants m⁻²) with different treatments as reported in Table 1.

2.2 | Weather Conditions and Irrigation

During the tomato growth cycle, maximum and minimum temperatures and rainfall were recorded daily by the meteorological stations of ARPAE (Agenzia Prevenzione Ambiente Energia, Emilia-Romagna) located near the fields. Rainfall distribution and maximum and minimum 10-day period mean temperatures during the 2020, 2021 and 2022 cropping seasons are reported in Figure S2.

TABLE 1 | Location and treatments of the tomato field trials in Italy.

Growing seasons	2020
Farms	Ganazzoli Trombini Contarini
Treatments	Control Biochar 200 g m ⁻² Biochar 200 g m ⁻² + Micosat F1 (2 g)
Growing seasons	2021
Farms	Ganazzoli Trombini Contarini
Treatments	Control Biochar 200 g m ⁻² Biochar 200 g m ⁻² + Micosat F1 (2 g) Biochar 200 g m ⁻² + AMF_MA (2 g) Biochar 200 g m ⁻² + AMF_MA (2 g) and MC_B (4 g)
Growing seasons	2022
Farm	Ganazzoli
Treatments	Control Biochar 200 g m ⁻² Biochar 200 g m ⁻² + Micosat F1 (2 g) Biochar 200 g m ⁻² + AMF_MA (2 g) Biochar 200 g m ⁻² + AMF_MA (2 g) and MC_B (4 g)

In 2020, a total of 325, 345 and 383 mm of irrigation water and rainwater were applied in Contarini, Trombini and Ganazzoli fields, respectively. In 2021, a total of 314, 287 and 271 mm of irrigation water and rainwater were applied in Contarini, Trombini and Ganazzoli, while in 2022 a total of 382 mm was applied in the Ganazzoli field. In 2020 and 2021, the maximum temperatures (T° max) during the transplanting and growing period were higher in Parma than in Ferrara (Figure S2A–D). In 2022 in Parma, the T° max was 32°C during the period of transplantation (Figure S2E).

2.3 | Source and Application Procedure of Biochar and Biofertilizers

The biochar used in the experiments was produced and characterized as previously described (biochar A4 in Marmioli et al. 2018). Pyrogasification was conducted at temperatures between 500°C and 700°C for 1–2 h. The yield was approximately 20 kg per 100 kg of pellets. The resulting biochar had a pH of 8.11, electrical conductivity of 1.4 mS cm⁻¹, bulk density of 0.37 g cm⁻³ and moisture content of 6.29% (fresh weight). It contained 95.63% organic matter (dry weight), 4.4% ash (dry weight), and metal concentrations (Cd, Cu, Ni, Fe, Pb and Zn) below international safety standards. Phytotoxicity tests showed no inhibition of germination or seedling growth. The biochar was applied at 200 g m⁻² before plant transplantation with landfill: manually spread to the first layers of soil and then buried at about 10 cm of depth. Different types of

microbial consortia were used through the field trials also in combination with biochar: (i) Micosat F1, a consortium composed of mycorrhizae, beneficial bacteria of the rhizosphere and saprophytic fungi (composed of *Trichoderma harzianum* TH 01, *Glomus mosseae* GP11, *Glomus coronatus* GU53, *Glomus caledonium* GM24, *Septoglomus viscosum* GC41, *Rhizophagus irregularis* RI31, *Bacillus amyloliquefaciens* BA41, *Pseudomonas fluorescens* PA29, *Azospirillum brasilense* AB86 and *Komagataella pastoris* PP59), a commercial product kindly provided by CCS Aosta (Aosta, Italy), was applied at the quantity of 4 g for each plant (for more details see Caldara et al. 2024); (ii) mycorrhizae (*R. intraradices*) (AMF_MA) were purchased from MycAgro lab, Bretenière, France (<http://www.mycagrolab.com>). This product is an arbuscular mycorrhizal fungus used in agriculture, known to improve root structure and enhance nutrient absorption, particularly phosphorus, promoting plant growth, stress resistance and soil health in a sustainable agriculture perspective (Wahab et al. 2023). The granular inoculum of AMF_MA was added to the soil at the quantity of 2 g for each plant and (iii) microbial consortia B (MC_B) was designed as part of the project Horizon 2020 SIMBA (Sustainable Innovation of Microbiome Applications in the Food System), as previously described (Tabacchioni et al. 2021; Hett et al. 2022). MC_B is composed of *A. vinelandii* DSM 2289, *R. aquatilis* BB23/T4d, *Bacillus* sp. BV84, *B. amyloliquefaciens* LMG 9814 and *P. fluorescens* DR54. This consortium was added to the soil at a quantity of 4 g for each plant close to the plant roots, 1 week after the transplant. The details of treatments and locations are summarized in Table 1. The treatment with MC_B combined with biochar and AMF_MA (biochar_MC_B-AMF_MA) was applied in 2021 in the three locations, and in 2022 only in the Ganazzoli field trials.

Fertilisation interventions occurred both before and after the transplant. The N, P and K requirements were calculated based on soil analysis and the expected fruit yield (under these environmental conditions). Nitrogen was administered in organic form while phosphate was used in mineral form. The amounts of chemical fertilisers (N, P₂O₅ and K₂O) used are listed in Table S1. The amount of P and K fertilisers were equal in all treatments; while the amount of N in the plots with biochar and microbes were decreased by 20% as compared to the 'control' plots.

Every year before transplanting, the Ganazzoli farm (conventional) applied an herbicide mixture consisting of glyphosate 3 kg L⁻¹ ha⁻¹, pendimetalin 1.1 kg L⁻¹ ha⁻¹ and metribuzin 0.5 kg L⁻¹ ha⁻¹. For organically grown tomato (Trombini and Contarini farms), no weed control treatment was performed.

2.4 | Physiological, Morphological and Production Traits

The relative chlorophyll content and leaf transpiration rate (LTR) were monitored throughout vegetative growth. Chlorophyll content was measured in vivo with the SPAD-502 chlorophyll meter (Konica Minolta Business Solution Italia Spa, Milan, Italy); 10 measurements, on five expanded leaves in each plot, were taken along the entire length of the leaf and the average was recorded.

The LTR was measured using the portable AP4 porometer (Delta-T Devices, Cambridge, UK) (scm⁻¹) on five expanded leaves in each plot.

At harvest, several traits were measured concerning final production, plant health status and fruit characteristics. Furthermore, other data were recorded: total refractometric index (g°Brix ha⁻¹), number of commercial fruits (%), average commercial fruit weight (g), number of unripened fruits (%) and soluble solid (°Brix). The Brix degree was determined using a digital refractometer (HI 96814; Hanna Instruments, Villafranca Padovana, Italy). The test was performed at room temperature and was repeated three times. Major and minor defects were evaluated on samples of 100 fruits randomly selected from commercially valuable fruits; rotten (%); rotten/broken fruits; apical rot (%) and bursted (%).

2.5 | Soil Analysis

2.5.1 | Chemical Analysis

For soil analysis, a sample up to 30 cm depth was taken 1 month before transplantation and the samples were immediately analysed for the main physical and chemical properties, as reported in Table S2. These analyses were conducted in all fields.

2.5.1.1 | Total Carbon. A sieved sample (φ 2 mm) was ground in a mortar prior to analysis. Total carbon (TC) and inorganic carbon (IC) were analysed with a total organic carbon (TOC) analyser (Model SSM 5000A; Shimadzu Corp., Kyoto, Japan). TOC content was calculated as the difference between TC and IC.

2.5.1.2 | Characterization of Dissolved Carbon and N Forms. Dissolved carbon and N forms were measured as described before (Ghidotti et al. 2017; Vassura et al. 2023). Dissolved organic carbon (DOC) of the water-soluble organic matter was measured with a Shimadzu TOC-L analyser (Shimadzu Corp., Kyoto, Japan). Quantification of each analysis is presented here as a mean of two to three injections of 100 μL, where the coefficient of variance for the replicate injections was < 2%. Dissolved N (DN) concentrations were measured with a TN module coupled with a TOC-L analyser (Shimadzu Corp., Kyoto, Japan).

2.5.2 | Biological Analysis

The rhizosphere soil was collected and analysed as previously described (Caldara et al. 2024). Briefly, rhizospheric soil was collected from three plants in duplicate; samples were kept at -80°C until DNA extraction. DNA was extracted from 250 mg of each sample using NucleoSpin Soil (Macherey-Nagel, Duren, Germany); DNA was visualised by electrophoresis on agarose gels to test for its integrity and quantified spectrophotometrically using a VARIAN Cary 50 UV-VIS device (Agilent Technologies, Santa Clara, CA, USA). Finally, DNA from each sample was used to amplify the genes encoding 16S rRNA (V3-V4 region) and 18S (ITS2 region). The PCR protocol was described in detail

in Graziano et al. (2022). After amplification, next-generation sequencing (NGS) was performed by BMR Genomics Srl (Padua, Italy) using standard procedures as described in Graziano et al. (2022).

2.6 | Statistical Evaluation

Standard statistical analysis was performed using the software Past 4.03 (Copyright Hammer 1999–2020). Data are presented as mean $\pm$ SD and analysed by ANOVA and Bonferroni–Holm post hoc test, or by two-way ANOVA and Tukey's post hoc test. A p value ≤ 0.05 was considered statistically significant. Then, all parameters were evaluated together by a multivariate analysis (MANOVA). To check the robustness of the effects of our treatments it was necessary to repeat the field trials across different years in different locations. The major potential confounding variables were soil type, weather conditions and management practices, since the work was based on the same cultivar (tomato Heinz 1648), grown in different locations on different type of soils, in conventional and one biological farms. The tomato plants were grown for 3 years in a row and treated with the same products, subjected to identical sampling methods and experimental observations. Whenever a statistically significant difference was detected, the post hoc Dunn's test was applied to identify the differences among the pairs of samples. The standardised values of each trait were visualised through a heatmap using the Heatmapper software (<http://www.heatmapper.ca/>, accessed on 02/2024).

2.7 | Economic Evaluation

The economic analysis aimed to assess farmers' profitability when applying biochar and biofertilizers as partial substitutes for conventional fertilisers. Economic analysis was carried out with a double perspective: market and carbon sequestration. The market perspective aimed to evaluate agricultural profitability in a business-as-usual scenario, while the carbon sequestration perspective assessed the feasibility for farms to participate in voluntary carbon markets. The increasing number of carbon registries, coupled with advancements in measurement and verification methods, indicated an expansion of voluntary carbon markets, offering both environmental and economic opportunities for farms (Oldfield et al. 2022).

Table 2 illustrated the average cultivation costs for the two types of tomato cultivation, as estimated through the field notebook. Revenues included in the analysis are due to the market value of the tomato production, which relies on market prices and yield. We assumed an average selling price of €151.90 Mg⁻¹ for organic tomatoes and €108.50 Mg⁻¹ for conventional production, according to EU commission data (<https://agridata.ec.europa.eu/extensions/DashboardFruitandVeg/FruitandVegetablePrices.html>, accessed on 06/2024). Payments from the Common Agricultural Policy (CAP) have been omitted from this analysis. The cost of biochar was estimated at €500 Mg⁻¹, under the assumption of bulk delivery and certified matter, while the cost of biofertilizers was derived from the expenses incurred during the field experiments.

TABLE 2 | Specific costs of tomato cultivation for the current situation (€ ha⁻¹).

Crop operations	Organic		Conventional	
Other inputs	381.28	6%	381.28	7%
Insurance	600.00	9%	600.00	11%
Fertilisers	1371.39	21%	929.25	17%
Direct machinery costs	1637.64	25%	1534.79	28%
Phytosanitary products	432.12	7%	594.54	11%
Hiring labour	835.85	13%	276.52	5%
Crop services	56.38	1%	56.38	1%
Seeds/plants	1204.69	18%	1204.69	22%
Total	6519.35	100%	5577.44	100%

Note: Economic data collected from experimental trials by Experimental Farm 'Stuard'.

The economic analysis was conducted for five scenarios:

1. Baseline, that represents the current (observed) use of fertilisers;
2. biochar, where we estimated the economic effects of the distribution of biochar;
3. biochar_Micosat F1, where the biochar is supplemented with Micosat F1;
4. biochar_AMF_MA, where biochar is supplemented with arbuscular mycorrhizal fungi (AMF);
5. biochar_MC_B_AMF_MA, where the biochar and the biofertilizer of scenario 'Biochar_AMF_MA' is supplemented with microbial consortia B (MC_B). For each scenario, we calculated the tomato gross margin, that is the difference between the crop total revenue (value of the commercial output) and the direct production costs. Yields detected for each farm over the 3 years of experiments (2020, 2021, 2022) have been classified according to the organic (Trombini and Contarini farms) and conventional (Ganazzoli farm) production practices; data presented are the averages of the 3 years compared to the control condition or baseline/first scenario.

To represent as realistically as possible the introduction of biochar and biofertilizers in agricultural practices, in Scenarios 2–5, we assumed a reduction in the use of conventional fertilisers from current use (baseline) to 70% for the fertilisation and to 80% for the fertilisers distributed by micro-irrigation. These fertiliser reductions are consistent with experiments carried out in the field. Moreover, due to the stability characteristics of biochar in the soil (Palansooriya et al. 2019; Quilliam et al. 2013), we assumed a repeated application of biochar in the same field every 3 years. This means that the cost sustained for biochar application in the first year is shared over 3 years.

3 | Results and Discussion

3.1 | Chemical Analysis of Soils

The results obtained from the carbon analysis of the soils taken from the three different farms over the 3 years are shown in Table 3. Total organic carbon (TOC) has been identified among the soil characteristics as describing the variance of bacterial richness, along with pH and texture class (Plassart et al. 2019). The TOC values in control soils ranged from 1.5% to 2.7%, which, combined with pH > 7, places these

soils within the range of European arable soils (Plassart et al. 2019). The loading of biochar into the soil did not significantly increase the TOC (Vassura et al. 2023) of the treated soils as compared to the control. The variability of the treated soils (1.5%–1.9% Ganazzoli; 1.1%–4.7% Trombini; 1.1%–2.8% Contarini) could be explained by changes inherent to the soil systems and agricultural practices (probably for Trombini 2021). DOC and DN (essentially nitrates, Vassura et al. 2023) are reported in Table 3. DOC ranged from 150 to 350 mg kg⁻¹ and DN from 20 to 200 mg kg⁻¹ without any apparent trend among treatments; this is reflected by the analysis of the soil

TABLE 3 | Total organic carbon (TOC, %), dissolved organic carbon (DOC, mg 100 g⁻¹), dissolved nitrogen (DN, mg 100 g⁻¹).

Field trials	Treatments	Property	2020	2021	2022
Ganazzoli	Control	TOC	1.5 ± 0.2	1.61 ± 0.07	1.85 ± 0.05
		DOC	18 ± 2	15 ± 1	22 ± 2
		DN	2.9 ± 0.9	9.2 ± 0.5	5.2 ± 0.4
	Biochar	TOC	2.4 ± 0.4	1.8 ± 0.1	1.82 ± 0.05
		DOC	22 ± 1	21.2 ± 0.7	25 ± 1
		DN	2.3 ± 0.8	7.8 ± 0.4	6.2 ± 0.5
	Biochar_Micosat F1	TOC	1.8 ± 0.2	1.6 ± 0.1	1.68 ± 0.04
		DOC	21 ± 1	14.5 ± 1.0	24 ± 1
		DN	2.8 ± 0.5	10.3 ± 0.6	3.8 ± 0.3
	Biochar_AMF_MA	TOC	n.t.	1.90 ± 0.09	1.79 ± 0.05
		DOC	n.t.	24 ± 2	20 ± 1
		DN	n.t.	12.0 ± 0.5	4.8 ± 0.4
	Biochar_AMF_MA_MC_B	TOC	n.t.	2.0 ± 0.1	1.95 ± 0.05
		DOC	n.t.	14.8 ± 0.9	26 ± 1
		DN	n.t.	7.8 ± 0.3	4.5 ± 0.3
Trombini	Control	TOC	1.1	4.48 ± 0.08	n.t.
		DOC	16 ± 2	29.5 ± 4.0	n.t.
		DN	20.4 ± 0.4	7 ± 1	n.t.
	Biochar	TOC	1.2	4.7 ± 0.2	n.t.
		DOC	21 ± 1	29 ± 3	n.t.
		DN	4.6 ± 0.5	5.5 ± 0.7	n.t.
	Biochar_Micosat F1	TOC	1.2	4.71 ± 0.07	n.t.
		DOC	19 ± 2	31 ± 2	n.t.
		DN	8.6 ± 0.1	5.8 ± 0.5	n.t.
	Biochar_AMF_MA	TOC	n.t.	4.65 ± 0.04	n.t.
		DOC	n.t.	30 ± 3	n.t.
		DN	n.t.	6.3 ± 0.4	n.t.
	Biochar_AMF_MA_MC_B	TOC	n.t.	4.6 ± 0.1	n.t.
		DOC	n.t.	29 ± 3	n.t.
		DN	n.t.	5.5 ± 0.4	n.t.

(Continues)

TABLE 3 | (Continued)

Field trials	Treatments	Property	2020	2021	2022
Contarini	Control	TOC	2.57 ± 0.06	1.11 ± 0.07	n.t.
		DOC	33.6 ± 0.8	15 ± 5	n.t.
		DN	4.2 ± 0.5	7.4 ± 0.8	n.t.
	Biochar	TOC	2.66 ± 0.03	1.0 ± 0.1	n.t.
		DOC	35 ± 2	14 ± 5	n.t.
		DN	3.60 ± 0.5	8.1 ± 0.9	n.t.
	Biochar_Micosat F1	TOC	2.9 ± 0.2	1.2 ± 0.1	n.t.
		DOC	33 ± 1	17 ± 3	n.t.
		DN	3.0 ± 0.4	10.5 ± 0.5	n.t.
	Biochar_AMF_MA	TOC	n.t.	2.59 ± 0.05	n.t.
		DOC	n.t.	15 ± 5	n.t.
		DN	n.t.	8 ± 1	n.t.
	Biochar_AMF_MA_MC_B	TOC	n.t.	1.12 ± 0.09	n.t.
		DOC	n.t.	15 ± 5	n.t.
		DN	n.t.	11 ± 1	n.t.

Note: These parameters were collected in the three field trials when treatment with biochar alone or in combination with microorganisms, in the 3 years (2020–2021–2022) of cultivation were performed.
Abbreviation: n.t., not tested.

microbiome, which showed similar distribution among the treatments (Section 3.2) at least at the sampling time when the tomatoes were almost mature and with the amounts of biochar employed in this study. Microbial activity could be related to DOC and DN (Liu et al. 2024). In other studies, the carbon content increased upon the addition of biochar (Chagas et al. 2022), and this change was reported to stimulate microbial growth (Deshoux et al. 2023; Siedt et al. 2021). Microbial stimulation was also achieved immediately after adding biochar to the soil and decreased over time (Soria et al. 2021). The combined addition of biochar and N fertilisers can change soil enzymes and microbial activities (Song et al. 2020). However, in these trials, no changes in TOC, DOC, DN or distribution of microbiome populations were detected (see next section).

3.2 | Rhizospheric Soil Microbiome

Rhizospheric soil samples were collected in the second half of July (see Table S3) when the tomato plants reached their final ripening stage. Bacteria and fungi were analysed considering their phyla, or classes, along with their relative distribution for each field and year. Considering the three fields and the 3 years, the seven most abundant phyla (Figure 1A), which explain more than 90% of the microbial population, were: Actinobacteria, Proteobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes and Verrucomicrobia. The composition reflects a general distribution of the bacteria phyla in European soils (Catania et al. 2022; Labouyrie et al. 2023), and was compatible with southern European soils (Plassart et al. 2019), although the general distribution seemed related to the type of land use (Lee et al. 2020). In Ganazzoli, Proteobacteria were always the

most abundant phylum (24%–33%, percentages are rounded values); this phylum was reported to be rather resilient to different intensities in land use (Yang et al. 2021). Actinobacteria decreased from 30% (2020) to 8% (2022), and Acidobacteria increased from 17% (2020) to 23% (2022), along with Bacteroidetes, which also increased from 4% to 12% (Table S4). In the Contarini field, Proteobacteria were the most abundant in 2020 and 2021, followed by Actinobacteria in 2020 and Acidobacteria in 2021. The concentration of Actinobacteria decreased significantly in the 2 years (from 20% in 2020 to 6% in 2021, p -value 0.0002 with Tukey's post hoc test), while Gemmatimonadetes showed an opposite trend (Table S4) although not statistically significant. In the Trombini field, Proteobacteria had the lowest percentages (17% in 2020) compared to the other two fields, and Actinobacteria was the more abundant phylum (30% in 2020). When performing a PERMANOVA, each field resulted significantly different according to both location and year (see Tables S5 and S6 for p -values), a result also visible with a PCA (Figure 1B,C). When the location was considered (Figure 1B), PC1 and PC2 variations were mainly correlated with Proteobacteria, Actinobacteria and Gemmatimonadetes. When the year was considered (Figure 1C), the abundance of Actinobacteria, Proteobacteria and Acidobacteria showed the greatest variability. Strains belonging to these phyla regulate biogeochemical cycles, decompose biopolymers, secrete exopolysaccharides and promote plant growth (Boubekri et al. 2022; Kalam et al. 2020; Spain et al. 2009; van Bergeijk et al. 2020). When the same analysis considered the treatments, no significant differences were recorded either in the presence of biochar alone or when biochar was combined with microbial consortia. However, the biochar supplement appears to determine variations in the bacterial distribution when applied at higher rates

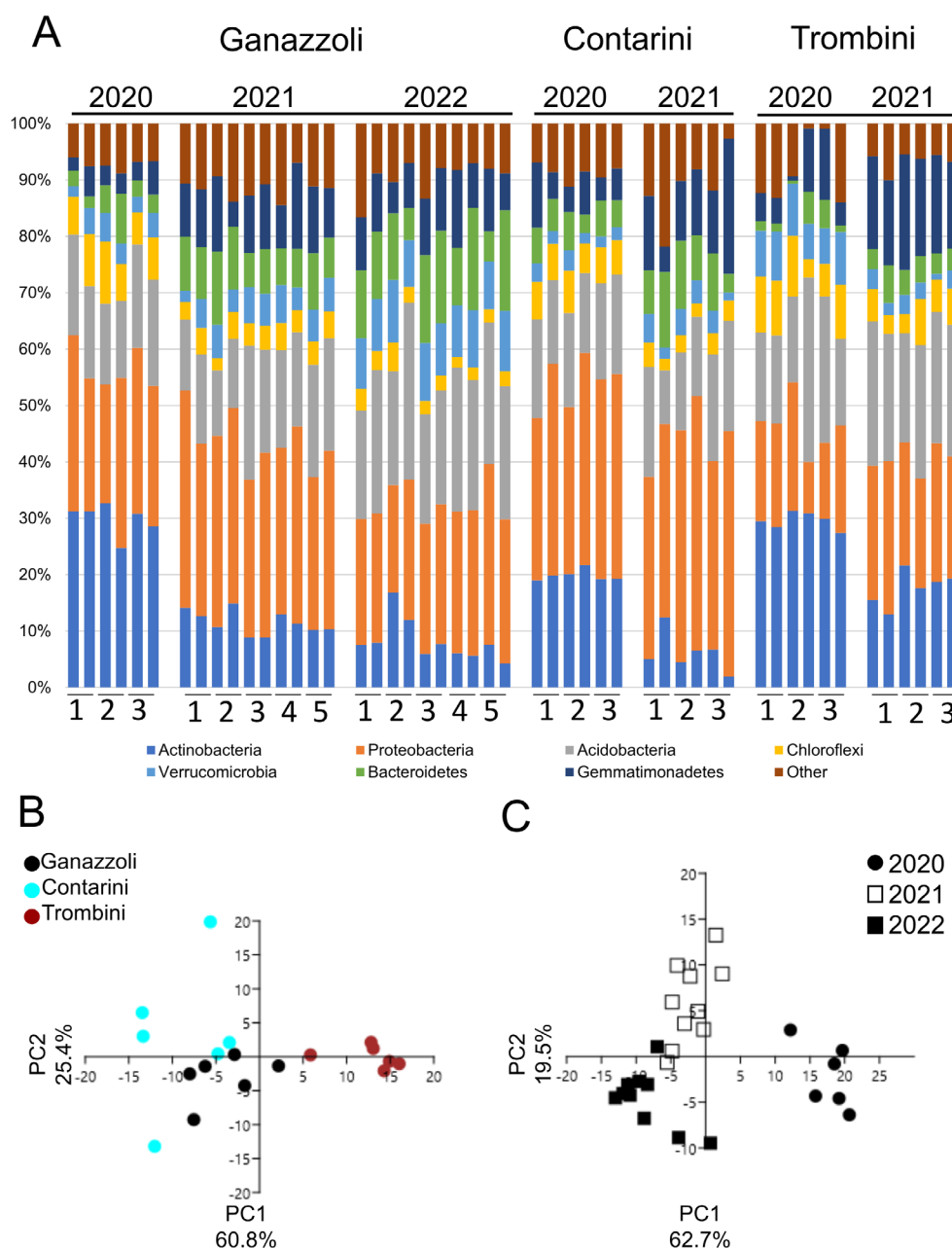


FIGURE 1 | Characterisation of rhizospheric bacterial communities. (A) Rhizospheric bacterial communities at the level of phyla in soil collected from the three field trials of tomatoes. Treatments were: Control (1), Biochar (2), biochar_Micosat F1 (3), biochar_AMF_MA (4), biochar_MC_B_AMF_MA (5). The graph reports the relative abundance (based on operative taxonomic units [OTUs]) of the most represented classes found in the rhizospheric soil in duplicate presented alongside and grouped by a grey line at the bottom of the image and just above the number indicating the type of treatment. The colours are ordered according to the legend reported at the bottom of the figure. (B) PCA of rhizospheric data considering the Ganazzoli (black), Contarini (light blue) and Trombini (brown) fields trials in the year 2021 and (C) considering the data from the Ganazzoli field trials for 3 years: 2020 (dots), 2021 (open squares), 2022 (closed squares) as indicated in the figure.

than those used in this study (Deshoux et al. 2023; Plassart et al. 2019). In these conditions, biochar influences the presence of specific phyla, but changes are also linked to soil type (Song et al. 2019). The addition of microbes to the soil failed, under our conditions, to modify the indigenous community in the long term (Caldara et al. 2024; Fierer 2017; Graziano et al. 2022). α -Analysis revealed a Shannon diversity index always above 4 for each field and year, with a Chao-I index above 200 in 2020 and 2022, but lower in 2021 (Table S7). These indices were not different in the Ganazzoli field for the 3 years but were lower in

2021 for the Contarini and Trombini fields when compared to 2020 (Table S7). As the soil microbial diversity is an indicator of soil resilience (Mocali et al. 2022; Yang et al. 2021), a lower microbial biodiversity could indicate a lower capability of soil to perform in these two last fields.

When the top seven classes of bacteria were considered, Acidobacteria, Actinobacteria, Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria were among the main five present, explaining almost 50% of the bacterial

distribution (Figure S3). The other classes were registered among Deltaproteobacteria, Thermoleophilia, Gemmatimonadetes and Chloracidobacteria, representing approximately 10% of the total bacteria present (Figure S3). PERMANOVA confirmed that the distribution of the classes was different according to the field and year (Tables S6 and S7), but not to treatment. Actinobacteria decreased between three- and six-fold over the 3 years in both Ganazzoli and Contarini farms. Actinobacteria strains solubilise K and P, which are beneficial for plant growth and yield; moreover, this class of microbes acts as biocontrol agents, mitigates the effects of abiotic stress, and acts as plant probiotics (Boubekri et al. 2022; van Bergeijk et al. 2020). Their positive influence on soil quality and plant performance was confirmed in several crops (Boukhatem et al. 2022; Naylor et al. 2017). Although lower amounts of these bacteria were recorded in the Ganazzoli and Contarini fields this did not influence the yield. In this work, samples were taken only during the ripening season to concentrate on possible long-term changes of the autochthonous microbiota, although it is not known how the concentration of the rhizospheric microbes can change throughout the growing period. In the Trombini farm, the main changes for 2020 and 2021 were recorded for Betaproteobacteria, which decreased 50%, and Gemmatimonadetes, which increased two-fold (Table S4).

Fungi are key components of soil agroecosystems, as they are influenced by climate conditions (Bakar et al. 2020; Marčiulyrienė et al. 2022), even if they are stable under drought, a condition where fungi positively support the growth of the bacterial community (de Vries et al. 2018; Hestrin et al. 2022). Fungal

diversity and homogenisation can be compromised in arable lands (Banerjee and van der Heijden 2023). Considering all fields for all the locations, Ascomycota was the main phylum present (70%), followed by Basidiomycota (11%) and Mortierellomycota (5%) (data not shown). The most abundant class, for each year and location, was Sordariomycetes, with approximately 50% of all classes, followed by Dothideomycetes (15%), Agaricomycetes (11%), Mortierellomycetes (8%), Tremellomycetes (5%), Eurotiomycetes (5%) and Leotiomyces (2%) (Figure 2A; Figure S4). Other papers report that these representative classes share similar distributions in agricultural soils (Dang et al. 2018). In any case, PCA revealed that the classes responsible for most variations were Sordariomycetes, Agaricomycetes and Tremellomycetes (Figure 2B). Strains within Sordariomycetes, such as *Trichoderma*, act as biocontrol agents and increase plant resistance to biotic and abiotic stress (Woo et al. 2023). Agaricomycetes recycle organic matter, and some strains may degrade hydrocarbons in the soil (Mohammadi-Sichani et al. 2017; Ruiz-Dueñas et al. 2021). PERMANOVA confirmed that the different treatments did not induce a major changes in the indigenous microbial population, and that the observed differences were related to the field and year (Tables S6 and S7). None of the treatments significantly changed the diversity or richness indices, which were similar in all samples analysed (Table S7). This is not surprising, as it is known that fungal networks are more resilient than bacterial ones (de Vries et al. 2018; Tang et al. 2023) and that plant-associated fungi can help bacteria to be more resilient to abiotic stress (Hestrin et al. 2022). The Shannon diversity index was always above 3 for each field and year, while the Chao-1 index was around 90 (Table S8). These

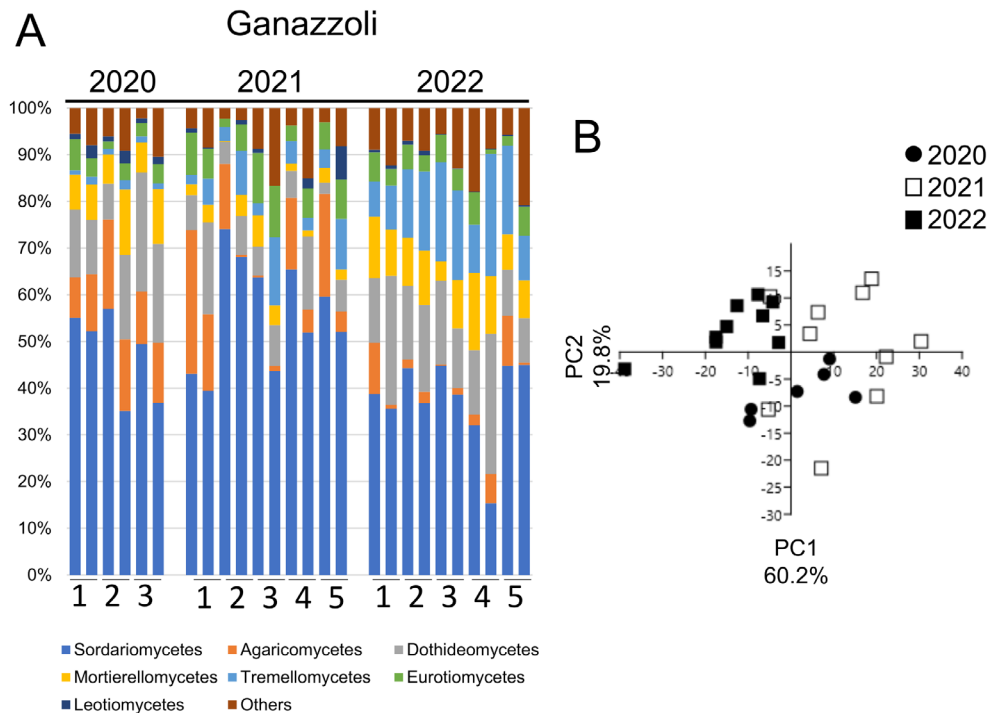


FIGURE 2 | Characterisation of rhizospheric fungal communities. (A) Rhizospheric fungal communities at the level of class collected in the Ganazzoli field trials. Treatments were: Control (1), Biochar (2), biochar_Micosat F1 (3), biochar_AMF_MA (4), biochar_MC_B_AMF_MA (5). The graph reports the relative abundance (based on operative taxonomic units [OTUs]) of the most represented classes found in the rhizospheric soil in duplicate and presented alongside and grouped by a grey line at the bottom of the image just above the number indicating the type of treatment. The colours are ordered according to the legend reported at the bottom of the figure. (B) PCA of rhizospheric data from the Ganazzoli field for 3 years 2020 (dots), 2021 (open squares), 2022 (closed squares) as reported in the figure.

diversity indices usually decline when soil is subject to intensive agricultural use (Xue et al. 2023). The soils analysed in this work displayed good diversity indices (Orrù et al. 2021; Tsiafouli et al. 2015).

3.3 | Results of Field Trials Performed Along Two Seasons (2020–2021) With Biochar and Micosat

The efficacy of biochar was tested when supplemented alone or in combination with Micosat F1 in three different fields (Ganazzoli, Contarini and Trombini) over two consecutive growing seasons. Leaf chlorophyll content and LTR index were evaluated during growth. At harvest, the quality parameters of the tomato fruits were analysed. All the collected data are shown in Table S9.

The photosynthetic capacity was greater in the Contarini and Trombini ($p=0.00919$) field trials treated with biochar combined with Micosat F1, 10.3% and 5.7%, respectively, as compared to control conditions. Despite this, considering the parameters linked to productivity in 2020, the treatment biochar_Micosat F1 determined an increase with respect to the control, in total refractometry ($\text{g}^\circ\text{Brix ha}^{-1}$) of tomatoes collected from Ganazzoli (3.0%) and Contarini (15.8%) field trials and a decrease in rotten fruits (%), -11.6% , -18.6% and -51.0% in Ganazzoli, Contarini and Trombini, respectively. (Figure 3; Table S9). Furthermore, the treatment biochar_Micosat F1 in the Ganazzoli and Trombini field trials determined an increase in the Brix degree (4.5% – 2.0% Ganazzoli and Trombini, respectively) and a decrease in the amount of bursted fruits

(%) (-48.8% and -42.5% Ganazzoli and Trombini, respectively) respect to the control condition. Data collected in 2021 showed that biochar in the three fields determined a decrease in the number of unripened tomatoes (-51.9% , -55.4% and -11.5% Ganazzoli, Contarini and Trombini, respectively) and an increase in their average weight (g) ($p=0.01881$, in the Ganazzoli field 3.4%), as compared to the control condition. The biochar supplement determined an increase in commercial products (3.8%) in Contarini field trial and a decrease in Brix degree (-6.2%) in the Ganazzoli field trials. The presence of biochar_MC_B_AMF_MA determined an increase in total refractometry ($\text{g}^\circ\text{Brix ha}^{-1}$) in tomato fruits collected in the Ganazzoli and Contarini field trials with respect to the control condition (14.2% and 12.7% , in Ganazzoli and Contarini, respectively). Furthermore, the treatment biochar_Micosat F1 in the Trombini (organic) field determined an increase in the Brix degree (4.5%) and a decrease in % of apical rot (-16.0%) and % of burst tomatoes (-69.7%) respect to the control condition.

As reported in Table S9 and Figure 4A, in 2021 tomato production increased in the three fields' trials; fruits had a lower average weight than those obtained in 2020, but fewer were the defective fruits, and a high marketable yield is the most important goal in processing tomato production. It was reported that organic tomato cultivation yields a lower marketable product as compared to conventional farming (Rocchetti et al. 2022; Ronga et al. 2015). In our studies, the combined use of biochar and Micosat F1, both in organic and conventional cultivation fields, led to an increase in marketable tomato production as compared to the untreated plants.

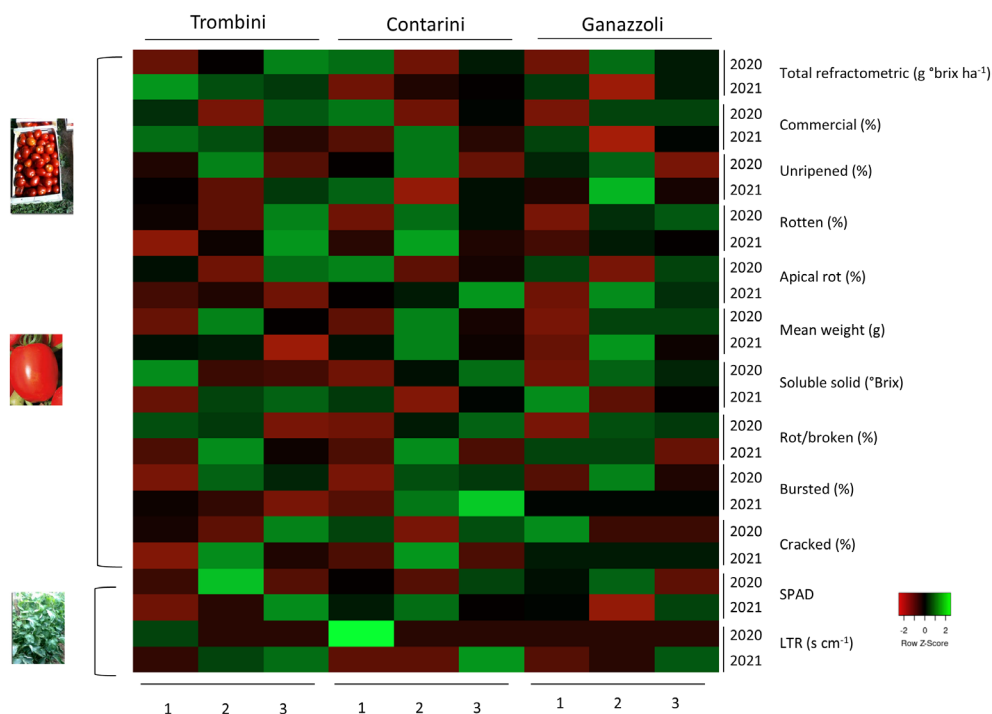


FIGURE 3 | Physiological and fruit quality traits from the 2020 and 2021 seasons for Trombini, Contarini and Ganazzoli field trials. Heatmap represent standardised data calculated as: Measure – average/standard deviation; traits with values below the average measurements are in red, traits with values above the average correspond to a scale of green, as reported in the figure. Treatments were: Control (1), Biochar (2), biochar_Micosat F1 (3).

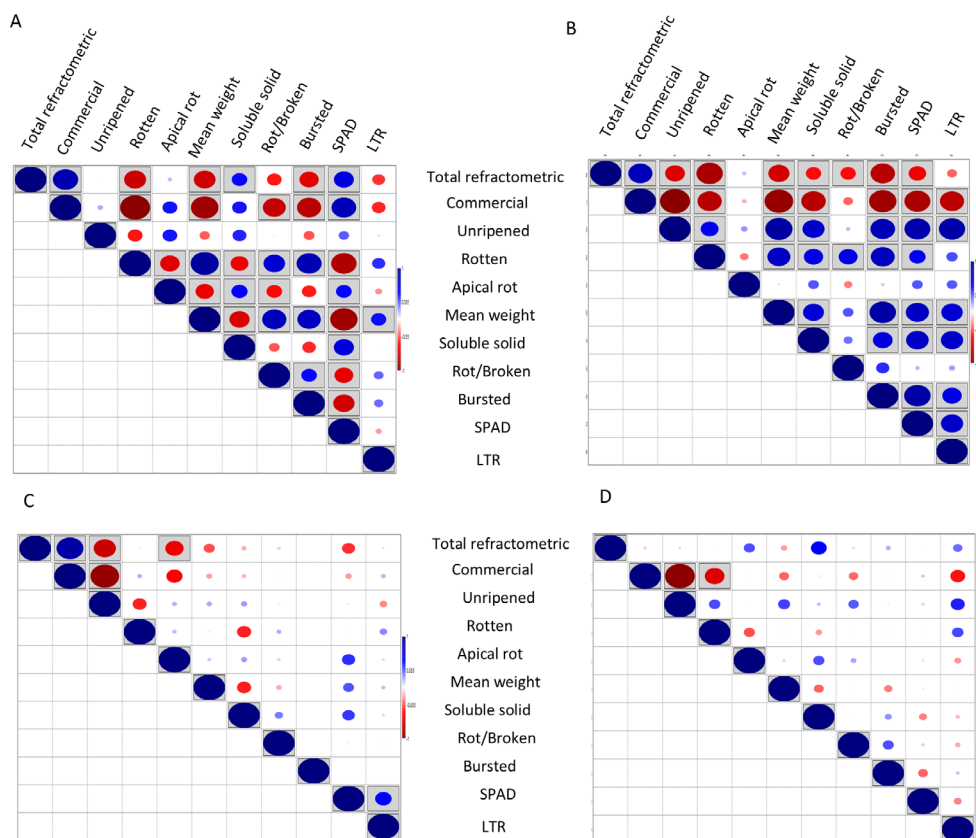


FIGURE 4 | Pearson correlation analysis. This is reported for each trait evaluated over the 2 years in Ganazzoli (A) and Contarini (B) field trials in 2020 and 2021 Pearson's correlation coefficient was calculated for each trait evaluated in Ganazzoli field trials over the 2 years (C) 2021 and (D) 2022. Correlations are expressed as a range of values within the $[-1, 1]$ interval. The value -1 indicates a perfect non-linear (negative) relationship, 1 is a perfect positive linear relationship and 0 is intermediate between positive or negative linear interdependency. The colour intensity and the size of the circle are proportional to the correlation coefficients. In the right side of the correlogram, the legend colour shows the correlation coefficients and the corresponding colours. The boxes highlighted in grey identify statistically significant correlations ($p < 0.05$), while non-highlighted circles indicate non-significant correlations.

Regarding fruit quality, the Brix degree is a measure of total soluble solids correlated with the sugar content and is a very relevant parameter for tomato canning companies. Indeed, since soluble solids represent a product's potential sweetness (an aspect of quality), Brix degree readings can interest many throughout the value chain. The Brix degree increased, although not significantly, in biochar_Micosat F1 treatment in all fields (Table S9). Furthermore, Micosat F1 contains bacteria that produce siderophores, molecules that make Fe^{3+} bioavailable for plants; Fe^{3+} is involved in chlorophyll biosynthesis in leaves, thus increasing photosynthesis (Caldara et al. 2024). In fact, chlorophyll content is considered a major indicator of photosynthetic efficiency and tomato yield (Della Lucia et al. 2022). The data obtained from the analyses of various growth and production traits agree with other studies (Guo, Yu, et al. 2021; Yang et al. 2023), in which the addition of microorganisms improved the performance of biochar-based amendment on plant growth, yield and fruit quality. Therefore, these studies suggest that the use of biochar in combination with Micosat F1 is a sustainable fertilisation strategy in conventional and organic agriculture. Climatic conditions,

soil properties, water and N availability are the main factors that affect yield and the profitability of fresh and processed tomato production (Ronga, Caradonia, et al. 2020).

The data obtained from each field trial were evaluated by Pearson's correlation analysis (Figure 4A,B; Figure S5). Ganazzoli and Contarini field trials showed some common significant correlations (Figure 4A,B); while the correlations identified in the Trombini experimental field are not in common with the other two fields (Figure S5). The marketability of the products was correlated with several traits. In general, positive correlations observed were: commercial product (%) and total refractometry ($\text{g}^\circ\text{Brix ha}^{-1}$); the average weight of tomatoes (g), % of both rotten and bursted product and finally, % of scalded product and % of rotten. The positive correlation between the marketability of products and various other parameters (Figure 4A,B) agreed with the results shown previously (Barrios-Masias and Jackson 2014; Ronga, Galligani, et al. 2019). Otherwise, negative correlations were identified between the % of commercial products and the average weight of tomatoes and the % of blanched tomatoes and between the % of rotten products and the % of unripened fruits.

3.4 | Results of the Ganazzoli Field Trial in Two Seasons (2021–2022) With Biochar, Mycorrhizae and Microbial Consortia

After evaluating the effects of biochar_Micosat F1 on tomato growth and production, in 2021 other treatments were added. In particular, mycorrhizae (AMF_MA) and a newly developed microbial consortium (MC_B) in combination with biochar were applied. As in the previous year (2020), physiological analyses were performed during the growing period (chlorophyll content and leaves transpiration); traits regarding final production, plant health status and fruit characteristics were measured, and results are reported in Table S9. Considering data from all field trials of 2021, the most interesting results were from the Ganazzoli field trials; in particular, the condition biochar_MC_B_AMF_MA determined the highest commercial production (0.8%) and total refractometer (14.22%), compared to control and biochar alone ($p=0.00113$). In this condition, the number of unripened fruits was also reduced ($p=0.004145$) (−30.1%).

For this reason, the same field scheme and types of analyses were applied in 2022 (Figure 5; Table S9). The results confirmed the positive effect of biochar_MC_B_AMF_MA, with the highest values for total and commercial production and Brix index, as compared to the control condition or to biochar alone. Positive effects in the marketable production of tomato were also reported by Vassura et al. (2023) when soil was amended with biochar, AMF_MA and MC_B. A series of Pearson's correlation analyses were performed starting from the data presented previously. In 2021, total refractometry ($g^{\circ}Brix\ ha^{-1}$) was positively correlated with the marketability of the product (commercial %) and the SPAD index, while it was negatively correlated with apical rot (%) and unripened fruits (%) (Figure 4C). A positive correlation

between tomato marketability and the leaf photosynthetic activity (SPAD index) was previously reported (Barrios-Masias and Jackson 2014). The chlorophyll content was closely related to the N present in the soil. The combined application of biochar and microorganisms can promote colonisation of soil by microorganisms and the transformation of N and other soil nutrients as solubilisation of P, thus ensuring nutrient uptake and the normal process of photosynthesis in tomato plants, thereby promoting the accumulation of photosynthates and increasing tomato yield (Wang et al. 2021). The percentage of commercial products was inversely correlated with the unripened products (%). This same result was observed in 2022, when the percentage of commercial products was inversely correlated also with rotten (%) products (Figure 4D). The data highlighted that the presence of biochar and microorganisms promotes plant growth and increases the marketability of the products (Hayat et al. 2010). All data obtained from the field trials at Ganazzoli farm were analysed by PCA, and the results are shown in Figure 6. In PCA, PC1 explained 52.3% of the variance, and is positively correlated with total and commercial production and Brix index, while PC2 explained 26.3% of the variance, and is correlated with rotten products and apical rot. Indeed, the combined treatment with biochar_MC_B_AMF_MA clustered along PC1, separated from the other treatments independently from the year of cultivation. The presence of biochar as a carrier of MC was beneficial in the case of tomato, confirming previous results obtained in pot experiments with other species of PGPM (Pavlicevic et al. 2022). These results may be influenced by the properties of the microorganisms in the consortia such as biological N fixation, synthesis of phytohormones (IAA, GA3 and cytokinin), and increased availability of micro- and macronutrients (P and iron) (Bolan et al. 2023; Graziano et al. 2022). Therefore, the presence of the newly developed consortia MC in combination with AMF

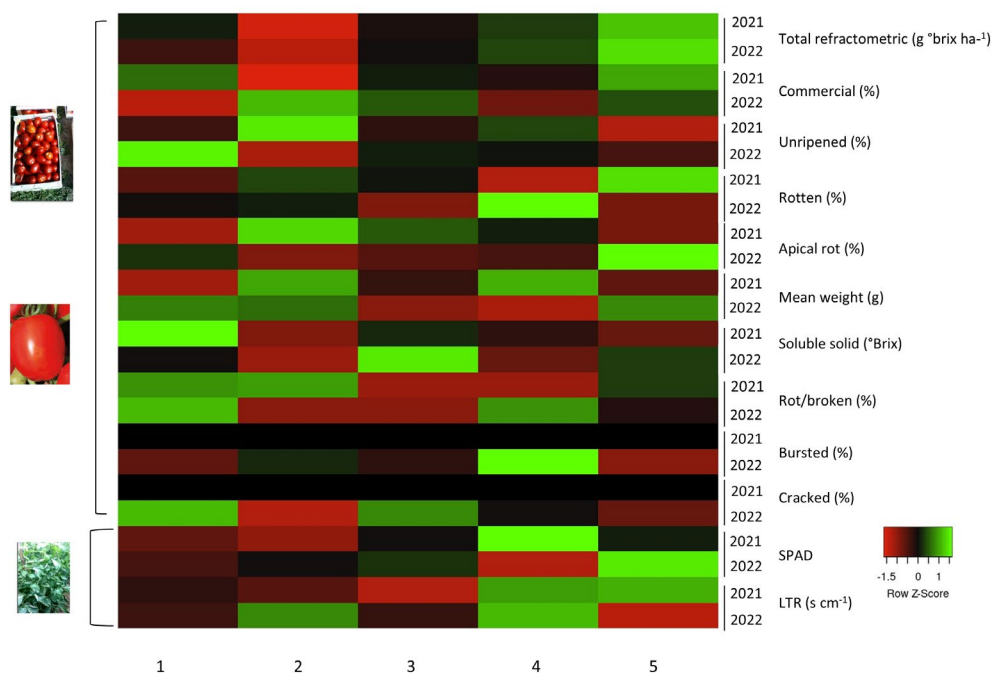


FIGURE 5 | Physiological and fruit quality traits from the 2021 and 2022 seasons for the Ganazzoli field. Heatmap is obtained on standardised data calculated as: Measure – average/standard deviation. Traits with value below the average are in red, while traits above the average correspond to a scale of green, as reported in the figure. Treatments were: Control (1), Biochar (2), biochar_Micosat F1 (3), biochar_AMF_MA (4), biochar_MC_B_AMF_MA (5).

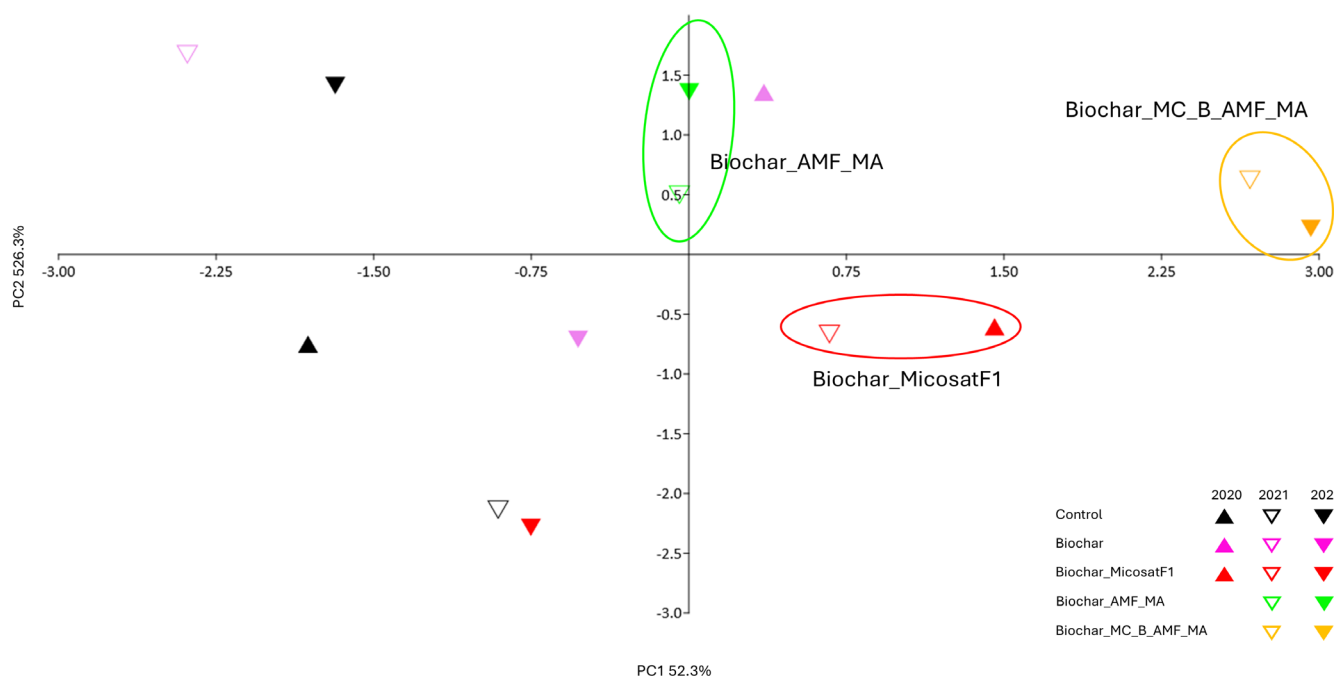


FIGURE 6 | PCA ordination diagram of tomato traits for samples grown under the different treatments in 2020, 2021 and 2022 in the Ganazzoli field trials. The percentage of total variance as explained by each axis is shown. Treatments are indicated with the following colour code: Control, black; Biochar, pink; Biochar_MicosatF1, red; Biochar_AMF_MA, green; Biochar_MC_B_AMF_MA, orange. Filled triangles indicate samples of 2020, empty triangle samples of 2021, and filled inverted triangles samples of 2022.

determined an increase in marketable tomato fruits and a decrease in unripened ones. Moreover, the combination of PGPM and AMF enhanced drought stress tolerance and yield in maize and tomato plants, promoted water use efficiency and mineral associated- and soil-organic carbon (Gulli et al. 2025; Khan et al. 2024; Krishna et al. 2022; Li et al. 2024, 2025).

3.5 | Economic and Environmental Impacts of the Biofertilizers and Biochar

Figure 7A,B illustrates the fertilisation costs of farming tomatoes in our economic analysis scenarios, comparing the organic and conventional (integrated) production agricultural practices. Overall, organic tomato cultivation incurs higher costs when compared to conventional production (+17%). Fertilisers account for 21% and 17% of total costs for organic and conventional production, respectively. Since in the Po Valley, processed tomato fertilisation is operated through irrigation, that is, fertirrigation, the cost of fertilisers accounts for only the nutrient compounds, excluding machinery and labour requirements. Moreover, partial substitution of conventional fertilisers with biochar and biofertilizers resulted in a relevant increase in the fertilisation cost, mainly due to the biochar. According to the scenario, 20%–47% of the total fertilisation cost is represented by biochar, under the hypothesis of one-time application over a period of 3 years. Biofertilizers cover about 20% of the total fertilisation cost, except for the biochar_MC_B_AMF_MA scenario, where the combined application of AMF and MC_B contributes over 30%.

The results of field experiments revealed that the application of biochar, without biofertilizers, negatively affects the tomato

yield, with a slight decrease of 6% compared to the baseline (data from Table S9 were grouped according to the different scenarios and averaged over the 3 years). On the contrary, the use of biochar in combination with biofertilizers has a positive effect on the yield. This confirms the results of meta-analysis studies (Ye et al. 2020), according to which ‘the use of biochar alone did not increase crop yield regardless of the control considered’. On average, in the scenario biochar_MicosatF1 and biochar_AMF_MA, the yield increases by 1% and up to 12%, respectively. In the scenario biochar_MC_B_AMF_MA, we can observe the highest yield increase, that is, +12% for organic tomato (55 Mgha⁻¹ compared to 49.65 Mgha⁻¹ in the baseline) and +23% for the conventional production (122.26 Mgha⁻¹ compared to 99.70 Mgha⁻¹ in the baseline). For conventional and organic production, we compared the average yields calculated for all farms and parcels during the period of the experiments.

Figure 7C–E reports the level of gross margin for each scenario and the corresponding variation with respect to the baseline. In both agricultural practices, the application of biochar, without the use of biofertilizers, is the worst scenario in terms of gross margin, indicating that the higher costs of fertilisation and lower yields concur to determine negative profitability in substituting part of conventional fertilisers with biochar. It is worth noting that the field experiments covered a 3-year period, while the literature suggests that the period for appreciating the soil improving property of biochar is more extended (Ye et al. 2020).

Combining biochar with Micosat F1 produces a relevant reduction in gross margin for organic tomatoes and a modest increase for conventional production. Scenarios biochar_AMF_MA and

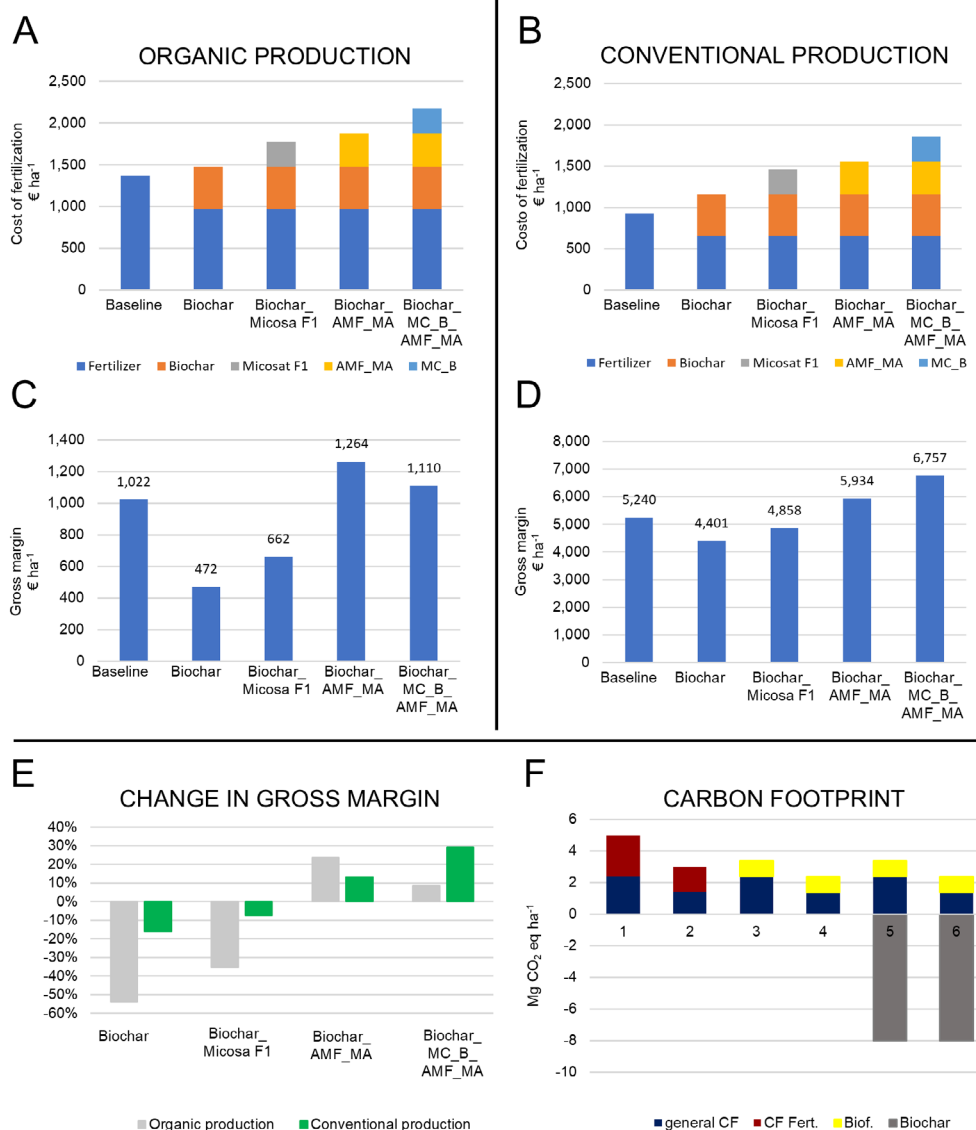


FIGURE 7 | Economic and environmental evaluation of biofertilizers and biochar adoption. Cost of fertilisers by agricultural practice and economic scenario in organic production (A) and conventional production (B); tomato gross margin across the scenarios in organic production (C) and conventional production (D); (E) change in tomato gross margin compared to the baseline in organic production and conventional production; and (F) carbon footprint (expressed in $\text{Mg CO}_2 \text{ eq ha}^{-1}$) of the different cultivation approaches: (1) The general CF of a conventional farm (dark blue) is combined with the cost of fertilisation (dark red); (2) The general CF costs of a biological farm (dark blue) is combined with the cost of fertilisation (dark red); (3) The general CF of a conventional farm (dark blue) is combined with the cost of biofertilizers (yellow); (4) The general CF costs of a biological farm (dark blue) is combined with the cost of biofertilizers (yellow); (5) Scenario 3 is combined with the improved CF given by the addition of biochar (grey) into the soil; (6) Scenario 4 is combined with the improved CF given by the addition of biochar (grey) into the soil. Data in the figure displays the overall $\text{CO}_2 \text{ eq}$ saved per ha when biofertilizers and biochar are combined (data are described in detail in the main text).

biochar_MC_B_AMF_MA are more beneficial, with gross margin that increases by 31% and 16%, respectively, in the case of organic production, and 13.2% and 29% in the case of conventional production, compared to their respective baselines. Consequently, by applying biochar in combination with AMF and MC_B, the yield increase offsets the fertilisation cost. Organic tomato obtains the highest gross margin per hectare in the scenario biochar_AMF_MA, thanks to a more than proportional increase in yield than in the total cost of production; in the scenario biochar_MC_B_AMF_MA, the marginal benefit due to the increase in the yield is lower than in the previous scenario. On the contrary, for conventional production, the marginal benefit obtained by the combined use of biochar and

biofertilizer in the scenario biochar_MC_B_AMF_MA is higher compared to the scenario biochar_AMF_MA.

Improvement in carbon sequestration in agricultural soils can be seen as an environmental advantage for society and an opportunity for farms. Applying biochar to the soil results in a long-term stable carbon sequestration, which contributes to the mitigation of climate change. The economic analysis evaluated this potential benefit, assuming that the farms using biochar, as a soil improver, can obtain carbon credits to sell to voluntary carbon credit markets. These voluntary markets promote the enhancement of ecosystem services, rewarding the carbon credit supplier with a price defined in negotiations with firms requiring

credit to offset the carbon emission produced by their economic activity. In this analysis, we assume a market price for carbon credits of €30/tCO₂eq⁻¹ (1 credit = 1 tCO₂eq), much lower than the corresponding price in the EU Emission Trading System (EU ETS) market (Maluccio et al. 2021). Biochar soil application provides farms with a carbon sequestration of 7.48 tCO₂eq and a potential additional economic benefit of €224 ha⁻¹ for sequestering CO₂ over 3 years. The yearly economic benefit is low but non-negligible. For organic farms, revenue from carbon credits contributes to increasing the gross margin in scenarios biochar, biochar_Micosat F1, biochar_AMF_MA and biochar_MC_B_AMF_MA by 16%, 11%, 6% and 7%, respectively, compared to the gross margin without carbon credits (Figure 6C). In the case of conventional production, the contribution of the carbon credits is lower and does not exceed +2% in all scenarios compared to the situation without carbon credits (Figure 6C). This is mainly due to the high proportion of the gross saleable production, that is, the market value of the tomato production. Carbon credits can act as an incentive to encourage the substitution of conventional fertilisers with biochar. However, the current low carbon credit market price could hinder farmers from shifting to low carbon agronomic practices.

In addition to the economic benefits of using biochar, there is also an environmental aspect that needs to be considered. Conventional production has been estimated to 'cost', or has a carbon footprint (CF), of approximately 5 MgCO₂-eq ha⁻¹ (Ronga, Galligan, et al. 2019; Wang et al. 2020), while organic farming consumes 3 MgCO₂-eq ha⁻¹; the largest difference is due to the use of pesticides that are missing in organic cultivation (Figure 6F). The difference is quite high, but it is slightly reduced if we consider that the average yield for organic farming, for example for 2021 in Northern Italy, was 14 Mg ha⁻¹ less than the conventional system, which in 2021 reached 80 Mg ha⁻¹ (Ismea 2022 and data from <https://oipomodoronorditalia.it/>). The CF considers field emissions, diesel production, lubricant production, irrigation, soil tillage and pesticide application. The impact of fertilisation (both production and distribution) is similar for both systems and was estimated to be between 40% and 55% of cultivation (Roussis et al. 2023; Solimene et al. 2023; Wang et al. 2020), and if we assume an average of 47%, this implies that the impact of fertilisation is around 2.4 MgCO₂-eq ha⁻¹ for the conventional field and 1.4 MgCO₂-eq ha⁻¹ for organic farming. The impact of producing and distributing microbial biofertilizers was previously estimated to be around 1 MgCO₂-eq ha⁻¹ (Caldara et al. 2024; Tensi et al. 2023) when applied at 10 kg ha⁻¹, this means that substituting conventional fertilisers with these products would save 1.4 and 0.4 MgCO₂-eq ha⁻¹ in conventional and organic systems, respectively. The main CO₂ saving would come from the use of biochar; indeed, this amendment when produced from wood chips by slow pyrolysis and applied at 2 Mg ha⁻¹, resulted in a negative CF value of -8 MgCO₂-eq ha⁻¹ (Caldara et al. 2024). Therefore, by combining these calculations, it results that a conventional farm substituting a conventional fertiliser with biofertilizers and biochar can save 9.4 MgCO₂-eq ha⁻¹, and in an organic farm 8.4 MgCO₂-eq ha⁻¹ (Figure 6F). Besides the improvement of the CF, it is important to note that conventional fertilisers can cause water eutrophication (Hasler et al. 2015). Therefore, their usage limitation can further support a healthy environment.

4 | Conclusions

The use of biofertilizers and soil improvers, as biochar, is considered an important tool for the transition to a more sustainable crop management and for a reduction of the chemical inputs in agriculture. For this, several commercial products based on AMF and PGPM consortia are continuously released onto the market, making it necessary to evaluate their effectiveness in different environments and growing conditions. This study, conducted on a conventional farm and two organic farms over a period of 3 years, showed how the simultaneous addition of biochar and microbial consortia determined an increase in the marketability of the products. Therefore, the presence of these 'tools', biofertilizers and soil improvers such as biochar, can pave the way towards greater sustainability for tomato cultivation and an improvement of the soil in terms of chemical and microbiological composition. Furthermore, the use of biofertilizers and biochar has a positive environmental effect, as it has led to the reduction of fertilisers (-20%), pesticides, CO₂ emissions and pollutants. Indeed, the cultivation carried out with microbes and biochar led to a reduction in waste and resulted in a better quality of the marketable product with fewer contaminants, combined with an increase in the gross margins, also owing to the earned carbon credits. The experiments presented in this work analysed the effects of biofertilizers and biochar only at the end of the growing seasons. It will be interesting to evaluate the long-lasting effect of having biochar in the soil, as it was previously reported that this amendment increases soil carbon sequestration, crop yield, soil fertility, and microbial activity even years after being added to the soil. This is an interesting approach that could help promote the future of soil health.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The sequencing data presented in this study are openly available at NCBI database, BioProject under accession number PRJNA1167691 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1167691>). The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.