



The relevance of biochar and co-applied SynComs on maize quality and sustainability: Evidence from field experiments

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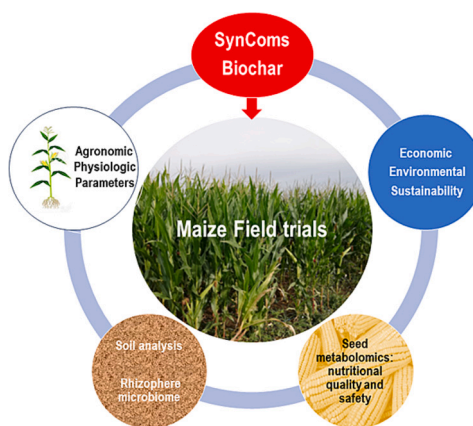
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HIGHLIGHTS

- SynComs can enhance the function of the soil/plant ecosystem.
- SynComs did not modify the indigenous microbial community in maize field.
- Biochar and SynComs determined interesting effects on kernel metabolome.
- Biochar and SynComs could partially replace the intense use of chemical fertilizers.

GRAPHICAL ABSTRACT



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ABSTRACT

Adoption of sustainable maize cropping practices is urgently needed. Synthetic microbial communities (SynComs) made of plant growth-promoting microorganisms (PGPMs), coupled with biochar from residual biomass,

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 From field to fork

offer an environmentally compatible alternative to inorganic fertilizers and may improve soil fertility. This article extends in a two-year field trial with preliminary results obtained in previous pot experiments, monitoring plant physiology, soil biology and chemistry, and kernel metabolomics. Here, we report the synergistic effect of the co-application of biochar, SynComs, and arbuscular mycorrhizal fungi on the soil microbiome, maize growth, and kernel metabolomic profile. SynComs application did not affect the diversity and richness of soil microbial communities; therefore, it posed a low risk of long-term effects on soil microbial ecology. With SynComs and biochar co-application to the soil, the physiology of maize plants was characterized by higher chlorophyll content, ear weight, and kernel weight. The combination of SynComs and biochar also affected the kernel metabolome, resulting in enriched health-beneficial and anti-stress metabolites. Since the preliminary evidence on the environmental and economic impact of these new associations was more favorable than that of conventional fertilizers, it seems reasonable that their large-scale implementation can eventually favor the transition to more sustainable agriculture.

1. Introduction

Sustainable agriculture is a multifaceted concept with economic, environmental, and social dimensions. Many approaches can be pursued to develop each dimension; however, solutions that include them all are few. The exploitation of alternative biofertilizers and amendments is one of these virtuous strategies and is recognized as a potential alternative to chemical fertilizers (Ammar et al., 2023). Indeed, chemical fertilizers and pesticides have helped increase agricultural production following the Green Revolution (Tilman et al., 2002); however, this has led to overexploitation of soils with nutrients and elements that affect soil geochemical cycles and microbial biodiversity (Rockström et al., 2009; Steffen et al., 2015). The consequences have been a negative impact on overall human and environmental health, affecting the equilibrium between humans, plants, animals, and nature (Bindraban et al., 2015; Destoumieux-Garzón et al., 2018; Chandini et al., 2019; Rong et al., 2021). Among biofertilizers, plant growth-promoting microorganisms (PGPM) are effective in improving the performance of many plant species (Reed and Glick, 2023) because of their unique biological activities. The application of several soil microorganisms, including species of bacteria and fungi (e.g., rhizobia, *Azotobacter* sp., *Bacillus* sp., *Azospirillum* sp., *Aerobacter* sp., *Burkholderia* sp., *Pseudomonas* sp., *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., and *Glomus* sp.) can reduce the need for chemical inputs and improve the quality and safety of harvested products and processed foodstuffs, while providing beneficial ecosystem services (Maćik et al., 2020; Maitra et al., 2021; Banerjee and van der Heijden, 2022; Chaudhary et al., 2022). Most bioinoculants are composed of a single beneficial microbe, defined as a PGPM or arbuscular mycorrhizal fungus (AMF) (de Souza et al., 2020; De Palma et al., 2022). The characterization of PGPM properties and their ability to interact with plant tissues usually requires testing at the laboratory level before transference to the field. A pot experiment under controlled conditions is the most common procedure to demonstrate the direct effect of individual microbial strains or consortia without any ‘environmental’ disturbance. Usually, pot experiments last for a few weeks and provide results regarding the germination and vegetative growth stages but not on the entire final performance. Previous studies have shown low reproducibility between pot experiments and field trials under identical treatment conditions (Nkebiwe et al., 2016; Symanczik et al., 2023). There are several possible explanations for the discrepancy. The most significant factors include the timing and application method, physical and chemical properties of the soil, fertilization method, and interactions with microbial populations in the soil. These interactions determine biological competition such as niche overlap, resource availability, and predation. In addition, typical biotic and abiotic stresses in the field also play a role (Symanczik et al., 2023). The effect of microbial consortia in ‘real’ agricultural soil can vary depending on the crop, chemical composition, environmental conditions, and indigenous microbial community of the soil. Their efficacy may be affected by varying environmental conditions, coexistence with other ‘indigenous’ microorganisms, and interaction with the host plant (Trivedi et al., 2020; Hett et al., 2023), as well as by their viability and persistence in

the soil after application (Iosa et al., 2024). Synthetic microbial Communities (SynComs) represent a promising strategy (Callens et al., 2022; Vaccaro et al., 2022) and are generated combining different microbial genera with different functional attributes (Riva et al., 2022; Mapelli et al., 2023). Under unfavorable environmental conditions, SynComs can enhance the function of the soil–plant ecosystem and improve crop yield and product quality (De Palma et al., 2022; Shayanathan et al., 2022). The main concern is that the application of SynComs could disturb the native microbiome and activate complex networks between inoculated and native species, which could unpredictably influence the balance of the biotic components of soil (Liu et al., 2023). This is critical considering that soil microbiome functions are positively correlated with microbial diversity and that the latter is strongly related to the ‘one health’ concept (Banerjee and van der Heijden, 2022). However, arable lands display lower microbial diversity (Banerjee et al., 2024); therefore, when using microbial consortia, it is crucial to evaluate their impact on resident microbial communities and general soil biodiversity. Well-established methods based on metagenomic analyses, targeted next generation sequencing (NGS), and sequencing of conserved sequences allow verification of the composition of the soil and rhizosphere microbiomes (Nwachukwu and Babalola, 2022).

Biochar is gaining importance as a carbon storage material that can improve soil quality (Beesley et al., 2013), as a soil amendment, and as a carrier of beneficial nutrients and microorganisms, facilitating PGPM growth and survival due to its morphology and physicochemical characteristics (Marmioli et al., 2018; Zhang et al., 2018a; Marmioli et al., 2022; Tan et al., 2022). Plant biomass-derived biochar has been recognized for several decades as an ecofriendly amendment that can improve soil quality, increase carbon sequestration, and reduce greenhouse gas (GHG) emissions (Vijay et al., 2021; Murtaza et al., 2023). The application of biochar in pot experiments has been reported to be beneficial for the growth of maize roots (Graziano et al., 2022; Yan et al., 2022) as it alters the physicochemical properties (Sohi et al., 2010) and increases soil fertility (Ding et al., 2016).

A systematic review of maize cultivation using biostimulants (Ocwa et al., 2024) reported a few examples of the use of biochar combined with AMF and PGPM. When biochar was applied together with AMF, composed of different *Glomus* species, and a consortium of *Bacillus* spp. (Wolna-Maruwka et al., 2021), or applied with humic acid and *Alcaligenes* spp. AZ9 (Hussain et al., 2019), an increase in yield was observed. Furthermore, a combination of PGPMs with biochar may lead to unexpected outcomes requiring further investigation. The properties of biochar affect different soil health indicators, such as pH, soil organic matter, and soil stability, along with microbial biomass, richness, and diversity (Deshoux et al., 2023; He et al., 2021). The influence of biochar alone or in combination with SynComs is not fully elucidated, and its negative effects are yet to be determined. There is a consensus on the requirements for more field trials in different countries and under different agronomic conditions to validate the effects of microbial consortia–biochar before its large-scale application in sustainable crop production (Ocwa et al., 2024).

In addition to environmental issues, the sustainability of agricultural

ecosystems should consider food security (OECD and FAO, 2023) as its fourth dimension. Adoption of alternative fertilizers and amendments may affect production at both the quantitative (yield) and qualitative (nutritional quality) levels; the first aspect has been the subject of many studies (Dos Santos Lopes et al., 2021; Chouhan et al., 2021; Ocwa et al., 2024), whereas the second needs to be further investigated, particularly regarding seed composition (Berta et al., 2014). The role of SynComs and biochar in modifying crop quality in terms of nutritional content by triggering the synthesis of beneficial metabolites is poorly understood. Characterization of the metabolomic profile of raw materials, such as cereal seeds or flour, has been used to elucidate the changes in the interactions between plants and microbes. Many investigations have focused on the response of plants to biotic or abiotic stresses, but few have evaluated the effects of PGPM on the plant metabolome in terms of nutrient composition and bioactive compounds that are advantageous to both plant and consumer health (Berta et al., 2014; Saia et al., 2019; Riboni et al., 2023).

Existing research reports data on SynComs that were carefully designed (Tabacchioni et al., 2021) and tested on wheat and maize in pot experiments (Graziano et al., 2022; Hett et al., 2022) or under different environmental conditions on maize in a two-year field trial (Hett et al., 2023). Here, for the first time, these newly developed SynComs were used, in combination with biochar and AMF, as alternative green biofertilizers in maize field trials. The chemical composition and microbiota of the soil were monitored during plant growth and maturation. Maize plants were monitored at morphological and physiological levels (photosynthesis, transpiration, and yield) during their entire growth cycle, seeds collected at maturity were milled, and the flour metabolome was analyzed. The rhizosphere microbiota was characterized by targeted NGS sequencing of conserved bacterial and fungal sequences, followed by bioinformatics analysis. The kernel metabolomic profile was derived by high-pressure liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS) using both target and untargeted approaches. The results showed the complete compatibility of SynComs and biochar with the rhizosphere microbiota. Within each growing season, SynComs applied in combination with biochar positively affected chlorophyll content (CHL) and yield traits. The metabolomic signature of maize kernels under the combined application of SynComs, biochar, and AMF showed enrichment of health-related and anti-stress metabolites. The results suggested that co-application of SynComs, biochar, and AMF as ‘green biofertilizers’ can improve maize yield and quality while maintaining sustainable agronomic practices. The economic and environmental impacts of the transition to this new type of fertilizer in agriculture are discussed, considering different agronomic scenarios.

2. Methods

2.1. Plant materials and experimental field trials

Field experiments were conducted during two growing seasons (2020 and 2021) in a silty clay loam soil at the experimental farm ‘Azienda Stuard’ (Parma, Italy; Lat. 44.4802300N, Long. 10.1603000E; 58 m above sea level). The experiments followed the relevant institutional, national, and European guidelines and legislation. Commercially available seeds of *Zea mays* cv DKC6587 (Dekalb®, Monsanto Agricoltura Italia SpA, Milan, Italy) were sown at a density of 8 plants/m² in 8.4 m² plots, keeping a distance of 17.8 cm between seeds in a row and 70 cm between rows, borders and passage within and around the plots were ensured for operations. Nine treatments with four replicates (2020) and 12 treatments with three replicates (2021) were distributed using a split-plot design, as described in Section 2.3. In 2020, we did not observe a large variability among the plots, and we decided to reduce the number of replicates and increase the number of combinations. This allowed us to focus on the more promising conditions, and the decision was supported by data from pot trials, as described by Graziano et al.

(2022). This choice allowed us to have a clearer view of the effects of the microbial consortia (B or C) and AMF alone or in combination with biochar. Moreover, when four replicates are used, only effect differences >2.02 Standard Deviation (SD) will be detected (maximum false negative rate (β) of 20 % and a maximum false positive rate (α) of 5 %), and when three replicates are used, the differences detected will have to be >2.38 SD. The difference between the two conditions was minimal. To lower this value, the number of replicates needs to be increased drastically, which was not feasible in our field trials, because different growing conditions needed to be tested.

The sowing dates for the two years were May 5, 2020, and April 24, 2021. Nitrogen (N) fertilizer (with 46 % urea) was applied once, 170 kg/ha in 2020 and 85 kg/ha in 2021. This corresponded to 100 % and 50 % of the normal fertilization plan for maize, respectively. Phosphates were excluded during the study period. Soil samples between 0 and 30 cm depth were collected before seedbed preparation and at harvest time during both growing seasons for physicochemical analysis, as presented in Table S1, S2. At the end of each growing season, the plants were harvested manually on October 7, 2020, and September 30, 2021.

2.2. Weather conditions and irrigation scheme

Maximum and minimum temperatures (°C) and precipitation (mm) were recorded daily by an automatic weather station installed near the experimental field (Fig. S1). Irrigation was performed with drip tapes, which have the advantage of compensating for water shortages during the most critical phases of the maize life cycle, considering the actual requirements of the plant, especially during the driest periods. The drip tape was placed in every two rows (1.5 m). The agrometeorological data collected from field trials in 2020 and 2021 are shown in Fig. S1. A general trend was observed in 2020, when higher rainfall (especially during the pre- and post-anthesis periods) and lower average minimum and maximum temperature values were recorded compared to 2021. The plots were drip-irrigated as necessary to optimize maize production, and considering both rain and irrigation, the total amount of water received was similar in both years.

2.3. Source of biofertilizers and biochar

SynComs were designed as previously described (Tabacchioni et al., 2021). Microbial Consortia B MC_B includes *Azotobacter vinelandii* DSM 2289, *Bacillus* spp. BV84, *Bacillus amyloliquefaciens* LMG 9814, *Pseudomonas fluorescens* DR5, *Rahnella aquatilis* BB23/T4d. MC_C is composed of *Azotobacter chroococcum* LS132, *B. amyloliquefaciens* LMG 9814, *Pseudomonas fluorescens* DR54, *Burkholderia ambifaria* MCI 7, and *Rahnella aquatilis* BB23/T4d. Each strain was grown in a pilot scale bioreactor according to the procedure previously described (Tabacchioni et al., 2021). The formulation of MC_B and MC_C was made by adding all single strains in a 1:1 ratio to reach at least >10⁶ CFU for each strain included in the consortiums. The growth, concentration, production, and stabilization of the SynComs were described in detail in Hett et al. (2023). AMF (MycAgro lab., Bretenière, France) in granular form contains *Rhizophagus intraradices* (10 propagules/g containing spores) and mineral solid particles (clay, zeolite) (Fracasso et al., 2020); it was supplied at 3 g to each plant (0.24 tons/ha). MICOSAT F (F1) in granular form was provided by CCS Aosta Srl (Aosta, Italy) (<https://www.micosat.it/prodotto/micosat-uno/>); it was supplied at 3 g to each plant (0.24 tons/ha).

Biochar (CHAR) was produced from woody pellet by using an experimental pyrolysis equipment (Iridenergy srl, Parma, Italy). Briefly, in Marmiroli et al. (2018) it is reported that pyrogasification was carried out at 500–700 °C for 1–2 h. The yield was about 20 kg/100 kg of pellet, and the biochar produced has a pH of 8.11, electrical conductivity of 1.4 mS/cm, bulk density of 0.37 g/cm³, moisture content 6.29 % fresh weight, organic matter 95.63 % dry weight, ash 4.4 % dry weight, metals (Cd, Cu, Ni, Fe, Pb and Zn) below international guidelines.

Phytotoxicity tests revealed no inhibition of germination or seedling growth. It was applied before sowing at a rate of 0.2 kg/m² (2 tons/ha) and buried to a depth of 10 cm.

For each microbial treatment, the required number of seeds and specific products were distributed using a pneumatic seeder machine containing two compartments, one for the seeds and other for the consortia. Each compartment had a nozzle whose opening could be regulated to allow the deposition of one seed at a time and the desired amount of granular or powdered material. The microbial treatments and biochar as a carrier were used in different combinations, as follows: 1) season 2020: Conventional condition (Control), Micosat (F1), AMF, CHAR, CHAR_AMF, CHAR_F1, CHAR_MC_B_AMF, CHAR_MC_C_AMF, CHAR_MC_C_F1; 2) season 2021: Conventional condition (Control), CHAR, MC_B, MC_C, AMF, MC_B_AMF, MC_C_AMF, CHAR_MC_B, CHAR_MC_C, CHAR_AMF, CHAR_MC_B_AMF, CHAR_MC_C_AMF. Overall, the treatments CHAR, AMF, CHAR_AMF, CHAR_MC_B_AMF, and CHAR_MC_C_AMF remained consistent throughout the two seasons. Further details of the treatments, and the concentrations used are summarized in Table S3.

2.4. Morphological, physiological, and production traits

CHL and leaf transpiration rate (LTR) were measured at the vegetative growth stage (V6), flowering (R1) and maturity (R4); plant height (PH) was measured at R1 and R4. At harvest (R6), number of ears (E), plant weight (gr) (PW), stock weight (gr) (SW), ear weight (gr) (EW), production at 15 % humidity (t/ha) (Y15), yield per plant (YPL), grain moisture % (GM), test weight (kg/hL) (TW), 1000 grain weight (gr) (TGW) were measured. CHL was measured with the SPAD-502 chlorophyll meter (Konica Minolta Business Solution Italia Spa, Milan, Italy), taking ten measurements along the length of five expanded leaves in each plot. The leaf transpiration rate was measured on five expanded leaves in each plot using the portable AP4 porometer (Delta-T Devices, Cambridge, UK).

Throughout the plant growth cycle, constant visual inspection was performed to detect any pathological evidence on the leaves; however, no signs of infection were detected. At harvest, the presence of aflatoxins in grains was analyzed using the B-TeZ ELISA AFLA B1 kit (BioTeZ, Berlin-Buch GmbH, Germany). In all the samples, aflatoxin levels were below the detection limit of the method (data not shown).

Standard statistical analyses of agronomic and physiologic data were performed using the software Past 4.03 (© Copyright Hammer 1999–2020).

2.5. Soil analysis

Soil chemical analyses were carried out according to standardized protocols as previously described (Caldara et al., 2024). For organic carbon (C), total nitrogen (N), and phosphorus (P), protocols reported in the ‘D.M. Politiche Agricole 13/09/99 Met. VII3’ were adopted, while for all other elements (calcium, Ca; iron, Fe; zinc, Zn; potassium, K) the protocols defined by LEI/MP/N. 30 2020 Rev.0 were applied. Soil pH and conductivity (CE) were measured as reported by “D.M. Politiche Agricole 13/09/99 Met. 248”.

2.6. Rhizospheric soil DNA extraction and 16S rRNA gene amplicon sequencing

Rhizospheric soils were sampled during the two growing seasons at the end of the trial for metagenomic analysis (September 3, 2020; September 1, 2021) and kept at –80 °C. DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen Group, Hilden, Germany) following the manufacturer’s instructions. The quantity and quality of DNA were evaluated using the NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA) and Qubit 4.0 fluorometer (Invitrogen, Carlsbad, CA), and by PCR, using 16S rRNA primers pairs, 314F and 805R, that target the V3-

V4 region. The PCR conditions consisted of 1 min at 94 °C followed by 25 cycles of 30 s at 94 °C, 30 s at 55 °C, 45 s at 72 °C with a final incubation for 7 min at 72 °C.

For each sample, two independent DNA extractions were performed and then combined in an equimolar ratio, resulting in a composite DNA sample. The construction and sequencing of the Illumina 16S rRNA gene library were performed using the Illumina MiSeq platform and the 300 PairedEnds strategy at Matis Ltd. (Reykjavik, Iceland). The Illumina MiSeq protocol is available at <https://web.uri.edu/gsc/files/16s-metagenomic-library-prep-guide-15044223-b.pdf>. Raw sequence data reported in this study are publicly available in the National Center for Biotechnology Information (NCBI) ‘Sequence Read Archive’ (SRA), under the accession number PRJNA1023724.

2.7. Bioinformatic and statistical analysis of 16S rRNA gene sequencing data

The pipeline for 16S rRNA amplicon sequence data analysis followed previously published procedures (Cangioli et al., 2022). Samples with reads lower than 5000 were discarded. Briefly, after trimming, paired-end sequences were clustered into Amplicon Sequence Variants (ASVs) following the DADA2 pipeline (version 1.16) (Callahan et al., 2016). The sequences were then filtered to remove chimeras. Taxonomic assignment was carried out against the SILVA NR99rel138 database (Quast et al., 2013) using the ‘DECIPHER’ R package (version 2.18.1) (Wright et al., 2012) as implementation of DADA2 (SSU version 138 available at: <http://www2.decipher.codes/Downloads.html>). The count tables of the annotated ASVs were processed by the ‘Phyloseq’ R package (version 1.34.0) in R environment version 4.0.5 (McMurdie and Holmes, 2012).

The rarefaction curve were generated using ‘ggplot2’ (version 3.3.3) (Wickham, 2011) and ‘ranacapa’ (version 0.1.0) (Kandlikar et al., 2018) R packages using the ‘ggrrare()’ function on the phyloseq object. The Shannon and Simpson alpha diversity indices were calculated and plotted using the function ‘diversity()’ within ‘microbiome’ R package (version 1.12.0) (Shetty and Lahti, 2019). Good’s coverage and Evenness indices were calculated through the R functions ‘goods()’ and ‘evenness()’, respectively, within the ‘microbiome’ R package (version 1.12.0). Multivariate analysis (PCA, Principal Component Analysis) was carried out after a *vst* transformation using the following functions: *varianceStabilizingTransformation()*, *plotPCA()*, and *ASVs*. The R package ‘ggplot2’ (version 3.3.3) was used to generate relative abundance plots. The effect of treatments and the relationships between the taxonomic composition of the microbiota and the agronomic and physiological data were evaluated by using permutational multivariate analysis of variance (PERMANOVA) using the R packages ‘ggplot2’ (version 3.3.3), ‘vegan’ (version 2.5–7) and ‘pairwiseAdonis’ (version 0.0.1) with the functions ‘adonis2()’ and ‘pairwise.adonis()’, respectively. To test the differential occurrence of ASVs among microbiota from different treatments, a differential counts analysis was performed, using the ‘DESeq2’ R package (version 1.30.0) functions: *phyloseq_to_deseq2()*, *estimateSizeFactors()*, *DESeq()*, and *results()* (Love et al., 2014). The use of PCA is justified by the fact that this analysis is an unsupervised multivariate statistical technique used for pattern recognition. It applies an orthogonal transformation to transform a set of potentially correlated variables into a set of linearly uncorrelated variables, referred to as principal components. This technique is often employed to investigate how a small number of principal components can reveal the underlying structure among several variables by identifying the components that account for the largest variance portion among the original variables. It also aims to preserve as much of the original information as possible, while ensuring that the components remain uncorrelated with one another. PERMANOVA is a non-parametric multivariate permutation test for variance. It is a robust and flexible technique used for testing the differences between groups. This method is useful for analyzing ecological community data (species counts and presence/absence), microbiome data (relative abundance of

species), and genetic data (gene expression levels or mutation counts).

2.8. Metabolomic analysis

Maize kernels were harvested at maturity and kept at 4 °C before being subjected to global metabolic profiling by HPLC-HRMS, as previously reported (Benincasa et al., 2020). Semi-polar metabolites were extracted from 20 mg lyophilized maize flour using 0.75 mL cold 50 % (v/v) methanol, 0.1 % (v/v) formic acid, and spiked with 5 µg/mL formononetin for internal standard. After shaking for 40' at 20 Hz using a Mixer Mill 300 (Qiagen, Valencia, CA, USA), samples were centrifuged for 15 min at 20,000 ×g at 4 °C. Thereafter, 0.6 mL supernatant was subjected to HPLC-HRMS.

Non-polar metabolites were extracted from 30 mg of lyophilized flour using 1 mL of 25 % (v/v) methanol, 50 % (v/v) chloroform, 25 % (v/v) 50 mM Tris-HCl, spiked with 10 µg/mL DL- α -tocopherol acetate as internal standard. After centrifugation, the organic hypo-phase was dried with a Speed Vac concentrator; the residue was resuspended in ethyl acetate (100 µL), transferred to HPLC tubes, and subjected to HPLC-HRMS. Subsequently, both untargeted and targeted analyses were performed on mass chromatograms. Untargeted metabolomics was performed using SIEVE software (ThermoFisher Scientific, Waltham, MA, USA) as previously reported (Dono et al., 2020). Targeted metabolite identification was performed by comparing chromatographic and spectral properties with authentic standards (when available) and reference spectra, in house database, literature data, and on the basis of the m/z accurate masses, as reported in the Pubchem database (<http://pubchem.ncbi.nlm.nih.gov/>) or on the Metabolomics Fiehn Lab Mass Spectrometry Adduct Calculator (<http://fiehnlab.ucdavis.edu/staff/kind/Metabolomics/MS-Adduct-Calculator/>), in the case of adduct detection. Data were analyzed by one-way ANOVA, and Tukey's pairwise t -test. A p -value ≤ 0.05 was considered statistically significant.

2.9. Searching tools to suggest economic and environmental consideration

Economic evaluations examine the financial viability of farmers using biochar and biofertilizers as partial replacements for traditional fertilizers. This analysis was conducted from two distinct perspectives: (i) market-based and (ii) carbon sequestration from an ecological perspective. Four possible scenarios were designed: (A1) conventional cultivation applying NPK for fertilization, (A2) fertilization with 50 % N (as in our study), (B) use of biofertilizers with 50 % N, and (C) same as (B) but with biochar. First, to analyze them we need to consider the cost of fertilization. The price of ammonium nitrate (N) is approximately 0.4 €/kg, triple-phosphate (P) is roughly 0.5 €/kg, and K is 0.4 €/kg (<http://teseo.clal.it/>, consulted on December 1, 2024). To calculate the cost of applying NPK, we must consider the application of P at 60 kg/ha, N at 170 kg/ha, and K at 20 kg/ha (Moungsree et al., 2022). The cost of N (with 46 % urea) was approximately 0.5 €/kg and was applied at 85 kg/ha. These microbial fertilizers, priced around 30 €/kg, cost 300 €/ha when applied at a rate of 10 kg/ha (Tensi et al., 2024), whereas biochar has an average price of 0.2 €/kg (Haeldermans et al., 2020; Struhs et al., 2020) and was applied at 2 ton/ha. All other costs related to agronomic operations (irrigation, sowing, etc.) were not considered, because there was little or no difference between traditional fertilization and the addition of SynComs.

To calculate the emissions expressed in CO₂ eq/ton, we considered literature data (Fantin et al., 2017; Li et al., 2021; Moungsree et al., 2022; Supasri et al., 2020; Ma et al., 2012). Additionally, the production of SynComs resulted in a total carbon footprint (CF) of 1 ton CO₂ eq/ha (Tensi et al., 2024), whereas biochar has a negative carbon output (its production traps CO₂) at rates of -0.47 to -25 ton CO₂ eq/ha, depending on the application rate, raw material, and production technology used (Brassard et al., 2018; Hamedani et al., 2019). In this study, biochar was produced from wood chips using slow pyrolysis and applied at 2 ton/ha, resulting in a CF of -10 ton CO₂ eq/ha.

3. Results

3.1. Soil chemical analysis

The total N content was similar in all plots in both growing seasons (Tables S1, S2), and the total organic C was higher in 2021. However, in both years, no differences were observed between the treatments. In general, the concentrations of these elements averaged those in healthy soil. The presence of plants was accompanied by an increase in Electrical Conductivity (EC), dry matter, and amounts of Ca, Fe, Zn, and K compared with bare soil. Overall, the Ca values measured in 2021 are higher than those measured in 2020. In 2021, the highest Ca values were observed for the CHAR_B_AMF and CHAR_C_AMF treatments, whereas in 2020, the CHAR treatment was associated with the highest amount of Ca and the highest pH value. In 2021, the pH values were lower than those in 2020 under all conditions. At the tested biochar concentration, there was no significant increase in pH as the pH of soil (7.8–8.6) and biochar (8–8.2; see biochar A4 in (Marmioli et al., 2018)) were quite close.

An increase in Ca and a decrease in pH offer important agronomic benefits to many soils, at least in the short-to-medium term (Rawat et al., 2018). Moreover, the addition of biochar to the soil can improve maize growth by stimulating plant N uptake (Peng et al., 2021); in a small amount, biochar did not significantly affect the chemical composition of the bulk soil, as previously reported (Vassura et al., 2023).

3.2. Agronomic and physiological parameters

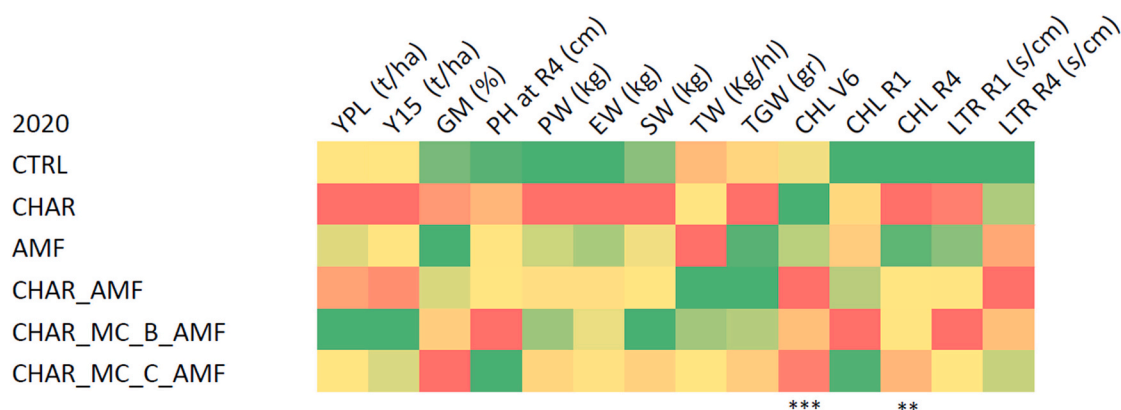
Maize developed vigorously throughout the two growing seasons with no signs of phytosanitary problems. Physiological and morphological data from all plots in both seasons are listed in Tables S4 and S5; data regarding the six common conditions in the two seasons are shown in Fig. 1. The final yield in both seasons was not significantly affected by the treatments or the reduction in fertilization (50 % less N and no P). In 2020, an overall higher yield was obtained compared to that in 2021, which was in line with the yield registered in the Italian region of Emilia-Romagna in the two seasons (Table S4, S5). This can be explained by the lower amount of rain and higher temperatures recorded in 2021. Significant differences in CHL were observed in 2020. At stage V6, the highest CHL values were observed in plants treated with CHAR_AMF, CHAR_MC_B_AMF, and CHAR_MC_C_AMF, compared to CHAR alone, and at stage R4, CHAR had significantly higher CHL values than the control. No significant differences were observed in 2021 (Fig. 1). However, the CHAR_MC_C_AMF treatment had the highest values for PW and TGW in 2021 (Table S5).

The correlations between the physiological and agronomic traits of the plants and the composition of the soil were studied using PCA (Fig. 2), which allowed for a full comparison between soil chemical composition, plant response, and rhizobiome formation. PC1 explained 36.9 % of the variance and was positively correlated with plant height and soil Fe and K content. PC2 explained 25.2 % of the variance and was positively correlated with TGW and LTR at maturity and some soil parameters such as Ca, P content, and EC. The treatment with CHAR_MC_C_AMF in both seasons clustered along PC2 was separate from the CHAR_AMF and AMF treatments. The treatment with CHAR_MC_B_AMF clustered along PC1 with the control.

3.3. Characterization of the rhizosphere microbiota

The 16S rRNA gene amplicon sequencing generated a satisfactory number of reads for each sample (Supplementary Dataset 1). A total of 4383 and 8482 Amplicon Sequence Variants (ASVs) were obtained in samples from 2020 and 2021, respectively, and assigned to bacterial taxonomy (Supplementary Dataset 1). Goods' coverage values of the selected samples were >0.99 for all samples, enough to compute reliable alpha-diversity indices. The Shannon index was approximately 5.76 for

A



B



Fig. 1. Agronomic and physiologic data collected during the maize field trials in 2020 (A) and 2021 (B). For each trait, a data standardization was performed, and color code scale from green (indicating reduction) to red (indicating elevation) indicates the variation in the range from -2 to $+2$. Production per plant (kg) (YPL), Production at 15 % humidity (t/ha) (Y15), Grain moisture% (GM), Plant height (cm) (PH), Plant weight (gr) (PW), Ear weight (gr) (EW), Stock weight (gr) (SW), Test weight (Kg/hl) (TW), and Thousand grain weight (gr) (TGW). Chlorophyll content (CHL); leaf transpiration rates (s/cm) (LTR). Vegetative growth stage (V6), flowering (R1), and maturity (R4). CTRL- no addition of biofertilizers or biochar; MC_B and MC_C, synthetic microbial consortia; AMF, mycorrhizae; CHAR, Biochar. Asterisks indicate traits showing significant differences among treatments (ANOVA, Tukey's post hoc test; ** $p < 0.01$, *** $p < 0.001$).

the samples collected in 2020 and slightly less in 2021, although not significantly, where an average Shannon index of 5.1 was measured (Table S6). No significant differences in alpha diversity were observed between treatments ($p > 0.05$), indicating that the application of SynCom did not affect native rhizosphere diversity (Table S6).

Regarding the taxonomic distribution of ASVs, those associated with Proteobacteria (18 %–20 %), Actinobacteria (19 %–30 %), Chloroflexi (5 %–13 %), Firmicutes (7 %–8 %), Verrucomicrobiota (3 %–6 %), and Acidobacteria (7 %–8.5 %) (Fig. 3A, B) were the most abundant in both seasons. Actinobacteria displayed the highest variability in both years, as their abundance was higher in 2020, reaching 30 %, whereas Proteobacteria was stable in both years.

Non-metric Multidimensional Scaling (nMDS), PERMANOVA, and PCA of differentially abundant ASVs (DESeq2 analysis) were then used to determine whether treatments with SynComs significantly altered the native rhizosphere microbiota. Owing to the lack of statistically significant differences among treatments or sampling times, the results indicated that the native microbiota was resilient to the treatments (Fig. 3C, D, Fig. S2 and Table S7), although in 2021, CHAR_AMF, MC_B_AMF, and

CHAR_MC_C_AMF were distributed slightly differently (variance 7.4 % and 4.4 %, respectively) compared to the other samples (Figs. 3D and S2 B). This could be related to the higher beta diversity recorded for these samples, rather than a different microbial distribution. As expected, owing to the lack of significant differences among treatments, no relationships between the rhizosphere microbiota and agronomic and physiological data were found in the first and second seasons of treatment at the flowering or harvesting stages (Tables S7, S8, and Fig. S3), suggesting a negligible effect of SynComs on the maize rhizosphere microbiota under the experimental conditions.

3.4. Untargeted metabolomics of maize kernels

Global metabolic profiles of kernels in both seasons were evaluated using HPLC-HRMS. Polar, semi-polar, and non-polar metabolome chromatograms were subjected to untargeted analyses to retrieve the set of differentially accumulated metabolites (DAM) in the samples under study, as reported in Tables S9 and S10. In samples from the 2020 season, 177 polar/semi-polar and 240 non-polar DAMs showing

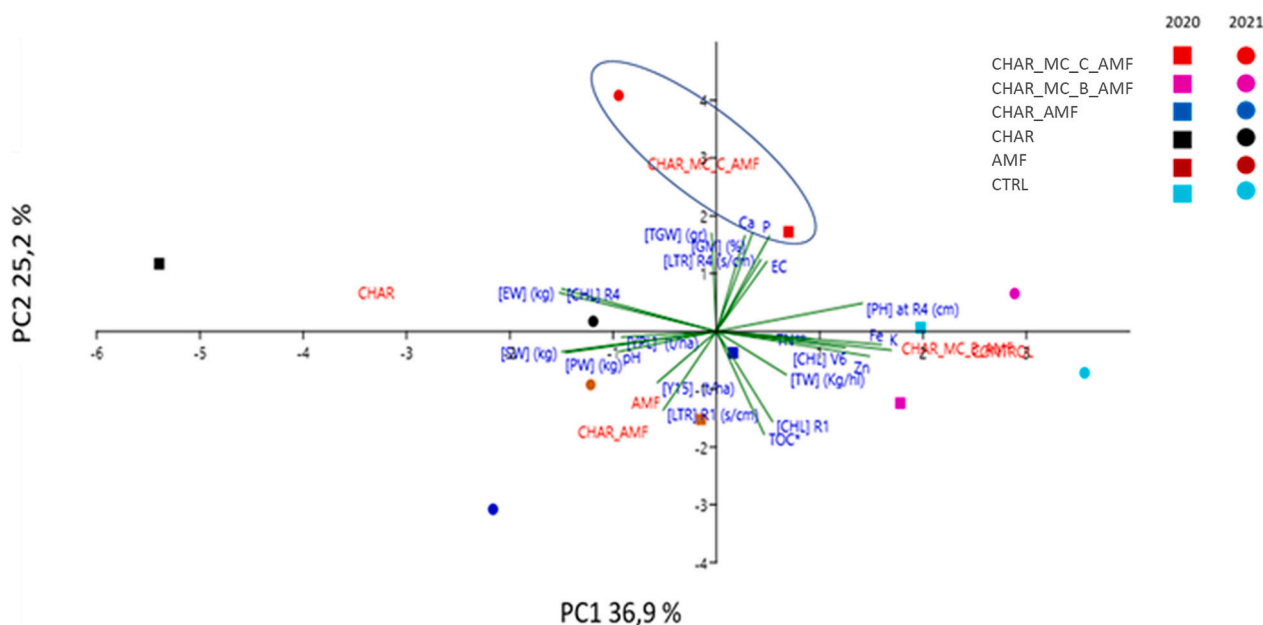


Fig. 2. Principal component analysis (PCA) ordination biplot diagram of maize traits for samples grown under the different treatments in 2020 and 2021. The biplots of the PCA model are calculated using the average values of the investigated parameters for each season. The two first components of the PCA model represented more than half of variation in the dataset (62.1 %). The percentage of total variance as explained by each axis is reported. Symbols' legend for treatments are boxed on the top right. CTRL- no biofertilizers or biochar; MC_B and MC_C, synthetic microbial consortia; AMF, mycorrhizae; CHAR, biochar. In particular, the red circle and square indicate CHAR_MC_C_AMF treatment in 2020 and 2021, the pink circle and square indicate CHAR_MC_B_AMF treatment in 2020 and 2021, the blue circle and square indicate CHAR_AMF treatment in 2020 and 2021, the black circle and square indicate CHAR treatment in 2020 and 2021, the brown circle and square indicate AMF treatment in 2020 and 2021, and sky-blue circle and square indicate the control condition in 2020 and 2021, respectively.

statistically significant alterations (according to pairwise Tukey's *t*-test) were obtained (Table S9), whereas in samples from the 2021 season, 696 polar/semi-polar and 246 non-polar DAMs were significantly altered (Table S10). Using a multivariate bioinformatics approach (PCA), a general overview of the metabolomic profile was obtained when PCA was performed according to treatments and metabolites (Fig. 4). Although it was not possible to obtain a clear separation between treatments in 2020, kernels from plants supplemented with MC_C (CHAR_MC_C_AMF and CHAR_MC_C_F1) were placed slightly farther apart from the other samples on the upper left and right sides of the PCA plot (Fig. 4A). The PCA performed according to the metabolites (Fig. 4B) highlighted those responsible for the variance within the samples that were selected for identification.

The same analysis was performed on the 2021 dataset according to treatments (Fig. 4C) and metabolites (Fig. 4D). A clear separation was observed only for the kernels of plants supplemented with MC_B and CHAR located further along PC2, indicating metabolomic differences (Fig. 4C).

3.5. Targeted metabolomics of polar and non-polar compounds

Using a custom metabolomics database, a targeted analysis was performed on both polar and non-polar species to quantify the levels of a set of primary and secondary metabolites with pro-nutritional (vitamins, phenylpropanoids, benzoxazinoids, etc.) and anti-nutritional (alkaloids, etc.) properties, and quantification of each metabolite in the different samples is listed in detail in Table S11. The selected metabolites accounted for 70 polar species, including primary and secondary metabolites, and 45 non-polar primary (different lipid classes) and secondary (isoprenoids) metabolites. A heatmap depicting the relative abundance of each metabolite compared to the control condition for both years is shown in Fig. 5. In 2020 samples, six DAMs were found in CHAR_MC_B_AMF, five in CHAR_MC_C_AMF, and five in CHAR_MC_C_F1, mostly in the phenolic acid, phenylpropanoid, and benzoxazinoid groups (Fig. 5). Notably, CHAR_MC_C_AMF,

CHAR_MC_B_AMF, and CHAR_AMF samples clustered separately from the control, CHAR, and AMF.

In 2021, 12 DAMs were identified. Specifically, the classes of amino acids, acids (except syringic acid), and benzoxazinoids displayed positive alterations, whereas phenylpropanoids decreased in two samples (AMF in 2020 and CHAR in 2021), as in the case of apigenin. In all the treatments, a significant decrease in azelaic acid, a molecule with anti-inflammatory, antioxidant, and bactericidal effects, was observed (Zhou et al., 2018). Other DAMs increased in 2020 and 2021; rutin and HMBOA-4-Glc over-accumulated in SynComs-treated kernels compared to the control.

Targeted analysis of the non-polar metabolome revealed specific alterations in the MC_B and MC_C treatments (Table S11). In 2020, 10 DAMs were detected in at least one condition compared to the control, many of which belonged to the classes of triglycerides and fatty acids. The CHAR_MC_B_AMF treatment produced the highest number of overabundant compounds. In 2021, 12 DAMs differentiated at least one of the treatments from the control; fatty acids and glycerolipids increased, whereas isoprenoids decreased. Notably, α -tocotrienol and phytofluene were slightly over-accumulated in treatments with AMF and CHAR_MC_C_AMF, respectively. Linolenic acid, undetected in the control, was over-accumulated in all treatments, except for CHAR and MC_C.

Comparing the results of the two seasons for both untargeted and targeted analyses, the set of DAMs that exhibited a conserved profile of over-accumulation or decumulation in maize samples grown in the presence of SynComs included both unknown species and annotated molecules of primary and secondary metabolism (Table 1). For example, D-pipecolic acid and a group belonging to different lipid classes showed a higher content in SynComs-treated maize than in control; tricaproin (C6:0), a triglyceride, was more abundant in SynComs-treated than in control maize kernels. Notably, fatty acids (e.g., 3E,9Z,12Z-octadecatrienoic acid) were decumulated in SynComs samples, whereas phospholipids and diacylglycerols (DGs) showed opposite tendencies.

Fig. 6 displays an overview of the main pathways of primary and

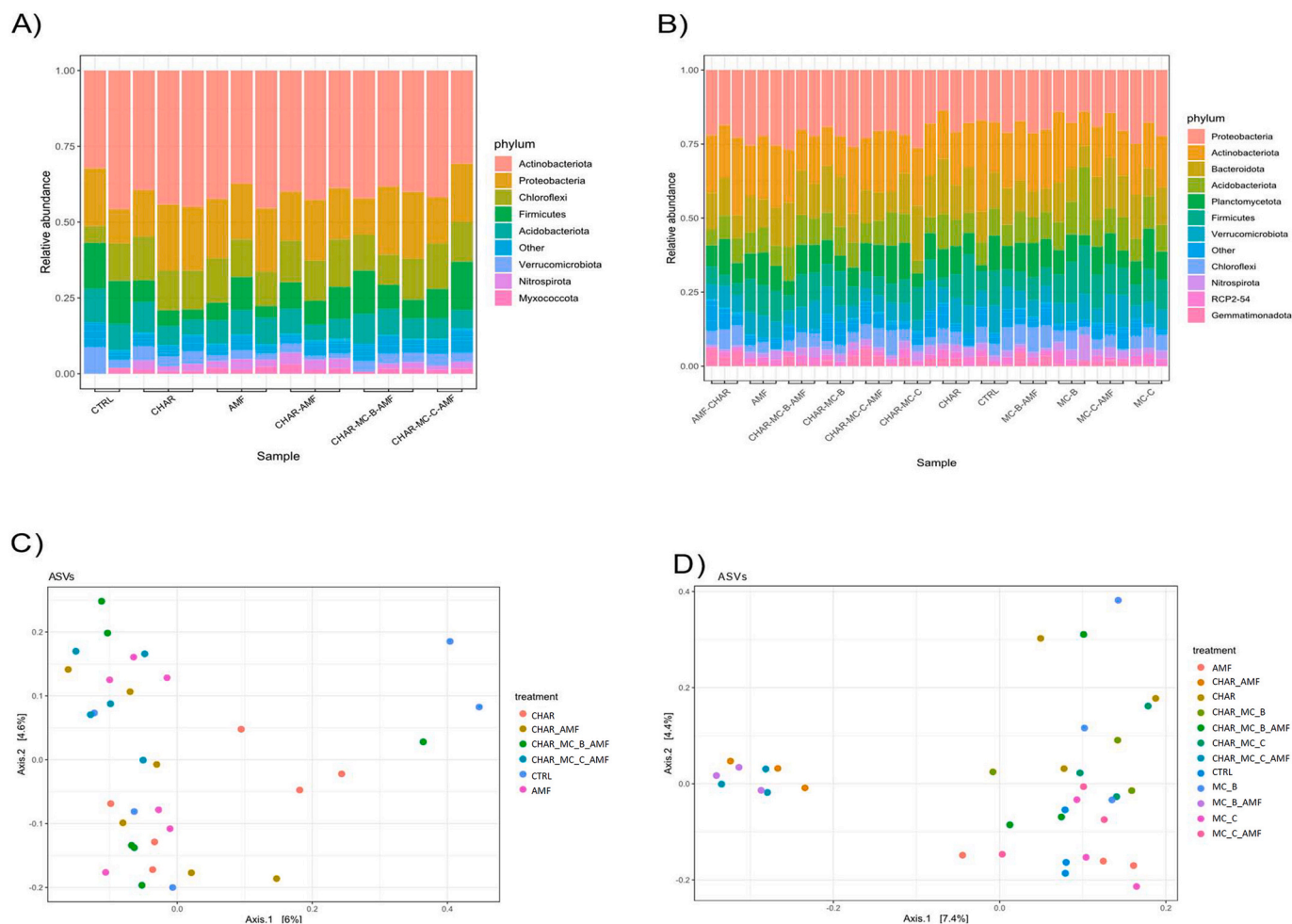


Fig. 3. Relative abundances of taxonomies and rhizospheric maize samples distribution in duplicate according to bacterial community. Abundances of detected ASVs are reported at phylum level. Panel (A) refers to the first season of field trials (2020), whereas Panel (B) refers to the second season of field trials (2021). The colors are ordered according to the legend reported at the bottom of the figure. Principal coordinates analysis (PCoA) of rhizosphere bacterial community was performed using Bray–Curtis distance as ordination method. Colors indicate different treatments applied to maize samples in field trials of season 2020 (C) and 2021 (D). Legends of content of fig. A, B, C, D are placed on their right-sides. CTRL- no biofertilizers or biochar; MC_B and MC_C, synthetic microbial consortia; AMF, mycorrhizae; CHAR, biochar.

secondary metabolism in which the selected metabolites are involved, and their potential roles are reported, which will be further discussed in the discussion section. The set of DAMs for the two seasons could represent potential metabolic biomarkers of SynCom–plant interactions, as well as crop quality.

3.6. Economic and environmental considerations

To make economic and environmental considerations, four different scenarios were considered: (A1) conventional cultivation with NPK cultivation fertilizers, (A2) fertilization with 50 % N, (B) use of biofertilizers with 50 % N, and (C) same as (B) but with biochar. The costs of fertilizer, biochar, or SynComs were calculated following the doses indicated in the Materials and Methods and are reported in Table S12. The fertilization costs (per hectare) were calculated to be 91 € when NPK was applied, 42.5 € when 50 % N was used, 342.5 € when 50 % N was combined with SynComs, and 742.5 € when biochar was also applied. The economic differences were in favor of conventional cultivation or even better of the application of a lower N input, a condition that did not affect yield in our study (Fig. 7B, Table S12). This scenario may change if government support was provided to producers that reduce CO₂ emissions. If we consider the ecological impact, the situation changes significantly. Indeed, when NPK was applied at conventional

concentrations (Scenario A1), 3.5 ton CO₂ eq/ha was produced, and the number diminished if only N was applied (1.5 ton CO₂ eq/ha in Scenario A2). The presence of biofertilizer alone increased this value (2.5 ton CO₂ eq/ha in Scenario B); however, when combining the emissions of N, biofertilizer, and biochar (Scenario C), the emissions were only –7.5 ton CO₂ eq/ha. Therefore, Scenario C strongly diminished the CO₂ emissions.

4. Discussion

4.1. Impacts of biochar, SynComs, and AMF on plant performance

SynComs, alone or in combination with biochar, affected plant growth and yield. When comparing the two seasons, the effects of environmental conditions were more relevant than the treatments. In the 2020 season, SynComs with biochar and AMF had a significant impact on CHL during vegetative growth, whereas at maturity, plants grown with biochar had higher CHL than the control plants. In addition, the overall performance of plants improved when grown with CHAR_MC_C_AMF, with the highest value of PW, plant yield, and TKW. In 2020, an increase in vegetative growth and nutrient uptake was evident in all inoculated plants, whereas in 2021, an increase in yield was observed for MC_C_AMF, but not for MC_C. This uneven

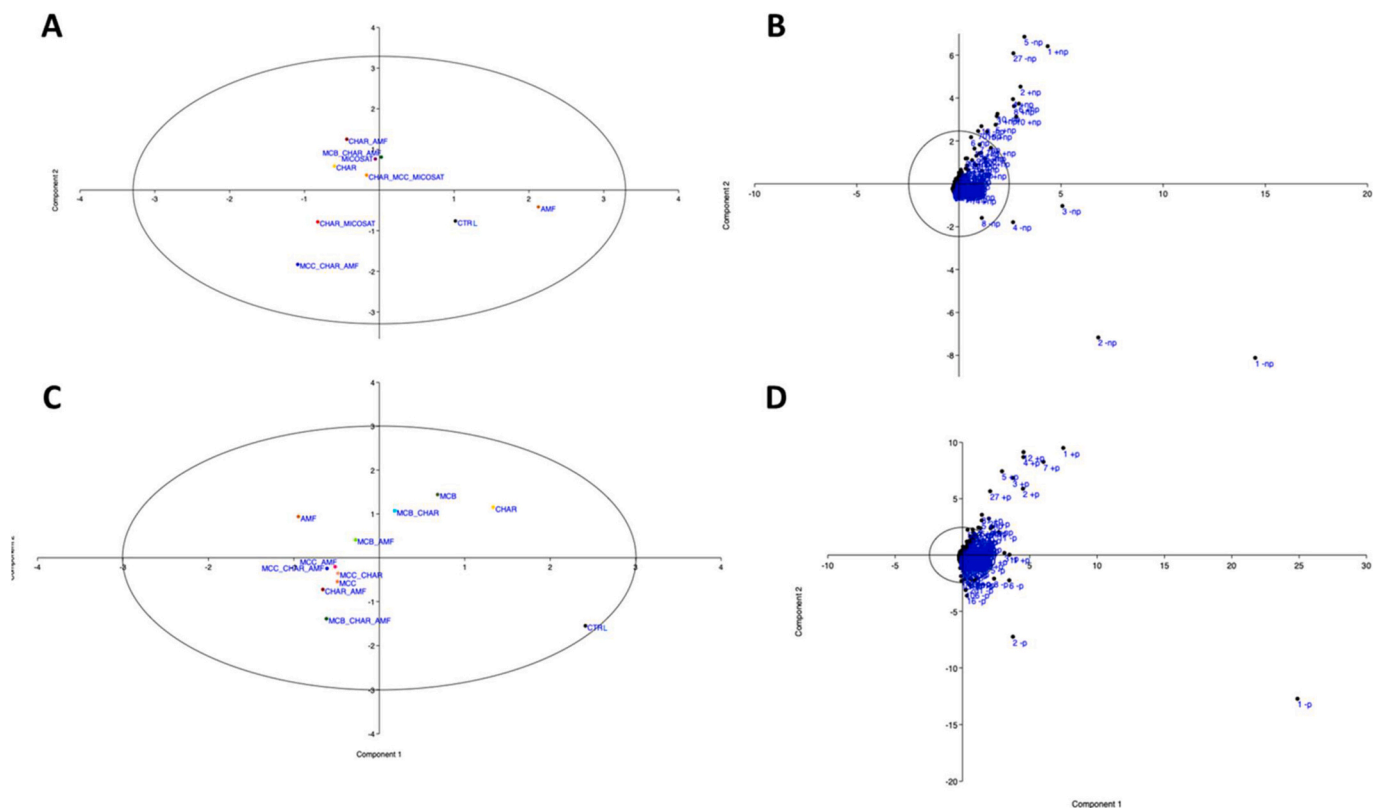


Fig. 4. Principal component analysis (PCA), according to the treatments (A, C) and the metabolites (B, D), of untargeted polar and non-polar metabolomes of maize kernels collected after field trials in 2020 (A, B) and 2021 (C, D). (A) Components 1 and 2 explain >89 % of total variance (74.19 % and 15.31 %, respectively). (B) Components 1 and 2 explain >99 % of total variance (98.61 % and 0.80 %, respectively). (C) Components 1 and 2 explain >88 % of total variance (83.21 % and 5.48 %, respectively), whereas in (D) Components 1 and 2 explain almost the total percentage of variance (99.33 % and 0.48 %, respectively). CTRL- no biofertilizers or biochar; MC_B and MC_C, synthetic microbial consortia; AMF, mycorrhizae; CHAR, Biochar.

performance of SynComs may be related to the antagonism between inoculants and native soil microbiota, as well as to environmental variables such as temperature, humidity, and soil physicochemical structure and composition. Considering the agro-meteorological conditions experienced by the maize plants during the two field trials (Fig. S1), plants experienced mild heat stress in 2021, and rainfall was half as that of 2020. Under field conditions, both biotic and abiotic factors can limit the efficiency of microbial inoculants (Hett et al., 2023; Symanczik et al., 2023; Nguyen, 2018), which may explain why field trials show significant differences within themselves and from pot experiments.

4.2. Rhizosphere microbial ecology

The union of microbial species with complementary functions has been exploited in the last decade owing to their advantages over single species (Liu et al., 2023). In the rhizosphere of plants, the microbiome is frequently modelled by reducing microbial diversity but increasing the presence of some classes, such as Bacteroidetes, Proteobacteria, and other copiotrophs (Ling et al., 2022). Minor changes were observed in the two growing seasons (e.g., Actinobacteria were more abundant in 2020 than in 2021, which could be related to variations in soil pH). The main phyla were Proteobacteria, Actinobacteria, Chloroflexi, Firmicutes, Verrucomicrobiota, and Acidobacteria, which are key groups in the rhizosphere bacteriome (Berg et al., 2021; Ling et al., 2022; Mishra et al., 2022). Bacteria from these phyla play key roles in regulating biogeochemical cycles, breaking down biopolymers, producing exopolysaccharides, and enhancing plant growth (Boubekri et al., 2022; Kalam et al., 2020; van Bergeijk et al., 2020). The bacterial phyla RCP2-54 and Gemmatimonadota have been previously found in the maize rhizosphere and have been reported to be negatively correlated

with ammonium fertilization in maize (Muhammad et al., 2022). Plactomycetes have also been found in the maize rhizosphere (Zhao et al., 2021). The soil microbiota of the maize rhizosphere had a composition similar to the overall distribution of bacterial phyla in European soils (Catania et al., 2022; Labouyrie et al., 2023) and was aligned with the characteristics of southern European soils (Plassart et al., 2019). Unlike previous reports (Cornell et al., 2021), it appears that the complexity and diversity of the rhizosphere ecosystem can handle small changes in species richness (Johnson et al., 2019). An inoculant can decrease microbial diversity (including increased dominance by some native groups) when its presence increases resource availability, or when it becomes dominant and suppresses native taxa (Johnson et al., 2019). A meta-analysis focused on the effect of bioinoculants showed significant results on resident soil microbial diversity in only 62 % of the cases analyzed (Cornell et al., 2021). In this work, the application of SynComs did not significantly change the taxonomic composition of the native rhizosphere microbiota. Previous studies have reported a temporary shift in soil microbiome after SynComs inoculation, as the shift was no longer evident a few weeks after application (Berg et al., 2021). Overall, the evaluation of the long-term effects of bioinoculants in agricultural soils requires further study to better understand and predict their potential success and integration with the resident microbiome in agricultural soils over several years (Mawarda et al., 2020; O'Callaghan et al., 2022). Many factors can favor or reduce the persistence of a microbial inoculum, such as soil characteristics (structure, pH, temperature, moisture, and mineral and organic components), interactions with the resident microbiome, species-specific interactions with plant roots, type of inoculum (single strain, consortium), and delivery systems. Studies have revealed variable responses in terms of inoculum persistence and detectability in the field (days and months), which are not

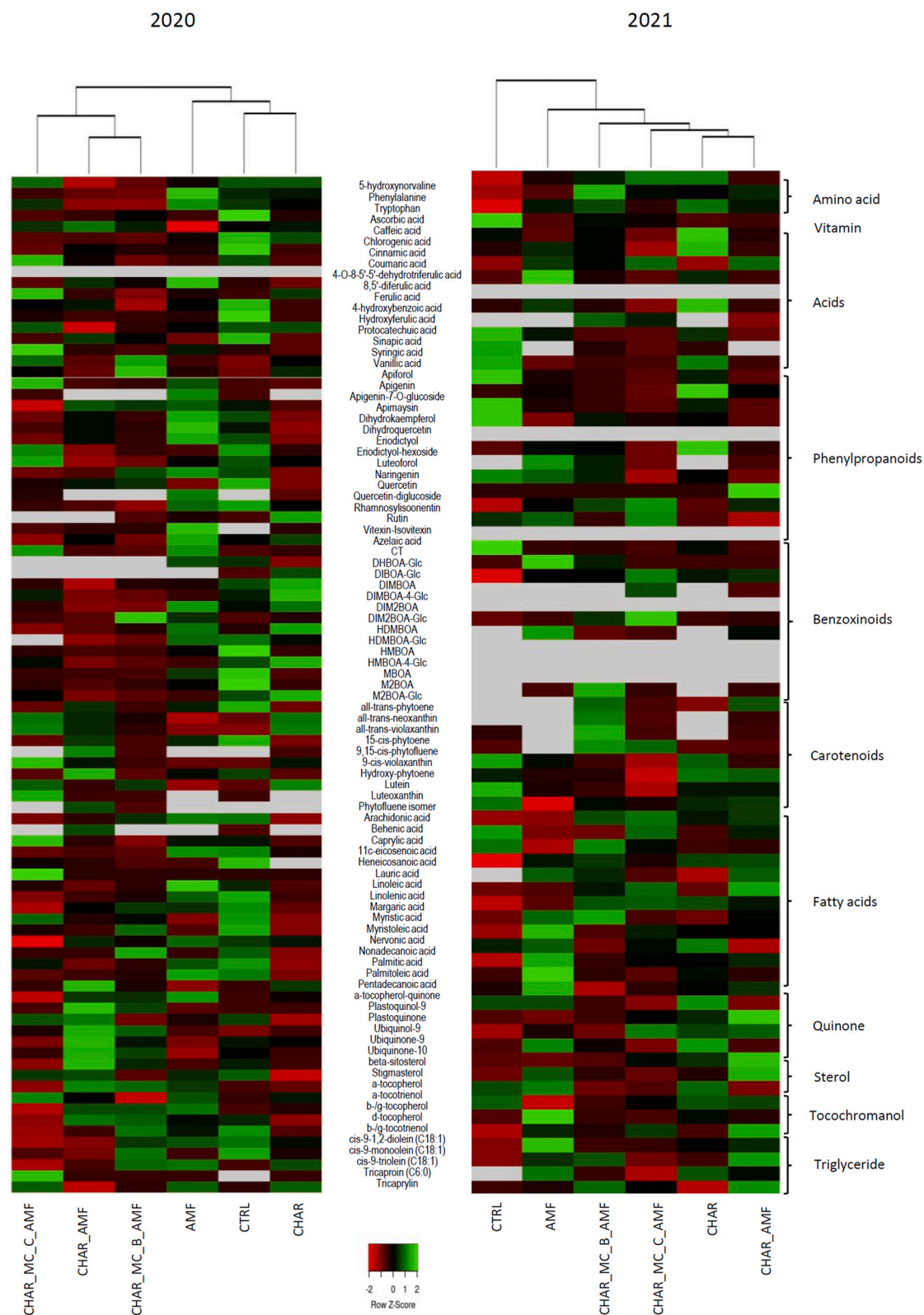


Fig. 5. Heat map representation of HPLC-HRMS-targeted metabolomics. Polar and non-polar ionic species detected in kernels of maize cultivated in 2020 and 2021 from plants grown in presence or absence of microbial consortia B and C, and in combination with AMF and CHAR, were analyzed. The rows display ionic species, and the columns represent the different treatments. A clustered heat map is a two-dimensional representation of data where individual values contained in a matrix are represented as colors. Color code scale from green to red indicates the variation in the range from -2 to $+2$ of decumulated and over-accumulated metabolites, compared to the control, whereas grey refers to undetected species. CTRL- no biofertilizers or biochar; MC.B and MC.C, synthetic microbial consortia; AMF, mycorrhizae; CHAR, biochar. This method groups similar rows and columns of the matrix together based on a chosen similarity measure, with Pearson correlation. The resulting clusters are represented as dendrograms.

Table 1

List of differentially accumulated metabolites identified as potentially metabolic biomarkers for SynComs plant interactions and crop quality. Data come from both untargeted and targeted metabolomics analyses of maize kernel samples collected in field trials of seasons 2020 and 2021.

m/z	Fraction	Voltage	Metabolite ID	2020	2021
130.0864	Polar	pos	D-Pipecolic acid	over-	over-
267.0130	Polar	pos	Nf	over-	over-
279.2318	Polar	pos	3E,9Z,12Z-octadecatrienoic acid	down-	down-
520.3399	Polar	pos	PC(18:2(9Z,12Z)/0:0) 1-linoleoyl-2-(1-enyl-stearoyl)-sn-glycero-3-phosphocholine	over-	over-
521.3432	Polar	pos	Nf	over-	over-
564.3307	Polar	neg	Nf	over-	over-
565.3340	Polar	neg	Nf	over-	over-
296.0659	Polar	pos	Glycerophosphocholine	over-	over-
476.2783	Polar	neg	LysoPE(0:0/18:2(9Z,12Z)) 2-linoleoyl-sn-glycero-3-phosphoethanolamine (glycerophospholipid)	over-	over-
522.3558	Polar	pos	PC(O-16:1(11Z)/2:0) 1-(11Z-hexadecenyl)-2-acetyl-sn-glycero-3-phosphocholine (Glycerophospholipids)	over-	over-
566.3466	Polar	neg	PS (21:0/0:0) 1-heneicosanoyl-glycero-3-phosphoserine (Glycerophospholipids)	over-	over-
891.2847	Polar	neg	Nf	over-	over-
576.5063	Non polar	pos	Nf	over-	over-
187.0975	Polar	neg	Azelaic acid	down-	down-
387.2740	Non polar	pos	Tricaproin (C6:0)	over-	over-
358.1132	Polar	pos	HMBOA-4-Glc	over-	over-
611.1606	Polar	pos	Rutin	over-	over-

always related to the long-term effects on the resident microbiome (Mawarda et al., 2020). Further studies are required to assess the resilience of resident microbiomes in terms of their functional characteristics.

Although no long-term differences were detected in the microbial population, the application of biochar to soil is expected to have long-term positive effects. Recent reviews have shown that long-term biochar application can increase soil organic carbon sequestration, increase crop yield, improve soil fertility, increase microbial activity, and sequester excess nutrients and heavy metals (Jiang et al., 2024; Gross et al., 2024; Waheed et al., 2025).

4.3. Kernel metabolites, chemistry, and function as defense, food, feed

Plants can counteract environmental stimuli by producing useful compounds (Medeiros et al., 2021; Nephali et al., 2021). Studies have been conducted on metabolic reprogramming in the rhizosphere of wheat and sorghum (Carlson et al., 2019; Mashabela et al., 2022), on the effect of biochar on the leaf metabolome (He et al., 2024), and on the cross-talk between plants and the soil microbiome through root exudates (Kimotho and Maina, 2024); however, few reports are available on SynCom-induced metabolic perturbation in maize kernels (Berta et al., 2014; Ocwa et al., 2024).

The DAMs detected in the two years showed an increased presence of glycerophospholipids, which are the main constituents of cell membranes (Reszczyńska and Hanaka, 2020), act as signaling molecules (Welti et al., 2007) and are involved in plant defense against pathogens (Cavaco et al., 2021). Glycerophosphocholine, a water-soluble choline molecule, reportedly prevents cognitive decline and promotes longevity in patients with Alzheimer's disease (Liu et al., 2022). Among these

glucoheptonic acids, 3E,9Z,12Z-octadecatrienoic acid and 12-oxo-10E-octadecenoic acid were decumulated during both seasons.

Pathways for the biosynthesis of pipecolic acid have been identified in several monocot and dicot species (Aliferis et al., 2014; Návarová et al., 2013), including economically and nutritionally important cereals (García-Secco et al., 2017; Moller, 1976). Studies have shown that pipecolic acid accumulates strongly in response to phytopathogens in leaves but also as a compatible solute to protect cells from stressful conditions (Arruda and Barreto, 2020; Kiyota et al., 2015). In the kernel context, its amount increased under SynComs treatment conditions, indicating an attempt to increase resistance to environmental stress. High temperature and drought stress in some critical phases of the maize growth cycle determine physiological and metabolic alterations (amyloplast biogenesis, endosperm cell division, and starch biosynthesis), causing a decrease in grain size, which is responsible for yield reductions (Waqas et al., 2021); and an increase in protective molecules may counterbalance this negative trend.

Kernels from SynCom-treated plants showed an increased amount of HMBOA-4-Glc compared to that of the control. This compound belongs to the family of benzoxazinoids (BXs), which are indole-derived plant defense compounds (e.g., antifeedant, insecticidal, antimicrobial, and allelopathic) (Niculaes et al., 2018) found in wild and cultivated *Poaaceae*, and maize is considered a model species to study their role (Kokubo et al., 2017). BXs are abundant in young root and shoot tissues where they are glucosylated and stored in vacuoles or released as root exudates (Schütz et al., 2019). Lower levels were detected in wheat and rye seeds; however, no clear evidence was found in maize (Zhou et al., 2018). In humans, anti-allergenic and anti-inflammatory effects have been observed. However, BXs have also been reported to suppress appetite and reduce the cellular uptake of glucose. Furthermore, BXs seem to influence the gut microbial community (Adhikari et al., 2015).

The kernels of SynCom-treated plants displayed over-accumulation and decumulation of rutin and azelaic acid, respectively. Rutin is a dietary flavonoid found in many vegetables and fruits. Its therapeutic effects are attributed to its antioxidant and anti-inflammatory properties. Studies have demonstrated its broad range of beneficial health properties, such as the prevention of neurodegenerative disorders, cardiovascular diseases, and skin cancer. However, the health benefits of rutin depend on its quantity and bioavailability (Frutos et al., 2019). Azelaic acid, which is less abundant in kernels of SynCom-treated maize, is a general marker of lipid peroxidation rather than an immune signal and accumulates in response to biotic stress (Wu et al., 2013). Fatty acids, organic acids, and phenylpropanoids were the main DAM classes observed (Fig. 6). They can be considered metabolomic markers for SynCom-induced plant priming, which may impact both plant adaptations to biotic and abiotic stress and the health-related properties of maize utilized for human nutrition.

4.4. Carbon footprint (CF) in transition to biofertilizers

Agriculture accounts for approximately 30 % of global GHG emissions, and the four main commodities—maize, wheat, rice, and barley—account for approximately 70 % of the total. Maize CF was calculated in different environments and was estimated to be in the range of 300–1000 kg CO₂ eq/ton of grain (Fantin et al., 2017; Li et al., 2021; Mounsgree et al., 2022; Supasri et al., 2020).

Chemical fertilizers account for approximately 70 % of GHG emissions per ton of grains (Fantin et al., 2017; Holka et al., 2017; Mounsgree et al., 2022), whereas the contribution of all the other phases (sowing, tillage, harvesting, and transport) to the Global Warming Potential (GWP) is approximately 15 % (for pesticides, ~5 %). The chemistry employed for maize cultivation affects both the environment (acidification, eutrophication of freshwater, and marine eutrophication) and human health (toxicity, carcinogenicity, photochemical ozone formation, and resource depletion) (Fantin et al., 2017). Overall, from an economic perspective, the co-application of biochar with SynComs

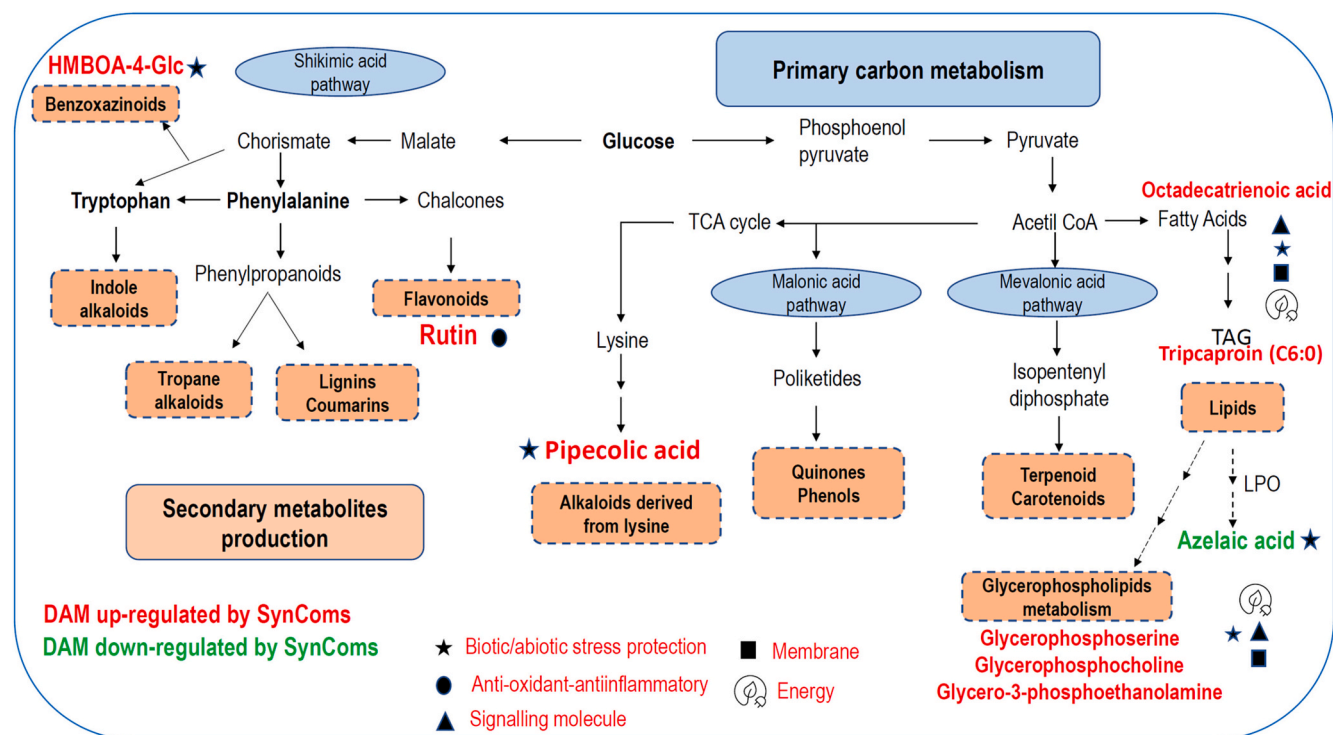


Fig. 6. Overview of the main pathways in primary and secondary metabolism involving the selected set of metabolites. The differentially abundant metabolites (DAMs) are indicated with the red font if over-accumulated and in green font if decumulated in samples treated with SynComs as compared to the control. The biological role of each metabolite is indicated by different symbols reported in the legend at the bottom of the figure.

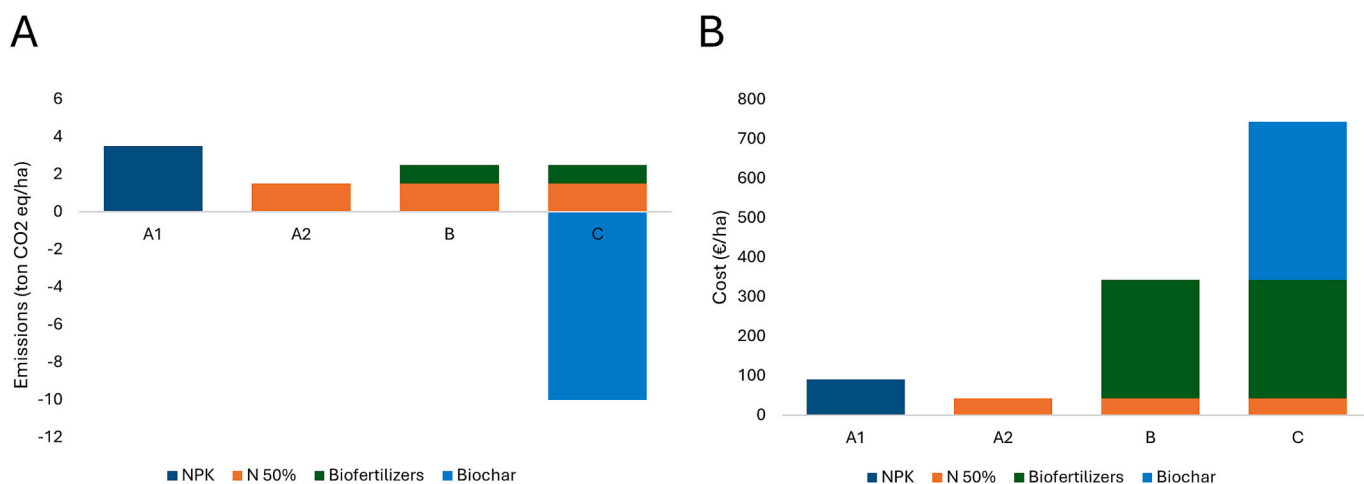


Fig. 7. Impact of the use of biofertilizers and biochar on the fertilization cost (A) and emissions (in terms of CO₂ eq/ton grains) as compared to conventional fertilizer. Three scenarios are compared: Scenario (A): fertilization with normal NPK fertilization; Scenario (B): fertilization with 50 kg/ha N and 10 kg/ha of biofertilizers; and Scenario (C): fertilization with 50 kg/ha N and 10 kg/ha biofertilizers, with delivery by biochar at 2 ton/ha. This comparison was possible because: the yields between the plots in the three conditions were similar, no disease was observed in plants and grains, and no chemical weeding was necessary. The approach used to discuss the data obtained, in a sustainability perspective, is more analytical than empirical, and the data reported are not necessarily related with the input/output of the field experiment but mainly referred from various literature and averaged for simplification.

remains at the research and development stage, as the costs are still high. However, the evaluation changes if the environmental impact is considered, as the combination of biochar and biofertilizer reduced the CO₂ emissions by three-fold, lowering the risk to human and environmental health. Similar calculations have been previously reported for wheat (Caldara et al., 2024). To date, the use of biofertilizers has been explored; in fact, the European Union has recently regulated their use (European Union Regulation, 2019), whereas biochar has been used as a soil amendment for some years (European Union Regulation, 2008). The

work presented here shows encouraging results in terms of both production yield and, more importantly, the environmental impact. Therefore, it is expected that, in the coming years, this new sustainable and circular mode of cultivation will be more widely employed, which will contribute to a strong reduction in the production costs of biofertilizers, favoring their large-scale use. In particular, if their distribution, as in this study, is performed with machines already used in agriculture for conventional cultivation, no additional costs must be considered for their application to crops.

5. Conclusion

In conclusion, the application of SynComs did not affect the biodiversity of the native rhizosphere microbial community, thus representing an environmentally friendly tool for more sustainable maize cultivation. SynComs affected kernel quality, with the metabolomic profile of maize kernels enriched in anti-stress metabolites and molecules advantageous to human health. Our results indicated that SynComs and biochar can partially replace the intense use of chemical fertilizers, pesticides, and insecticides. As suggested by Life Cycle Analysis studies on maize production in several countries, the main hotspots for all impact categories are fertilizers and pesticides (Boone et al., 2016; Fantin et al., 2017; Holka et al., 2017; Hou et al., 2021; Król-Badziak et al., 2021; Li et al., 2021; Moungsree et al., 2022; Supasri et al., 2020; Zhang et al., 2018b; Zhong et al., 2020). The transition to more sustainable agriculture is necessary and possible, and there are suitable alternatives to chemical fertilizers in terms of biofertilizers (such as SynComs) and novel amendments (such as biochar) to reduce CF. However, a gross inspection of heterogeneous literature data referring to a crop (maize) that has heterogeneous behavior in different countries did not show similar economic advantages.

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CRedit authorship contribution statement

Mariolina Gulli: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Lisa Cangioli:** Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Sarah Frusciante:** Visualization, Software, Methodology, Investigation, Data curation. **Sara Graziano:** Software, Methodology, Investigation, Data curation. **Marina Caldara:** Methodology, Investigation. **Alessia Fiore:** Formal analysis. **Alexandra M. Klonowski:** Software, Investigation, Data curation. **Elena Maestri:** Project administration. **Andrea Brunori:** Writing – review & editing, Methodology. **Alessio Mengoni:** Writing – review & editing, Writing – original draft. **Anne Pihlanto:** Project administration, Funding acquisition. **Gianfranco Diretto:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis. **Nelson Marmiroli:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis. **Annamaria Bevivino:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

- Adhikari, K.B., Tanwir, F., Gregersen, P.L., Steffensen, S.K., Jensen, B.M., Poulsen, L.K., Nielsen, C.H., Høyer, S., Borre, M., Fomsgaard, I.S., 2015. Benzoxazinoids: cereal phytochemicals with putative therapeutic and health-protecting properties. *Mol. Nutr. Food Res.* <https://doi.org/10.1002/mnfr.201400717>.
- Aliferis, K.A., Faubert, D., Jabaji, S., 2014. A metabolic profiling strategy for the dissection of plant defense against fungal pathogens. *PLoS One* 9, e111930. <https://doi.org/10.1371/JOURNAL.PONE.0111930>.
- Ammar, E.E., Rady, H.A., Khattab, A.M., Amer, M.H., Mohamed, S.A., Elodamy, N.I., Al-Farga, A., Aioub, A.A.A., 2023. A comprehensive overview of eco-friendly biofertilizers extracted from living organisms. *Environ. Sci. Pollut. Res.* <https://doi.org/10.1007/s11356-023-30260-x>.
- Arruda, P., Barreto, P., 2020. Lysine catabolism through the saccharopine pathway: enzymes and intermediates involved in plant responses to abiotic and biotic stress. *Front. Plant Sci.* <https://doi.org/10.3389/fpls.2020.00587>.
- Banerjee, S., van der Heijden, M.G.A., 2022. Soil microbiomes and one health. *Nat. Rev. Microbiol.* 2022 21:1 21, 6–20. doi:<https://doi.org/10.1038/s41579-022-00779-w>.
- Banerjee, S., Zhao, C., Garland, G., Edlinger, A., García-Palacios, P., Romdhane, S., Degruene, F., Pescador, D.S., Herzog, C., Camuy-Velez, L.A., Bascompte, J., Hallin, S., Philippot, L., Maestre, F.T., Rillig, M.C., van der Heijden, M.G.A., 2024. Biotic homogenization, lower soil fungal diversity and fewer rare taxa in arable soils across Europe. *Nat. Commun.* 2024 15:1 15, 1–10. doi:<https://doi.org/10.1038/s41467-023-44073-6>.
- Beesley, L., Marmiroli, M., Pagano, L., Pighi, V., Fellet, G., Fresno, T., Vamerli, T., Bandiera, M., Marmiroli, N., 2013. Biochar addition to an arsenic contaminated soil increases arsenic concentrations in the pore water but reduces uptake to tomato plants (*Solanum lycopersicum* L.). *Sci. Total Environ.* 454–455, 598–603. <https://doi.org/10.1016/j.scitotenv.2013.02.047>.
- Benincasa, P., D'Amato, R., Falcinelli, B., Troni, E., Fontanella, M.C., Frusciante, S., Guiducci, M., Beone, G.M., Businelli, D., Diretto, G., 2020. Grain endogenous selenium and moderate salt stress work as synergic elicitors in the enrichment of bioactive compounds in maize sprouts. *Agronomy* 2020, Vol. 10, Page 735 doi: <https://doi.org/10.3390/AGRONOMY10050735>.
- Berg, G., Kusstatscher, P., Abdelfattah, A., Cernava, T., Smalla, K., 2021. Microbiome modulation—toward a better understanding of plant microbiome response to microbial inoculants. *Front. Microbiol.* 12, 650610. <https://doi.org/10.3389/FMICB.2021.650610/BIBTEX>.
- van Bergeijk, D.A., Terlouw, B.R., Medema, M.H., van Wezel, G.P., 2020. Ecology and genomics of Actinobacteria: new concepts for natural product discovery. *Nat. Rev. Microbiol.* 18, 546–558. <https://doi.org/10.1038/s41579-020-0379-y>.
- Berta, G., Copetta, A., Gamalero, E., Bona, E., Cesaro, P., Scarafoni, A., D'Agostino, G., 2014. Maize development and grain quality are differentially affected by mycorrhizal fungi and a growth-promoting pseudomonad in the field. *Mycorrhiza* 24, 161–170. <https://doi.org/10.1007/s00572-013-0523-x>.

- Bindraban, P.S., Dimkpa, C., Nagarajan, L., Roy, A., Rabbinge, R., 2015. Revisiting fertilisers and fertilisation strategies for improved nutrient uptake by plants. *Biol. Fertil. Soils* 51, 897–911. <https://doi.org/10.1007/s00374-015-1039-7>.
- Boone, L., Van Linden, V., De Meester, S., Vandecasteele, B., Muylle, H., Roldán-Ruiz, I., Nemeček, T., Dewulf, J., 2016. Environmental life cycle assessment of grain maize production: an analysis of factors causing variability. *Sci. Total Environ.* 553, 551–564. <https://doi.org/10.1016/j.scitotenv.2016.02.089>.
- Boubekri, K., Soumare, A., Mardad, I., Lyamlouli, K., Ouhdouch, Y., Hafidi, M., Kouisni, L., 2022. Multifunctional role of Actinobacteria in agricultural production sustainability: a review. *Microbiol. Res.* <https://doi.org/10.1016/j.micres.2022.127059>.
- Brassard, P., Godbout, S., Pelletier, F., Raghavan, V., Palacios, J.H., 2018. Pyrolysis of switchgrass in an auger reactor for biochar production: a greenhouse gas and energy impacts assessment. *Biomass Bioenergy* 116, 99–105. <https://doi.org/10.1016/j.biombioe.2018.06.007>.
- Caldara, M., Gulli, M., Graziano, S., Riboni, N., Maestri, E., Mattarozzi, M., Bianchi, F., Careri, M., Marmiroli, N., 2024. Microbial consortia and biochar as sustainable biofertilisers: analysis of their impact on wheat growth and production. *Sci. Total Environ.* 917, 170168. <https://doi.org/10.1016/j.scitotenv.2024.170168>.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13:7 13, 581–583. <https://doi.org/10.1038/nmeth.3869>.
- Callens, K., Fontaine, F., Sanz, Y., Bogdanski, A., D'Hondt, K., Lange, L., Smidt, H., van Overbeek, L., Kotic, T., Maguin, E., Meisner, A., Sarand, I., Sessitsch, A., 2022. Microbiome-based solutions to address new and existing threats to food security, nutrition, health and agrifood systems' sustainability. *Front. Sustain. Food Syst.* 6, 537. <https://doi.org/10.3389/FSUFS.2022.1047765/BIBTEX>.
- Cangioli, L., Mancini, M., Napoli, M., Fagorzi, C., Orlandini, S., Vaccaro, F., Mengoni, A., 2022. Differential response of wheat rhizosphere bacterial community to plant variety and fertilization. *Int. J. Mol. Sci.* 23. <https://doi.org/10.3390/ijms23073616>.
- Carlson, R., Tugizimana, F., Steenkamp, P.A., Dubery, I.A., Labuschagne, N., 2019. Differential metabolic reprogramming in *Paenibacillus alvei*-primed *Sorghum bicolor* seedlings in response to *fusarium pseudograminearum* infection. *Metabolites* 9. <https://doi.org/10.3390/metabo9070150>.
- Catania, V., Bueno, R.S., Alduina, R., Grilli, E., La Mantia, T., Castaldi, S., Quatrini, P., 2022. Soil microbial biomass and bacterial diversity in southern European regions vulnerable to desertification. *Ecol. Indic.* 145, 109725. <https://doi.org/10.1016/j.ecolind.2022.109725>.
- Cavaco, A.R., Ana, Matos, R., Figueiredo, A., 2021. Speaking the language of lipids: the cross-talk between plants and pathogens in defence and disease. *Cell. Mol. Life Sci.* 78, 4399–4415. <https://doi.org/10.1007/s00018-021-03791-0>.
- Chandini, R.K., Kumar, Randeep, Kumar, Ravendra, Om, P., 2019. The impact of chemical fertilizers on our environment and ecosystem. In: *Research Trends in Environmental Sciences*, pp. 71–86.
- Chaudhary, P., Singh, S., Chaudhary, A., Sharma, A., Kumar, G., 2022. Overview of biofertilizers in crop production and stress management for sustainable agriculture. *Front. Plant Sci.* 13. <https://doi.org/10.3389/FPLS.2022.930340>.
- Chouhan, G.K., Jaiswal, D.K., Gaurav, A.K., Mukherjee, A., Verma, J.P., 2021. PGPM as a potential bioinoculant for enhancing crop productivity under sustainable agriculture. In: *Biofertilizers: Vol 1: Advances in Bio-Inoculants*. Elsevier, pp. 221–237. <https://doi.org/10.1016/B978-0-12-821667-5.00009-9>.
- Cornell, C., Kokkoris, V., Richards, A., Horst, C., Rosa, D., Bennett, J.A., Hart, M.M., 2021. Do bioinoculants affect resident microbial communities? A meta-analysis. *Front. Agron.* 3, 753474. <https://doi.org/10.3389/fagro.2021.753474>.
- De Palma, M., Scotti, R., D'Agostino, N., Zaccardelli, M., Tucci, M., 2022. Phyto-friendly soil bacteria and fungi provide beneficial outcomes in the host plant by differentially modulating its responses through (in)direct mechanisms. *Plants* 11, 2672. <https://doi.org/10.3390/PLANTS11202672/S1>.
- Deshoux, M., Sadet-Bourgeteau, S., Gentil, S., Prévost-Bouré, N.C., 2023. Effects of biochar on soil microbial communities: a meta-analysis. *Sci. Total Environ.* 902, 166079. <https://doi.org/10.1016/j.scitotenv.2023.166079>.
- Destoumieux-Garzón, D., Mavingui, P., Boetsch, G., Boissier, J., Darriet, F., Duboz, P., Fritsch, C., Giraudoux, P., Roux, F. Le, Morand, S., Paillard, C., Pontier, D., Sueur, C., Voituren, Y., 2018. The one health concept: 10 years old and a long road ahead. *Front. Vet. Sci.* 5, 14. <https://doi.org/10.3389/FVETS.2018.00014/BIBTEX>.
- Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L., Zheng, B., 2016. Biochar to improve soil fertility. A review. *Agron. Sustain. Dev.* 2016 36:2 36, 1–18. <https://doi.org/10.1007/S13593-016-0372-Z>.
- Dono, G., Rambla, J.L., Frusciantè, S., Granell, A., Diletto, G., Mazzucato, A., 2020. Color mutations alter the biochemical composition in the San Marzano tomato fruit. *Metabolites* 10, 110. <https://doi.org/10.3390/METABO10030110>.
- European Union Regulation, 2008. No 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives (Text with EEA relevance) OJ L 312, 22.11.2008, <http://data.europa.eu/eli/dir/2008/98/oj> (version: 18/02/2024).
- European Union Regulation, 2019. No 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003 PE/76/2018/REV/1, <http://data.europa.eu/eli/reg/2019/1009/oj> (version 17/11/2024).
- Fantini, V., Righi, S., Rondini, I., Masoni, P., 2017. Environmental assessment of wheat and maize production in an Italian farmers' cooperative. *J. Clean. Prod.* 140, 631–643. <https://doi.org/10.1016/J.JCLEPRO.2016.06.136>.
- Fracasso, A., Telò, L., Lanfranco, L., Bonfante, P., Amaducci, S., 2020. Physiological beneficial effect of *Rhizoglyphus intraradices* inoculation on tomato plant yield under water deficit conditions. *Agronomy* 10. <https://doi.org/10.3390/agronomy10010071>.
- Frutos, M.J., Rincón-Frutos, L., Valero-Cases, E., 2019. Rutin. *Nonvitamin and Nonmineral Nutritional Supplements*, 111–117. <https://doi.org/10.1016/B978-0-12-812491-8.00015-1>.
- García-Seco, D., Chiappello, M., Bracale, M., Pesce, C., Bagnaresi, P., Dubois, E., Moulin, L., Vannini, C., Koebnik, R., 2017. Transcriptome and proteome analysis reveal new insight into proximal and distal responses of wheat to foliar infection by *Xanthomonas translucens*. *Sci. Rep.* 7, 10157. <https://doi.org/10.1038/s41598-017-10568-8>.
- Graziano, S., Caldara, M., Gulli, M., Bevinno, A., Maestri, E., Marmiroli, N., 2022. A metagenomic and gene expression analysis in wheat (*T. durum*) and maize (Z. mays) biofertilized with PGPM and biochar. *Int. J. Mol. Sci.* 23, 10376. <https://doi.org/10.3390/IJMS231810376/S1>.
- Gross, A., Bromm, T., Polifka, S., Fischer, D., Glaser, B., 2024. Long-term biochar and soil organic carbon stability—evidence from field experiments in Germany. *Sci. Total Environ.* 954, 176340. <https://doi.org/10.1016/j.scitotenv.2024.176340>.
- Haeldermans, T., Campion, L., Kuppens, T., Vanreppelen, K., Cuypers, A., Schreurs, S., 2020. A comparative techno-economic assessment of biochar production from different residue streams using conventional and microwave pyrolysis. *Bioresour. Technol.* 318, 124083. <https://doi.org/10.1016/J.BIORTECH.2020.124083>.
- Hamedani, Z., Mays, Del Zotto, L., Bocci, E., Colantoni, A., Villarini, M., 2019. Eco-efficiency assessment of bioelectricity production from Iranian vineyard biomass gasification. *Biomass Bioenergy* 127, 105271. <https://doi.org/10.1016/J.BIOMBIOE.2019.105271>.
- He, M., Xiong, X., Wang, L., Hou, D., Bolan, N.S., Ok, Y.S., Rinklebe, J., Tsang, D.C.W., 2021. A critical review on performance indicators for evaluating soil biota and soil health of biochar-amended soils. *J. Hazard. Mater.* 414, 125378. <https://doi.org/10.1016/j.jhazmat.2021.125378>.
- He, T., Chen, Lin, Wu, Y., Wang, J., Wu, Q., Sun, J., Ding, C., Zhou, T., Chen, Limin, Jin, A., Li, Y., Zhu, Q., 2024. Combined metabolome and transcriptome analyses of maize leaves reveal global effect of biochar on mechanisms involved in anti-herbivory to *Spodoptera frugiperda*. *Metabolites* 14. <https://doi.org/10.3390/metabo14090498>.
- Hett, J., Neuhoﬀ, D., Döring, T.F., Masoero, G., Ercole, E., Bevinno, A., 2022. Effects of multi-species microbial inoculants on early wheat growth and litterbag microbial activity. *Agronomy* 2022 (12), 899. <https://doi.org/10.3390/AGRONOMY12040899>.
- Hett, J., Döring, T.F., Bevinno, A., Neuhoﬀ, D., 2023. Impact of microbial consortia on organic maize in a temperate climate varies with environment but not with fertilization. *Eur. J. Agron.* 144, 126743. <https://doi.org/10.1016/J.EJA.2023.126743>.
- Holka, M., Bieńkowski, J.F., Jankowiak, J., Dąbrowicz, R., 2017. Life cycle assessment of grain maize in intensive, conventional crop production system. *Rom. Agric. Res.* 34.
- Hou, L., Yang, Y., Zhang, X., Jiang, C., 2021. Carbon footprint for wheat and maize production modulated by farm size: a study in the North China plain. *Int. J. Clim. Chang. Strateg. Manag.* 13, 302–319. <https://doi.org/10.1108/IJCCSM-10-2020-0110>.
- Hussain, A., Ahmad, M., Zahid Mumtaz, M., Nazli, F., Aslam Farooqi, M., Khalid, I., Iqbal, Z., Arshad, H., 2019. Impact of integrated use of enriched compost, biochar, humic acid and Alcaligenes sp. AZ9 on maize productivity and soil biological attributes in natural field conditions. *Ital. J. Agron.* 14, 101–107. <https://doi.org/10.4081/ija.2019.1413>.
- Iosa, I., Agrimonti, C., Marmiroli, N., 2024. Real-time PCR (qPCR) to discover the fate of plant growth-promoting rhizobacteria (PGPR) in agricultural soils. *Microorganisms* 12. <https://doi.org/10.3390/microorganisms12051002>.
- Jiang, Y., Li, T., Xu, X., Sun, J., Pan, G., Cheng, K., 2024. A global assessment of the long-term effects of biochar application on crop yield. *Curr. Res. Environ. Sust.* 7, 100247. <https://doi.org/10.1016/j.crsust.2024.100247>.
- Johnson, J.S., Spakowicz, D.J., Hong, B.Y., Petersen, L.M., Demkowicz, P., Chen, L., Leopold, S.R., Hanson, B.M., Agresta, H.O., Gerstein, M., Sodergren, E., Weinstock, G.M., 2019. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nat. Commun.* 10. <https://doi.org/10.1038/s41467-019-13036-1>.
- Kalam, S., Basu, A., Ahmad, I., Sayyed, R.Z., El-Enshasy, H.A., Dailin, D.J., Suriani, N.L., 2020. Recent understanding of soil acidobacteria and their ecological significance: a critical review. *Front. Microbiol.* 11, 580024. <https://doi.org/10.3389/FMICB.2020.580024/BIBTEX>.
- Kandlikar, G.S., Gold, Z.J., Cowen, M.C., Meyer, R.S., Freise, A.C., Kraft, N.J.B., Moberg-Parker, J., Sprague, J., Kushner, D.J., Curd, E.E., 2018. Ranacapa: an R package and shiny web app to explore environmental DNA data with exploratory statistics and interactive visualizations. *F1000Res* 7. <https://doi.org/10.12688/F1000RESEARCH.16680.1>.
- Kimotho, R.N., Maina, S., 2024. Unraveling plant–microbe interactions: can integrated omics approaches offer concrete answers? *J. Exp. Bot.* 75, 1289–1313. <https://doi.org/10.1093/JXB/ERAD448>.
- Kiyota, E., Pena, I.A., Arruda, P., 2015. The saccharopine pathway in seed development and stress response of maize. *Plant Cell Environ.* 38, 2450–2461. <https://doi.org/10.1111/PCE.12563>.
- Kokubo, Y., Nishizaka, M., Ube, N., Yabuta, Y., Tebayashi, S.I., Ueno, K., Taketa, S., Ishihara, A., 2017. Distribution of the tryptophan pathway-derived defensive secondary metabolites gramine and benzoxazinones in Poaceae. *Biosci. Biotechnol. Biochem.* 81, 431–440. <https://doi.org/10.1080/09168451.2016.1256758>.
- Król-Badziak, A., Pishgar-Komleh, S.H., Rozakis, S., Księżak, J., 2021. Environmental and socio-economic performance of different tillage systems in maize grain production:

- application of life cycle assessment and multi-criteria decision making. *J. Clean Prod.* 278, 123792. <https://doi.org/10.1016/J.JCLEPRO.2020.123792>.
- Labouyrie, M., Ballabio, C., Romero, F., Panagos, P., Jones, A., Schmid, M.W., Mikryukov, V., Dulya, O., Tedersoo, L., Bahram, M., Lugato, E., van der Heijden, M. G.A., Orgiazzi, A., 2023. Patterns in soil microbial diversity across Europe. *Nat. Commun.* 2023 14:1 14, 1–21. doi:<https://doi.org/10.1038/s41467-023-37937-4>.
- Li, S., Thompson, M., Mousavi, S., Dvorak, B., 2021. Life cycle and economic assessment of corn production practices in the western US Corn Belt. *Sustain. Prod. Consum.* 27, 1762–1774. <https://doi.org/10.1016/J.SPC.2021.04.021>.
- Ling, N., Wang, T., Kuzyakov, Y., 2022. Rhizosphere bacteriome structure and functions. *Nat. Commun.* 13, 1–13. <https://doi.org/10.1038/s41467-022-28448-9>.
- Liu, J.Y., Zheng, R.Q., Wang, Y., Liu, Y.H., Jiang, S., Wang, X.Z., He, K., Pan, X., Zhou, T., Li, T., Xia, Q., Zhang, W.N., 2022. The endogenous metabolite glycerophosphocholine promotes longevity and fitness in *Caenorhabditis elegans*. *Metabolites* 2022, Vol. 12, Page 177 12, 177. doi:<https://doi.org/10.3390/METAB12020177>.
- Liu, X., Mei, S., Salles, J.F., 2023. Inoculated microbial consortia perform better than single strains in living soil: a meta-analysis. *Appl. Soil Ecol.* 190, 105011. <https://doi.org/10.1016/J.APSOIL.2023.105011>.
- Lopes, M.J. dos S., Dias-Filho, M.B., Gurgel, E.S.C., 2021. Successful plant growth-promoting microbes: inoculation methods and abiotic factors. *Front. Sustain. Food Syst.* <https://doi.org/10.3389/fsufs.2021.606454>.
- Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550. <https://doi.org/10.1186/s13059-014-0550-8>.
- Ma, B.L., Liang, B.C., Biswas, D.K., Morrison, M.J., McLaughlin, N.B., 2012. The carbon footprint of maize production as affected by nitrogen fertilizer and maize-legume rotations. *Nutr. Cycl. Agroecosyst.* 94, 15–31. <https://doi.org/10.1007/s10705-012-9522-0>.
- Maçik, M., Gryta, A., Fraç, M., 2020. Biofertilizers in agriculture: an overview on concepts, strategies and effects on soil microorganisms. *Adv. Agron.* 162, 31–87. <https://doi.org/10.1016/BS.AGRON.2020.02.001>.
- Maitra, S., Brestic, M., Bhadra, P., Shankar, T., Praharaj, S., Palai, J.B., Shah, M.M.R., Berek, V., Ondrisik, P., Skalický, M., Hossain, A., 2021. Bioinoculants-natural biological resources for sustainable plant production. *Microorganisms* 2022 (10), 51. <https://doi.org/10.3390/MICROORGANISMS10010051>.
- Mapelli, F., Mengoni, A., Riva, V., Borin, S., 2023. Bacterial culturing is crucial to boost sustainable agriculture. *Trends Microbiol.* 31 (1), 1–4. <https://doi.org/10.1016/J.TIM.2022.10.005>.
- Marmiroli, M., Bonas, U., Imperiale, D., Lencioni, G., Mussi, F., Marmiroli, N., Maestri, E., 2018. Structural and functional features of chars from different biomasses as potential plant amendments. *Front. Plant Sci.* 9, 1119. <https://doi.org/10.3389/FPLS.2018.01119/BIBTEX>.
- Marmiroli, M., Caldara, M., Pantalone, S., Malcevski, A., Maestri, E., Keller, A.A., Marmiroli, N., 2022. Building a risk matrix for the safety assessment of wood derived biochars. *Sci. Total Environ.* 839, 156265. <https://doi.org/10.1016/J.SCITOTENV.2022.156265>.
- Mashabela, M.D., Tugizimana, F., Steenkamp, P.A., Piater, L.A., Dubery, I.A., Mhlongo, M.I., 2022. Untargeted metabolite profiling to elucidate rhizosphere and leaf metabolome changes of wheat cultivars (*Triticum aestivum* L.) treated with the plant growth-promoting rhizobacteria *Paenibacillus alvei* (T22) and *Bacillus subtilis*. *Front. Microbiol.* 13. <https://doi.org/10.3389/fmicb.2022.971836>.
- Mawarda, P.C., Le Roux, X., Dirk van Elsland, J., Salles, J.F., 2020. Deliberate introduction of invisible invaders: a critical appraisal of the impact of microbial inoculants on soil microbial communities. *Soil Biol. Biochem.* 148, 107874. <https://doi.org/10.1016/J.SOILBIO.2020.107874>.
- McMurdie, P.J., Holmes, S., 2012. PhyloSeq: a bioconductor package for handling and analysis of high-throughput phylogenetic sequence data. *Pac. Symp. Biocomput.* 235–246. https://doi.org/10.1142/9789814366496_0023.
- Medeiros, D.B., Brotman, Y., Fernie, A.R., 2021. The utility of metabolomics as a tool to inform maize biology. *Plant Commun.* <https://doi.org/10.1016/j.xplc.2021.100187>.
- Mishra, A., Singh, L., Singh, D., 2022. Unboxing the black box—one step forward to understand the soil microbiome: a systematic review. *Microb. Ecol.* 2022 85:2 85, 669–683. doi:<https://doi.org/10.1007/S00248-022-01962-5>.
- Moller, B.L., 1976. Lysine catabolism in barley (*Hordeum vulgare* L.). *Plant Physiol.* 57, 687–692. <https://doi.org/10.1104/pp.57.5.687>.
- Moungsree, S., Neamhom, T., Polprasert, S., Patthanaisaranukool, W., 2022. Carbon footprint and life cycle costing of maize production in Thailand with temporal and geographical resolutions. *Int. J. Life Cycle Assess.* 28, 891–906. <https://doi.org/10.1007/S11367-022-02021-4/FIGURES/5>.
- Muhammad, I., Yang, L., Ahmad, S., Zeeshan, M., Farooq, S., Ali, I., Khan, A., Zhou, X.B., 2022. Irrigation and nitrogen fertilization alter soil bacterial communities, soil enzyme activities, and nutrient availability in maize crop. *Front. Microbiol.* 13, 833758. <https://doi.org/10.3389/FMICB.2022.833758/BIBTEX>.
- Murtaza, G., Ahmed, Z., Eldin, S.M., Ali, B., Bawazeer, S., Usman, M., Iqbal, R., Neupane, D., Ullah, A., Khan, A., Hassan, M.U., Ali, I., Tariq, A., 2023. Biochar-soil-plant interactions: a cross talk for sustainable agriculture under changing climate. *Front. Environ. Sci.* <https://doi.org/10.3389/fenvs.2023.1059449>.
- Návarová, H., Bernsdorff, F., Döring, A.C., Zeier, J., 2013. Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. *Plant Cell* 24, 5123–5141. <https://doi.org/10.1105/TPC.112.103564>.
- Nephali, L., Moodley, V., Piater, L., Steenkamp, P., Buthelezi, N., Dubery, I., Burgess, K., Huyser, J., Tugizimana, F., 2021. A metabolomic landscape of maize plants treated with a microbial biostimulant under well-watered and drought conditions. *Front. Plant Sci.* 12. <https://doi.org/10.3389/fpls.2021.676632>.
- Nguyen, M., Luan, 2018. Biostimulant Effects of Rhizobacteria on Wheat Growth and Nutrient Uptake under Contrasted N Supplies. *Diss. Université de Liège, Liège, Belgique.*
- Niculaeas, C., Abramov, A., Hannemann, L., Frey, M., 2018. Plant protection by benzoxazinoids—recent insights into biosynthesis and function. *Agronomy.* <https://doi.org/10.3390/agronomy8080143>.
- Nkebiwe, P.M., Weinmann, M., Müller, T., 2016. Improving fertilizer-depot exploitation and maize growth by inoculation with plant growth-promoting bacteria: from lab to field. *Chem. Biol. Technol. Agric.* 3. <https://doi.org/10.1186/s40538-016-0065-5>.
- Nwachukwu, B.C., Babalola, O.O., 2022. Metagenomics: a tool for exploring key microbiome with the potentials for improving sustainable agriculture. *Front. Sustain. Food Syst.* 6, 886987. <https://doi.org/10.3389/fsufs.2022.886987>.
- O'Callaghan, M., Ballard, R.A., Wright, D., 2022. Soil microbial inoculants for sustainable agriculture: limitations and opportunities. *Soil Use Manag.* 38, 1340–1369. <https://doi.org/10.1111/SUM.12811>.
- Ocwa, A., Mohammed, S., Mousavi, S.M.N., Illés, Á., Bojtor, C., Ragán, P., Rátónyi, T., Harsányi, E., 2024. Maize grain yield and quality improvement through biostimulant application: a systematic review. *J. Soil Sci. Plant Nutr.* 24, 1609–1649. <https://doi.org/10.1007/s42729-024-01687-z>.
- OECD, FAO, 2023. Environmental Sustainability in Agriculture 2023. <https://doi.org/10.4060/cc9065en>.
- Peng, J., Han, X., Li, N., Chen, K., Yang, J., Zhan, X., Luo, P., Liu, N., 2021. Combined application of biochar with fertilizer promotes nitrogen uptake in maize by increasing nitrogen retention in soil. *Biochar* 3, 367–379. <https://doi.org/10.1007/s42773-021-00090-6>.
- Plassart, P., Prévost-Bouré, N.C., Uroz, S., Dequiedt, S., Stone, D., Creamer, R., Griffiths, R.I., Bailey, M.J., Ranjard, L., Lemaenceau, P., 2019. Soil parameters, land use, and geographical distance drive soil bacterial communities along a European transect. *Sci. Rep.* 9, 1–17. <https://doi.org/10.1038/s41598-018-36867-2>.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. <https://doi.org/10.1093/NAR/GKS1219>.
- Rawat, Jyoti, Sanwal, Pankaj, JS, 2018. Towards the mechanisms of nutrient solubilization and fixation in soil system. In: Meena, V. (Ed.), *Role of Rhizospheric Microbes in Soil*. Springer, Singapore, pp. 229–257.
- Reed, L., Glick, B.R., 2023. The recent use of plant-growth-promoting bacteria to promote the growth of agricultural food crops. *Agriculture (Switzerland)*. 13, 1089. <https://doi.org/10.3390/agriculture13051089>.
- Reszczyńska, E., Hanaka, A., 2020. Lipids composition in plant membranes. *Cell Biochem. Biophys.* 78, 401–414. <https://doi.org/10.1007/s12013-020-00947-w>.
- Riboni, N., Bianchi, F., Mattarozzi, M., Caldara, M., Gulli, M., Graziano, S., Maestri, E., Marmiroli, N., Careri, M., 2023. Ultra-high performance liquid chromatography-ion mobility-high-resolution mass spectrometry to evaluate the metabolomic response of durum wheat to sustainable treatments. *J. Agric. Food Chem.* 71, 15407–15416. <https://doi.org/10.1021/acs.jafc.3c04532>.
- Riva, V., Mapelli, F., Bagnasco, A., Mengoni, A., Borin, S., 2022. A meta-analysis approach to defining the culturable core of plant endophytic bacterial communities. *Appl. Environ. Microbiol.* 88 (6). <https://doi.org/10.1128/aem.02537-21>.
- Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F.S., Lambin, E., Lenton, T.M., Scheller, M., Folke, C., Schellnhuber, H.J., Nykvist, B., De Wit, C.A., Hughes, T., Van Der Leeuw, S., Rodhe, H., Sörlin, S., Snyder, P.K., Costanza, R., Svedin, U., Falkenmark, M., Karlberg, L., Corell, R.W., Fabry, V.J., Hansen, J., Walker, B., 2009. Planetary boundaries: exploring the safe operating space for humanity. *Ecol. Soc.* 14 (2). <https://www.jstor.org/stable/26268316>.
- Rong, Bing, L., Gong, K., Yuan, Duan, F., Ying, Li, S., Kun, Zhao, M., He, J., Zhou, W., Bin, Yu, Q., 2021. Yield gap and resource utilization efficiency of three major food crops in the world—a review. *J. Integr. Agric.* 20, 349–362. [https://doi.org/10.1016/S2095-3119\(20\)63555-9](https://doi.org/10.1016/S2095-3119(20)63555-9).
- Saia, S., Fraggaso, M., De Vita, P., Beleggia, R., 2019. Metabolomics provides valuable insight for the study of durum wheat: a review. *J. Agric. Food Chem.* 67, 3069–3085. <https://doi.org/10.1021/acs.jafc.8b07097>.
- Schütz, V., Bigler, L., Girel, S., Laschke, L., Sicker, D., Schulz, M., 2019. Conversions of benzoxazinoids and downstream metabolites by soil microorganisms. *Front. Ecol. Evol.* 7. <https://doi.org/10.3389/fevo.2019.00238>.
- Shayanthan, A., Ordoñez, P.A.C., Oresnik, I.J., 2022. The role of synthetic microbial communities (SynCom) in sustainable agriculture. *Front. Agron.* 4, 58. <https://doi.org/10.3389/FAGRO.2022.896307/BIBTEX>.
- Shetty, S.A., Lahti, L., 2019. Microbiome data science. *J. Biosci.* 44, 1–6. <https://doi.org/10.1007/S12038-019-9930-2/TABLES/1>.
- Sohi, S.P., Krull, E., Lopez-Capel, E., Bol, R., 2010. A review of biochar and its use and function in soil. *Adv. Agron.* 105, 47–82. [https://doi.org/10.1016/S0065-2113\(10\)05002-9](https://doi.org/10.1016/S0065-2113(10)05002-9).
- de Souza, R.S.C., Armanhi, J.S.L., Arruda, P., 2020. From microbiome to traits: designing synthetic microbial communities for improved crop resiliency. *Front. Plant Sci.* 11. <https://doi.org/10.3389/FPLS.2020.01179>.
- Steffen, W., Richardson, K., Rockström, J., Cornell, S.E., Fetzer, I., Bennett, E.M., Biggs, R., Carpenter, S.R., De Vries, W., De Wit, C.A., Folke, C., Gerten, D., Heinke, J., Mace, G.M., Persson, L.M., Ramanathan, V., Reyers, B., Sörlin, S., 2015. Planetary boundaries: guiding human development on a changing planet. *Science* 347, 1259855. <https://doi.org/10.1126/science.1259855>.
- Struhs, E., Mirkouei, A., You, Y., Mohajeri, A., 2020. Techno-economic and environmental assessments for nutrient-rich biochar production from cattle manure: a case study in Idaho, USA. *Appl. Energy* 279, 115782. <https://doi.org/10.1016/J.APENERGY.2020.115782>.

- Supasri, T., Itsubo, N., Gheewala, S.H., Sampattagul, S., 2020. Life cycle assessment of maize cultivation and biomass utilization in northern Thailand. *Sci. Rep.* 10, 3516. <https://doi.org/10.1038/s41598-020-60532-2>.
- Symanczik, S., Lipp, C., Mäder, P., Thonar, C., Kundel, D., 2023. Limited effectiveness of selected bioeffectors combined with recycling phosphorus fertilizers for maize cultivation under Swiss farming conditions. *Front. Plant Sci.* 14, 1239393. <https://doi.org/10.3389/fpls.2023.1239393>.
- Tabacchioni, S., Passato, S., Ambrosino, P., Huang, L., Caldara, M., Cantale, C., Hett, J., Del Fiore, Antonella, Fiore, Alessia, Schlüter, A., Sczyrba, A., Maestri, E., Marmiroli, N., Neuhoff, D., Nesme, J., Sørensen, S.J., Aprea, G., Nobili, C., Presenti, O., Giovannetti, G., Giovannetti, C., Pihlanto, A., Brunori, A., Bevivino, A., 2021. Identification of beneficial microbial consortia and bioactive compounds with potential as plant biostimulants for a sustainable agriculture. *Microorganisms* 9, 1–23. <https://doi.org/10.3390/microorganisms9020426>.
- Tan, S., Narayanan, M., Thu Huong, D.T., Ito, N., Unpaprom, Y., Pugazhendhi, A., Lan Chi, N.T., Liu, J., 2022. A perspective on the interaction between biochar and soil microbes: a way to regain soil eminence. *Environ. Res.* 214. <https://doi.org/10.1016/j.envres.2022.113832>.
- Tensi, A.F., Ang, F., van der Fels-Klerx, H.J., 2024. Microbial applications and agricultural sustainability: a simulation analysis of Dutch potato farms. *Agric. Syst.* 214, 103797. <https://doi.org/10.1016/j.agry.2023.103797>.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. *Nature* 418:6898–6948, 671–677. doi:<https://doi.org/10.1038/nature01014>.
- Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., Singh, B.K., 2020. Plant–microbiome interactions: from community assembly to plant health. *Nat. Rev. Microbiol.* 18, 607–621. <https://doi.org/10.1038/s41579-020-0412-1>.
- Vaccaro, F., Cangioli, L., Mengoni, A., Fagorzi, C., 2022. Synthetic plant microbiota challenges in nonmodel species. *Trends Microbiol.* 30 (10), 922–924. <https://doi.org/10.1016/j.tim.2022.06.006>.
- Vassura, I., Fabbri, D., Rombolà, A.G., Rizzi, B., Menichetti, A., Cornali, S., Pagano, L., Reggiani, R., Vecchi, M.R., Marmiroli, N., 2023. Multi-analytical techniques to study changes in carbon and nitrogen forms in a tomato-cultivated soil treated with biochar and biostimulants. *Soil Environ. Health* 1, 100050. <https://doi.org/10.1016/J.SEH.2023.100050>.
- Vijay, V., Shreedhar, S., Adlak, K., Payyanad, S., Sreedharan, V., Gopi, G., Sophia van der Voort, T., Malarvizhi, P., Yi, S., Gebert, J., Aravind, P.V., 2021. Review of large-scale biochar field-trials for soil amendment and the observed influences on crop yield variations. *Front. Energy Res.* 9, 710766. <https://doi.org/10.3389/fenrg.2021.710766>.
- Waheed, A., Xu, H., Qiao, X., Aili, A., Yiremaikeybayi, Y., Haitao, D., Muhammad, M., 2025. Biochar in sustainable agriculture and climate mitigation: mechanisms, challenges, and applications in the circular bioeconomy. *Biomass Bioenergy* 193, 107531. <https://doi.org/10.1016/j.biombioe.2024.107531>.
- Waqas, M.A., Wang, X., Zafar, S.A., Noor, M.A., Hussain, H.A., Azher Nawaz, M., Farooq, M., 2021. Thermal stresses in maize: effects and management strategies. *Plants* 10, 293. <https://doi.org/10.3390/plants10020293>.
- Welti, R., Shah, J., Li, W., Li, M., Chen, J., Burke, J.J., Fauconnier, M.-L., Chapman, K., Chye, M.-L., Wang, X., 2007. Plant lipidomics: discerning biological function by profiling plant complex lipids using mass spectrometry. *Front. Biosci. (Landmark Ed)* 2, 2494–2506. <https://doi.org/10.2741/2250>.
- Wickham, H., 2011. ggplot2. *Wiley Interdiscip. Rev. Comput. Stat.* 3, 180–185. <https://doi.org/10.1002/WICS.147>.
- Wolna-Maruwka, A., Piechota, T., Niewiadomska, A., Kamiński, A., Kayzer, D., Grzyba, A., Pilarska, A.A., 2021. The effect of biochar-based organic amendments on the structure of soil bacterial community and yield of maize (*Zea mays* L.). *Agronomy* 11, 1286. <https://doi.org/10.3390/agronomy11071286>.
- Wright, E.S., Yilmaz, L.S., Noguera, D.R., 2012. DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. *Appl. Environ. Microbiol.* 78, 717–725. <https://doi.org/10.1128/AEM.06516-11>.
- Wu, L., Wang, S., Chen, X., Wang, X., Wu, Liancheng, Zu, X., Chen, Y., 2013. Proteomic and phytohormone analysis of the response of maize (*Zea mays* L.) seedlings to sugarcane mosaic virus. *PLoS One* 8, e70295. <https://doi.org/10.1371/journal.pone.0070295>.
- Yan, H., Cong, M., Hu, Y., Qiu, C., Yang, Z., Tang, G., Xu, W., Zhu, X., Sun, X., Jia, H., 2022. Biochar-mediated changes in the microbial communities of rhizosphere soil alter the architecture of maize roots. *Front. Microbiol.* 13, 1023444. <https://doi.org/10.3389/fmicb.2022.1023444/FULL>.
- Zhang, L., Jing, Y., Xiang, Y., Zhang, R., Lu, H., 2018a. Responses of soil microbial community structure changes and activities to biochar addition: a meta-analysis. *Sci. Total Environ.* 643, 926–935. <https://doi.org/10.1016/j.scitotenv.2018.06.231>.
- Zhang, W., Wang, C., Dong, M., Jin, S., Li, H., 2018b. Dynamics of soil fertility and maize growth with lower environment impacts depending on a combination of organic and mineral fertilizer. *J. Soil Sci. Plant Nutr.* 18, 556–575. <https://doi.org/10.4067/S0718-95162018005001701>.
- Zhao, Y., Fu, W., Hu, C., Chen, G., Xiao, Z., Chen, Y., Wang, Z., Cheng, H., 2021. Variation of rhizosphere microbial community in continuous mono-maize seed production. *Sci. Rep.* 11, 1–13. <https://doi.org/10.1038/s41598-021-81228-1>.
- Zhong, F., Jiang, D., Zhao, Q., Guo, A., Ullah, A., Yang, X., Cheng, Q., Zhang, Y., Ding, X., 2020. Eco-efficiency of oasis seed maize production in an arid region, Northwest China. *J. Clean. Prod.* 268, 122220. <https://doi.org/10.1016/j.jclepro.2020.122220>.
- Zhou, S., Richter, A., Jander, G., 2018. Beyond defense: multiple functions of benzoxazinoids in maize metabolism. *Plant Cell Physiol.* 58, 1528–1537. <https://doi.org/10.1093/pcp/pcy064>.