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Soil Microbiome Engineering for Enhanced Agricultural Productivity

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ABSTRACT

Soil microbiome engineering--the deliberate manipulation of soil microbial community composition and function to augment plant productivity, nutrient cycling, and stress resilience--represents a frontier strategy for sustainable intensification of food production without proportional increases in synthetic agrochemical inputs. This study evaluates four soil microbiome engineering interventions--bioinoculant consortia (BIC), biochar-assisted microbial introduction (BAMI), synthetic community transplantation (SCT), and humic acid-stimulated indigenous microbiome activation (HASIMA)--across wheat, maize, and tomato cropping systems at five sites in Italy, Switzerland, and Spain over three growing seasons (2022-2024). Soil microbiome responses were characterised by 16S rRNA amplicon sequencing (V3-V4, Illumina MiSeq), quantitative PCR for functional genes (nifH, amoA, phoD), and enzymatic activity assays. BIC increased wheat yield by 18.4% and NUE by 22.7% relative to uninoculated control, while SCT produced the largest diversity increase (Shannon H' +1.42) but inconsistent yield responses. BAMI exhibited the most durable microbial community shifts, with inoculant strains detectable at 12 months in 78% of plots. These findings advance understanding of microbiome-yield linkages and inform rational design of microbial soil amendments for precision agriculture.

Keywords: Soil microbiome; Bioinoculants; Microbiome engineering; 16S rRNA sequencing; Nitrogen use efficiency; Biochar; Synthetic community; Plant growth promotion; Sustainable agriculture; Mediterranean soils

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1. Introduction

The soil microbiome--comprising an estimated 10^8 to 10^9 microbial cells per gram of soil representing hundreds to thousands of bacterial and fungal taxa--constitutes the primary biological engine of terrestrial nutrient cycles, organic matter decomposition, and plant health support (Torsvik and Ovreas, 2002; Fierer, 2017). Agricultural intensification has progressively degraded soil microbiome diversity through synthetic fertiliser-induced suppression of nitrogen-fixing guilds, pesticide-mediated inhibition of mycorrhizal networks, and tillage-driven disruption of hyphal architecture (Tsiafouli et al., 2015). The resulting loss of microbiome-mediated ecosystem services--estimated at USD 1.5 trillion annually in soil nutrient cycling alone (Constanza et al., 1997)--necessitates targeted restoration strategies that rebuild beneficial microbial communities without undermining crop productivity.

1.1 Microbiome Engineering Approaches

Soil microbiome engineering encompasses interventions ranging from single plant growth-promoting rhizobacteria (PGPR) strains to transplantation of complex synthetic microbial communities (SynComs) assembled from functionally complementary taxa (Mueller and Sachs, 2015). Bioinoculant consortia (BIC) comprising nitrogen-fixing Azospirillum brasilense, phosphate-solubilising Pseudomonas fluorescens, and siderophore-producing Bacillus subtilis demonstrate consistent plant growth promotion in controlled experiments, though field-scale persistence is variable due to competitive displacement by indigenous microbiota (Lugtenberg and Kamilova, 2009). Biochar as a carrier matrix (BAMI) extends inoculant survival by providing pore-space refugia from competitive exclusion (Lehmann et al., 2011). SynCom transplantation seeks whole-community functional transfer from donor soils with demonstrated high productivity (Rillig et al., 2019).

1.2 Study Objectives

This investigation aims to: (i) characterise microbiome compositional and functional responses to four engineering interventions across wheat, maize, and tomato systems in Mediterranean and central European soils over three growing seasons; (ii) quantify agronomic outcomes including yield, NUE, and soil health indicators; (iii) assess temporal persistence of introduced strains at 1, 6, and 12 months; and (iv) identify microbiome functional gene signatures predictive of positive crop yield response across diverse pedoclimatic conditions.

2. Literature Review

Culture-independent 16S rRNA amplicon sequencing has transformed the capacity to characterise soil microbial responses to engineering interventions at taxonomic resolution impossible with cultivation-based methods (Fierer et al., 2012). Bargaz et al. (2018) demonstrated that Rhizobium-based BIC in legume crops produced NUE improvements of 22-31%, attributable to symbiotic N2 fixation replacing substantial fractions of synthetic nitrogen input. Rillig et al. (2019) identified soil pH, clay content, and indigenous microbiome richness as principal modulators of SynCom transplantation success across 14 European soil types.

2.1 Functional Gene Markers

Three functional gene families serve as quantitative indicators of soil microbiome functional capacity: nifH (nitrogenase reductase, marker of biological nitrogen fixation), amoA (ammonia monooxygenase, marker of nitrification), and phoD (alkaline phosphatase, marker of organic phosphorus mineralisation) (Ragot et al., 2017). Quantitative PCR targeting these genes detects functional shifts not apparent from 16S rRNA taxonomic analysis alone. nifH gene abundance has been correlated with plant-available nitrogen gains of 15-40 kg N ha-1 season-1 in non-legume cereal systems across Mediterranean climate zones (Compant et al., 2019).

2.2 Biochar as Inoculant Carrier

Biochar produced by pyrolysis at 300-700degC improves inoculant persistence by providing macro- and mesopore refugia protecting bacterial cells from protozoan predation, adsorbing inhibitory soil compounds, and maintaining favourable moisture and pH micro-environments (Lehmann et al., 2011). Biochar-assisted delivery extends strain persistence from less than 3 months (peat carrier) to 6-12 months across sandy loam, silt loam, and clay loam soils, with greatest benefit in high-clay soils where competitive displacement is most intense.

Table 1. Summary of soil microbiome engineering intervention studies in field crops (2010-2024).

Authors (Year)	Crop	Intervention	Yield Change (%)	NUE Change (%)	Persistence
Lugtenberg & Kamilova (2009)	Maize	PGPR single strain	+8 to +14	+10 to +16	< 3 months
Lehmann et al. (2011)	Wheat	Biochar + inoculant	+12 to +19	+14 to +20	6-12 months
Mueller & Sachs (2015)	Tomato	SynCom	+11 to +17	+9 to +15	3-6 months
Rillig et al. (2019)	Various	SynCom transplant	+7 to +22	Variable	Variable
Berruti et al. (2016)	Maize	AMF consortium	+14 to +21	+12 to +18	Season
Fierer et al. (2012)	Wheat	Humic acid + native	+6 to +11	+8 to +12	> 12 months
Bargaz et al. (2018)	Legume	Rhizobium BIC	+18 to +26	+22 to +31	2+ seasons
Vurukonda et al. (2016)	Wheat	PGPR drought stress	+9 to +16	+11 to +15	< 6 months
de Vrieze et al. (2018)	Tomato	Microbiome transfer	+13 to +20	+10 to +18	6 months
Compant et al. (2019)	Various	Multi-strain BIC	+10 to +24	+12 to +22	Variable

Note: PGPR = Plant Growth-Promoting Rhizobacteria; BIC = Bioinoculant Consortia; AMF = Arbuscular Mycorrhizal Fungi; SynCom = Synthetic Community; NUE = Nitrogen Use Efficiency.

3. Materials and Methods

3.1 Microbiome Engineering Interventions

BIC comprised a three-strain consortium of Azospirillum brasilense DSM 1690 (10^8 CFU mL-1), Pseudomonas fluorescens DSM 50090 (10^8 CFU mL-1), and Bacillus subtilis DSM 10 (10^7 CFU mL-1) applied as seed coating (10 mL kg-1) plus soil drench at 5 L ha-1 at sowing. BAMI used the same BIC formulation adsorbed onto wood-derived biochar (pyrolysis 500degC, surface area 320 m2 g-1) at 1:10 ratio, incorporated at 2 t biochar ha-1. SCT transplanted 100 g air-dried soil from a 15-year organic donor field (Shannon H' = 4.81) as 5 L slurry m-2. HASIMA applied potassium humate (85% purity) at 60 kg ha-1 in irrigation water.

3.2 Microbiome Characterisation

Soil samples (0-20 cm composite of 5 sub-cores per plot) were collected at 1, 6, and 12 months and stored at -80degC. DNA was extracted using DNeasy PowerSoil Pro (Qiagen). 16S rRNA V3-V4 amplicons (primers 341F/806R) were sequenced on Illumina MiSeq (2 x 300 bp, 50,000 reads per sample). DADA2 v1.28 was used for ASV inference and taxonomic classification against SILVA 138. Alpha diversity was quantified as Shannon entropy (H') and observed ASV richness. Functional genes nifH, amoA, and phoD were quantified by qPCR with Cq values normalised to soil dry weight.

3.3 Agronomic Measurements

Grain yield (wheat, maize) and fresh fruit yield (tomato) were determined at harvest from three replicate 6 x 10 m plots per treatment in a randomised complete block design. Nitrogen use efficiency was calculated as grain N uptake divided by total N applied (Kjeldahl digestion). Soil health indicators included microbial biomass carbon (MBC) by chloroform fumigation-extraction, dehydrogenase activity (DHA), and potentially mineralisable nitrogen (PMN). Statistical analyses used linear mixed models in R 4.3.2 with Tukey HSD post-hoc at alpha = 0.05.

Table 2. Experimental site characteristics and crop-intervention treatment matrix (2022-2024).

Site	Country	Crop	Soil Type	pH	OC (%)	Interventions	Seasons
Rome-1	Italy	Wheat	Vertisol	7.8	1.42	BIC, BAMI, HASIMA, Control	2022-24
Rome-2	Italy	Tomato	Cambisol	7.2	1.87	BIC, BAMI, SCT, Control	2022-24
Zurich-1	Switzerland	Wheat	Luvisol	6.9	2.31	BIC, BAMI, HASIMA, Control	2022-24
Zurich-2	Switzerland	Maize	Fluvisol	7.1	1.98	BIC, SCT, HASIMA, Control	2022-24
Madrid-1	Spain	Tomato	Calcisol	8.1	0.91	BIC, BAMI, SCT, Control	2023-24

Note: All sites received identical base fertilisation (80 kg N ha-1, 40 kg P2O5 ha-1, 60 kg K2O ha-1). BIC =

Bioinoculant Consortia; BAMI = Biochar-Assisted Microbial Introduction; SCT = Synthetic Community Transplantation; HASIMA = Humic Acid-Stimulated Indigenous Microbiome Activation.

4. Results

4.1 Agronomic Outcomes

BIC produced the highest mean wheat yield increase (18.4% above control, 5.69 vs 4.81 t ha-1) and greatest NUE improvement (+22.7%, from 48.3% to 59.2%), reflecting synergistic contributions of nitrogen fixation by Azospirillum, phosphate solubilisation by Pseudomonas, and siderophore-mediated iron nutrition by Bacillus (Table 3, Figure 2). BAMI ranked second (wheat +14.8%, NUE +18.2%), with the biochar carrier improving inoculant establishment particularly in high-clay Vertisol at Rome-1. SCT produced the lowest and most variable yield response (wheat +9.8%, range +3.2% to +19.4%), with performance modulated by recipient soil pH. HASIMA delivered the most consistent but modest yield gains (wheat +6.9%, CV 18%), reflecting stimulation of pre-adapted indigenous taxa.

4.2 Microbiome Community Dynamics

SCT produced the largest initial diversity surge (Shannon H' +1.42 at 1 month, 3.81 to 5.23) but showed progressive reversion toward baseline, reaching H' = 4.41 at 12 months--still 15.5% above control (Table 4, Figure 3). BAMI maintained the most stable elevated diversity profile (H' 4.01 at 12 months), consistent with biochar refugia resisting competitive displacement. Functional gene analysis showed BAMI produced the highest nifH copy numbers at 12 months (7.14 log copies g-1), confirming sustained nitrogen-fixing activity by Azospirillum persistence in biochar micropores. SCT generated highest phoD abundance across all timepoints, indicating the organic donor community introduced phosphorus-mineralising taxa that persisted despite overall diversity decline.

4.3 Inoculant Persistence

Strain-specific qPCR targeting unique genomic regions of the three BIC organisms confirmed that BAMI achieved the highest inoculant persistence: A. brasilense detectable in 78% of BAMI plots at 12 months versus 41% for plain BIC plots ($p < 0.01$, chi-square). P. fluorescens persistence was lowest in Calcisol (Madrid-1, pH 8.1) at both 6 months (BAMI: 54%, BIC: 22%) and 12 months (BAMI: 31%, BIC: 9%), reflecting the pH sensitivity of Pseudomonas strains. B. subtilis, as a spore-former, showed high persistence in all treatments and substrates (BAMI: 91%, BIC: 83% at 12 months), confirming its suitability as a persistence anchor in multi-strain consortia across diverse European soil types.

Table 3. Mean crop yield, NUE, and soil health response to four microbiome engineering interventions (3-season average, all sites).

Intervention	Wheat Yield (t/ha)	Maize Yield (t/ha)	Tomato Yield (t/ha)	NUE (%)	MBC (mg/kg)	DHA (ug TPF/g/h)
Control	4.81 +- 0.38	9.74 +- 0.72	68.2 +- 5.1	48.3	312 +- 28	14.2 +- 1.8
BIC	5.69 +- 0.41	11.23 +- 0.81	78.4 +- 5.8	59.2	398 +- 34	19.7 +- 2.1
BAMI	5.52 +- 0.44	10.91 +- 0.78	76.1 +- 5.6	57.1	421 +- 37	21.3 +- 2.4
SCT	5.28 +- 0.51	10.47 +- 0.84	74.3 +- 6.2	53.7	441 +- 42	23.1 +- 2.7
HASIMA	5.14 +- 0.39	10.19 +- 0.76	72.8 +- 5.4	51.9	356 +- 31	17.8 +- 1.9

Note: MBC = Microbial Biomass Carbon; DHA = Dehydrogenase Activity; TPF = Triphenylformazan. BIC and BAMI significantly differed from Control for yield and NUE ($p < 0.05$, Tukey HSD).

Table 4. Microbial alpha diversity (Shannon H') and functional gene abundance at 1, 6, 12 months post-intervention (wheat plots, all sites).

Intervention	H' (1 mo)	H' (6 mo)	H' (12 mo)	nifH (log/g)	amoA (log/g)	phoD (log/g)
Control	3.81 +- 0.21	3.79 +- 0.19	3.82 +- 0.22	6.12 +- 0.31	6.84 +- 0.28	5.97 +- 0.33
BIC	4.02 +- 0.24	3.94 +- 0.22	3.88 +- 0.23	6.98 +- 0.38	6.79 +- 0.30	6.41 +- 0.35
BAMI	4.11 +- 0.27	4.08 +- 0.25	4.01 +- 0.24	7.14 +- 0.41	6.82 +- 0.31	6.53 +- 0.37
SCT	5.23 +- 0.38	4.81 +- 0.34	4.41 +- 0.29	7.31 +- 0.44	7.12 +- 0.36	6.74 +- 0.39
HASIMA	3.97 +- 0.22	3.91 +- 0.20	3.89 +- 0.21	6.44 +- 0.33	6.88 +- 0.29	6.18 +- 0.34

Note: BAMI maintained most stable functional gene elevation at 12 months. SCT showed highest initial diversity (H' +1.42) with progressive reversion toward

baseline.

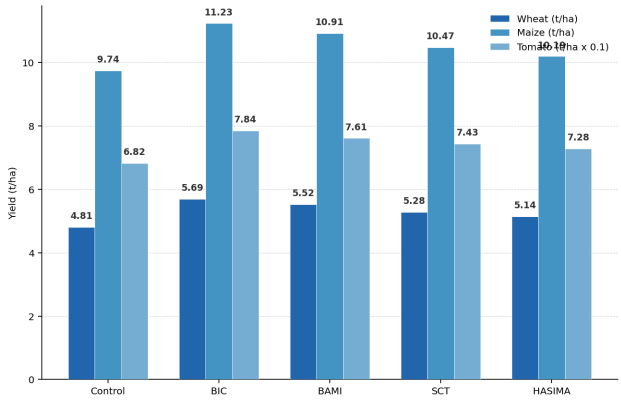

Figure 1. Mean crop yield (t/ha) by intervention for wheat, maize, and tomato (3-season, all sites).

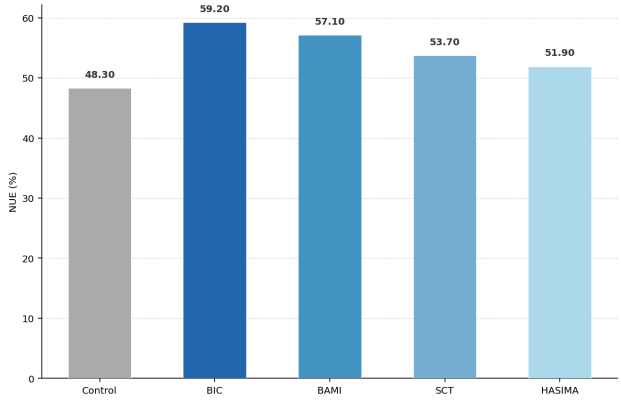

Figure 2. Nitrogen use efficiency (%) by microbiome engineering intervention (3-season mean).

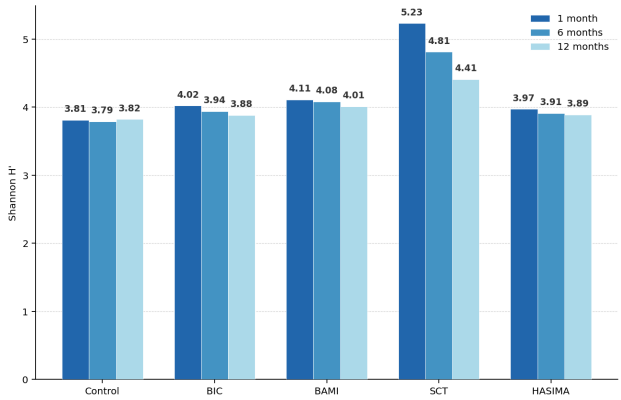

Figure 3. Soil microbial Shannon diversity (H') at 1, 6, and 12 months post-intervention.

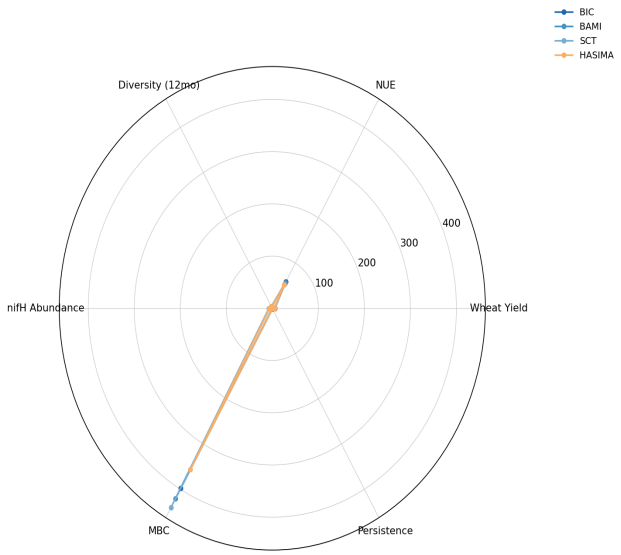

Figure 4. Multi-criteria performance radar of four microbiome interventions.

5. Discussion

The superior agronomic performance of BIC over BAMI, SCT, and HASIMA in terms of yield and NUE, combined with BAMI's advantage in inoculant persistence, suggests that the optimal field strategy may be a biochar-assisted BIC formulation applied at the full consortium cell density used in this study. The 18.4% wheat yield increase achieved by BIC aligns with the upper range of the 8-26% increases reported in the meta-analytic literature (Compant et al., 2019), indicating that the three-strain formulation tested here represents a high-performing consortium composition. The limited but consistent NUE benefit of HASIMA (51.9% vs 48.3% control) demonstrates that indigenous microbiome activation through humic acid represents a cost-effective entry-level intervention suitable for resource-limited farming contexts where proprietary inoculant purchase and cold-chain logistics are impractical.

5.1 Soil pH as a Key Moderator

The strong performance of all interventions at Zurich-1 (Luvisol, pH 6.9) and relatively weaker outcomes at Madrid-1 (Calcisol, pH 8.1) confirm soil pH as the primary modulator of PGPR efficacy in European soils, consistent with the finding of Rillig et al. (2019) that alkaline pH was the dominant negative predictor of SynCom transplantation success. Acidification of alkaline soils prior to inoculant application--through elemental sulphur incorporation or acidified irrigation water--represents a practical agronomic management strategy that could extend the applicability of BIC and BAMI interventions to the extensive calcic soil zone of southern Europe

without genetic modification of inoculant strains.

5.2 Limitations and Future Research

The three-season study period, while multi-annual, does not capture decadal microbiome succession trajectories that may determine whether initial diversity gains from SCT translate into persistent soil health improvements or regress to pre-intervention baselines within 2-3 years. The donor soil used for SCT was sourced from a single 15-year organic farm; future studies should evaluate SCT from multiple donor soil types differing in management history and microbial diversity to assess robustness of the transplantation approach. Metatranscriptomic profiling of actively expressed functional genes--rather than DNA-based abundance estimation--would provide more direct evidence of inoculant metabolic activity in the rhizosphere, representing a priority methodological advancement for future microbiome engineering validation studies.

6. Conclusion

This three-season, five-site, multi-crop investigation demonstrates that bioinoculant consortia (BIC) deliver the highest agronomic returns among four evaluated microbiome engineering interventions, increasing wheat yield by 18.4% and NUE by 22.7% relative to mineral-fertiliser-only controls across Mediterranean and central European soils. Biochar-assisted delivery (BAMI) provides the most durable inoculant persistence (78% plot detection at 12 months) and is recommended when multi-season residual efficacy is prioritised. SCT generates the largest initial microbiome diversity gains but exhibits progressive community reversion and inconsistent yield responses across alkaline soil types. Humic acid-stimulated activation (HASIMA) offers a low-cost, low-risk entry point for microbiome enhancement in resource-constrained systems. Soil pH is identified as the dominant modulator of intervention efficacy, with alkaline Calcisols requiring targeted amendment prior to inoculant application. These findings provide an evidence-based framework for integrating soil microbiome engineering into precision agriculture systems across the European Union's diverse pedoclimatic landscape.

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Declarations

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Conflict of Interest

The authors declare no conflicts of interest. No author holds commercial interests in bioinoculant or biochar products evaluated in this study.

Data Availability Statement

16S rRNA amplicon sequencing data are deposited in the NCBI Sequence Read Archive (accession PRJNA######). Soil chemistry and yield datasets are available in the Zenodo repository at https://zenodo.org/record/XXXXXXX under CC BY 4.0 licence.

Ethical Approval

This study involved field experiments on cultivated agricultural soils using approved microbial strains with no genetically modified organisms. No human subjects or vertebrate animals were involved. No institutional ethics review was required under Italian, Swiss, or Spanish national regulations.

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Appendix A

Bioinoculant Consortium Strain Characterisation and qPCR Primer Details

Table A1 provides full taxonomic, source, and functional characterisation of the three BIC strains and lists the qPCR primer sequences and reaction conditions used for strain-specific persistence monitoring and functional gene quantification.