Supplementary Information

For

Mechanism of synthetic communities in improving acidic soil and promoting crop growth

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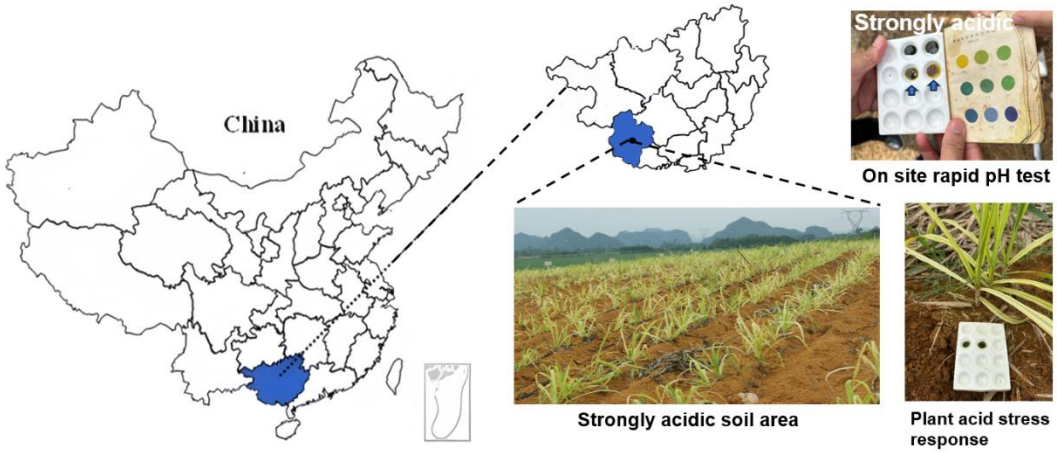

Fig. S1. Strongly acidic red soil area

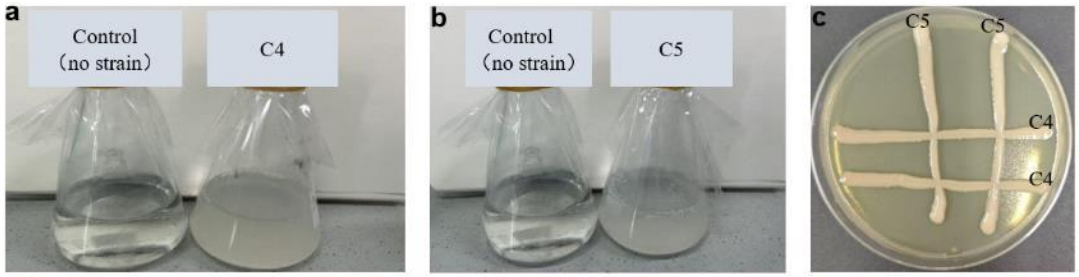

Fig. S2. The growth of C4 and C5 in low-carbon (0.05%, w/v) medium (**a-b**) and verification of their resistance effects (**c**).

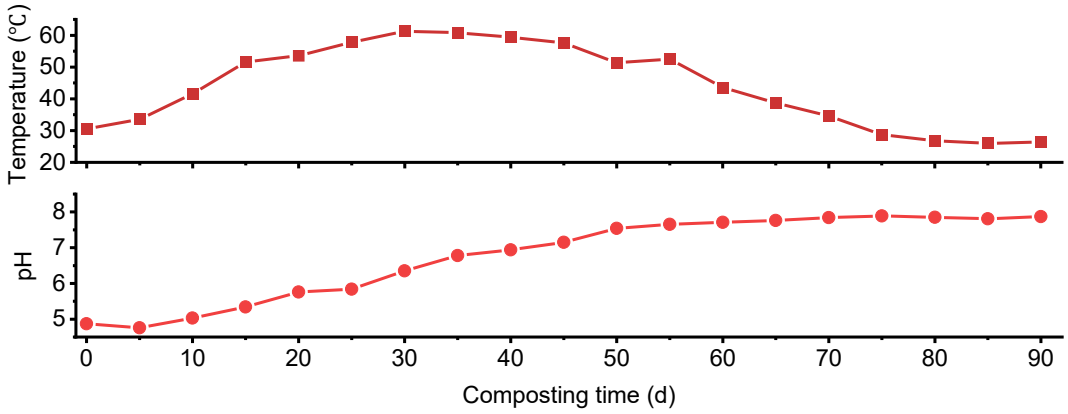

Fig. S3. Temperature and pH changes during the FM composting process

Text S1

Optimization experiment of improving Acidic soil with Microbial agents.

Soil culture experiments were carried out based on the screened C4 and C5 bacteria in order to obtain the optimal application method of the bacteria to pot. Meanwhile, the Bacillus available on the market was employed to soil improvement as a comparison. The incubation test method refers to previous research (Rong et al. 2024). In short, the soil (100 g) water content was adjusted to 60% of the saturation threshold using sterile deionized water. Each treatment was replicated three times, and the samples were placed in an incubator in the dark at 25℃. The soil samples were collected at 30 d for pH and SOC analysis. Additionally, fresh soil samples were manually homogenized with a sterilized spatula, diluted, and plated on LB agar to count culturable viable bacteria.

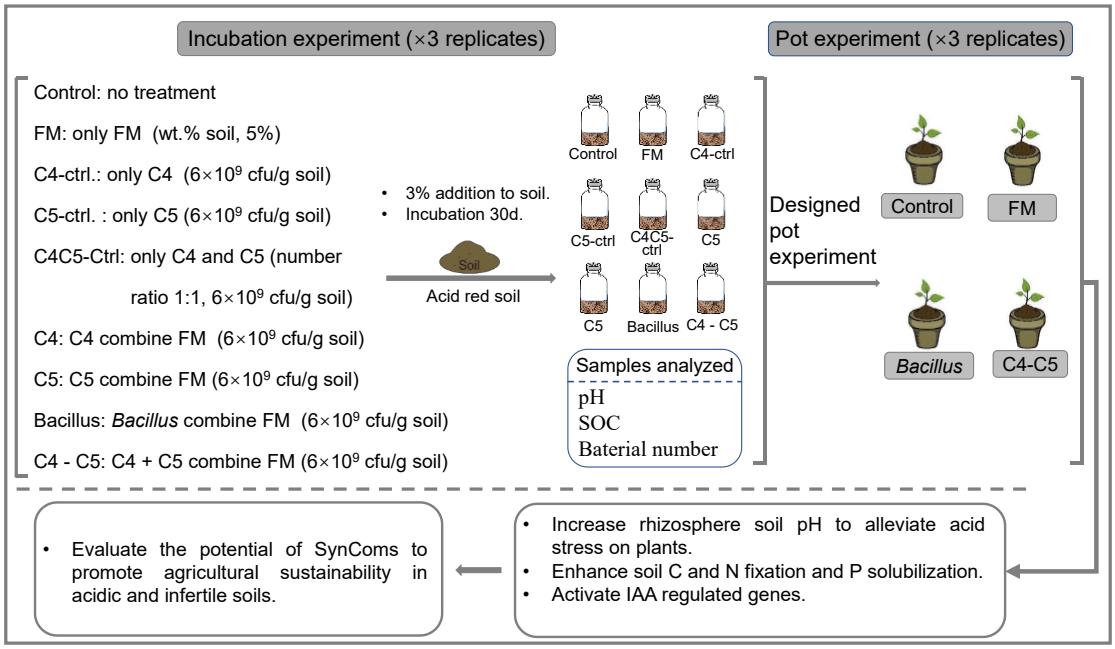

Fig. S4. Schematic of experimental design, methods and objective.

After improvements, a significant increase in soil pH was observed (Fig. S5 a). The FM combined with microorganisms demonstrated a more effective enhancement of soil pH, with the C4-C5 treatment yielding the most favorable results. The SOC increased across all

treatments (Fig. S5 b), indicating a positive response to microbial biomass as a source of carbon input. Furthermore, it was noted that the combination of FM with microbial agents was more effective in increasing soil SOC and promoting the colonization and proliferation of microbial biomass within the soil (Fig. S5 c). Overall, microbial agents not only enhanced soil pH and organic carbon levels but also, when combined with FM, facilitated the colonization of strains in the soil. This forms the foundation for our subsequent design of pot experiments.

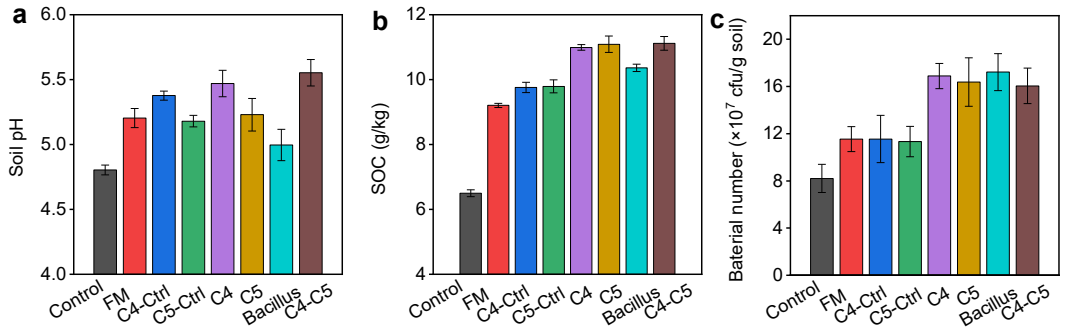

Fig. S5. Soil pH (a), SOC (b) content and bacterial number (c) under different treatments.

Text S2

The *cbbL* gene determination

DNA was extracted from soil samples using PrimeScript™ RT reagent Kit (RR047A, Takara, Japan). The *cbbL* gene (K2f, ACCAYCAAGCCSAAGCTSGG; V2r, GCCTTCSAGCTTGCCSACCRC) was amplified on a QuantStudio 6 Flex (Thermo Fisher, USA). The reaction system (30 μL) included Forward Primer (10 μM, 0.5 μL), Reverse Primer (10 μM, 0.5 μL), template DNA (2 μL) and ddH_2O (27 μL). The cbbL gene standard curve was produced by the continuously diluted plasmid method, and there was no inhibition of qPCR according to the amplification efficiency and other parameters of the standard curve (Fig. S6a). A dissolution curve (Fig. S6b) was used to test the amplification specificity. An amplification efficiency of 86.00 % was obtained with an R2 value of 0.99. The gene copies were calculated based on the Ct (cycle threshold) value from QuantStudio 6 Flex.

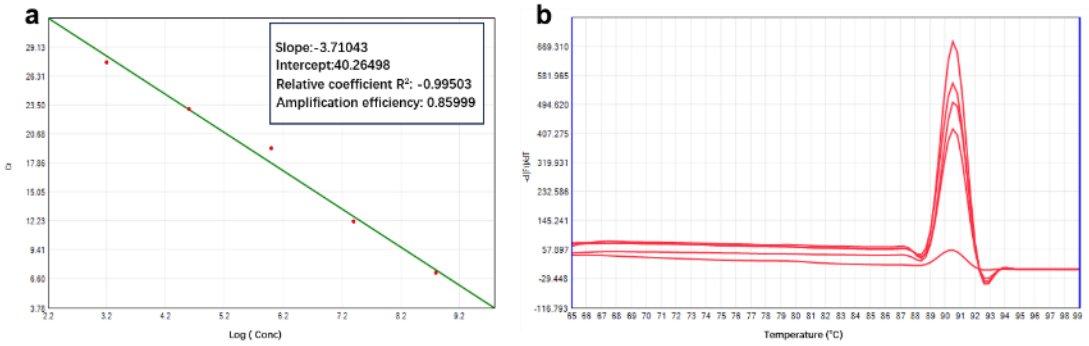

Fig. S6. Standard curve of *cbbL* gene standard amplification (**a**). Standard curve equation is: Y=-3.71043X+40.26498, R^2=0.99. Amplification dissolution curve of *cbbL* gene samples (**b**).

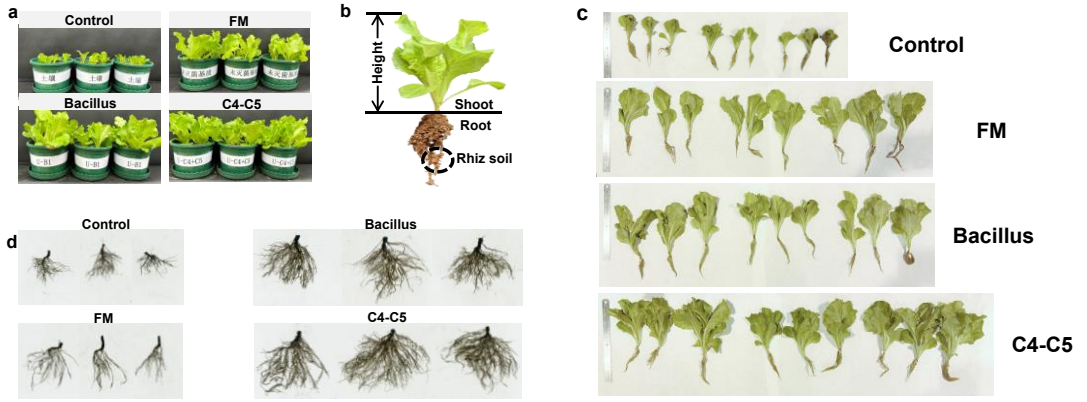

Fig. S7. Pot experiment. growth characteristics (**a-c**) and roots morphology (**c**) at harvest.

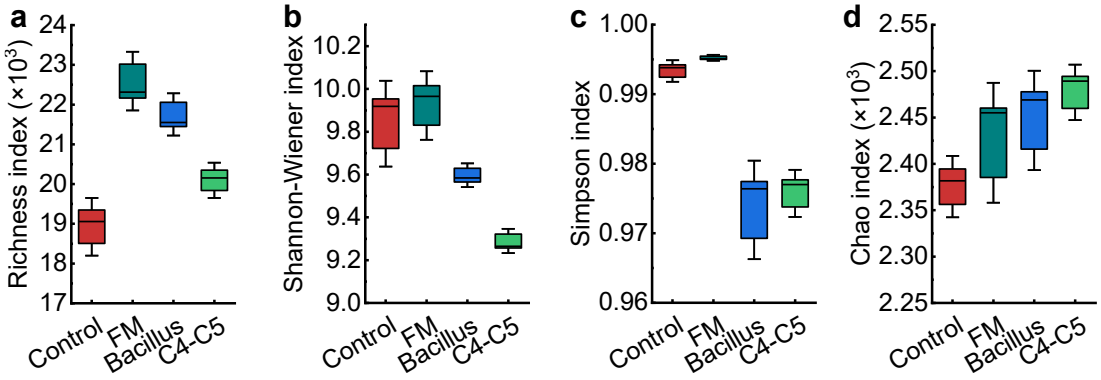

Fig. S8. α diversity index of Richness (**a**) Shannon- wiener (**b**) Simpson (**c**) and Chao (**d**).

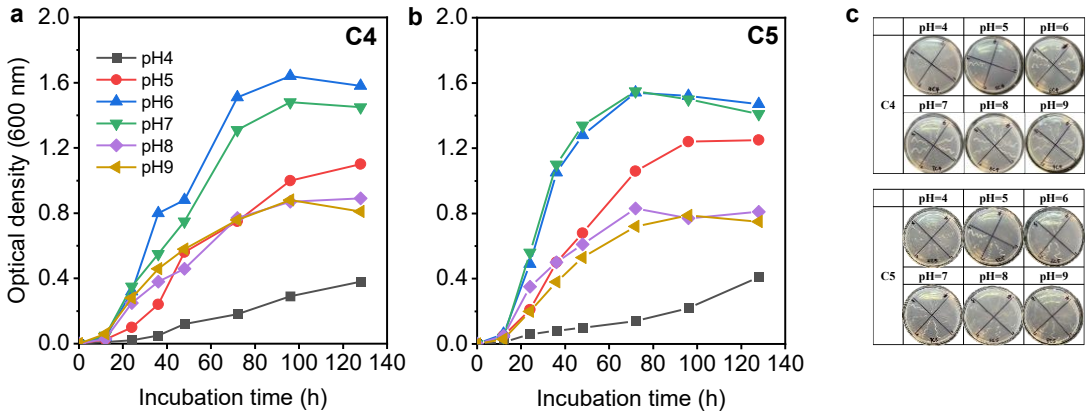

Fig. S9. Growth curves (**a-b**) and plate experiments (**c**) of C4 and C5 in different initial pH.

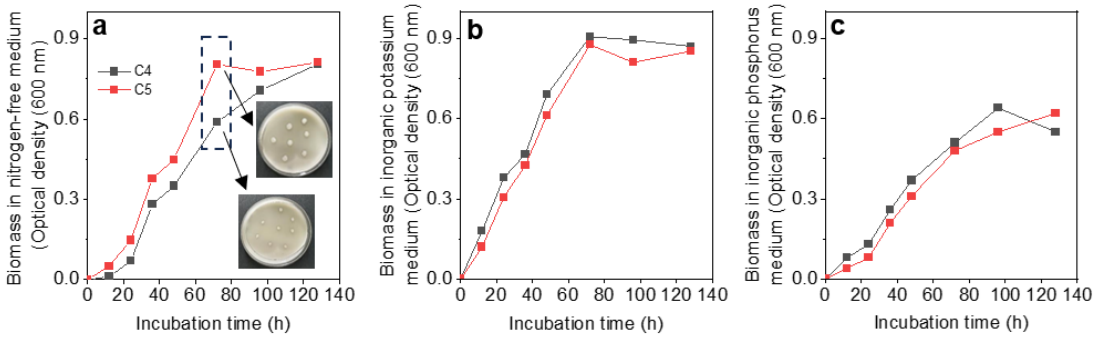

Fig. S10. The biomass of C4 and C5 bacteria in Ashby medium (**a**), inorganic phosphorus medium (**b**) and inorganic potassium medium (**c**).

Table S1

Medium composition with 0.1%, 0.07%, 0.05% carbon content, respectively.

Composition	Mass	Composition	Mass
$MnSO_4$	0.5 g	NH_4Cl	0.5 g
Na_2HPO_4	0.02 g	$FeCl_3$	0.02 g
KH_2PO_4	10.0 g	$Na_2S_2O_3$	10.0 g
$MgSO_4$	1.0 g	NaCl	1.0 g
$CaCl_2$	1 L	Double distilled H_2O	1 L
	2.75g (0.1%)		
Glucose	1.925g (0.07%)		
	1.375g (0.05%)		

Table S2

Physical and chemical properties of FM.

Item	FM
pH	7.87
Organic matter (g/kg)	262.90
Organic carbon (g/kg)	152.49
Available N (mg/kg)	52.58
Available P (mg/kg)	24.25
Available K (mg/kg)	13.11
C/N	16.3:1

Table S3

The abundance of microorganisms at the phylum level (%).

	Soil	D1	D3	D6
Bacillota	19.75	**93.56**	92.09	**49.05**
Actinomycetota	18.99	0.51	0.28	0.22
Pseudomonadota	23.89	**3.28**	5.79	**50.50**
Chloroflexota	8.62	0.01	0.02	0.01
Bacteroidota	6.72	0.07	0.21	0.13
Patescibacteria	0.83	0.00	0.00	0.02
Acidobacteriota	11.65	0.03	0.03	0.06
Others	9.56	2.54	1.58	0.02

Table S4

Indole-3-acetic acid concentration (mg/L) in different treatment.

Incubation time (d)	*Bacillus* sp.	C5	C4	C4-C5
0	0	0	0	0
1	3.35 ± 0.16	7.98 ± 0.10	2.32 ± 0.11	6.63 ± 0.22
2	4.04 ± 0.47	15.57 ± 0.31	4.63 ± 0.32	18.79 ± 1.37
3	4.29 ± 1.42	25.76 ± 0.92	5.42 ± 0.97	29.98 ± 2.10
4	7.59 ± 0.76	30.30 ± 4.27	5.91 ± 0.35	32.71 ± 1.79
5	8.92 ± 0.85	38.13 ± 1.15	6.65 ± 0.94	34.32 ± 1.98
6	8.67 ± 0.34	37.44 ± 2.18	8.33 ± 0.78	35.53 ± 1.10

Table S5

Plant height, fresh weight and chlorophyll content in different treatments.

	Control	FM	Bacillus	C4-C5
Height (mm)	66.89 ± 4.40	139.56 ± 9.29	152.78 ± 13.01	147.56 ± 12.23
Fresh weigh (g)	1.15 ± 0.19	4.49 ± 0.53	6.30 ± 0.93	7.22 ± 0.56
Chlorophyll a+b (mg/g)	7.87 ± 0.03	9.56 ± 0.15	11.18 ± 0.10	10.53 ± 0.51

Table S6

Root length and root surface area in different treatments.

	Control	FM	Bacillus	C4-C5
Length (cm)	85.87 ± 18.77	107.64 ± 41.11	271.54 ± 24.15	287.60 ± 25.94
Surface area (cm^2)	17.71 ± 4.25	21.08 ± 6.23	73.41 ± 4.66	92.85 ± 10.02

Table S7

Soil pH

	Control	FM	Bacillus	C4-C5
pH at harvest	4.73 ± 0.06	5.33 ± 0.44	5.36 ± 0.10	5.50 ± 0.03
pH before transplant	4.91 ± 0.03	5.38 ± 0.07	5.33 ± 0.05	5.37 ± 0.10

Table S8

Total abundance of C cycle genes in different treatments.

	genes abundance ($\times 10^3$ TPM)
Control	26.15 ± 0.35
FM	25.40 ± 0.79
Bacillus	26.84 ± 0.40
C4-C5	27.51 ± 0.80

Table S9

Genes abundance of *acdA*, *carB*, *atpA*, *accC*, *atpD* in different treatment.

genes		Control	FM	Bacillus	C4-C5
acdA	Level 1	1272.33	864.83	861.58	827.16
	Level 2	1148.83	889.21	845.62	788.16
	Level 3	1119.86	873.60	871.65	862.24
carB	Level 1	543.80	670.92	657.70	667.37
	Level 2	572.32	691.89	638.70	663.34
	Level 3	611.07	704.26	631.66	707.38
atpA	Level 1	528.55	594.77	678.58	628.92
	Level 2	573.77	635.77	689.51	653.09
	Level 3	591.61	683.17	643.65	687.92
accC	Level 1	458.06	510.59	487.58	560.20
	Level 2	482.83	524.59	534.51	552.20
	Level 3	503.36	515.92	469.51	584.94
atpD	Level 1	424.3093	509.9062	504.6584	534.9022
	Level 2	450.1463	555.6925	521.51	511.9178
	Level 3	485.0479	572.9446	508.6584	550.402

Table S10

Hot map of nitrogen metabolic pathways

	Control	FM	Bacillus	C4-C5
Assimilatory nitrate reduction	6	6	7	6
Dissimilatory nitrate reduction	13	14	14	14
Denitrification	5	5	5	5
Nitrification auxiliary	2	2	2	2
Nitrogen fixation	4	5	5	6
Nitrification	5	6	5	6
Organic degradation and synthesis	14	16	14	16
Total	49	54	52	55

Table S11

Hot map of phosphorus metabolic pathways

	Control	FM	Bacillus	C4-C5
Organic phosphoester hydrolysis	7	8	8	8
Phosphonate and phosphinate metabolism	21	21	20	21
Pentose phosphate pathway	8	8	8	8
Phosphotransferase system	42	45	43	45
Purine metabolism	21	22	22	22
Pyrimidine metabolism	16	16	16	16
Pyruvate metabolism	6	6	6	6
Transporters	7	7	7	7
Two component system	13	13	14	13
Others	5	5	5	5
Total	146	151	149	151

Reference

Rong, Qun, Dingtian Lu, Kai Zhong, Shu Yang, Zhongyi Li, and Chaolan Zhang. 2024. Mechanism of antimony oxidation and adsorption using immobilized *Klebsiella aerogenes* HC10 in soil, *Science of the Total Environment*, 956: 177404.