

Supplementary Material

A multi-attribute approach to evaluating the impact of biostimulants on crop performance

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Sequencing data availability

Amplicon sequencing raw data are available at EMBL-EBI project number PRJEB59860.

Supplementary Table

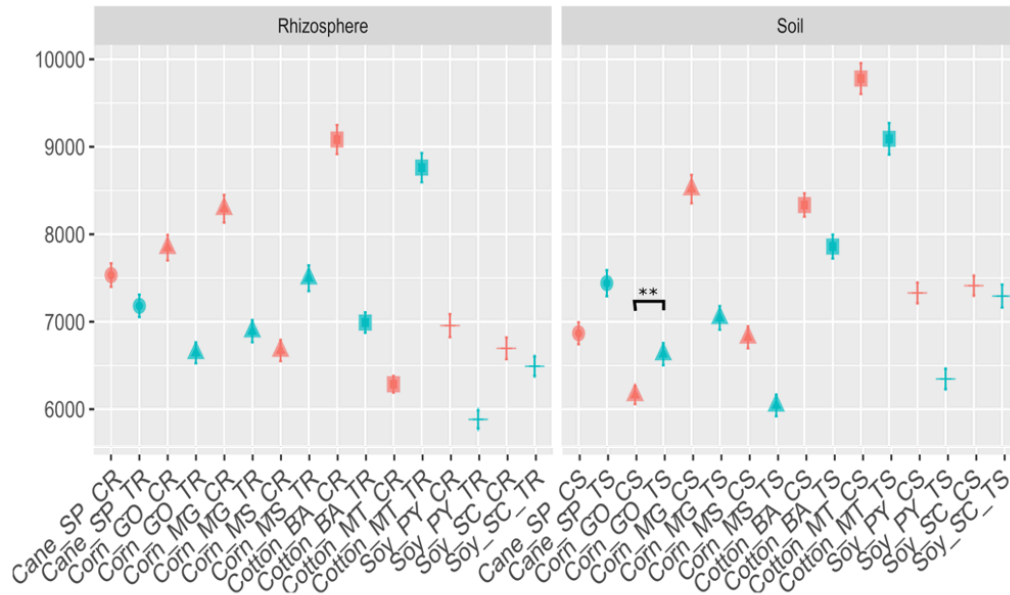
Supplementary Table 1. Indicator variable values (control vs. biostimulant plots) and calculated impact and performance indices related to the assessment dimensions of Crop development, production, and soil quality, obtained in seven reference farms studied in relation to the adoption of plant biostimulant technology. For details on specific variable measurement units, refer to Figure 1.

Case studies	Corn_MG		Corn_GO		Corn_MS		Soy_SC		Soy_PY		Cotton_MT		Sugarcane_SP	
Dimensions / indicators	Variable values	Indices	Variable values	Indices	Variable values	Indices	Variable values	Indices	Variable values	Indices	Variable values	Indices	Variable values	Indices
<u>Crop development, production</u>														
Stand	3.7-3.9	0.77	2.98-3.0	0.71	3.2-3.3	0.74	9.7-10.3	0.78	8.6-9.4	0.81	7.68-7.73	0.71	11.6-12.8	0.82
Rooting	12-14	0.87	280-345	0.91	12.5-14.0	0.83	2.63-3.11	0.88	12-15	0.91	33.7-41.3	0.90	25-34	0.95
Plant vigor 1	318-533	0.99	16-18.5	0.86	16.6-19.9	0.89	7.1-7.3	0.74	7.3-7.8	0.78	27.1-30.4	0.83	2.8-2.81	0.71
Plant vigor 2	14-16	0.85	13.3-13.5	0.73	13.2-15.3	0.86	36.2-38.6	0.78	2.6-5.2	1.0	6.7-7.0	0.76	16.9-18.0	0.78
Plant vigor 3	107.4-112.8	0.76	13.2-14.1	0.78	74-79	0.78	14.0-16.4	0.87	27.4-50.2	1.0	13.1-14.3	0.81	1.36-1.38	0.72
Plant vigor 4	211-235	0.82	193-197	0.73	166-178	0.79	56.7-58.5	0.74	26.8-30.4	0.84	47.1-49.3	0.76	190-200	0.77
Product quality	nd	nd	8.0-8.7	0.80	296-310	0.76	135-136	0.71	182.6-190.4	0.75	nd	nd	136.3-150.9	0.82
Production	nd	nd	134.7-141.9	0.77	33-45	0.95	43.9-49.6	0.84	11.42-13.2	0.86	3.17-3.42	0.79	69.6-76.3	0.81
Net revenue	nd	nd	11719-12214	0.75	2871-3784	0.94	7243-8051	0.82	1884-2047	0.80	42220-45298	0.79	7935-8454	0.78
<u>Soil chemistry</u>														
Organic Matter	3.25-3.18	0.68	3.50-3.10	0.55	1.60-2.58	0.99	2.68-3.29	0.91	3.18-3.78	0.88	3.21-2.99	0.61	3.36-3.24	0.66
pH impact	nd	nd	5.2-5.7	0.99	4.7-5.3	0.99	5.2-5.4	0.99	5.2-5.5	0.99	5.5-5.6	0.99	nd	nd
pH perform.				0.75		0.63		0.66		0.73		0.74		
P impact	nd	nd	31.4-17.5	0.10	132-76.6	0.11	16.7-19.7	0.89	15.4-38.1	1.0	75.4-100.8	0.96	nd	nd
P perform.				0.45		0.99		0.50		0.79		0.83		
K impact	nd	nd	2.0-1.7	0.46	3.3-4.1	0.93	6.2-7.4	0.90	5.7-31.3	1.0	3.3-3.4	0.75	nd	nd
K perform.				0.44		0.83		1.0		0.10		0.74		
Mg impact	nd	nd	38.8-20.2	0.08	2.7-10.6	1.0	13.0-18.7	0.98	7.9-22.4	1.0	23.0-23.7	0.75	nd	nd

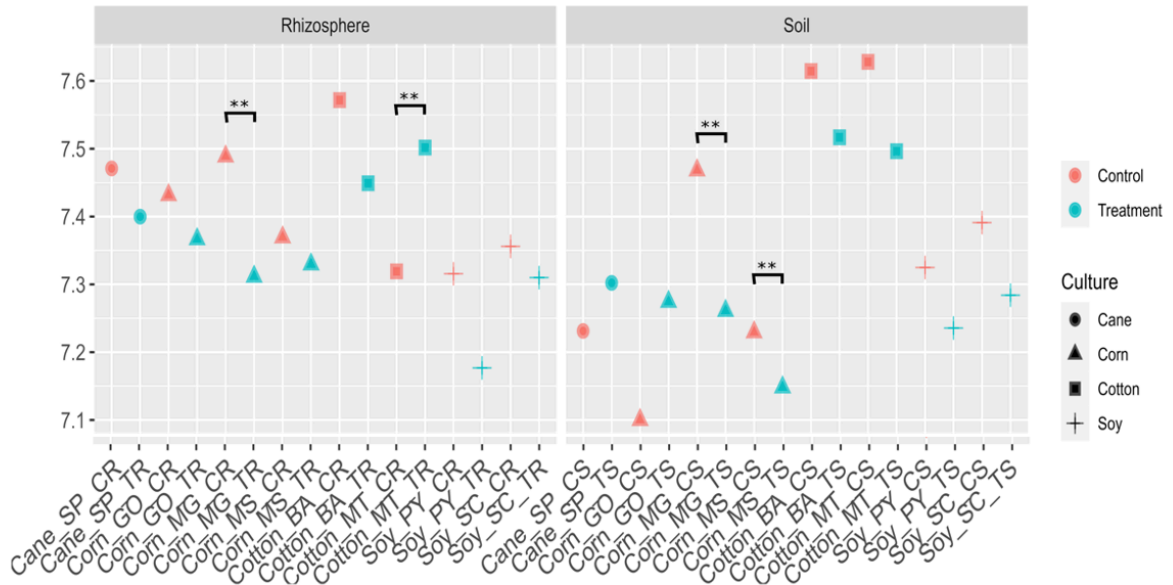
Mg perform.				0.82		0.99		0.82		0.82		0.82		
H + Al impact	nd	nd	42.8-50.4	0.48	57.6-56.3	0.72	62.5-64.4	0.66	63.9-55.1	0.85	57.6-49.3	0.86	nd	nd
H + Al perform.				0.16		0.10		0.02		0.11		0.17		
Total bases impact	nd	nd	103.2-65.2	0.21	17.0-61.3	1.0	69.3-89.5	0.93	47.8-135.2	1.0	77.2-80.1	0.75	nd	nd
Total bases perform.				0.94		0.94		0.97		0.99		0.97		
CEC impact	nd	nd	146-115.6	0.41	74.6-117.6	0.99	131.8-153.9	0.87	111.7-190.3	0.99	134.8-129.4	0.65	nd	nd
CEC perform.				0.99		0.99		0.99		0.99		0.99		
Bases saturation impact	nd	nd	70.7-56.4	0.41	22.8-52.1	1.0	52.6-58.2	0.82	42.8-71.0	0.99	57.3-61.9	0.79	nd	nd
Bases saturation perform.				0.49		0.44		0.51		0.65		0.55		
<u>Soil physics</u>														
Compaction impact	2.44-2.18	0.82	2.65-2.52	0.76	2.43-2.24	0.79	3.42-1.78	1.0	1.88-1.59	0.87	1.68-1.58	0.76	2.17-2.16	0.68
Compaction performance		0.68		0.64		0.67		0.73		0.75		0.75		0.70
<u>Soil biology</u>														
β-Glycosidase impact	145-131.3	0.55	123-174	0.98	26.7-98.8	1.0	125.1-107.6	0.47	82.0-117.4	0.98	81.5-92.7	0.86	67.7-72.8	0.80
β-Glycosidase performance		0.71		0.90		0.55		0.60		0.65		0.52		0.40
Arylsulfatase impact	86.9-130.9	0.99	70-130	1.0	6.0-75.2	1.0	147.0-159.8	0.47	60.9-85.3	0.98	57.9-52.2	0.54	90.9-96.2	0.78
Arylsulfatase performance		0.90		0.90		0.56		0.60		0.63		0.38		0.70

Supplementary Figures

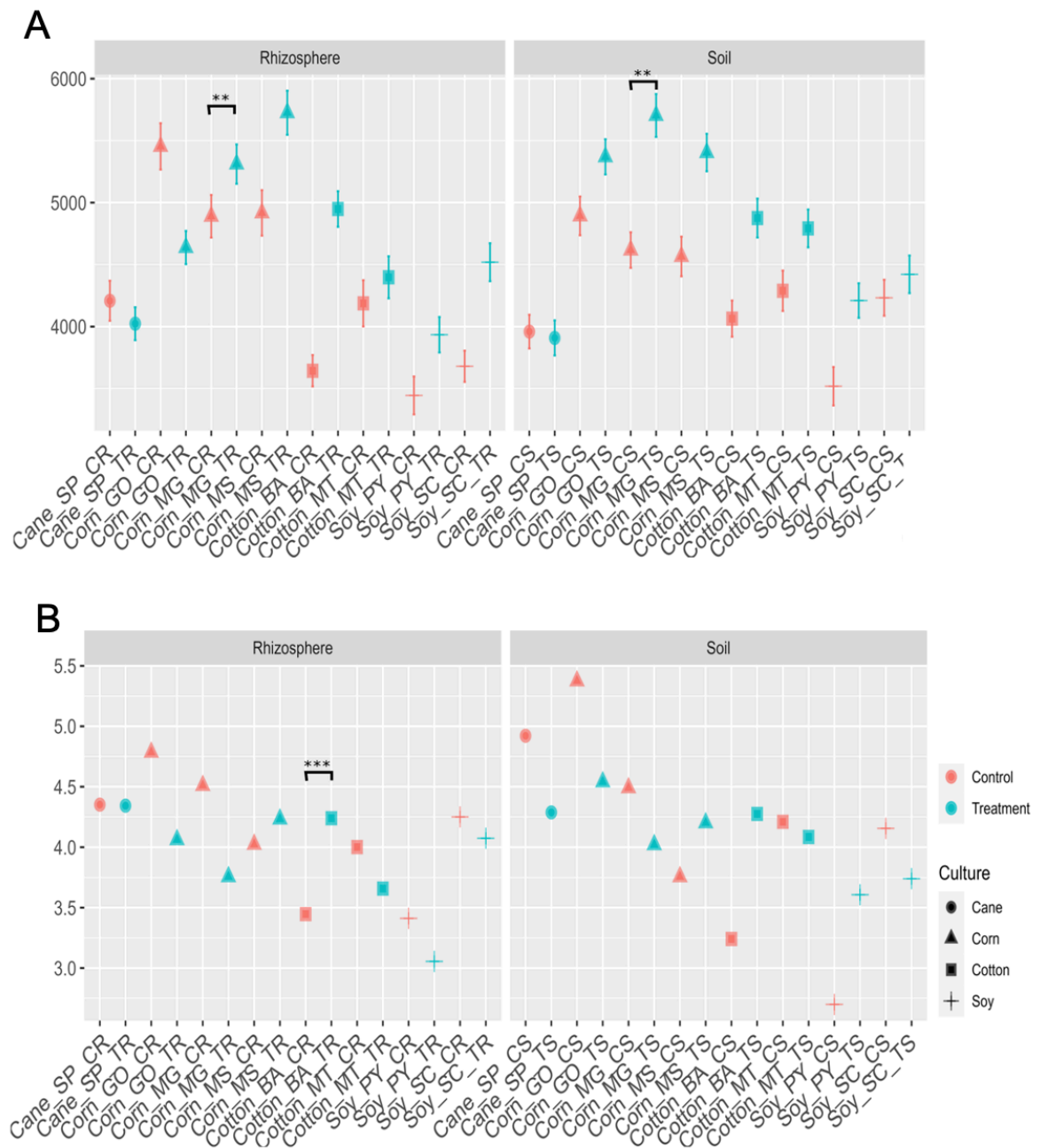
A



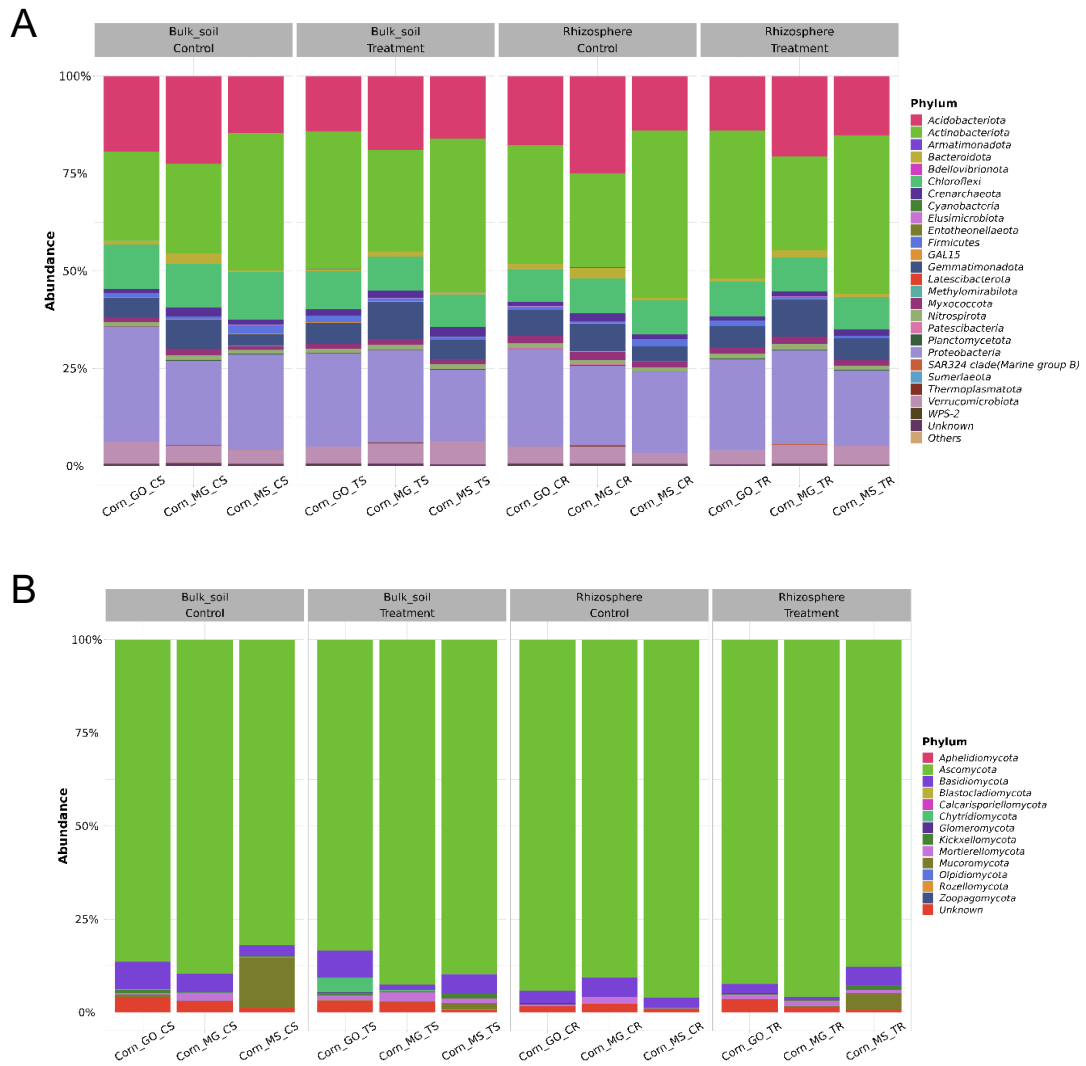
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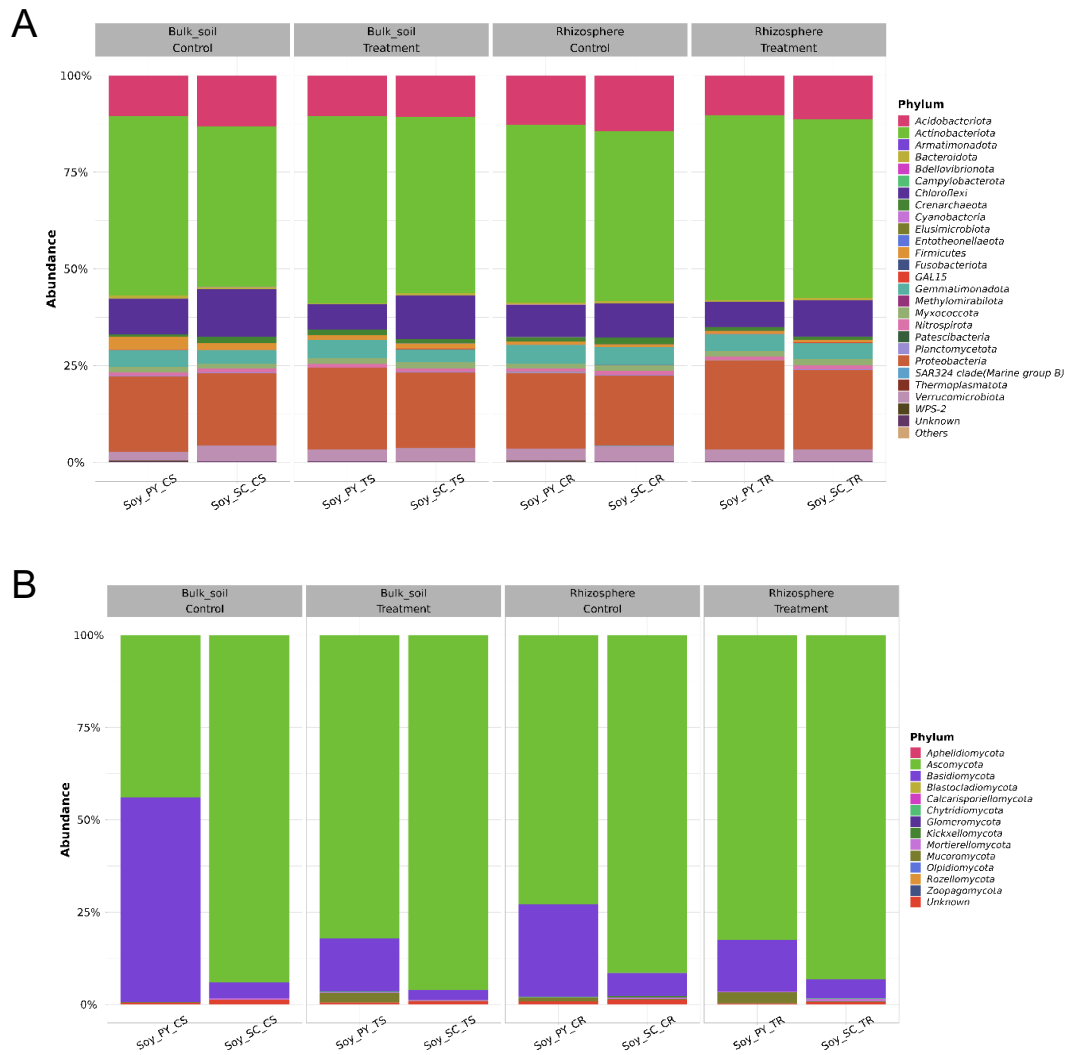
Supplementary Figure 1. Alpha diversity analysis of bacterial communities using Chao1 (A) and Shannon (B) indices. The microbiome was analyzed based on their 16S rRNA gene amplicon sequencing. The lines in the graph represent the mean values of Chao1 and Shannon indices for each sample considering 3 replicates. Error bars represent standard deviations. Each crop is represented by a different color as indicated in the key. Locations and all four treatments are indicated, control e(CR) or treatment (TR). Significant differences are indicated by ** (pvalue< 0.05) or *** (pvalue< 0.01) according to T test.



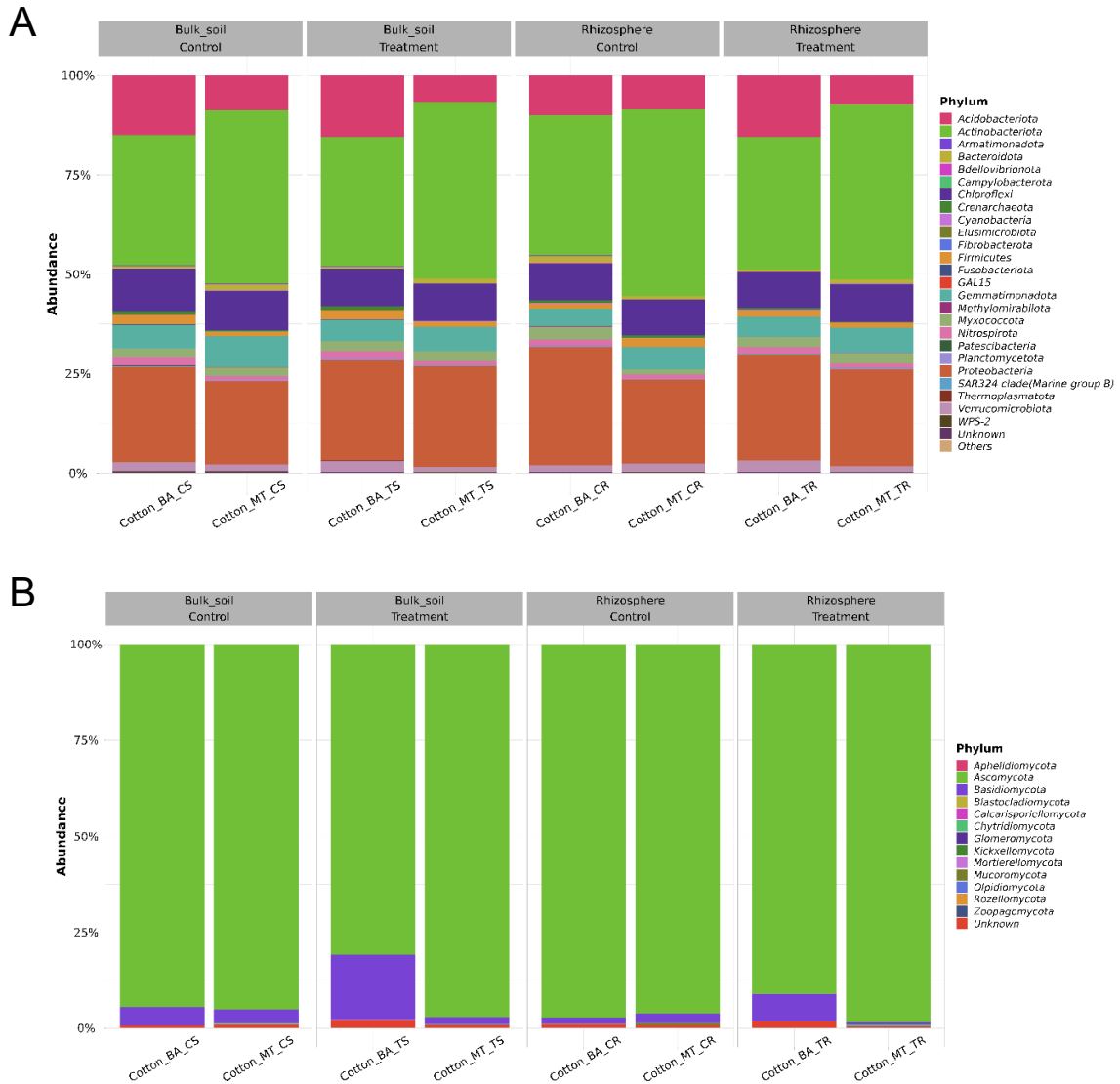
Supplementary Figure 2. Alpha diversity analysis of fungal communities using Chao1 (A) and Shannon (B) indices. The microbiome was analyzed based on their ITS amplicon sequencing. The lines in the graph represent the mean values of Chao1 and Shannon indices for each sample considering 3 replicates. Error bars represent standard deviations. Each crop is represented by a different color as indicated in the key. Locations and all four treatments are indicated, control (CR) or treatment (TR)). Significant differences are indicated by ** (pvalue< 0.05) or *** (pvalue< 0,01) according to T test.



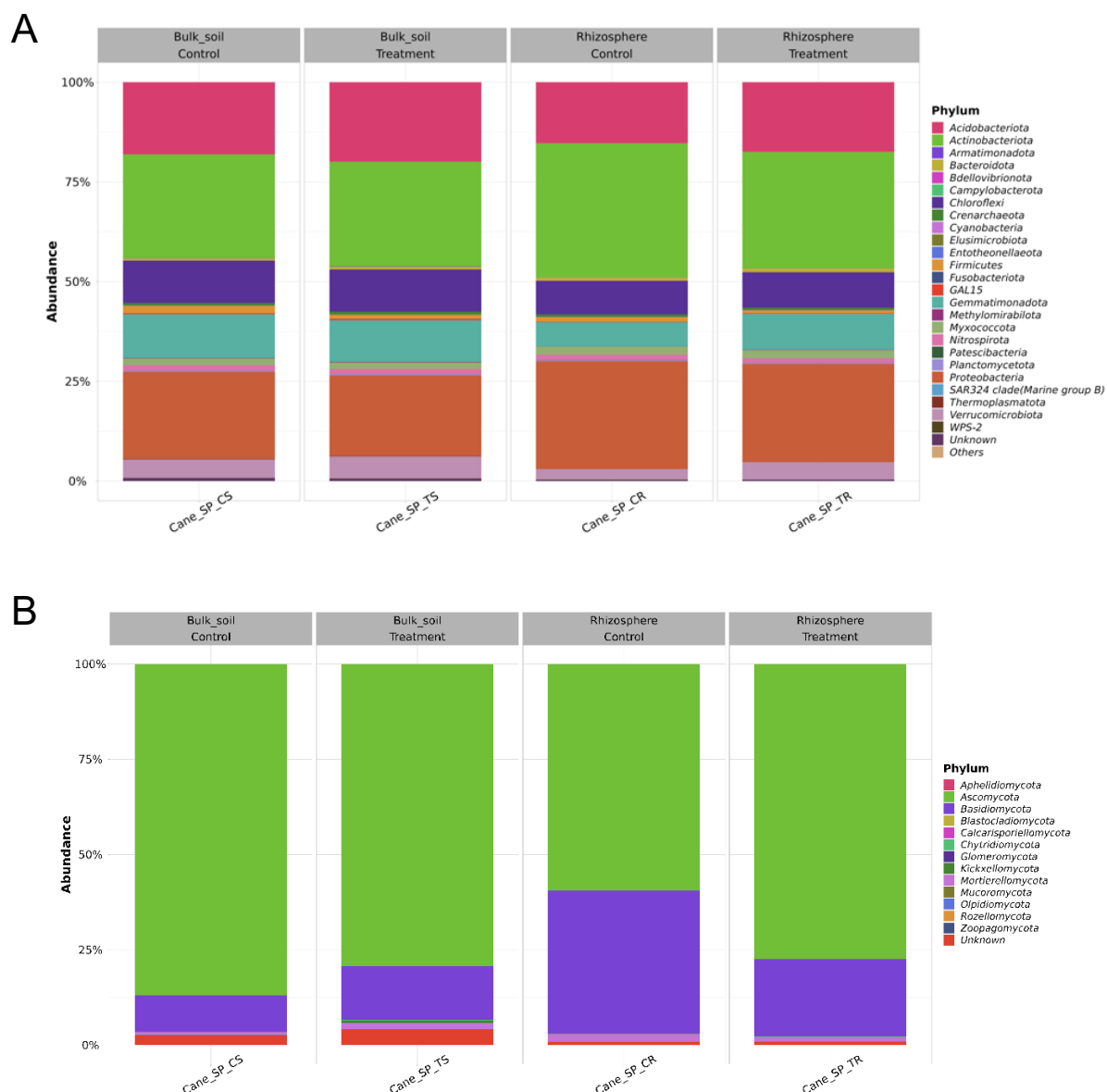
Supplementary Figure 3. Relative abundance of bacterial (A) and fungal (B) phyla in the microbiome associated with corn production systems in GO, MG and MS. Each bar represents one of the four treatments (average of 3 replicates) per location, i.e., control rhizosphere (CR), control soil (CS), treatment rhizosphere (TR) and treatment soil (TS). The color-coding in the bars corresponds to different phyla, as indicated in the key.



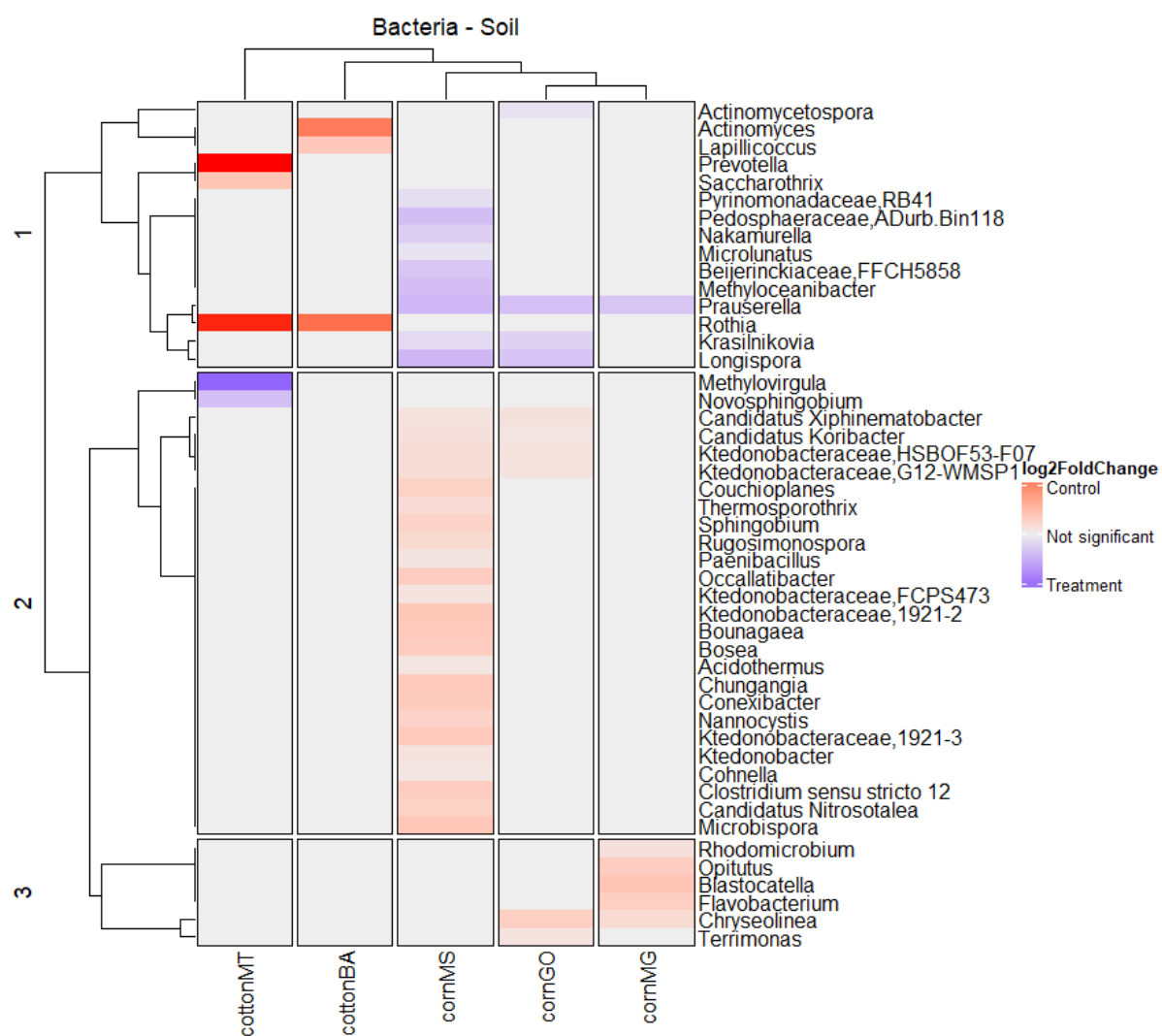
Supplementary Figure 4. Relative abundance of bacterial (A) and fungal (B) phyla in the microbiome associated with soy bean production systems in SC and PY. Each bar represents one of the four treatments (average of 3 replicates) per location, i.e., control rhizosphere (CR), control soil (CS), treatment rhizosphere (TR) and treatment soil (TS). The color-coding in the bars corresponds to different phyla, as indicated in the key.



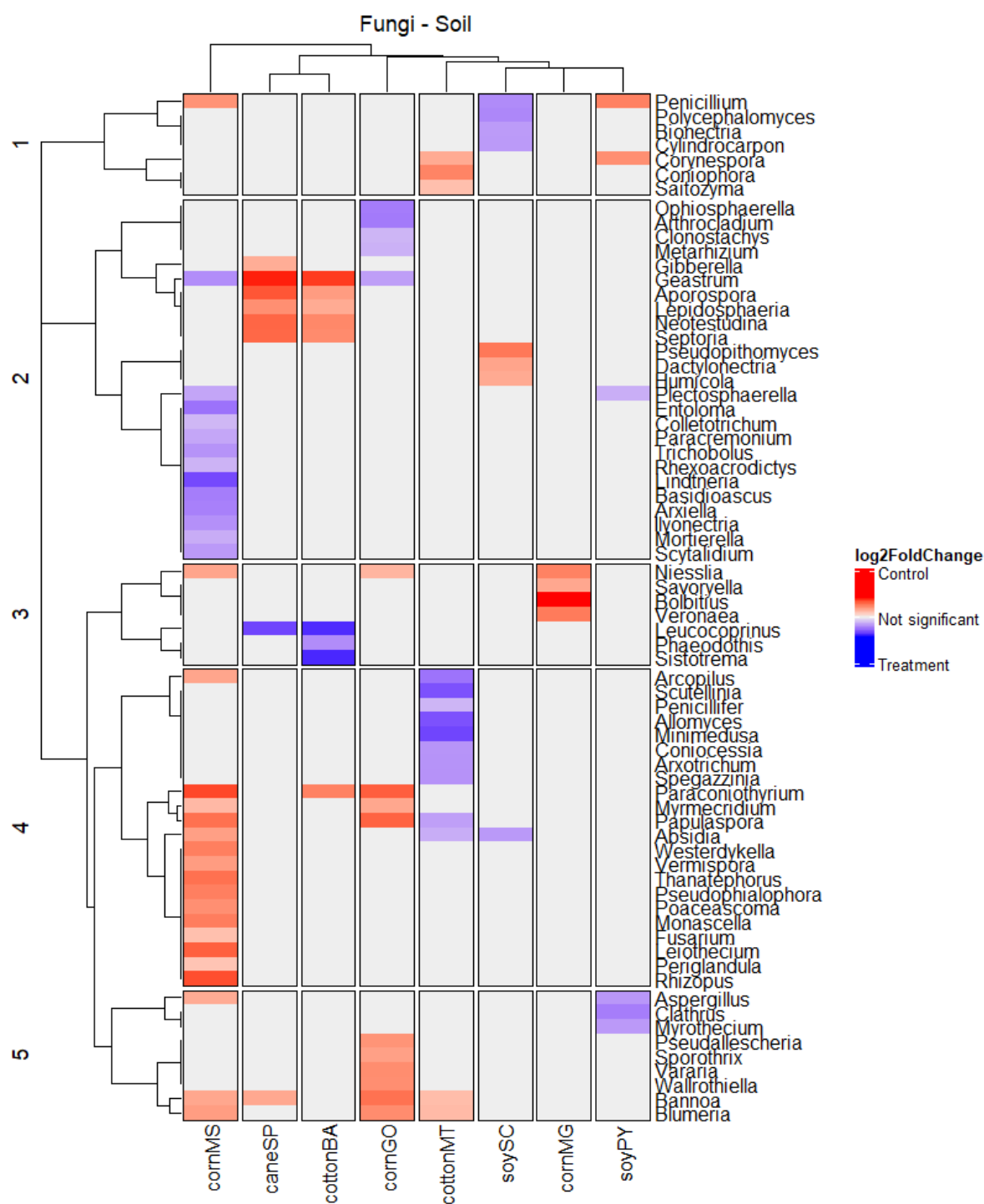
Supplementary Figure 5. Relative abundance of bacterial (A) and fungal (B) phyla in the microbiome associated with cotton production systems in MT and BA. Each bar represents one of the four treatments (average of 3 replicates) per location, i.e., control rhizosphere (CR), control soil (CS), treatment rhizosphere (TR) and treatment soil (TS). The color-coding in the bars corresponds to different phyla, as indicated in the key.



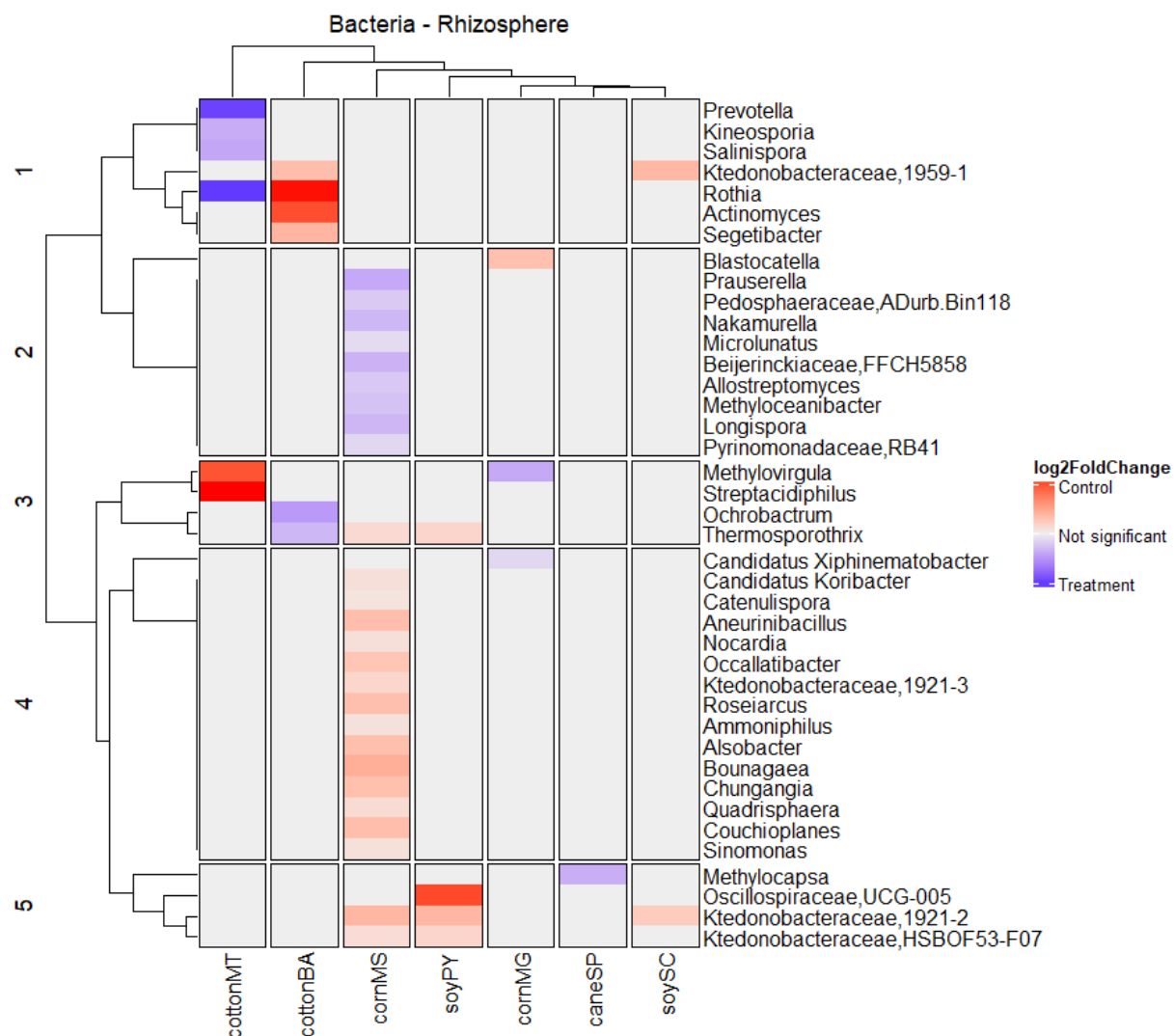
Supplementary Figure 6. Relative abundance of bacterial (A) and fungal (B) phyla in the microbiome associated with sugar cane production systems in SP. Each bar represents one of the four treatments (average of 3 replicates) per location, i.e., control rhizosphere (CR), control soil (CS), treatment rhizosphere (TR) and treatment soil (TS). The color-coding in the bars corresponds to different phyla, as indicated in the key.



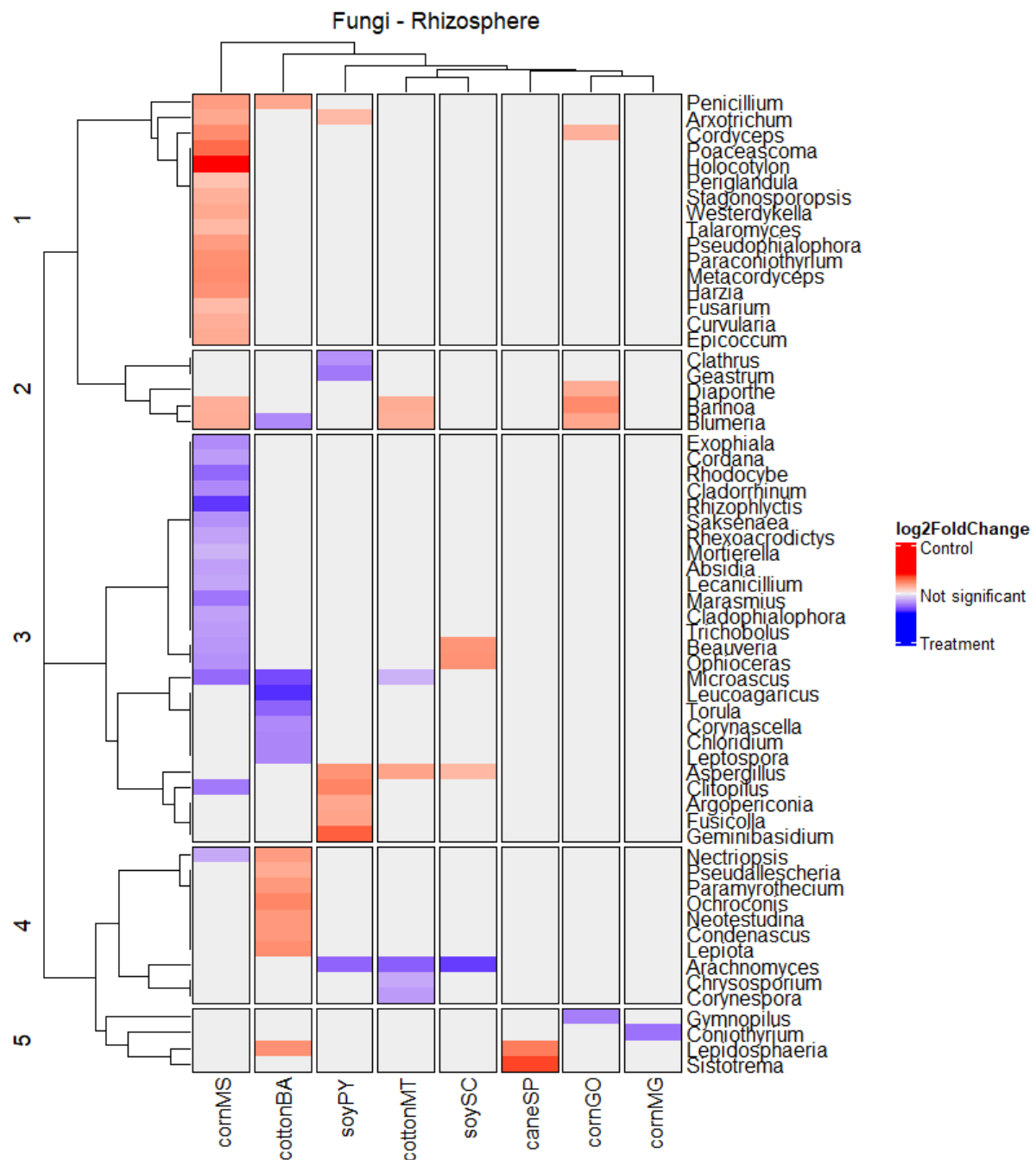
Supplementary Figure 7. Heatmap displaying changes in abundance of soil bacterial taxa significantly (p -value < 0.05) enriched in control (red) or in the biostimulant (blue) treatment. The intensity of the color represents the magnitude of the log2 fold change. Only treatments with significant differences between treatments are shown in the figure.



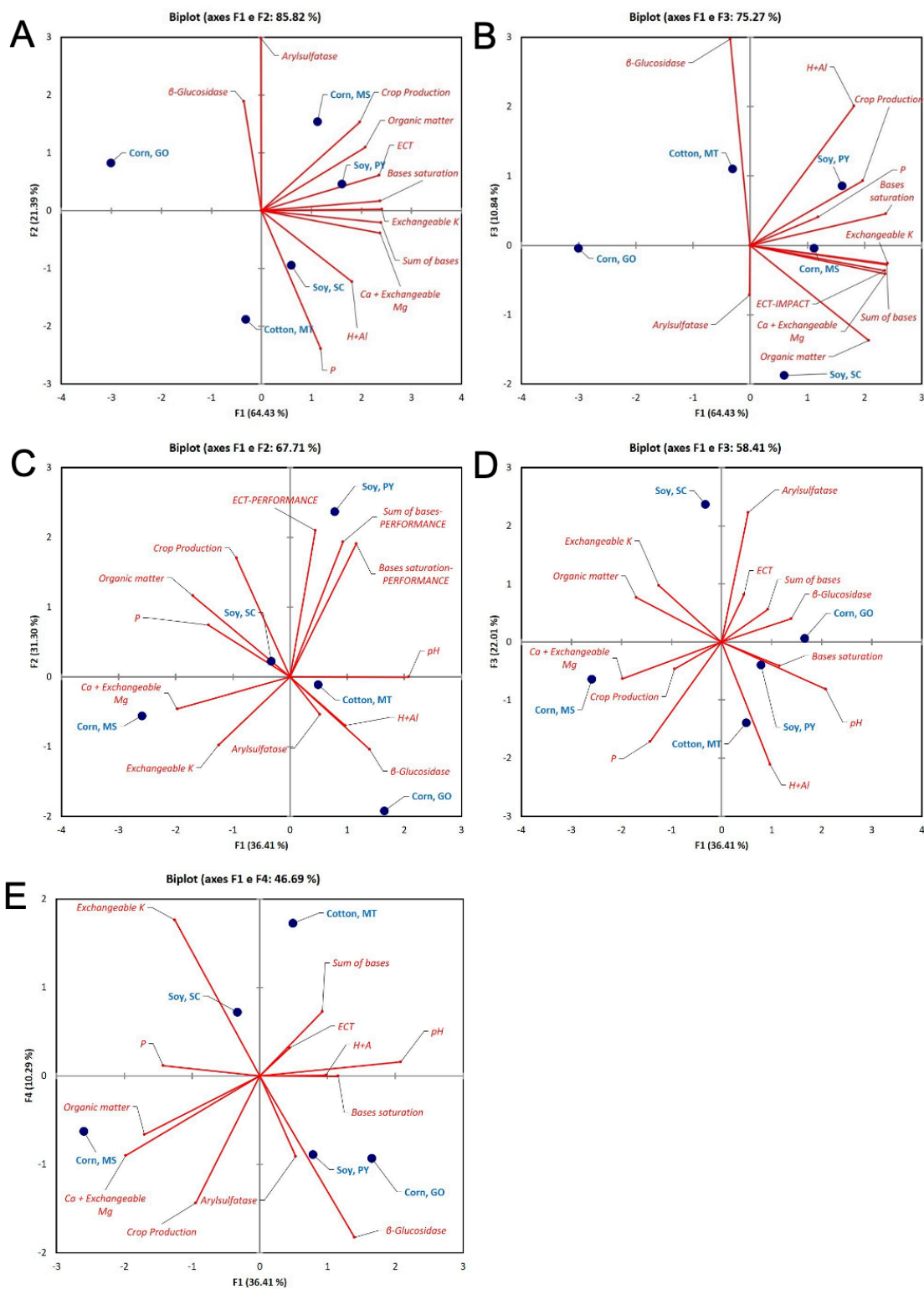
Supplementary Figure 8. Heatmap displaying changes in abundance of soil fungal taxa significantly (p -value < 0.05) enriched in control (red) or in the biostimulant (blue) treatment. The intensity of the color represents the magnitude of the log2 fold change. Only treatments with significant differences between treatments are shown in the figure.



Supplementary Figure 9. Heatmap displaying changes in abundance of rhizosphere bacterial taxa significantly (p -value < 0.05) enriched in control (red) or in the biostimulant (blue) treatment. The intensity of the color represents the magnitude of the log2 fold change. Only treatments with significant differences between treatments are shown in the figure.



Supplementary Figure 10. Heatmap displaying changes in abundance of rhizosphere fungal taxa significantly (p -value < 0.05) enriched in control (red) or in the biostimulant (blue) treatment. The intensity of the color represents the magnitude of the log2 fold change. Only treatments with significant differences between treatments are shown in the figure.



Supplementary Figure 11. Biplots of the Principal Components Analysis for impact values (A and B) and for technical performance (C, D and E) of biostimulant use showing the correlation circle of the variables (indicators, red vectors) and the observations cloud (case studies, blue dots) with normalized values.