

Supplementary Information

Microbiome-inspired phyllospheric synthetic communities increase microbial diversity and productivity of field-grown plants of *Agave tequilana*

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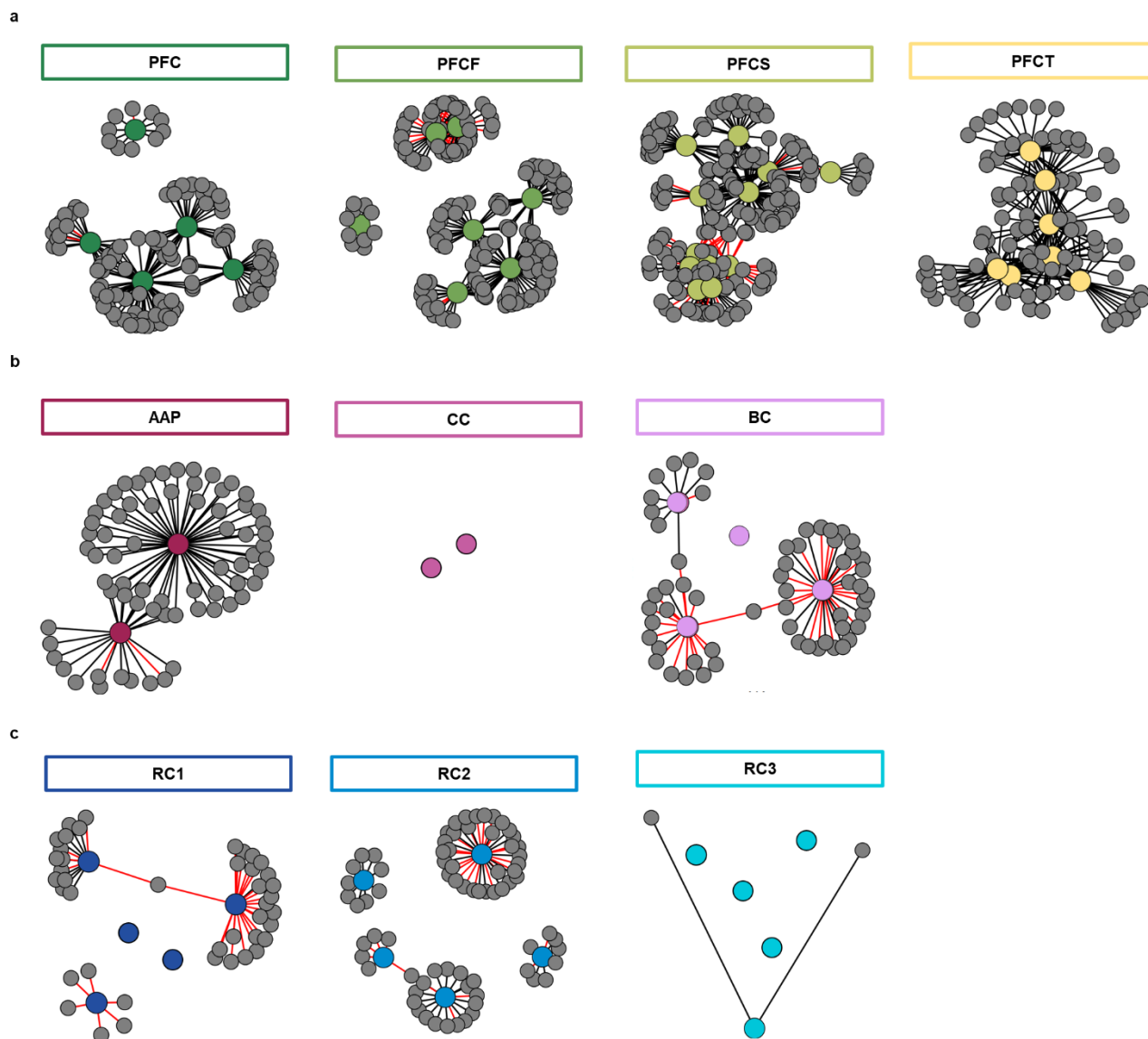


Fig. S1. Network position of the syncoms members in the correlation network of the phyllosphere of *A. salmiana*. The OTUs matching the syncom members and their interactions were extracted from previously constructed correlation networks , V. M. F.-N, Citlali Fonseca-García, Damaris Desgarennnes and L. P. P.-M. *in press*).

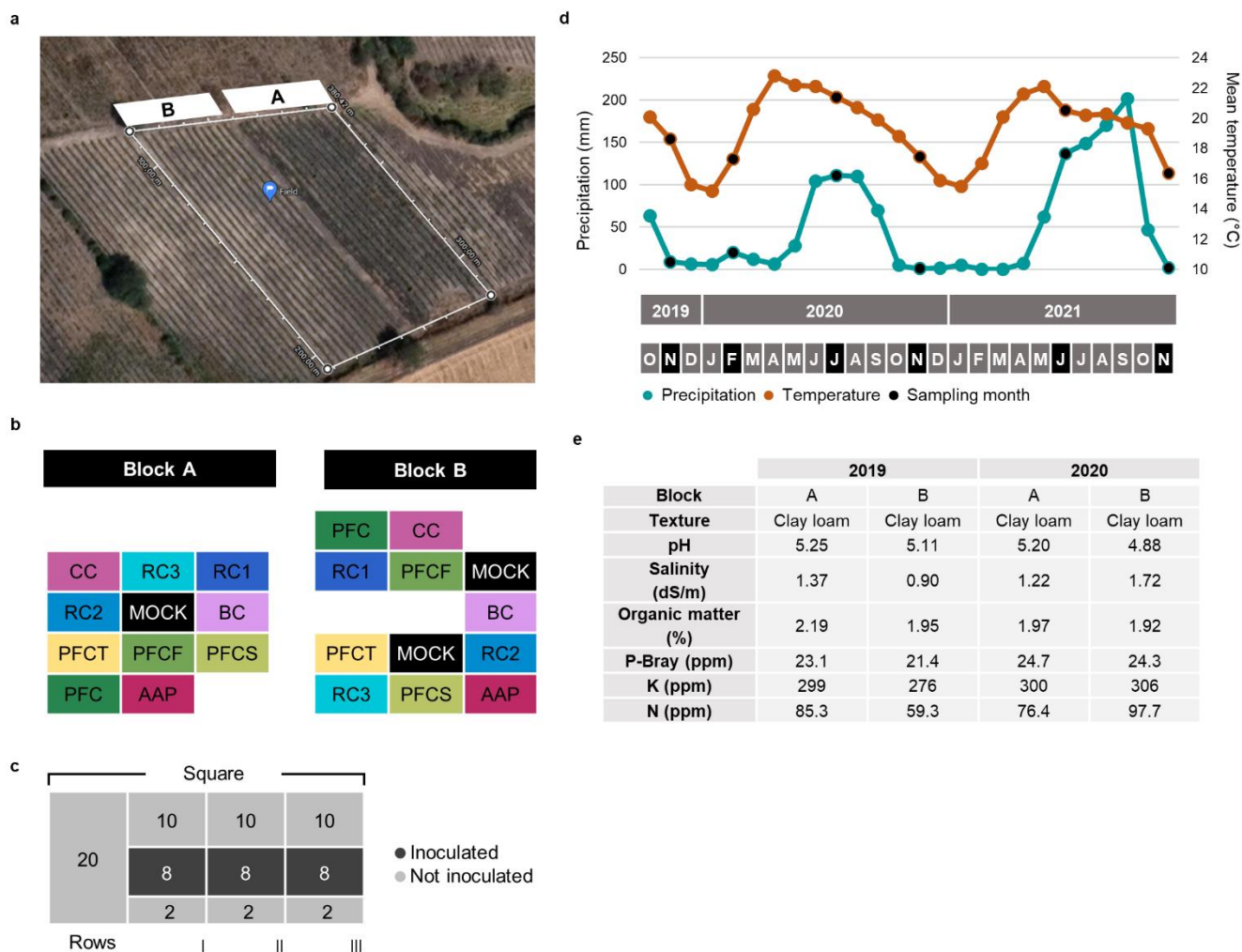


Fig. S2. Field of *A. tequilana* plants in Pénjamo, Gto., Mexico. a) location, b) spatial distribution of blocks and treatments, c) plants treated per square, d) Precipitation and mean temperature by month (Data provided by Comisión Nacional del Agua (CONAGUA), Mexico), e) Soil physicochemical characteristics.



Fig. S3. Sampling strategy. *A. tequilana* plants were evaluated in rainy (June or July) and dry months (November, February). Icons represent the array of analysis performed.

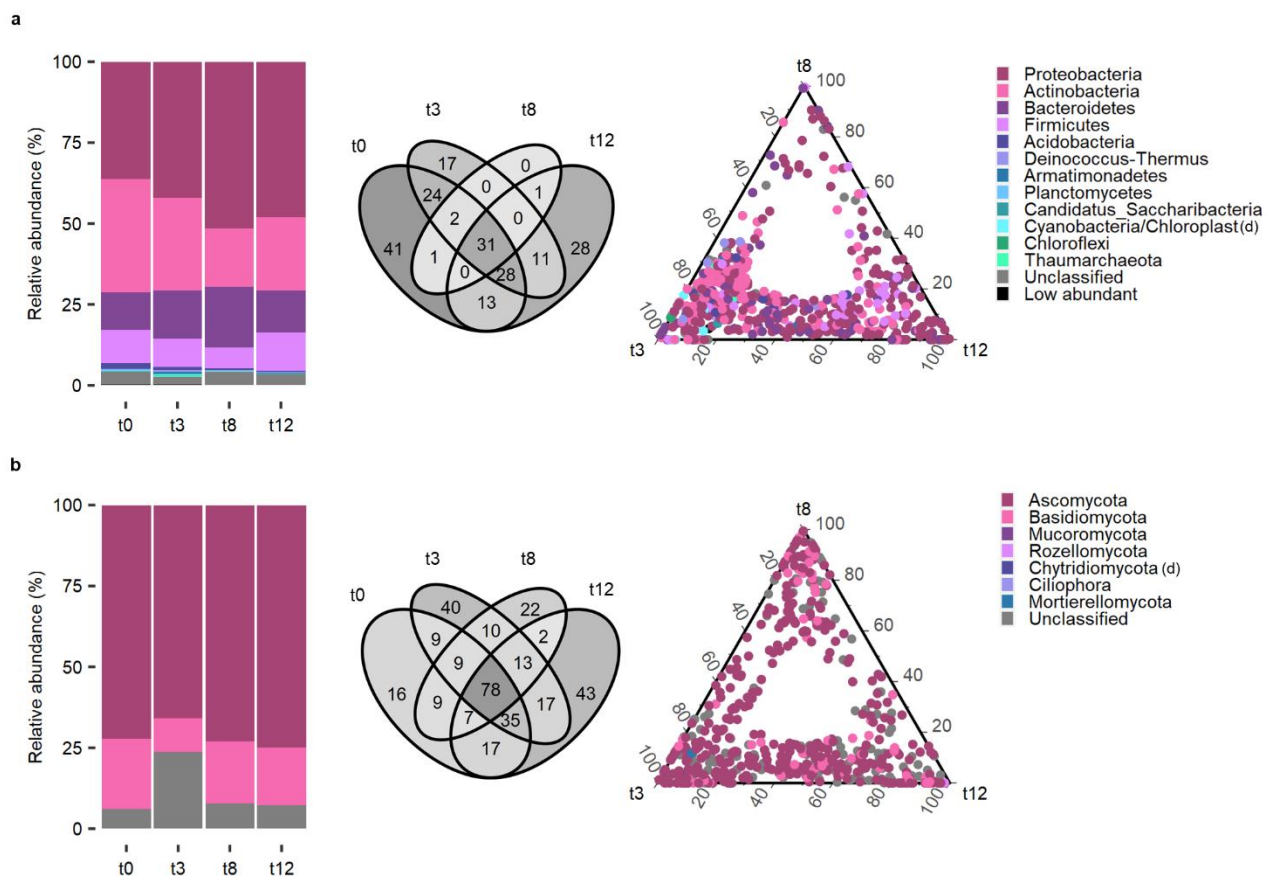


Fig. S4. Phylum-level classification, core, and enrichment analysis of prokaryotic (a) and eukaryotic (b) phyllospheric communities across sampling seasons. Bars represent the phylum-level relative abundance of OTUs. Venn diagrams show the number of shared OTUs (50% prevalence). Ternary plots show the ratio of the mean relative abundance of differential OTUs (Kruskal-Wallis test/Dunn test: $FC \geq |1.5|$ and adjusted $p\text{-value} \leq 0.05$). Phylum-level enrichments are indicated with letters. d: enriched in dry season. r: enriched in rainy season (Wilcox test: $FC \geq |1.5|$ and adjusted $p\text{-value} \leq 0.05$). Sampling seasons. t0:November 2019. t3:February 2020. t8:July 2020. t12:November 2020.

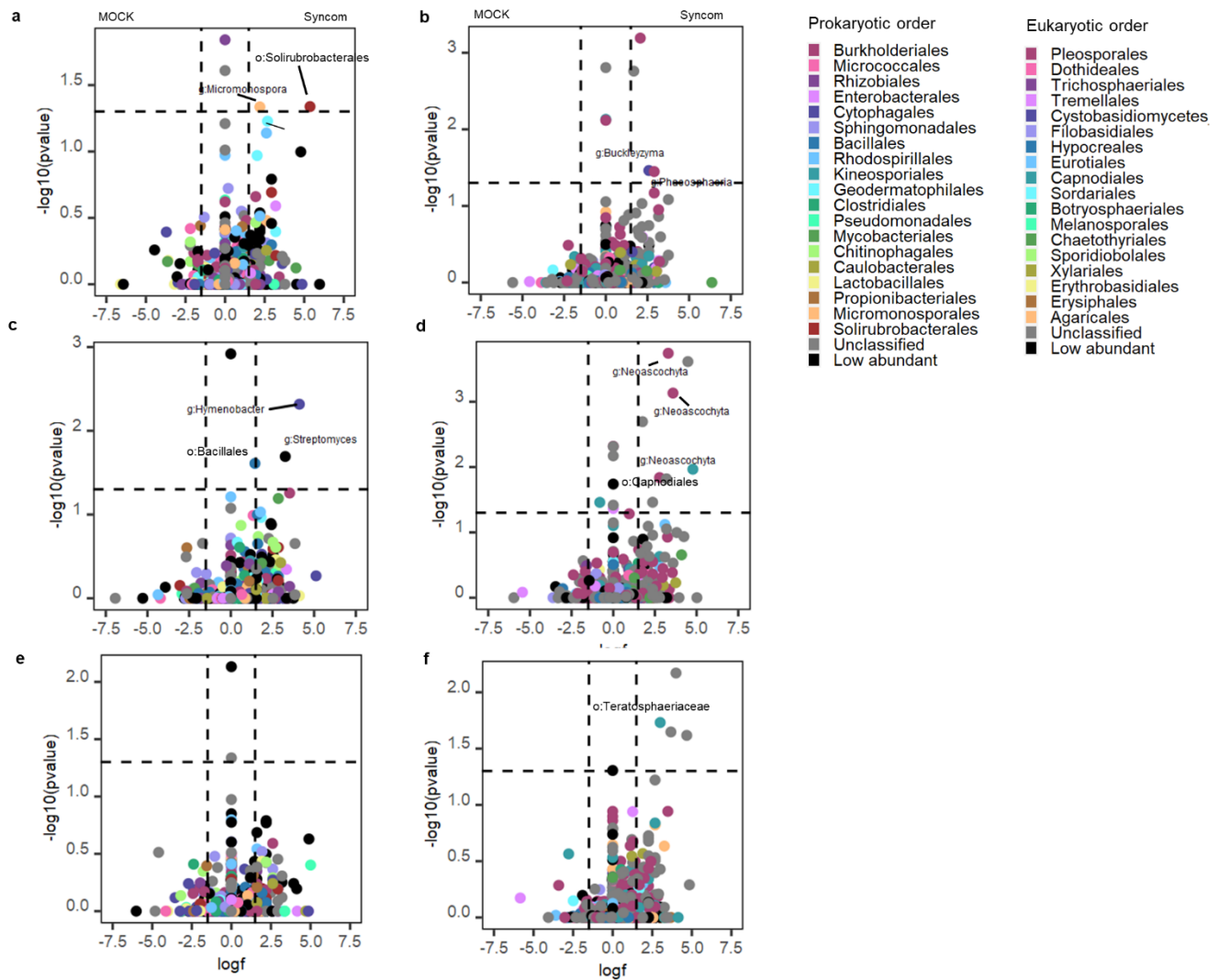


Fig. S5. Differential OTUs between non-inoculated plants and plants inoculated with PFC, PFCS and RC2 syncoms. Scatter plots represent the fold change ($\log_2$) and adjusted p-value of the prokaryotic (a,c,e) and eukaryotic (b,d,f) OTUs of non-inoculated plants compared to planta inoculated with PFC (a,b), PFCS (c,d) and RC3 (e,f) syncoms. Differential OTUs with a fold change ≥ 1.5 and adjusted p-value ≤ 0.05 were selected. OTUs are colored based on their order-level classification. .

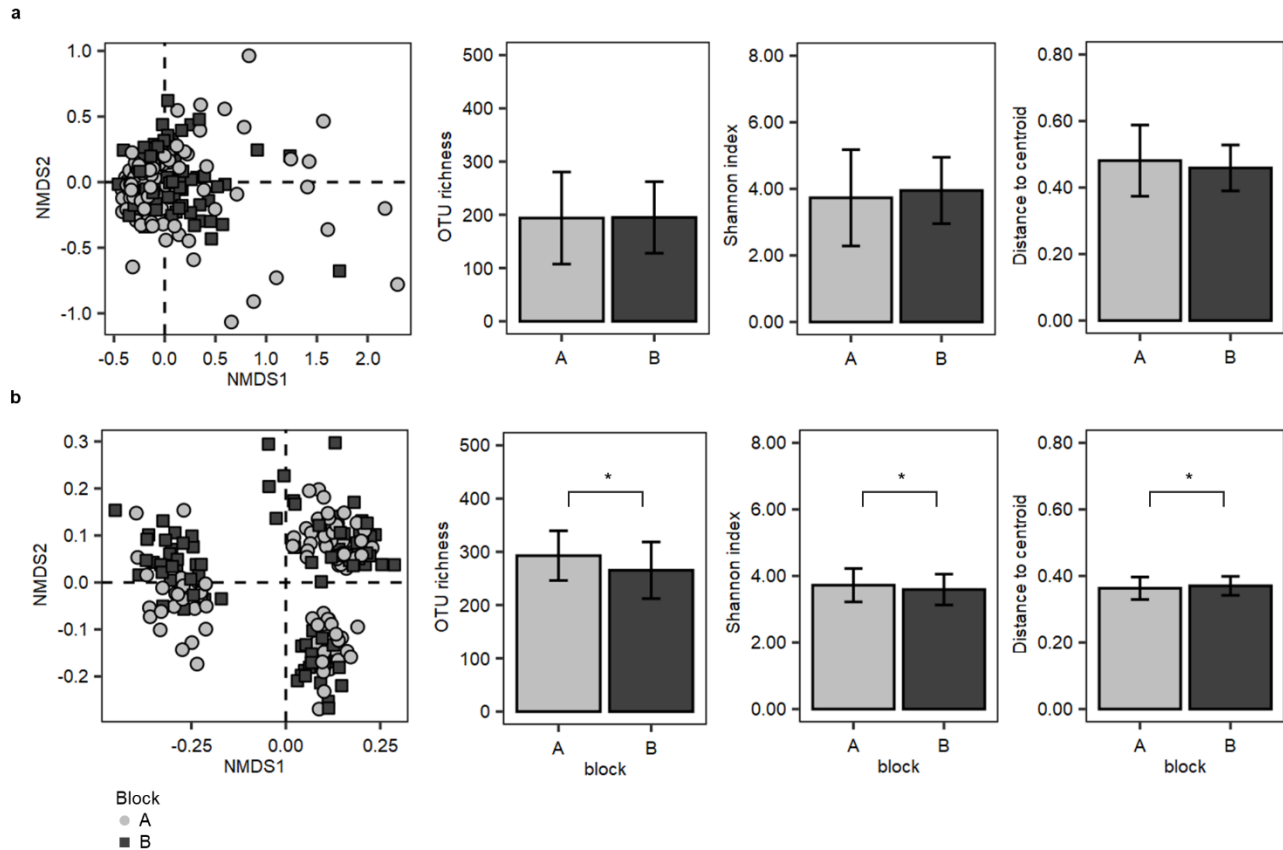


Fig S6. Diversity of the prokaryotic (a) and eukaryotic (b) phyllospheric communities by block.

Nonmetric multidimensional scaling ordinations (NMDS) were calculated using binary Bray-Curtis dissimilarities between samples. Bars represent the mean $\pm$ sd. of the observed OTU richness, Shannon index, and distance to the centroid of the samples between blocks (A and B). Asterisks indicate a significant change between blocks (Wilcox test: $p \leq 0.05$).

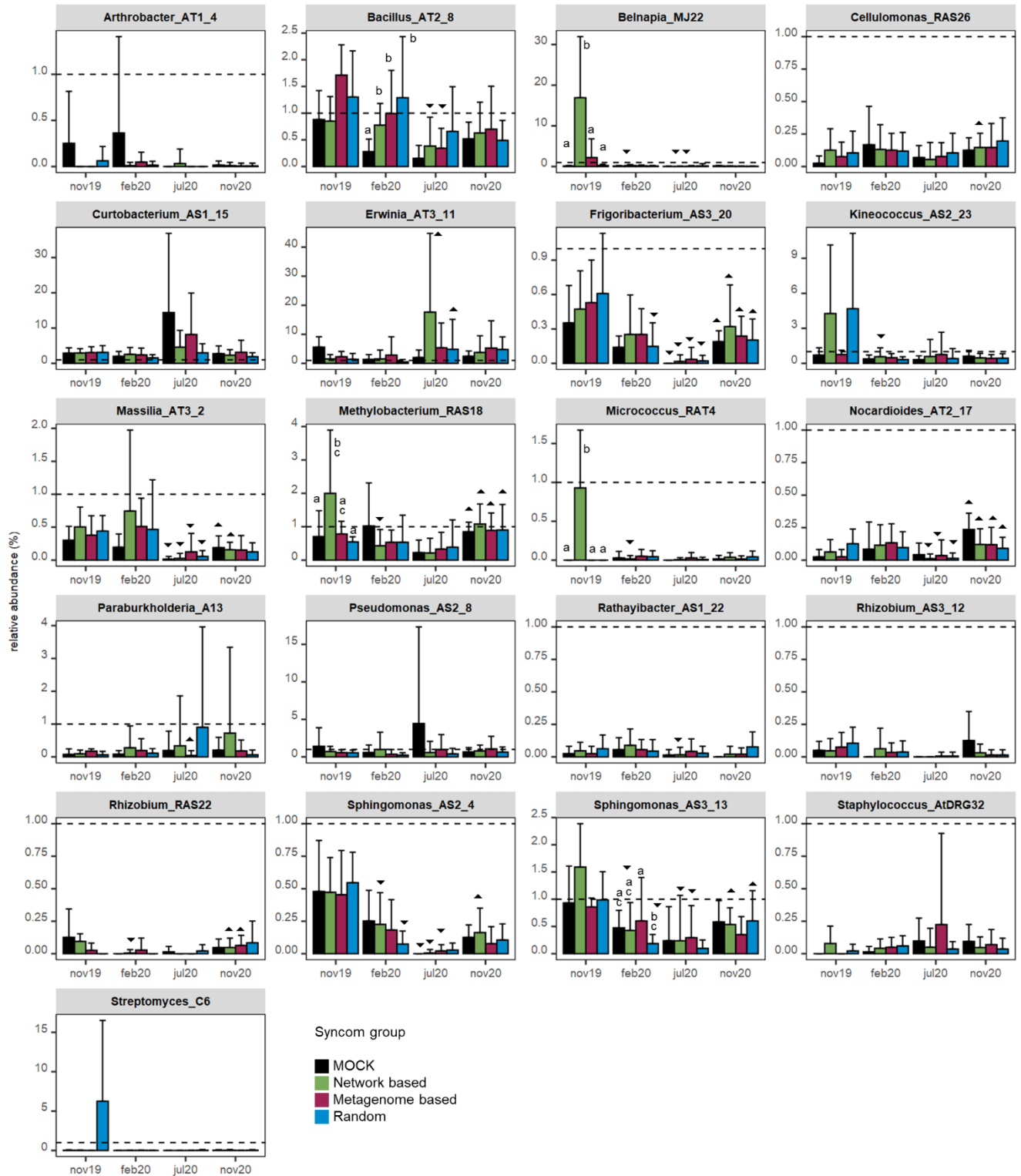


Fig. S7. Detection of inoculated strains on amplicon sequence data. Bar plots represent the mean relative abundance + sd. of the OTUs matching the inoculated strains from November 2019 to November 2020. Data was grouped based on the syncom design. Letters indicate significant differences in the relative abundance of a strain between syncom designs (Kruskal-Wallis test/Dunn test: $FC \geq |1.5|$ and adjusted p-value ≤ 0.05). Up and down arrow heads indicate significant increases and decreases, respectively, in the relative abundance of a strain compared to the previous sampling season (Kruskal-Wallis test/Dunn test: $FC \geq |1.5|$ and adjusted p-value ≤ 0.05)

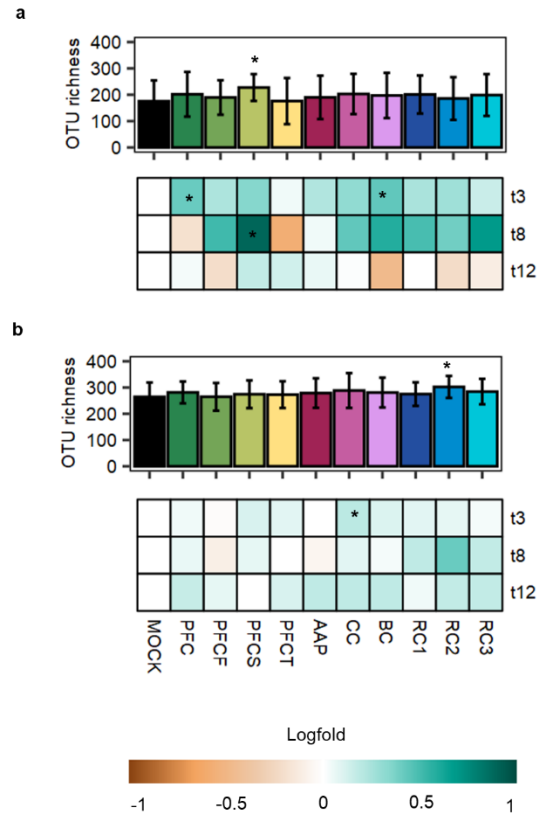


Fig. S8. OTU richness of phyllospheric communities by syncom treatment. Bars represent the mean $\pm$ sd of the OTU richness of prokaryotic (a) and eukaryotic (b) communities by syncom treatment and the heatmaps below show the log₂-fold change of the OTU richness between the syncom treatments and the control (MOCK) in each sampling season (t3, t8, t12). Asterisks indicate a significant change between treatment and control (Wilcox test: $p \leq 0.05$). Sampling seasons: t0:November 2019. t3:February 2020. t8:July 2020. t12:November 2020

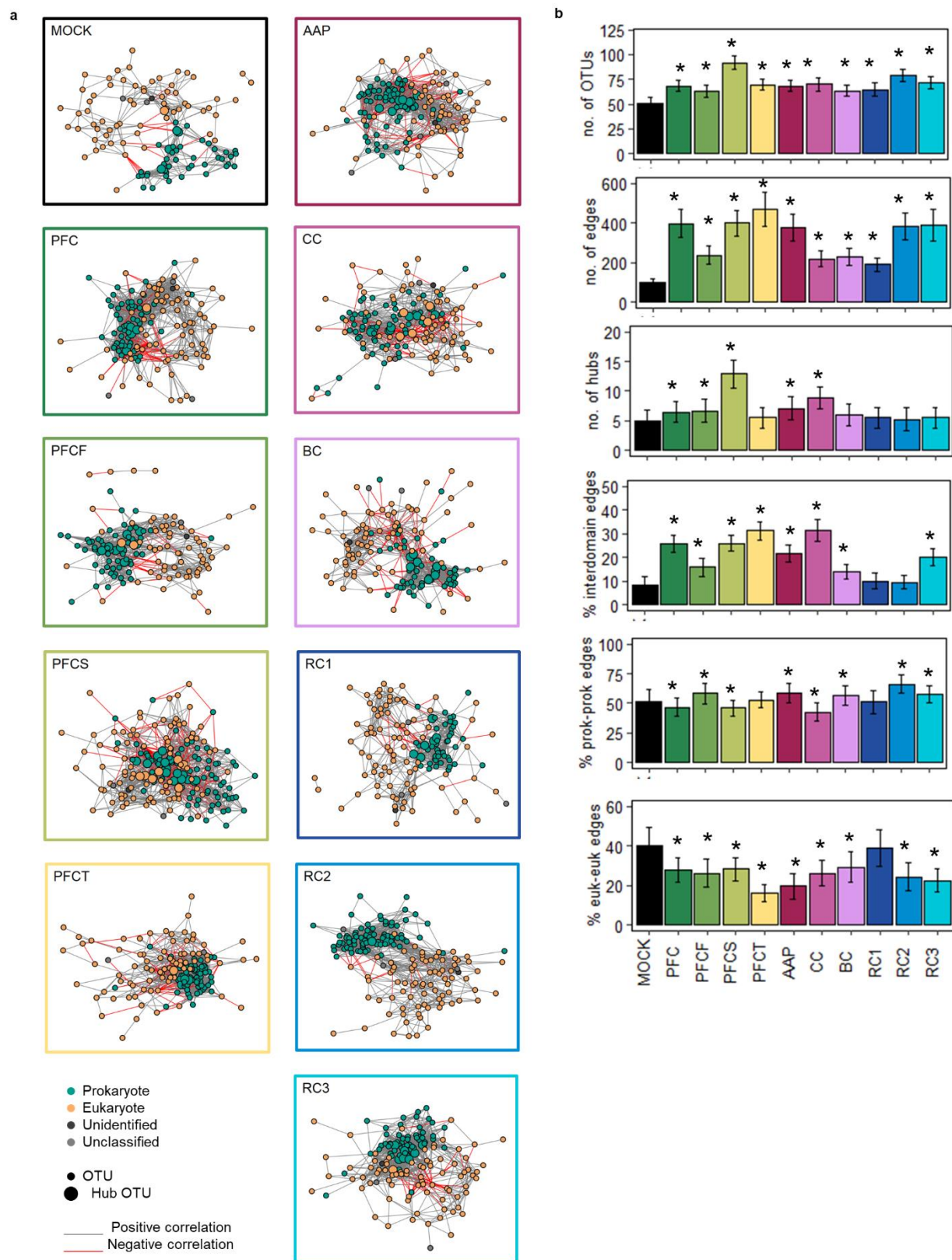
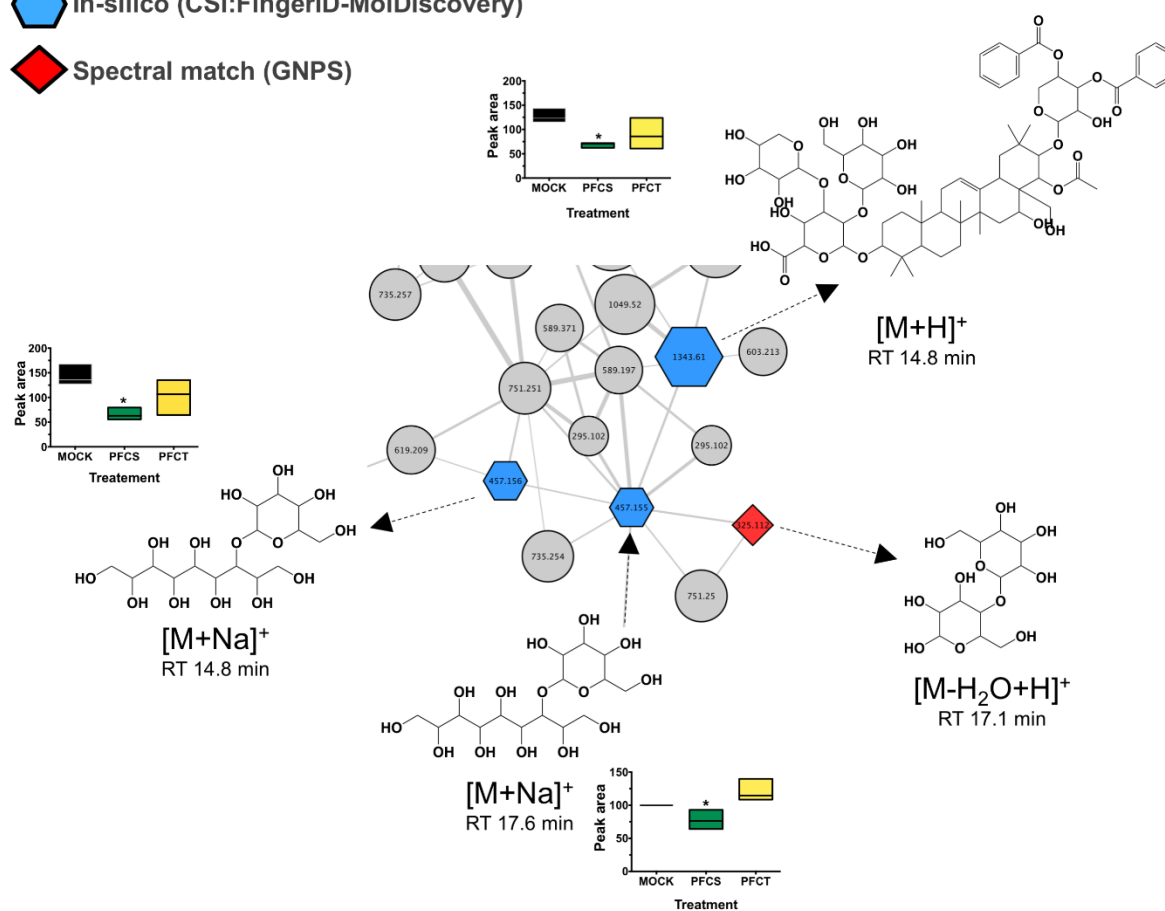


Fig. S9. Network analysis of phyllospheric microbial communities. a) Correlation networks of microbial communities of plants inoculated with synthetic communities. Vertices represent OTUs and edges represent significant correlation (Spearman cor. $\rho \geq |0.6|$) and p-value ≤ 0.01). Vertices are colored based on the OTU Domain and edges based on their weight. Red was used for negative correlations and black for positive correlations. Bigger OTUs represent microbial hubs. b) Network metrics across 100 random networks per syncom treatment. Asterisks indicate significant differences (Wilcox test: p-value ≤ 0.01) between a treatment and the non-inoculated plants (MOCK).

 In-silico (CSI:FingerID-MolDiscovery)

 Spectral match (GNPS)

2001



14

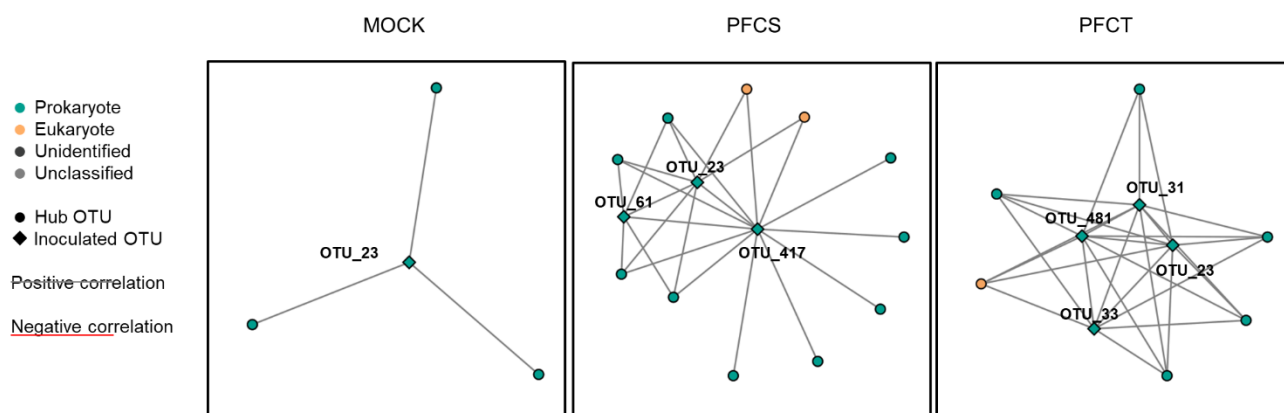


Fig. S11. Correlation between inoculated and hub OTUs. Vertices represent OTUs and edges represent significant correlation (Spearman cor. $\rho \geq |0.6|$) and $p\text{-value} \leq 0.01$). Vertices are colored based on the OTU kingdom and edges based on their weight. Red was used for negative correlations and black for positive correlations. OTU_23: *Methylobacterium* (*Methylobacterium* sp. RAS18), OTU_61: *Sphingomonas* (*Sphingomonas endophytica* AS3.13), OTU_417: Microbacteriaceae (*Frigoribacterium endophyticum* AS3.20), OTU_31: Bacillaceae (*Bacillus niacini* AT2.8), OTU_33: *Kineococcus* (*Kineococcus radiotolerans* AS2.23), OTU_481: *Massilia* (*Massilia aurea* AT3.2).

Table S1. Syncom members and their relevance in correlation networks.

Species	Strain	Host	Network				Metagenome			Random		
			PFC	PFCF	PFCS	PFCT	AAP	CC	BC	RC1	RC2	RC3
<i>Belnapia rosea</i>	MJ22	as	h.at/h.as	h.at/h.as	h.at/h.as	h.at/h.as	h.at/h.as					
<i>Methylobacterium</i> sp.	RAS18	as	d.at/d.as	d.at/d.as	d.at/d.as	d.at/d.as	d.at/d.as					
<i>Micrococcus aloeverae</i>	RAT4	at	d.at/c. as	d.at/c. as	d.at/c. as	d.at/c. as						
<i>Nocardioides cavernae</i>	AT2.17	at	d.at/h.as	d.at/h.as	d.at/h.as	d.at/h.as						
<i>Staphylococcus hominis</i>	AtDRG32	at	h.at/c.as	h.at/c.as	h.at/c.as	h.at/c.as						
<i>Aureobasidium</i> sp.	AT3.8	at		- /d.as	- /d.as							
<i>Cladosporium</i> sp.	cutV	as		c.at/h.as	c.at/h.as							
<i>Sphingomonas endophytica</i>	AS3.13	as			d.at/c.d.as					d.at/c.d.as		
<i>Sphingomonas aerolata</i>	AS2.4	as			*							
<i>Sphingomonas melonis</i>	AS2.3	as										
<i>Cellulomonas cellasea</i>	RAS26	as			-/h.as							
<i>Frigoribacterium endophyticum</i>	AS3.20	as			-/h.as							
<i>Kineococcus radiotolerans</i>	AS2.23	as				c.d.at/-				c.d.at/-		
<i>Bacillus niacini</i>	AT2.8	at				c.d.at/c.d.as						
<i>Massilia aurea</i>	AT3.2	at				d.at/-						
<i>Leptolyngbya</i> sp.	AT32P	at						-				
<i>Nodosilinea</i> sp.	AT21F	at						-				
<i>Pantoea brenneri</i>	A11	as							-/h.as		-/h.as	
<i>Erwinia gerundensis</i>	AT3.11	at							-			
<i>Paraburkholderia caledonica</i>	A13	as							-			
<i>Pseudomonas</i> sp.	AS2.8	as							-			
<i>Rhizobium</i> sp.	RAS22	as								-	-	
<i>Paenibacillus</i> sp.	A5	as								-		-
<i>Rathayibacter caricis</i>	AS1.22	as								-		
<i>Arthrobacter</i> sp.	AT1.4	at									-	
<i>Bhargavaea ginsengi</i>	C13	as									-	
<i>Streptomyces cacaoi</i>	C6	as									-	
<i>Curtobacterium oceanosedimentum</i>	AS1.15	as										-/c.as
<i>Kytococcus sedentarius</i>	RAS25	as										-
<i>Phyllobacterium</i> sp.	A21	as										-
<i>Rhizobium soli</i>	AS3.12	as										-

Host species. as: *Agave salmiana*. at: *Agave tequilana*. Network relevance. h: consistent hub genus. c: consistent highly central genus. d: consistent highly connected OTU.

(*) Five different OTUs of *Sphingomonas* were highly connected and central in *A. salmiana* compared to one in *A. tequilana*

Bacillus niacini / *Neobacillus niacini* (current)

Methylobacterium sp. / *Methylorubrum* (current)

Table S2. Prokaryotic and eukaryotic orders enriched in each season

Data	Order	Abundance in dry (%)	Abundance in rainy (%)	p.value	FC	Enriched in
Prokaryotic	g:Gp16	0.114	0.039	6.62E-05	1.55	dry
	o:Abditibacteriales	0.054	0.007	7.00E-04	2.86	dry
	o:Acidimicrobiales	0.041	0.002	8.56E-04	4.46	dry
	o:Blastocatellales	0.019	0.006	4.88E-02	1.81	dry
	o:Gammaproteobacteria_incertae_sedis	0.022	0.002	3.37E-03	3.58	dry
	o:Kineosporiales	3.072	0.974	6.68E-18	1.66	dry
	o:Ktedonobacterales	0.058	0.017	2.81E-02	1.81	dry
	o:Nakamurellales	0.266	0.024	6.02E-14	3.46	dry
	o:Nitrosomonadales	0.017	0.002	1.66E-02	3.17	dry
	o:Nitrospirales	0.015	0.004	4.61E-02	2.00	dry
	o:Rubrobacterales	0.069	0.024	2.32E-03	1.51	dry
	o:Sphingobacteriales	1.124	0.383	1.35E-13	1.55	dry
	o:Xanthomonadales	0.695	0.146	6.36E-12	2.25	dry
	o:Caulobacterales	0.622	3.052	2.74E-07	-2.29	rainy
	o:Agaricostilbales	0.023	0.008	1.86E-04	1.50	dry
	o:Cantharellales	0.024	0.002	1.56E-03	3.36	dry
Eukaryotic	o:Chaetothyriales	0.690	0.162	2.53E-16	2.09	dry
	o:Cystobasidiomycetes	2.471	0.584	8.12E-21	2.08	dry
	o:Cystofilobasidiales	0.127	0.002	4.99E-16	5.75	dry
	o:Erysiphales	0.466	0.030	1.27E-23	3.95	dry
	o:Lecanorales	0.029	0.010	2.85E-04	1.54	dry
	o:Microstromatales	0.169	0.012	5.49E-15	3.84	dry
	o:Orbiliales	0.023	0.001	9.17E-06	5.29	dry
	o:Ostropales	0.008	0.002	2.68E-02	2.26	dry
	o:Phyllachorales	0.009	0.001	3.99E-04	3.99	dry
	o:Sporidiobolales	0.283	0.083	1.38E-05	1.78	dry
	o:Trichosphaeriales	7.260	2.084	8.05E-22	1.80	dry
	o:Ustilaginales	0.297	0.003	1.34E-17	6.65	dry
	o:Agaricales	0.062	0.677	6.13E-23	-3.45	rainy
	o:Filobasidiales	3.301	9.769	4.90E-06	-1.57	rainy
	o:Melanosporales	0.254	1.015	1.34E-02	-2.00	rainy
	o:Pezizomycotina	0.005	0.015	6.17E-03	-1.63	rainy

dry: February 2020 (t3) and November 2020 (t12). rainy: July 2020 (t8)

Table S3. Number of prokaryotic OTUs enriched in each season

Phylum	Order	# OTUs enriched in dry	# OTUs enriched in rainy
NA	o:Unclassified	18	3
p:Proteobacteria	o:Rhizobiales	28	3
p:Proteobacteria	o:Burkholderiales	22	6
p:Bacteroidetes	o:Cytophagales	17	3
p:Proteobacteria	o:Sphingomonadales	17	0
p:Proteobacteria	o:Rhodospirillales	15	1
p:Actinobacteria	o:Micrococcales	14	1
p:Firmicutes	o:Bacillales	11	1
p:Actinobacteria	o:Propionibacteriales	7	0
p:Bacteroidetes	o:Chitinophagales	6	2
p:Proteobacteria	o:Rhodobacterales	6	0
p:Actinobacteria	o:Geodermatophilales	5	0
p:Firmicutes	o:Lactobacillales	5	0
p:Proteobacteria	o:Xanthomonadales	5	0
p:Actinobacteria	o:Mycobacteriales	4	0
p:Proteobacteria	o:Pseudomonadales	4	2
p:Proteobacteria	o:Sphingobacteriales	4	0
p:Proteobacteria	o:Caulobacterales	3	1
p:Abditibacteriota	o:Abditibacteriales	2	0
p:Firmicutes	o:Clostridiales	2	0
p:Deinococcus-Thermus	o:Deinococcales	2	0
p:Proteobacteria	o:Enterobacterales	2	0
p:Actinobacteria	o:Gaiellales	2	0
p:Actinobacteria	o:Kineosporiales	2	0
p:Actinobacteria	o:Micromonosporales	2	0
p:Proteobacteria	o:Myxococcales	2	1
p:Actinobacteria	o:Nakamurellales	2	0
p:Actinobacteria	o:Solirubrobacterales	2	0
p:Acidobacteria	g:Gp1	1	0
p:Acidobacteria	g:Gp16	1	0
p:Acidobacteria	g:Gp3	1	1
p:Actinobacteria	o:Acidimicrobiales	1	0
p:Proteobacteria	o:Aeromonadales	1	0
p:Acidobacteria	o:Blastocatellales	1	0
p:Proteobacteria	o:Cellvibrionales	1	0
p:Bacteroidetes	o:Flavobacteriales	1	0
p:Proteobacteria	o:Nitrosomonadales	1	0
p:Nitrospirae	o:Nitrospirales	1	0
p:Actinobacteria	o:Rubrobacterales	1	0
p:Proteobacteria	o:Nevskiales	0	1
Total		222	26

dry: February 2020 (t3) and November 2020 (t12). rainy: July 2020 (t8)

Table S4. Number of eukaryotic OTUs enriched in each season

Phylum	Order	# OTUs enriched in dry	# OTUs enriched in rainy
NA	Unclassified	112	55
p:Ascomycota	o:Pleosporales	37	32
p:Basidiomycota	o:Agaricales	0	15
p:Ascomycota	o:Capnodiales	8	15
p:Basidiomycota	o:Filobasidiales	1	3
p:Ascomycota	o:Xylariales	4	3
p:Ascomycota	o:Diaporthales	0	2
p:Ascomycota	o:Helotiales	2	2
p:Ascomycota	o:Botryosphaeriales	4	1
p:Basidiomycota	o:Corticiales	0	1
p:Ascomycota	o:Dothideales	4	1
p:Ascomycota	o:Eurotiales	1	1
p:Ascomycota	o:Hypocreales	13	1
p:Ascomycota	o:Melanosporales	0	1
p:Ascomycota	o:Onygenales	0	1
p:Ascomycota	o:Pezizomycotina	0	1
p:Basidiomycota	o:Russulales	1	1
p:Ascomycota	o:Sordariales	8	1
p:Basidiomycota	o:Agaricostilbales	2	0
p:Basidiomycota	o:Cantharellales	2	0
p:Ascomycota	o:Chaetothyriales	8	0
p:Basidiomycota	o:Cystobasidiomycetes	1	0
p:Basidiomycota	o:Cystofilobasidiales	1	0
p:Ascomycota	o:Erysiphales	6	0
p:Ascomycota	o:Lecanorales	1	0
p:Ascomycota	o:Lichenostigmatales	1	0
p:Basidiomycota	o:Microstromatales	2	0
p:Mucoromycota	o:Mucorales	2	0
p:Ascomycota	o:Myriangiales	2	0
p:Ascomycota	o:Pezizales	1	0
p:Basidiomycota	o:Sporidiobolales	3	0
p:Basidiomycota	o:Tremellales	7	0
p:Ascomycota	o:Trichosphaeriales	3	0
p:Basidiomycota	o:Ustilaginales	1	0
Total		238	137

dry: February 2020 (t3) and November 2020 (t12). rainy: July 2020 (t8)

Table S5. PERMANOVA on phyllospheric microbial communities during the first 12 months of experiment.

	Factor	16S					ITS2				
		df	SumOfSqs	R	F	P	df	SumOfSqs	R	F	P
Global	Season	3	7.352	0.142	12.431	0.001	3	7.103	0.274	24.563	0.001
	Block	1	0.496	0.010	2.518	0.002	1	0.355	0.014	3.687	0.001
	Treatment	10	2.092	0.040	1.061	0.188	10	1.233	0.047	1.279	0.004
	Season:Block	3	1.261	0.024	2.133	0.001	3	0.592	0.023	2.047	0.001
	Season:Treatment	30	5.781	0.112	0.978	0.639	30	2.958	0.114	1.023	0.314
	Block:Treatment	10	2.200	0.042	1.116	0.073	10	1.071	0.041	1.111	0.109
	Season:Block:Treatment	29	5.401	0.104	0.945	0.850	27	2.623	0.101	1.008	0.415
Nov2019	Block	1	0.221	0.075	1.547	0.012	1	0.183	0.099	1.985	0.001
	Treatment	10	1.469	0.499	0.997	0.526	10	0.939	0.511	0.939	0.783
Feb2019	Block	1	0.526	0.044	3.067	0.001	1	0.184	0.034	1.937	0.001
	Treatment	10	1.779	0.148	1.037	0.227	10	1.030	0.189	1.086	0.018
	Block:Treatment	10	1.814	0.151	1.058	0.164	9	0.932	0.171	1.092	0.017
Jul2019	Block	1	0.542	0.030	2.086	0.002	1	0.315	0.051	3.028	0.001
	Treatment	10	2.967	0.162	1.142	0.060	10	1.252	0.203	1.205	0.001
	Block:Treatment	10	2.834	0.155	1.091	0.138	10	1.078	0.175	1.038	0.218
Nov2020	Block	1	0.473	0.042	2.952	0.001	1	0.264	0.049	2.913	0.001
	Treatment	10	1.659	0.148	1.036	0.247	10	0.976	0.181	1.075	0.052
	Block:Treatment	10	1.695	0.152	1.059	0.194	10	0.963	0.179	1.062	0.072

Table S6. Syncom members and OTUs that match them.

Species	Strain	OTU match	Mean relative abundance (%)	Classification in RDP
<i>Arthrobacter</i> sp.	AT1.4	OTU_523	1.491	<i>Paeniglutamicibacter</i>
<i>Bacillus niacini</i>	AT2.8	OTU_31	0.655	NA
<i>Belnapia rosea</i>	MJ22	OTU_29	0.705	<i>Belnapia</i>
<i>Bhargavaea ginsengi</i>	C13	OTU_31	0.655	NA
<i>Cellulomonas cellasea</i>	RAS26	OTU_26	0.032	<i>Cellulomonas</i>
<i>Curtobacterium oceanosedimentum</i>	AS1.15	OTU_6	3.282	<i>Curtobacterium</i>
<i>Erwinia gerundensis</i>	AT3.11	OTU_3	5.315	<i>Pantoea</i>
<i>Frigoribacterium endophyticum</i>	AS3.20	OTU_417	0.154	NA
<i>Kineococcus radiotolerans</i>	AS2.23	OTU_33	0.670	<i>Kineococcus</i>
<i>Kitycoccus sedentarius</i> .	RAS25	OTU_1156	0.000*	<i>Kytococcus</i>
<i>Leptolyngbya</i> sp.	AT32P	OTU_778	0.000*	NA
<i>Massilia aurea</i>	AT3.2	OTU_481	0.223	<i>Massilia</i>
<i>Methylobacterium</i> sp.	RAS18	OTU_23	0.581	<i>Methylorubrum</i>
<i>Micrococcus aloeverae</i>	RAT4	OTU_260	0.052	<i>Micrococcus</i>
<i>Nocardioidea cavernae</i>	AT2.17	OTU_221	0.066	<i>Nocardioidea</i>
<i>Nodosilinea</i> sp.	AT21F	OTU_1527	0.000*	NA
<i>Paenibacillus</i> sp.	A5	OTU_1038	0.000*	<i>Paenibacillus</i>
<i>Pantoea brenneri</i>	A11	OTU_3	5.315	<i>Pantoea</i>
<i>Paraburkholderia caledonica</i>	A13	OTU_82	0.311	<i>Paraburkholderia</i>
<i>Phyllobacterium</i> sp.	A21	OTU_468	0.000*	<i>Phyllobacterium</i>
<i>Pseudomonas</i> sp.	AS2.8	OTU_25	0.854	<i>Pseudomonas</i>
<i>Rathayibacter caricis</i>	AS1.22	OTU_1842	0.032	<i>Rathayibacter</i>
<i>Rhizobium soli</i>	AS3.12	OTU_2630	0.029	NA
<i>Rhizobium</i> sp.	RAS22	OTU_501	0.022	<i>Rhizobium</i>
<i>Sphingomonas aerolata</i>	AS2.4	OTU_237	0.115	<i>Sphingomonas</i>
<i>Sphingomonas endophytica</i>	AS3.13	OTU_61	0.383	<i>Sphingomonas</i>
<i>Sphingomonas melonis</i>	AS2.3	OTU_8	0.000*	<i>Sphingomonas</i>
<i>Staphylococcus hominis</i>	AtDRG32	OTU_87	0.044	<i>Staphylococcus</i>
<i>Streptomyces cacaoi</i>	C6	OTU_272	0.096	<i>Streptomyces</i>
<i>Aureobasidium</i> sp.	AT3.8	OTU_6221	0.000*	<i>Dioscorea</i>
<i>Cladosporium</i> sp.	cutV	OTU_8236	0.000*	NA

Bacillus niacini / *Neobacillus niacini* (current). *Methylobacterium* sp. / *Methylorubrum* (current)

(*) Not detected after abundance and frequency filtering.

Table S7. Detected microbial hubs in the correlation networks of plants treated with syncoms.

MOCK	PFC	PFCF	PFCS	PFCT	AAP	CC	BC	RC1	RC2	RC3	order	family	genus	otu.id
hub	hub	hub	hub		hub	hub				hub	Kineosporiales	Kineosporiaceae	Quadrifphaera	POTU_20
hub											Rhizobiales	Aurantimonadaceae	Aureimonas	POTU_41
hub			hub		hub	hub	hub	hub	hub		Rhizobiales	Methylobacteriaceae	Methylobacterium	POTU_43
hub								hub			Micrococcales	Microbacteriaceae	Amnibacterium	POTU_210
hub			hub			hub					Pleosporales	NA	NA	FOTU_3557
	hub	hub		hub							Bacillales	Bacillaceae	Bacillus	POTU_14
	hub		hub		hub						Micrococcales	Intrasporangiaceae	NA	POTU_99
	hub					hub					Clostridiales	Peptostreptococcaceae	Terrisporobacter	POTU_127
	hub		hub								Propionibacteriales	Nocardiodaceae	Nocardioidea	POTU_271
	hub										Pleosporales	NA	NA	FOTU_147
		hub			hub	hub		hub			Bacillales	NA	NA	POTU_31
		hub									Geodermatophilales	Geodermatophilaceae	Klenkia	POTU_84
		hub						hub			Rhizobiales	Methylobacteriaceae	Microvirga	POTU_111
		hub	hub				hub			hub	Rhodobacterales	Rhodobacteraceae	Paracoccus	POTU_123
		hub							hub		Propionibacteriales	Nocardiodaceae	Nocardioidea	POTU_136
		hub									Rhodospirillales	Acetobacteraceae	Roseomonas	POTU_138
		hub					hub			hub	Cytophagales	Hymenobacteraceae	Hymenobacter	POTU_166
		hub									Pleosporales	Didymosphaeriaceae	Spegazzinia	FOTU_78
		hub	hub								NA	NA	NA	FOTU_223
			hub								Sphingomonadales	Sphingomonadaceae	Sphingomonas	POTU_18
			hub	hub	hub						Burkholderiales	Comamonadaceae	NA	POTU_24
			hub								Geodermatophilales	Geodermatophilaceae	Modestobacter	POTU_28
			hub			hub					Micrococcales	Micrococcaceae	NA	POTU_48
			hub								Sphingomonadales	Sphingomonadaceae	Sphingomonas	POTU_89
			hub								Rhizobiales	Methylobacteriaceae	Methylobacterium	POTU_113
			hub								Clostridiales	Peptostreptococcaceae	Romboutsia	POTU_114
			hub								Chitinophagales	Chitinophagaceae	Segetibacter	POTU_145
			hub								Sphingomonadales	Sphingomonadaceae	Sphingomonas	POTU_164
			hub								Sphingomonadales	Sphingomonadaceae	NA	POTU_167
			hub								Rhodobacterales	Rhodobacteraceae	Paracoccus	POTU_275
			hub								Pleosporales	NA	NA	FOTU_25
			hub								Pleosporales	NA	NA	FOTU_77
			hub								Pleosporales	Didymellaceae	NA	FOTU_98
			hub								Pleosporales	NA	NA	FOTU_130
			hub								Botryosphaeriales	Botryosphaeriaceae	Dothiorella	FOTU_159
			hub								Clostridiales	Clostridiaceae	Clostridium_sensu_stricto	POTU_16
			hub				hub				Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	POTU_40
			hub		hub						Propionibacteriales	Propionibacteriaceae	Microlunatus	POTU_302
			hub								Burkholderiales	Oxalobacteraceae	Massilia	POTU_481
			hub								NA	NA	NA	FOTU_1035
					hub						Micromonosporales	Micromonosporaceae	Actinoplanes	POTU_5
					hub						Micrococcales	Microbacteriaceae	Amnibacterium	POTU_125
					hub						Kineosporiales	Kineosporiaceae	Pseudokineococcus	POTU_322
					hub						Micrococcales	Microbacteriaceae	NA	POTU_417
						hub					Bacillales	Bacillaceae	NA	POTU_34
						hub					Micrococcales	Dermabacteraceae	Brachybacterium	POTU_437
						hub					Sphingomonadales	Sphingomonadaceae	Sphingomonas	POTU_726
						hub					Dothideales	Aureobasidiaceae	Aureobasidium	FOTU_5
						hub					NA	NA	NA	FOTU_42

	hub		Pleosporales	NA	NA	FOTU_175
	hub		NA	NA	NA	FOTU_2418
		hub	Sphingomonadales	Sphingomonadaceae	Sphingomonas	POTU_61
		hub	Bacillales	NA	NA	POTU_559
		hub	Geodermatophilales	Geodermatophilaceae	Geodermatophilus	POTU_1267
		hub	Geodermatophilales	Geodermatophilaceae	Blastococcus	POTU_1432
		hub	Rhizobiales	Methylobacteriaceae	Methylorubrum	POTU_23
		hub	Micrococcales	Intrasporangiaceae	NA	POTU_229
			Propionibacteriales	Nocardioidaceae	Marmoricola	POTU_463
			hub	Cytophagales	Hymenobacteraceae	POTU_12
			hub	Burkholderiales	Oxalobacteraceae	NA
			hub	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto
			hub	Micrococcales	Microbacteriaceae	NA
			hub	NA	NA	NA
			hub	Trichosphaerales	Trichosphaeriaceae	Nigrospora

Colored microbial families, genera and OTUs correspond to inoculated taxa in each syncom.

Table S8. Molecular characterization of phyllospheric bacteria and fungi.

Strain	Morphology	Gram staining	Amplicon	Sequencing strategy	Identification data base	Identification method	Isolation media	Host	Niche	Site	Sampling date	Genbank	Ref.
<i>Arthrobacter</i> sp. AT1.4	rods	positive	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	At	ph	Pe	sep-17	ON217558	4
<i>Aureobasidium</i> sp. AT3.8	yeast	NA	ITS partial	pJET1.2	NCBI PRJNA177353	MEGA alignment	TSA	At	ph	SF	sep-17	MT488313	4
<i>Bacillus niacini</i> AT2.8	rod	positive	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	At	ph	SF	sep-17	ON217559	4
<i>Belnapia rosea</i> MJ22	cocci	negative	16S-rRNA partial	pJET1.2	EZBioCloud	EZBioCloud server	Winogradsky	As	ph	SF	may-12	ON217573	2
<i>Bhargavaea ginseng</i> C13	cocci	positive	16S-rRNA partial	PCR product	EZBioCloud	MEGA alignment	TSA	As	ph	SF	may-13	ON217556	4
<i>Cellulomonas cellasea</i> RAS26	rods	positive	16S-rRNA partial	pGEM-T	EZBioCloud, genebank	MEGA alignment	Methanol /TSA	As	ph	SF	sep-17	ON217577	4
<i>Curtobacterium oceanosedimentum</i> AS1.15	rods	positive	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	As	ph	SF	sep-17	ON217560	4
<i>Cladosporium</i> sp. cutV	filamentous	NA	ITS partial	PCR product	NCBI PRJNA177353	MEGA alignment	cuticles / TSA	As	ph	SF	sep-17	MT488312	4
<i>Erwinia gerundensis</i> AT3.11	rods	negative	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	At	ph	SF	sep-17	ON217561	4
<i>Frigoribacterium endophyticum</i> AS3.20	rods	positive	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	As	ph	SF	sep-17	ON217562	4
<i>Kineococcus radiotolerans</i> AS2.23	cocci	positive	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	At	ph	Pe	sep-17	ON217563	4
<i>Kytococcus sedentarius</i> RAS25	cocci	positive	16S-rRNA partial	PCR product	EZBioCloud	EZBioCloud server	TSA	As	ph	SF	sep-17	ON217580	4
<i>Leptolyngbya</i> sp. AT32P	filamentous	variable	16S-rRNA partial	pGEM-T	EZBioCloud, genebank	MEGA alignment	Gromov	At	ph	Pe	sep-17	ON217574	1
<i>Massilia aurea</i> AT3.2	rods	negative	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	At	ph	Pe	sep-17	ON217564	4
<i>Methylobacterium</i> sp. RAS18	rods	negative	16S-rRNA partial	pGEM-T	EZBioCloud, genebank	MEGA alignment	Methanol /TSA	As	ph	SF	sep-17	ON217576	1
<i>Micrococcus aloeverae</i> RAT4	cocci	positive	16S-rRNA partial	pGEM-T	EZBioCloud, genebank	MEGA alignment	TSA	At	ph	Pe	sep-17	ON217578	4
<i>Nocardioidees cavernae</i> AT2.17	rods	positive	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	At	ph	Pe	sep-17	ON217565	4
<i>Nodosilinea</i> sp. AT21F	filamentous	variable	16S-rRNA partial	pGEM-T	EZBioCloud, genebank	MEGA alignment	Gromov	At	ph	Pe	sep-17	ON217575	1
<i>Paenibacillus</i> sp. A5	rods	positive	16S-rRNA partial	PCR product	EZBioCloud	MEGA alignment	TSA	As	ph	Ma	may-13	ON217555	4
<i>Pantoea brenneri</i> A11	rods	negative	16S-rRNA partial	PCR product	EZBioCloud	MEGA alignment	TSA	As	ph	Ma	may-13	ON217552	4
<i>Paraburkholderia caledonica</i> A13	rods	negative	16S-rRNA partial	PCR product	EZBioCloud	MEGA alignment	TSA	As	ph	Ma	may-13	ON217553	4
<i>Phyllobacterium</i> sp. A21	rods	negative	16S-rRNA partial	PCR product	EZBioCloud	MEGA alignment	TSA	As	ph	Ma	may-13	ON217554	4
<i>Pseudomonas</i> sp. AS2.8	rods	negative	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	As	ph	SF	sep-17	ON217566	4

<i>Rathayibacter caricis</i> AS1.22	rods	positive	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	As	ph	Pe	sep-17	ON217567	4
<i>Rhizobium soli</i> AS3.12	pleomorphic	negative	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	As	ph	SF	sep-17	ON217568	4
<i>Rhizobium</i> sp. RAS22	rods	negative	16S-rRNA partial	pGEM-T	EZBioCloud, genebank	MEGA alignment	Gromov / TSA	As	ph	SF	sep-17	ON217579	4
<i>Sphingomonas aerolata</i> AS2.4	rods	negative	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	As	ph	SF	sep-17	ON217571	4
<i>Sphingomonas endophytica</i> AS3.13	rods	negative	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	As	ph	SF	sep-17	ON217570	4
<i>Sphingomonas melonis</i> AS2.3	rods	negative	16S-rRNA partial	pJET1.2	EZBioCloud	MEGA alignment	TSA	As	ph	SF	sep-17	ON217569	4
<i>Staphylococcus hominis</i> AtDRG32	cocci	positive	16S-rRNA partial	pJET1.2	EZBioCloud	EZBioCloud server	TSA	At	le	invitro	NA	ON217572	3
<i>Streptomyces cacaoi</i> C6	filamentous	positive	16S-rRNA partial	PCR product	EZBioCloud	MEGA alignment	TSA	As	ph	SF	may-13	ON217557	4

Plant species. as: *Agave salmiana*. at: *Agave tequilana*. Niche. ph: phyllosphere. le: leaf endosphere. Site SF: San Felipe, Gto. Ma: El Magueyal, Gto. Pe: Penjamo, Gto.

Bacillus niacini / *Neobacillus niacini* (current)

Methylobacterium sp. / *Methylobacterium* (current)

Table S9. Culture conditions of the phyllospheric microbial strains used for syncoms.

Strain		Incubation time for log phase (hr)	Media
<i>Arthrobacter</i> sp.	AT14	24	TSB 1X
<i>Aureobasidium</i> sp.	AT38	24	TSB 1X
<i>Bacillus niacini</i>	AT28	24	TSB 1X
<i>Belnapia rosea</i>	MJ22	36	TSB 0.5X
<i>Bhargavaea ginsengi</i>	C13	48	TSB 1X
<i>Cellulomonas cellasea</i>	RAS26	72	TSB 0.5X
<i>Curtobacterium oceanosedimentum</i>	AS115	24	TSB 1X
<i>Erwinia gerundensis</i>	AT311	24	TSB 1X
<i>Frigoribacterium endophyticum</i>	AS320	20	TSB 1X
<i>Kineococcus radiotolerans</i>	AS323	48	TSB 1X
<i>Kytococcus sedentarius</i>	RAS25	36	TSB 0.5X
<i>Massilia aurea</i>	AT32	24	TSB 0.5X
<i>Methylobacterium</i> sp.	RAS18	35	TSB 1X
<i>Micrococcus aloeverae</i>	RAT4	24	TSB 1X
<i>Nocardioides cavernae</i>	AT217	24	TSB 1X
<i>Paenibacillus</i> sp.	A5	18	TSB 1X
<i>Pantoea brenneri</i>	A11	10	TSB 1X
<i>Paraburkholderia caledonica</i>	A13	20	TSB 1X
<i>Phyllobacterium</i> sp.	A21	20	TSB 1X
<i>Pseudomonas</i> sp.	AS28	18	TSB 1X
<i>Rathayibacter caricis</i>	AS122	20	TSB 0.5X
<i>Rhizobium soli</i>	AS312	24	TSB 1X
<i>Rhizobium</i> sp.	RAS22	48	TSB 1X
<i>Sphingomonas aerolata</i>	AS24	24	NB
<i>Sphingomonas endophytica</i>	AS313	35	TSB 1X
<i>Sphingomonas melonis</i>	AS23	20	TSB 1X
<i>Staphylococcus hominis</i>	AtDRG32	10	TSB 1X
<i>Streptomyces cacaoi</i>	C6	48	TSB 1X

TSB: Trypticase soy broth, NB: Nutrient broth

Bacillus niacini / *Neobacillus niacini* (current)*Methylobacterium* sp. / *Methylorubrum* (current)

Table S10. Viable count of the inoculated synthetic communities

Species	Strain	PFC	PFCF	PFCS	PFCT	AAP	CC	BC	RC1	RC2	RC3
<i>Belnapia rosea</i>	MJ22	3.0E+06	2.0E+06	1.0E+06	2.8E+08	5.0E+06					
<i>Methylobacterium sp.</i>	RAS18	1.5E+08	1.3E+07	2.1E+07	5.4E+07	1.3E+08					
<i>S. hominis</i>	AtDRG32	7.0E+06	1.0E+06	1.4E+07	5.0E+06						
<i>Nocardioideae cavernae</i>	AT217	4.5E+07	1.6E+07	1.2E+07	5.0E+06						
<i>Micrococcus aloeverae</i>	RAT4	4.5E+07	3.7E+07	2.8E+07	5.2E+07						
<i>Aureobasidium sp</i>	AT38		4.0E+06	<1.0E+06							
<i>Cladosporium sp</i>	cutV		<1.0E+06	<1.0E+06							
<i>S. aerolata</i>	AS24			1.0E+06							
<i>S. endophytica</i>	AS313			4.0E+06					<1.0E+06		
<i>Sphingomonas melonis</i>	AS23			1.6E+07							
<i>F. endophyticum</i>	AS320			5.2E+07							
<i>Cellulomonas cellasea</i>	RAS26			4.8E+08							
<i>Massilia aurea</i>	AT32				4.0E+05						
<i>K. radiotolerans</i>	AS223				3.1E+07				1.0E+06		
<i>Bacillus niacini</i>	AT28				<1.0E+06						
<i>Leptolyngbya sp.</i>	AT32P						NA				
<i>Nodosilinea sp.</i>	AT21F						NA				
<i>P. caledonica</i>	A13							1.4E+07			
<i>Pantoea brenneri</i>	A11									4.0E+07	
<i>Erwinia gerundensis</i>	AT311							3.0E+07			
<i>Pseudomonas sp.</i>	AS28							4.4E+07			
<i>Rathayibacter caricis</i>	AS122								3.0E+06		
<i>Paenibacillus sp</i>	A5								5.0E+06		1.0E+06
<i>Rhizobium sp.</i>	RAS22								7.0E+06	4.0E+06	
<i>Arthrobacter sp.</i>	AT14									3.0E+06	
<i>Bhargavaea ginsengi</i>	C13									<1.0E+06	
<i>Streptomyces cacaoi</i>	C6									<1.0E+06	
<i>C. oceanosedimentum</i>	AS115										1.0E+06
<i>Phyllobacterium sp</i>	A21										3.00E+06
<i>Rhizobium soli</i>	AS312										
<i>K. sedentarius</i>	RAS25										6.00E+06

Viable count in CFU/ml

Green: Pair of strains that could not be differentiated based on colony morphology.

Red: strain that could not be detected in the 10⁶ dilution.*Bacillus niacini* / *Neobacillus niacini* (current)*Methylobacterium sp.* / *Methylobacterium* (current)

Table S11. Sequencing statistics - from raw data to the measurable and rarefied OTU tables.

Amplicon	16S-rRNA-V4	ITS2
Paired reads quality trimming	TRIMMOMATIC LEADING:12 TRAILING:12 SLIDINGWINDOW:4:12 MINLEN:30 AVGQUAL:12	TRIMMOMATIC LEADING:12 TRAILING:12 SLIDINGWINDOW:4:12 MINLEN:30 AVGQUAL:12
Paired reads assemble	FLASH -minoverlap 10 -max overlap 303	FLASH -minoverlap 10 -max overlap 303
Assembled read quality filtering	VSEARCH --fastx_filter -stripriht 24 -stripleft 23 -minlen 250 -maxlen 256 -expected error 1	VSEARCH --fastx_filter -stripriht 18 -stripleft 39 -minlen 296 -maxlen 394 -expected error 1
Dereplication of assembled reads (by and across samples)	VSEARCH --derep_fulllength default parameters	VSEARCH --derep_fulllength default parameters
OTU clustering	VSEARCH --cluster_size default parameters similarity= 0.97	VSEARCH --cluster_size default parameters similarity= 0.95
Singleton removal	VSEARCH --sortbysize default parameters min size= 2	VSEARCH --sortbysize default parameters min size= 2
Chimera detection	VSEACH --uchime_denovo --uchime_ref default parameters	VSEACH --uchime_denovo default parameters
Mapping reads to OTUs (assembled reads pre-filtering)	VSEARCH --usearch_global default parameters similarity= 0.97	VSEARCH --usearch_global default parameters similarity= 0.95
Classification of OTUs	VSEARCH --sintax default parameters -bootstrap 0.80 Bases -RDP v18 (Cole, et al., 2014)	VSEARCH --sintax default parameters -bootstrap 0.80 -UNITE 8.3 (Abarenkov, et al., 2021)
Total of input paired reads	21,735,602	12,391,310
Total of reads to cluster	9,316,362	5,067,067
Size range of reads	250 - 256 bp	296 – 394 bp
Total of OTUs	33,220	8,945
Total of reads to map	11,851,383	11,805,492
Total of mapped reads	10,298,852 (86.9%)	11,474,938 (97.2%)
Total of Classified OTUs	31,419 (94.58%)	6991 (78.18%)
Total of OTUs after filtering	1,164	1,416
Number of libraries after filtering	234	199

Table S12. Putative down and up-regulated metabolites in leaves of plants treated with PFCS syncom

	Name ^a	Superclass ^b	Class ^b	Subclass ^b	Molecular Formula ^c	Adduct ^d	Theoretical mass	Experimental mass	Mass accuracy (ppm)	Retention Time (min)	Cosmic Score ^e	log ₂ (FC) ^f
Down-regulated	2-ethyl-4-fluoro-3-nitro-3H-1,2,4-triazole	Organoheterocyclic compounds	Heteroaromatic compounds	not_assigned	C4H7FN4O2	[M + H] ⁺	163.0631	163.0606	-15.4480	14.8487	ND	-2.3377
	Unknown	Organic acids and derivatives	Boronic acid derivatives	Boronic acids	ND	ND	ND	ND	ND	17.2335	ND	-0.7478
	Unknown	Organic acids and derivatives	Boronic acid derivatives	Boronic acids	ND	ND	ND	ND	ND	14.8622	ND	-1.5541
	N-bromo-N-diethoxyphosphorylethanamine	Organohalogen compounds	Alkyl halides	Alkyl bromides	C6H15BrNO3P	[M + Na] ⁺	281.9871	281.9849	-7.6351	14.0599	ND	-1.7122
	[2-Chloro-1-(3,4-diethoxyphenyl)-2,2-difluoroethyl]hydrazine	Benzenoids	Benzene and substituted derivatives	Halobenzenes	C12H17ClF2N2O2	[M + H] ⁺	295.1025	295.1015	-3.4463	13.6811	0.161	-2.3523
	2-Vinyl-9-[beta-d-ribofuranosyl]hypoxanthine	Organic oxygen compounds	Organooxygen compounds	Carbohydrates and carbohydrate conjugates	C12H14N4O5	[M + H] ⁺	295.1042	295.1015	-9.2035	14.7599	0.339	-2.6135
	1,3-Dimethyl-5-[(1-deoxo-L-arabino-1-ylidene)amino]-6-aminouracil	Organic oxygen compounds	Organooxygen compounds	Carbohydrates and carbohydrate conjugates	C11H18N4O6	[M + Na] ⁺	325.1124	325.1128	1.1842	14.8059	0.271	-1.8970
	Unknown	Lipids and lipid-like molecules	not_assigned	not_assigned	ND	ND	ND	ND	ND	17.2305	0.255	-2.4349
	1,3-Dimethyl-7-[3-(3-methyl-2,6-dioxopurin-7-yl)-2-oxopropyl]purine-2,6-dione	Organoheterocyclic compounds	Diazines	Pyrimidines and pyrimidine derivatives	C16H16N8O5	[M + H] ⁺	401.1322	401.1308	-3.5200	15.1628	0.711	-0.9235
	Unknown	Organic oxygen compounds	Organooxygen compounds	Carbohydrates and carbohydrate conjugates	ND	ND	ND	ND	ND	14.8384	ND	-2.3259
	6-[[4-(5-Carboxypentylamino)-6-(octylamino)-1,3,5-triazin-2-yl]amino]hexanoic acid	Lipids and lipid-like molecules	Steroids and steroid derivatives	Hydroxysteroids	C23H42N6O4	[M - H4O2 + H] ⁺	431.3134	431.3150	3.5264	20.0213	0.147	-0.8087
	5-[[[(2R,3S,4S,5R,6R)-6-[(2S,3S,4S,5R)-3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-5-oxopentanoic acid	Organic oxygen compounds	Organooxygen compounds	Carbohydrates and carbohydrate conjugates	C17H28O14	[M + H] ⁺	457.1557	457.1546	-2.4171	13.6865	0.297	-2.5773
	7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxynonane-1,2,3,4,5,6,8,9-octol	Organic oxygen compounds	Organooxygen compounds	Carbohydrates and carbohydrate conjugates	C15H30O14	[M + Na] ⁺	457.1533	457.1549	3.4562	14.8183	0.358	-2.5617
	tert-Butyl 4-(N-(2,2-difluoroethyl)-4-methoxyphenylsulfonamido)piperidine-1-carboxylate	Organic oxygen compounds	Organooxygen compounds	Carbohydrates and carbohydrate conjugates	C19H28F2N2O5S	[M + Na] ⁺	457.1584	457.1571	-2.8655	17.6482	0.489	-0.7839
	2-[[2-[N-(dimethylsulfamoyl)-4-fluoroanilino]acetyl]-[(4-methoxyphenyl)methyl]amino]-N-propan-2-ylbutanamide	Benzenoids	Benzene and substituted derivatives	Halobenzenes	C25H35FN4O5S	[M + Na] ⁺	545.2210	545.2205	-0.8639	17.9447	0.513	-1.2177
	N-[6-[[[(8aS)-7-amino-6-[(2R)-4,6-diamino-2,3-dihydroxycyclohexyl]oxy-4-hydroxy-3-(methylamino)-2,3,4,4a,6,7,8,8a-octahydropyrano[3,2-b]pyran-2-yl]oxy]-5-hydroxy-2-(hydroxymethyl)oxan-3-yl]-2-aminoacetamide	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C23H44N6O11	[M + H] ⁺	581.3146	581.3104	-7.3351	15.3744	0.351	-3.1176

	[4-[2-[4-(Dihydroxyamino)oxybutanoylamino]-3-[4-(dihydroxyamino)oxybutoxy]-3-oxopropyl]phenyl] 4-(dihydroxyamino)oxybutanoate	Organic oxygen compounds	Organoxygen compounds	Carbohydrates and carbohydrate conjugates	C21H34N4O14	[M + Na] ⁺	589.1969	589.1980	1.7549	14.8049	NA	-2.2023
	tert-butyl N-[5-amino-1-[[2-(butylamino)-1-(4-ethynylphenyl)-2-oxoethyl]-octylamino]-1,5-dioxopentan-2-yl]carbamate	Lipids and lipid-like molecules	Fatty Acyls	Fatty amides	C32H50N4O5	[M + Na] ⁺	593.3679	593.3679	-0.0522	14.7969	0.284	-2.1135
	Unknown	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	ND	ND	ND	ND	ND	13.7278	ND	-2.7743
	N-[1-[[[(2S)-1-[2-[(2S)-2-carbamoylpyrrolidine-1-carbonyl]pyrrolidin-1-yl]-3-methylbutan-2-yl]-methylamino]-3,3-dimethyl-1-oxobutan-2-yl]-1-propan-2-yl]piperidine-2-carboxamide	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C31H56N6O4	[M + Na] ⁺	599.4261	599.4243	-2.9094	17.1153	0.238	-1.2774
	Pimelauteide	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C29H52N6O9	[M - H ₂ O + H] ⁺	611.3768	611.3797	4.6796	20.0485	0.282	-0.8592
	6-[[[2'-O,3'-O-Isopropylidene-5'-adenosyloxy)methyl]-2'-O,3'-O-Isopropylideneuridine	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C26H33N7O10	[M + H ₃ N + H] ⁺	621.2633	621.2591	-6.7395	15.5005	0.218	-1.7340
	2-(2,4-Dibromophenoxy)-1-[4-[2-(2,4-dibromophenoxy)acetyl]-1,4-diazepan-1-yl]ethanone	Organic oxygen compounds	Organoxygen compounds	Carbohydrates and carbohydrate conjugates	C21H20Br4N2O4	[M + H] ⁺	680.8235	680.8215	-2.9024	14.8357	NA	-2.3036
	4-(2-Aminoethoxy)-2-[2-(2-aminoethoxy)-1-[bis(carboxymethyl)amino]ethyl]-3-[bis(carboxymethyl)amino]-2-[2-[bis(carboxymethyl)amino]ethyl-(carboxymethyl)amino]butanoic acid	Organic oxygen compounds	Organoxygen compounds	Carbohydrates and carbohydrate conjugates	C26H44N6O18	[M + H] ⁺	729.2790	729.2721	-9.5286	14.6217	0.754	-1.0402
	7-Deoxy-7-oxo-21-O-benzoyl-21-O-de(3-carboxy-1-oxo-2-propenyl)-2-demethyl-2-methoxy-24-methylhygrolidin	Benzenoids	Benzene and substituted derivatives	Benzoyl derivatives	C42H60O10	[M + Na] ⁺	747.4084	747.4087	0.3077	25.1323	0.497	-1.2948
	2,2'-(((19-amino-2,11,16,18-tetrahydroxy-5,9-dimethylcosane-6,7-diy)bis(oxy))bis(2-oxoethane-2,1-diy))disuccinic acid	Lipids and lipid-like molecules	Prenol lipids	Terpene glycosides	C34H59NO16	[M + H ₃ N + H] ⁺	755.4178	755.4210	4.2983	14.7939	0.143	-2.2204
	3-[acetyl-[(2S,3R,4R,5S,6R)-2-hydroxy-4,5-bis(phenylmethoxy)-6-(phenylmethoxymethyl)oxan-3-yl]amino]-2-hydroxy-N-undecylpropanamide	Lipids and lipid-like molecules	Fatty Acyls	Fatty amides	C43H60N2O8	[M + Na] ⁺	755.4247	755.4226	-2.7759	19.0287	0.948	-0.9375
	Unknown	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	ND	ND	ND	ND	ND	15.7036	ND	-1.7613
	(8S,11S,14S)-14-amino-N-[(4S)-4-amino-5-[[[(4S)-4-amino-5-[(3-amino-2-hydroxypropyl)amino]-5-oxopentyl]amino]-5-oxopentyl]-11-[(2R)-3-amino-2-hydroxypropyl]-17-hydroxy-10,13-dioxo-9,12-diazatricyclo[14.3.1.12,6]henicosa-1(20),2,4,6(21),16,18-hexaene-8-carboxamide	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C36H56N10O8	[M + H] ⁺	757.4360	757.4353	-0.9242	13.7501	ND	-3.1059
	Yuccalan	Lipids and lipid-like molecules	Steroids and steroid derivatives	Steroidal glycosides	C39H64O15	[M + H] ⁺	773.4324	773.4344	2.6117	20.0211	0.293	-0.7365
Up-regulated	(E)-9-oxododec-10-enoic acid	Lipids and lipid-like molecules	Fatty Acyls	Lineolic acids and derivatives	C12H20O3	[M - H ₄ O ₂ + H] ⁺	177.1279	177.1265	-8.1297	19.3663	0.045	0.7198
	15-Hexadecynoic acid	Lipids and lipid-like molecules	Fatty Acyls	not_assigned	C16H28O2	[M + Na] ⁺	275.1987	275.1992	1.7079	21.1416	0.078	1.1806
	Unknown	Organic acids and derivatives	Boronic acid derivatives	Boronic acids	ND	ND	ND	ND	ND	21.5436	ND	1.5629

Unknown	Benzenoids	Benzene and substituted derivatives	not_assigned	ND	ND	ND	ND	ND	23.3034	ND	1.2267
Unknown	Lipids and lipid-like molecules	Fatty Acyls	not_assigned	ND	ND	ND	ND	ND	20.5563	ND	1.2863
Unknown	Organic acids and derivatives	Boronic acid derivatives	not_assigned	ND	ND	ND	ND	ND	19.7677	ND	2.0117
15-Oxoicosa-11,13-dienoic acid	Lipids and lipid-like molecules	Prenol lipids	not_assigned	C20H34O3	[M - H ₂ O + H] ⁺	305.2481	305.2468	-4.2425	21.3891	0.013	1.0856
N-(oxolan-2-ylmethyl)-4-[2-(1,2,4-triazol-1-yl)ethyl]piperazine-1-carboxamide	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C14H24N6O2	[M + H] ⁺	309.2039	309.2059	6.4715	23.2271	0.166	1.0246
2-acetamido-N-[1-amino-5-(diaminomethylideneamino)-1-oxopentan-2-yl]-4-methylpentanamide	Lipids and lipid-like molecules	Fatty Acyls	Fatty acids and conjugates	C14H28N6O3	[M + H] ⁺	329.2301	329.2299	-0.5255	19.3978	0.389	0.7916
3-[2-[2-[2-(3-Aminopropoxy)ethoxy]ethylamino]ethoxy]ethoxypropan-1-amine	Organic nitrogen compounds	Organonitrogen compounds	Amines	C14H33N3O4	[M + Na] ⁺	330.2369	330.2344	-7.5612	24.1777	0.629	2.1924
1-[(5S,8R,9S,10R,13S,14S,17S)-6,6-difluoro-2-(1-hydroxyethyl)-13-methyl-2,3,4,5,7,8,9,10,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl]-2-hydroxyethanone	Lipids and lipid-like molecules	not_assigned	not_assigned	C22H34F2O3	[M + H] ⁺	385.2554	385.2564	2.5697	18.0373	0.031	1.1847
Unknown	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	ND	ND	ND	ND	ND	24.4875	ND	1.1438
Leupeptin acid	Lipids and lipid-like molecules	Fatty Acyls	Fatty acids and conjugates	C20H38N6O5	[M - H ₂ O + H] ⁺	425.2876	425.2886	2.3537	20.6134	0.308	1.3693
2-[[[(2S,5R)-2-[[[(2R)-azepan-2-yl]methoxycarbonyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2,2-difluoroacetic acid	Organic oxygen compounds	Organooxygen compounds	Alcohols and polyols	C16H24F2N4O6	[M + Na] ⁺	429.1561	429.1558	-0.6058	18.7059	ND	3.3251
1-[(2-Cyclopentylloxyphenyl)methyl]-3-[[4-(difluoromethoxy)-3-methoxyphenyl]methyl]urea	Benzenoids	Benzene and substituted derivatives	not_assigned	C22H26F2N2O4	[M + Na] ⁺	443.1758	443.1733	-5.6185	19.0655	0.173	1.0960
3-(3-butyl-2,6-dioxo-7-pentylpurin-8-yl)-N-[2-(diethylamino)ethyl]propanamide	Lipids and lipid-like molecules	Steroids and steroid derivatives	not_assigned	C23H40N6O3	[M + H] ⁺	449.3240	449.3265	5.5550	20.0213	0.364	3.0588
2-amino-N-[[5-[[[2-amino-5-(diaminomethylideneamino)pentanoyl]amino]methyl]piperazin-2-yl]methyl]-5-(1-aminoethylideneamino)pentanamide	Organoheterocyclic compounds	Azoles	not_assigned	C19H41N11O2	[M + H] ⁺	456.3523	456.3475	-10.4393	24.4573	ND	1.4410
(2S)-2-acetamido-N-3-[1-[4-[[[(3S)-5-ethyl-2-methyl-4-oxoheptan-3-yl]amino]-4-oxobutyl]triazol-4-yl]propyl]-3-methylbutanamide	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C26H46N6O4	[M + H] ⁺	507.3659	507.3686	5.4024	20.5957	0.704	1.5958
(2S)-2-[[[(2S,3S)-2-[[[(2S)-1-[[[(2S)-2,6-diaminohexanoyl]pyrrolidine-2-carbonyl]amino]propanoyl]amino]-3-methylpentanoyl]amino]-4-methylpentanoic acid	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C26H48N6O6	[M - H ₂ O + H] ⁺	523.3608	523.3624	3.1088	19.3543	0.42	1.0099
5-(2-ethoxyethylamino)-5-oxo-2-[16-(2H-tetrazol-5-yl)hexadecanoylamino]pentanoic acid	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C26H48N6O5	[M + H] ⁺	525.3764	525.3787	4.2179	20.5965	0.552	1.3507
2,6-Decanedicarboxylic acid di[N'-(4-butylsalicyloyl) hydrazide]	Lipids and lipid-like molecules	Steroids and steroid derivatives	Bile acids, alcohols and derivatives	C34H50N4O6	[M - H ₂ O + H] ⁺	593.3703	593.3691	-2.0493	25.8561	0.188	1.9563

(2S,3R)-2-acetamido-N-[(2S,4S,5S)-5-[[[(2S,3R)-2-acetamido-3-methylpentanoyl]amino]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methylpentanamide	Lipids and lipid-like molecules	Steroids and steroid derivatives	Hydroxysteroids	C34H50N4O5	[M + H] ⁺	595.3859	595.3842	-3.0165	24.4615	0.299	1.1886
N-[(1R)-2-oxo-2-[[11-oxo-11-(pyridin-4-ylmethylamino)undecyl]amino]-1-phenylethyl]dodecanamide	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C37H58N4O3	[M + H] ⁺	607.4587	607.4569	-3.0718	20.6163	0.591	1.3020
10-N,21-N-bis(2,2-dimethoxyethyl)-2,7,13,18-tetrazatricyclo[17.3.1.18,12]tetracos-1(23),8(24),9,11,19,21-hexaene-9,10,11,20,21,22,23,24-octamine	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C28H52N12O4	[M + H] ⁺	621.4313	621.4360	7.6710	19.3721	ND	0.9235
N-[2-[[[(2S)-6-[[[(4S)-1-(diaminomethylideneamino)-5-oxoheptan-4-yl]amino]-1-hydroxy-3,6-dioxohexan-2-yl]amino]-2-oxoethyl]hexadecanamide	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C32H60N6O6	[M + H] ⁺	625.4653	625.4673	3.3111	20.6178	0.433	1.3763
tert-butyl 2-[[3-[1-[4-[(2-amino-3-methylbutanoyl)-methylamino]-3-methoxy-5-methylheptanoyl]pyrrolidin-2-yl]-3-methoxy-2-methylpropanoyl]amino]-3-phenylpropanoate	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C37H62N4O7	[M - H4O2 + H] ⁺	639.4485	639.4456	-4.6071	19.3988	0.452	0.9190
Methyl 3-[8,12-bis(3-methoxy-3-oxopropyl)-3,7,13,17,18-pentamethyl-1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24-tetracosahydroporphyrin-2-yl]propanoate	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C37H64N4O6	[M - H2O + H] ⁺	643.4798	643.4783	-2.4492	20.5946	0.487	1.2778
2-[2-[2-[2-[2-[2-[2-[2-[4-(2-Methylheptan-2-yl)phenoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol	Lipids and lipid-like molecules	Prenol lipids	Terpene glycosides	C34H62O11	[M + H] ⁺	647.4370	647.4347	-3.6297	26.8553	0.396	1.6567
[1-decanoyloxy-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxypropan-2-yl] (9Z,12Z)-heptadeca-9,12-dienoate	Lipids and lipid-like molecules	Prenol lipids	Terpene glycosides	C36H64O10	[M + H] ⁺	657.4578	657.4566	-1.8632	19.4013	0.255	1.0020
Cemadotin	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C35H56N6O5	[M + K] ⁺	679.3949	679.3899	-7.3992	26.2119	0.236	1.2970
(2R)-2,6-diamino-N-[(2R)-1-[[[(2R)-1-[[[(2R)-1-[(2R)-1-amino-1-oxopropan-2-yl]amino]-1-oxo-3-phenylpropan-2-yl]amino]-1-oxo-3-phenylpropan-2-yl]amino]-3-methyl-1-oxobutan-2-yl]amino]-1-oxo-3-phenylpropan-2-yl]hexanamide	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C41H56N8O6	[M + H] ⁺	757.4401	757.4384	-2.2140	24.4615	0.426	1.0253

^a IUPAC or common name of the top candidate metabolite predicted by CSI:FingerID tool within SIRIUS software

^b Classification by the CANOPUS tool using the Classyfire ontology

^c Molecular formula annotated by ZODIAC tool within SIRIUS software

^d Adduct detected corresponding to the molecular formula predicted by ZODIAC

^e Confidence score assigned to the candidate metabolite predicted by CSI:FingerID tool

^f Log2 fold change (FC) with respect to the comparison of agave-treated vs. Non-treated agave or MOCK group

Abbreviations: ND, not determined; FC, fold-change.

Table S13. Putative down and up-regulated metabolites in leaves of plants treated with PFCT syncom

	Name ^a	Superclass ^b	Class ^b	Subclass ^b	Molecular Formula ^c	Adduct ^d	Theoretical mass	Experimental mass	Mass accuracy (ppm)	Retention Time (min)	Cosmic Score ^e	log2 (FC) ^f
Down-regulated	phosphono (Z)-3-propyloctadec-9-enoate	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C21H41O5P	[M + Na] ⁺	427.2589	427.2567	-5.3434	19.5787	0.099	-2.2793
	N-[(3S,6R)-6-[[[(6S,8aS)-7-amino-6-[(1R,4R)-4,6-diamino-2,3-dihydroxycyclohexyl]oxy-4-hydroxy-3-(methylamino)-2,3,4,4a,6,7,8,8a-octahydropyrano[3,2-b]pyran-2-yl]oxy]-5-hydroxy-2-(hydroxymethyl)oxan-3-yl]-2-aminoacetamide	Organoheterocyclic compounds	Diazines	Pyrimidines and pyrimidine derivatives	C23H44N6O11	[M + H] ⁺	581.3146	581.3104	-7.3351	15.3744	0.351	-1.8435
	Unknown	Organoheterocyclic compounds	Azacyclic compounds	not_assigned	ND	ND	ND	ND	ND	25.4588	ND	-1.7964
	(8S,11S,14S)-14-amino-N-[(4S)-4-amino-5-[[[(4S)-4-amino-5-[(3-amino-2-hydroxypropyl)amino]-5-oxopentyl]amino]-5-oxopentyl]-11-[(2R)-3-amino-2-hydroxypropyl]-17-hydroxy-10,13-dioxo-9,12-diazatricyclo[14.3.1.12,6]henicosa-1(20),2,4,6(21),16,18-hexaene-8-carboxamide	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	C36H56N10O8	[M + H] ⁺	757.4361	757.4353	-1.0364	13.7501	ND	-1.6667
	[2-Chloro-1-(3,4-diethoxyphenyl)-2,2-difluoroethyl]hydrazine	Organic oxygen compounds	Organooxygen compounds	Carbohydrates and carbohydrate conjugates	C12H17ClF2N2O2	[M + H] ⁺	295.1025	295.1015	-3.4463	13.6811	ND	-1.5622
	N,N-Dimethyl-N-[2-(beta-D-glucopyranosyloxy)ethyl]-1-dodecaneaminium	Lipids and lipid-like molecules	Fatty Acyls	Fatty amides	C22H45NO6	[M + H3N + H] ⁺	437.3591	437.3628	8.5902	18.4550	0.506	-1.3074
	5-[[[(2R,3S,4S,5R,6R)-6-[(2S,3S,4S,5R)-3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-5-oxopentanoic acid	Organic oxygen compounds	Organooxygen compounds	Carbohydrates and carbohydrate conjugates	C17H28O14	[M + H] ⁺	457.1557	457.1546	-2.4171	13.6865	0.297	-1.1072
	2-ethyl-4-fluoro-3-nitro-3H-1,2,4-triazole	Organoheterocyclic compounds	Heteroaromatic compounds	not_assigned	C4H7FN4O2	[M + H] ⁺	163.0631	163.0606	-15.4480	14.8487	ND	-1.0952
	5-(2,3-Dihydroxypropoxy)-5-oxopentanoic acid	Organic acids and derivatives	Carboxylic acids and derivatives	Dicarboxylic acids and derivatives	C8H14O6	[M + Na] ⁺	229.0688	229.0697	3.8460	20.3237	0.132	-1.087
Up-regulated	Unknown	Organic acids and derivatives	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	ND	ND	ND	ND	ND	16.7235	ND	1.0116
	2-[[[(2S,5R)-2-[[[(2R)-azepan-2-yl]methoxycarbamoyl]-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl]oxy]-2,2-difluoroacetic acid	Organic oxygen compounds	Organooxygen compounds	Alcohols and polyols	C16H24F2N4O6	[M + Na] ⁺	429.1562	429.1558	-0.7503	18.7058	ND	2.8592

^a IUPAC or common name of the top candidate metabolite predicted by CSI:FingerID tool within SIRIUS software

^b Classification by the CANOPUS tool using the Classyfire ontology

^c Molecular formula annotated by ZODIAC tool within SIRIUS software

^d Adduct detected corresponding to the molecular formula predicted by ZODIAC

^e Confidence score assigned to the candidate metabolite predicted by CSI:FingerID tool

^f Log2 fold change (FC) with respect to the comparison of agave-treated vs. Non-treated agave or MOCK group

Abbreviations: ND, not determined; FC, fold-change.

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