Supplementary Information for:

Variation in root exudate composition influences soil microbiome membership and functional potential

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***Running title*:** Root exudates influence soil microbiome dynamics

Supplementary Files

File S1: Exudate and Exometabolite data.

File S2: 16S rRNA gene amplicon sequencing summary, ANCOM statistics, and feature table; metagenome sequencing and assembly statistics, metagenome-assembled genome summaries and abundance data.

File S3: DRAM annotation outputs for MAGs.

File S4: Raw DRAM annotations (can accessed using https://doi.org/10.5281/zenodo.5639650)

File S5: Synthetic exudate calculations for sugars

File S6: Synthetic exudate calculations for organic acids + final exudate treatment calcualtions

Supplementary Figures

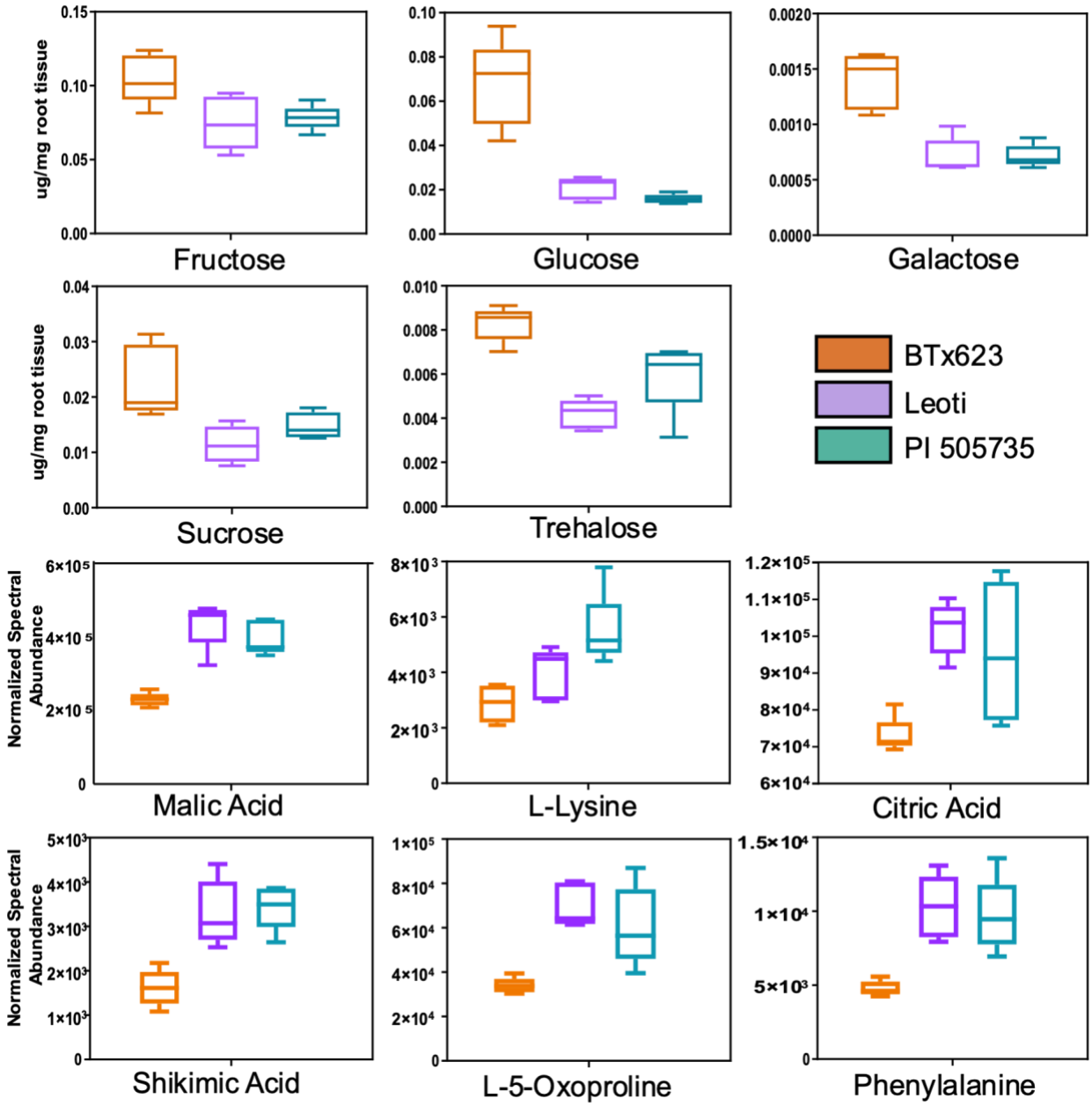

Figure S1. Normalized (to mg root weight) root exudate concentrations of three sorghum genotypes. Sorghum seedling (7 days old) sugar and organic acid concentrations were used to develop root exudate treatments, HST and HOT.

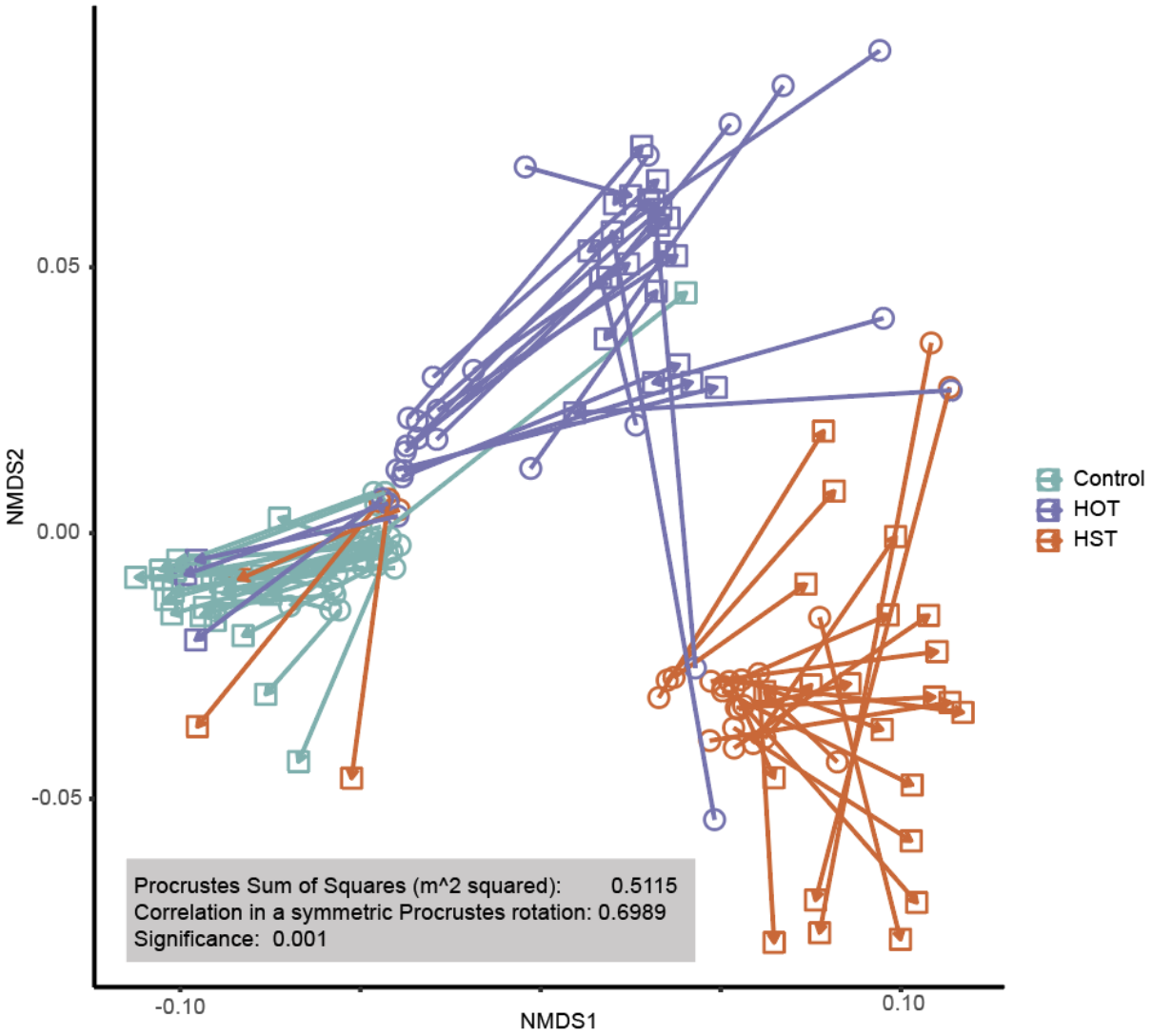

Figure S2. Procrustes ordination of exometabolite and 16S rRNA gene amplicon community NMDS ordinations showing the two datasets are significantly coordinated.

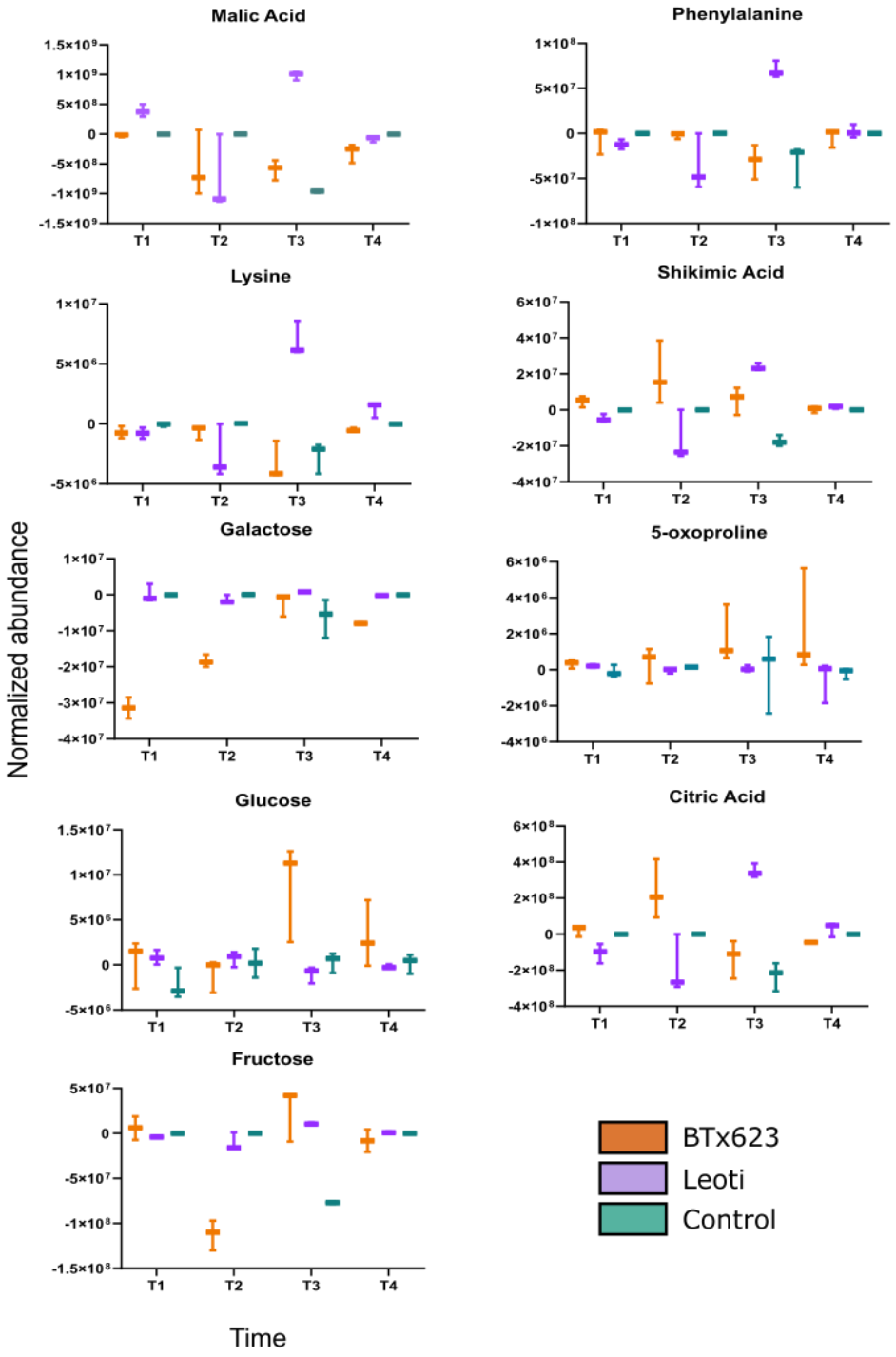

Figure S3. Consumption of added synthetic root exudate compounds. Time is representative of the 24h time period between exudate additions. Each value was calculated by subtracting the initial metabolite (M_T) sample from the following day from the final metabolite sample from the previous day (M_{T-1}) to calculate consumption. For instance, Day 1 M_T was subtracted from Day 0 M_{T-1} to calculate metabolite turnover for Time 1 (T1).

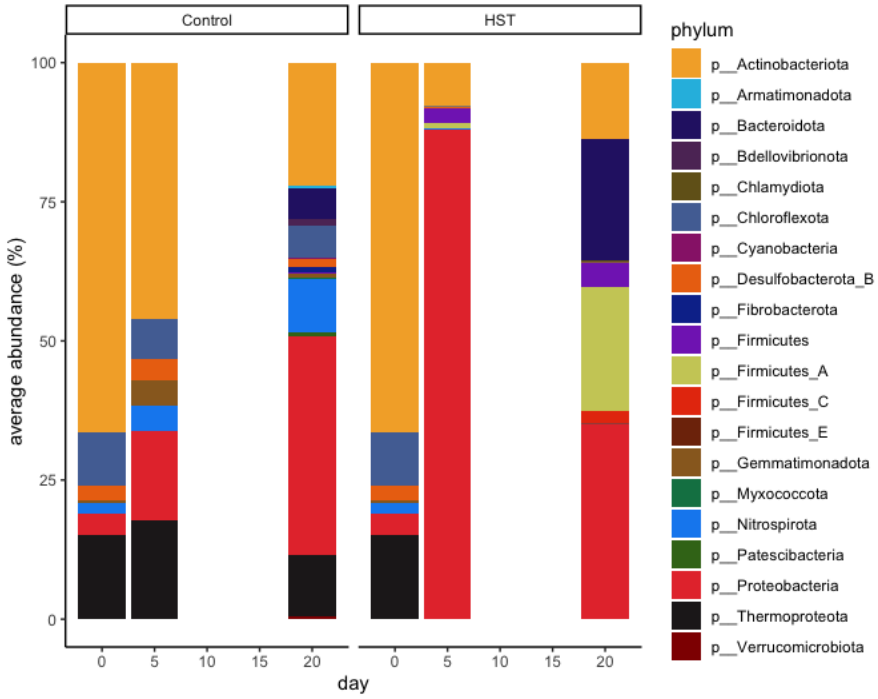

Figure S4. Phylum-level MAG abundance for control and HST microcosms at three timepoints. Values represent the average of replicates for each timepoint (n=3).

Supplementary Tables

Table S1. The recipe for the phosphate buffered minimal medium (pH 6.5) used in the microcosms.

Component	**Added to 1000mL**
Ammonium Chloride (NH_4Cl)	0.25 g
Sodium phosphate, dibasic (Na_2HPO_4)	0.56 g
Sodium phosphate, monobasic (NaH_2PO_4)	1.6 g

Table S2. Selected reaction monitoring transitions and instrument settings for each phytohormone. Product Ion 1 was used for quantitation and Product Ion 2 was used for qualification.

Compound Name	Abbreviation	Precursor Ion m/z	Product Ion 1 m/z	Product Ion 2 m/z	Polarity	Collision Energy (eV)
dihydrophaseic acid	DPA	280.9	237.1	170.9	-	20
Gibberellic Acid 3	GA3	344.9	239.1	220.9	-	22
Indole-3 carboxylic acid	ICA	161.9	88.9	116.8	+	-53
Salicylic Acid	SA	137.1	65.1	39.1	-	24
Salicylic Acid-d4	SA-d4	141.1	96.9		-	29
Indole-3- Acetyl alanine	IA-alanine	247.1	130	103	+	-22
Phaseic Acid	PA	279	139	121.9	-	19
Indole-3-Acetic Acid	IAA	175.9	129.9	102.9	+	-25
Indole-3-Acrylic Acid	IA-Acra	188	169.8	114.9	+	-19
Abscisic Acid	ABA	263	152.9	219.2	-	17
Abscisic Acid-d6	ABA-d6	269.1	159.1		-	18
Indole-3-Acetonitrile	IAnitrile	157.1	129.8	117	+	-15
Jasmonic Acid	JA	210.9	150.8	193	+	-18
Jasmonic Acid-d5	JA-d5	215.9	134.9		+	-16
Indole-3-Butyric Acid	IBA	204.1	117	130.1	+	-46
Gibberellic Acid 4	GA4	330.9	287	243.1	-	26
oxophytodienoic acid	OPDA	290.9	164.9	247.2	-	28
Methyl Jasmonate	meJA	225.4	104.9	91.1	+	-45
1-Aminocyclopropane Carboxylic Acid	ACC	101.9	56.2	30.3	+	-19
Benzoic Acid	BA	122.9	45.1	51	+	-47
Blumenol C Glucoside		373.3	193.2	134.9	+	-28
Blumenol B		227.2	107.1	139.1	+	-33

Table S3. Compounds and total amounts in each treatment used in microcosms experiment, see supplementary files S5 and S6 for calculations.

Compound	High Sugar Treament (ug/day)	High Sugar Treament (ug total for 5 days)	High Sugar Treament (mg total for 5 reps)	High Organic Acid Treament (ug/day)	High Organic Acid Treament (ug total for 5 days)	High Organic Acid Treament (mg total for 5 reps)
Malic acid	58122.08	290610.38	1453.05	312547.18	1562735.89	7813.68
Citric acid	18399.96	91999.79	460.00	68949.51	344747.54	1723.74
Phenylalanine	1281.11	6405.53	32.03	6374.09	31870.43	159.35
L-5-Oxoproline	8983.02	44915.11	224.58	44747.52	223737.58	1118.69
Shikimic acid	405.05	2025.25	10.13	2078.10	10390.52	51.95
L-Lysine	806.82	4034.08	20.17	2942.24	14711.21	73.56
Glucose	3243.70	16218.51	81.09	1004.44	5022.19	25.11
Sucrose	1079.57	5397.84	26.99	63.39	316.94	1.58
Fructose	5017.97	25089.85	125.45	3603.69	18018.45	90.09
Galactose	67.16	335.79	1.68	34.35	171.73	0.86
Trehalose	397.77	1988.83	9.94	200.73	1003.65	5.02