

Article Title: Soil microbiome disruption used as a method to investigate specific and general plant-bacterial relationships in three agroecosystem soils

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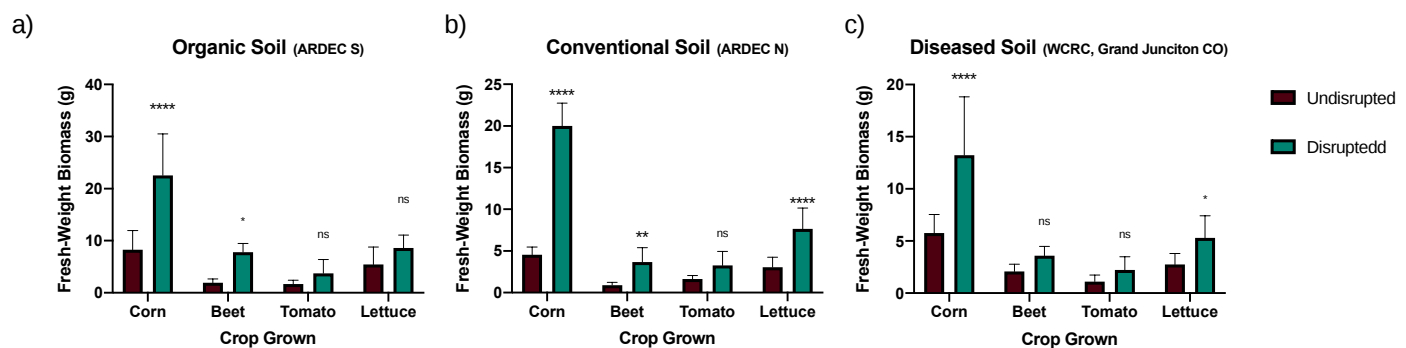
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Keywords: Agroecosystem, Rhizosphere, Microbiome, Autoclave disruption, Soil microbiology, 16S Sequencing

Supplementary Information

Supplemental Figure S1: Mean crop fresh-weight (FW) biomass (a) grown in organic soil (b) conventional soil and (c) diseased soil (n=10 / treatment, $\pm$ standard deviation). Significant increases in biomass of each crop are denoted by “*”, “**” or “****” above each. Sidak’s multiple comparison test using GraphPad (Vers 8.2.1).



Supplemental Table S1: Average Plant Fresh Weight (FW) Biomass and Percent Increases

Average Fresh Weight (g)		Crops Tested			
Soil	Treatment	Corn	Beet	Tomato	Lettuce
Organic	Unautoclaved	8.280	1.945	1.691	5.460
	Autoclaved	22.540	7.820	3.740	8.610
	% Increase	172.222	302.057	121.171	57.692
Conventional	Unautoclaved	4.550	0.913	1.630	3.050
	Autoclaved	20.000	3.760	3.270	7.650
	% Increase	339.560	311.829	100.613	150.820
Diseased	Unautoclaved	5.760	2.080	1.100	2.740
	Autoclaved	14.689	3.590	2.220	5.290
	% Increase	155.015	72.596	101.818	93.066

Supplemental Table S2: Average Plant Dry Weight (DW) Biomass and Percent Increases

Average Dry Weight (g)		Crops Tested			
Soil	Treatment	Corn	Beet	Tomato	Lettuce
Organic	Unautoclaved	2.080	0.340	0.280	0.931
	Autoclaved	4.460	1.430	0.770	1.680
	% Increase	114.423	320.588	175.000	80.451
Conventional	Unautoclaved	1.060	0.135	0.290	0.490
	Autoclaved	4.260	0.650	0.625	1.750
	% Increase	301.887	381.481	115.517	257.143
Diseased	Unautoclaved	1.120	0.350	0.169	0.472
	Autoclaved	3.211	0.530	0.259	1.090
	% Increase	186.706	51.429	53.254	130.932

Supplemental Table S3: Pairwise comparison of qPCR results for values of 16s rRNA copies per g FW rhizosphere or bulk soil (NS= Non “sterile” and soils were not autoclaved before experimentation S= Sterile and soils were autoclaved before experimentation)

16s rRNA copies / g FW Soil - Comparison of Disrupted Vs. Undisrupted Soils				
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Organic Soil				
NoPlant:Unautoclaved-Beet:Unautoclaved	-202242348	-662224516	ns	0.8530598
NoPlant:Unautoclaved-Corn:Unautoclaved	-99531654	-559513822	ns	0.9983772
NoPlant:Unautoclaved-Lettuce:Unautoclaved	-288537662	-748519831	ns	0.4746212
Tomato:Unautoclaved-NoPlant:Unautoclaved	323777128	-136205040	ns	0.3284444
NoPlant:Autoclaved-Beet:Autoclaved	-55721412	-515703580	ns	0.9999857

NoPlant:Autoclaved-Corn:Autoclaved	-136426493	-596408661	ns	0.9844926
NoPlant:Autoclaved-Lettuce:Autoclaved	22368828	-437613341	ns	1
Tomato:Autoclaved-NoPlant:Autoclaved	-34844228	-494826396	ns	0.9999998
Conventional Soil				
NoPlant:Unautoclaved-Beet:Unautoclaved	-111707062	-1810238653	ns	0.9999999
NoPlant:Unautoclaved-Corn:Unautoclaved	210997912	-1487533680	ns	0.9999823
NoPlant:Unautoclaved-Lettuce:Unautoclaved	764427629	-934103963	ns	0.8363816
Tomato:Unautoclaved-NoPlant:Unautoclaved	-827567533	-2526099125	ns	0.7698208
NoPlant:Autoclaved-Beet:Autoclaved	-1003978478	-2702510070	ns	0.5514078
NoPlant:Autoclaved-Corn:Autoclaved	-773297144	-2471828736	ns	0.8276529
NoPlant:Autoclaved-Lettuce:Autoclaved	-2046485876	-3745017467	**	0.0108405
Tomato:Autoclaved-NoPlant:Autoclaved	524863771	-1173667821	ns	0.9797232
Diseased Soil				
NoPlant:Unautoclaved-Beet:Unautoclaved	-86778106	-1254318809	ns	0.9999998
NoPlant:Unautoclaved-Corn:Unautoclaved	-1560104315	-2727645018	***	0.0039314
NoPlant:Unautoclaved-Lettuce:Unautoclaved	-50719652	-1218260356	ns	1
Tomato:Unautoclaved-NoPlant:Unautoclaved	81312294	-1086228409	ns	0.9999999
NoPlant:Autoclaved-Beet:Autoclaved	162085172	-1005455532	ns	0.9999546
NoPlant:Autoclaved-Corn:Autoclaved	160028973	-1007511731	ns	0.9999592
NoPlant:Autoclaved-Lettuce:Autoclaved	157904694	-1009636010	ns	0.9999636
Tomato:Autoclaved-NoPlant:Autoclaved	-220716905	-1388257608	ns	0.9994323

Supplemental Table S4: Pairwise comparison between values of Shannon's alpha diversity assigned to each treatment. (Above) Comparison of Shannon's α -diversity values between disrupted and undisrupted treatments. (Below) Comparison of Shannon's α -diversity values between communities detected in disrupted no plant treatments and disrupted plant treatments (e.g. Crop: Autoclaved= disrupted, Crop: Unautoclaved=undisrupted).

Shannon's Alpha Diversity Value Comparison of Disrupted Crop Vs. Undisrupted Crop Treatments

Organic Soil					
Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
Beet:Unautoclaved-Beet:Autoclaved	0.230	-0.403	0.863	0.943	ns
Corn:Unautoclaved-Corn:Autoclaved	-0.122	-0.755	0.511	0.999	ns
Lettuce:Unautoclaved-Lettuce:Autoclaved	-0.785	-1.493	-0.077	0.023	ns
Tomato:Unautoclaved-Tomato:Autoclaved	-0.128	-0.761	0.505	0.999	ns
SoilOnly:Unautoclaved-SoilOnly:Autoclaved	-0.176	-0.809	0.457	0.989	ns

Conventional Soil					
Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
Beet:Unautoclaved-Beet:Autoclaved	0.261	-0.617	1.138	0.981	ns
Corn:Unautoclaved-Corn:Autoclaved	-0.320	-1.104	0.465	0.886	ns
Lettuce:Unautoclaved-Lettuce:Autoclaved	0.167	-0.710	1.044	0.999	ns
Tomato:Unautoclaved-Tomato:Autoclaved	0.283	-0.594	1.160	0.969	ns
SoilOnly:Unautoclaved-SoilOnly:Autoclaved	0.534	-0.251	1.318	0.354	ns
Diseased Soil					
Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
Beet:Unautoclaved-Beet:Autoclaved	0.225	-0.602	1.052	0.991	ns
Corn:Unautoclaved-Corn:Autoclaved	0.849	0.022	1.676	0.041	*
Lettuce:Unautoclaved-Lettuce:Autoclaved	0.467	-0.360	1.294	0.608	ns
Tomato:Unautoclaved-Tomato:Autoclaved	0.717	-0.110	1.544	0.125	ns
SoilOnly:Unautoclaved-SoilOnly:Autoclaved	0.774	-0.053	1.601	0.079	ns
Shannon's Alpha Diversity Value Comparison of Disrupted Soil Alone Vs. Disrupted Crop Treatments					
Organic Soil					
Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
SoilOnly:Autoclaved-Beet:Autoclaved	0.241	-0.392	0.874	0.926	ns
SoilOnly:Autoclaved-Corn:Autoclaved	0.219	-0.414	0.852	0.957	ns
SoilOnly:Autoclaved-Lettuce:Autoclaved	-0.010	-0.643	0.623	1.000	ns
Tomato:Autoclaved-SoilOnly:Autoclaved	-0.021	-0.654	0.612	1.000	ns
Conventional Soil					
Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
SoilOnly:Autoclaved-Beet:Autoclaved	-0.317	-1.102	0.467	0.890	ns
SoilOnly:Autoclaved-Corn:Autoclaved	-0.482	-1.266	0.303	0.481	ns
SoilOnly:Autoclaved-Lettuce:Autoclaved	-0.393	-1.178	0.391	0.721	ns
Tomato:Autoclaved-SoilOnly:Autoclaved	0.336	-0.449	1.120	0.856	ns
Diseased Soil					
Tukey's multiple comparisons test	diff	lwr	upr	p-value	summary
SoilOnly:Autoclaved-Beet:Autoclaved	-0.561	-1.388	0.266	0.373	ns
SoilOnly:Autoclaved-Corn:Autoclaved	-0.523	-1.350	0.304	0.464	ns
SoilOnly:Autoclaved-Lettuce:Autoclaved	-0.534	-1.361	0.293	0.438	ns
Tomato:Autoclaved-SoilOnly:Autoclaved	0.649	-0.178	1.475	0.210	ns

Supplemental Table S5: Crop-specific and crop-shared bacterial abundance increases resulting from growth in disrupted agroecosystems.

Crop specific taxa	
<u>Crop</u>	<u>Taxa</u>
Beet	Acetobacteraceae_ <i>Roseococcus</i>
	Bacteriovoracaceae_ <i>Peredibacter</i>
	Chitinophagaceae_ <i>Flavisolibacter</i>
	Chitinophagaceae_ <i>Parasegetibacter</i>
	Chitinophagaceae_ <i>UTBCD1</i>
	Solimonadaceae_ <i>Solimonas</i>
Corn	Cytophagaceae_ <i>Sporocytophaga</i>
	Fibrobacteraceae_ <i>possible_genus_04</i>
	Pseudomonadaceae_ <i>Pseudomonas</i>
	Rhodothermaceae_ <i>unclassified</i>
Lettuce	(class: Berkelbacteria o: unclassified) unclassified_ <i>unclassified</i>
	(k: Archaea c: Woesearchaeia) unclassified_ <i>unclassified</i>
	(k: unclassified p: unclassified c: unclassified o: unclassified) unclassified_ <i>unclassified</i>
	(o: Micavibrionales) unclassified_ <i>unclassified</i>
	Acetobacteraceae_ <i>Rhodovastum</i>
	Acetobacteraceae_ <i>Roseomonas</i>
	Burkholderiaceae_ <i>Rhodoferax</i>
	Burkholderiaceae_ <i>unclassified</i>
	Caulobacteraceae_ <i>unclassified</i>
	Crocinitomicaceae_ <i>Fluviicola</i>
	Cytophagaceae_ <i>Cytophaga</i>
	Intrasporangiaceae_ <i>Knoellia</i>
	Opitutaceae_ <i>unclassified</i>
	Oscillatoriaceae_ <i>Oscillatoria_PCC-6304</i>
	Sporolactobacillaceae_ <i>unclassified</i>

Tomato	Tepidisphaeraceae_ <i>Tepidisphaera</i>
	Verrucomicrobiaceae_ <i>Brevifollis</i>
	(o: Candidatus_Peribacteria) unclassified_ <i>unclassified</i>
	(o:Candidatus_Pacebacteria) unclassified_ <i>unclassified</i>
	(p: BRC1) unclassified_ <i>unclassified</i>
	Archangiaceae_ <i>unclassified</i>
	Bacillaceae_ <i>Bacillus</i>
	Beijerinckiaceae_ <i>Chelatococcus</i>
	Caulobacteraceae_ <i>Asticcacaulis</i>
	Hydrogenedensaceae_ <i>unclassified</i>
	Microbacteriaceae_ <i>Yonghaparkia</i>
	Rhizobiaceae_ <i>Aminobacter</i>
	Rhodobacteraceae_ <i>Cereibacter</i>
	Sphingobacteriaceae_ <i>Mucilaginibacter</i>
	Thermoactinomycetaceae_ <i>Shimazuella</i>
	Verrucomicrobiaceae_ <i>unclassified</i>
	Xanthomonadaceae_ <i>Thermomonas</i>

Shared by two or more crops

<u>Crop</u>	<u>Taxa</u>
Beet + Corn	(c: Microgenomatia o: unclassified) unclassified_ <i>unclassified</i>
	Caulobacteraceae_ <i>Caulobacter</i>
Beet + Tomato	Paenibacillaceae_ <i>Ammoniphilus</i>
Beet + Lettuce	(c: Sericytochromatia o: unclassified) unclassified_ <i>unclassified</i>
	Kineosporiaceae_ <i>Quadrisphaera</i>
	Paenibacillaceae_ <i>Brevibacillus</i>
	Rubinisphaeraceae_ <i>SH-PL14</i>
	Sphingomonadaceae_ <i>unclassified</i>

	<i>Verrucomicrobiaceae_Roseimicrobium</i>
Corn + Tomato	<i>Xanthomonadaceae_Stenotrophomonas</i>
Corn + Lettuce	(o: Bacillales) <i>unclassified_unclassified</i>
	(o: Candidatus_Peribacteria) <i>unclassified_unclassified</i>
Tomato + Lettuce	<i>Bacillaceae_Anoxybacillus</i>
	<i>Beijerinckiaceae_alphaI_cluster</i>
	<i>Cyclobacteriaceae_unclassified</i>
	<i>env.OPS_17_unclassified</i>
	<i>Flavobacteriaceae_Flavobacterium</i>
	<i>Nodosilineaceae_Nodosilinea_PCC-7104</i>
	<i>Verrucomicrobiaceae_Verrucomicrobium</i>

Shared by Three or more crops

<u>Crop</u>	<u>Taxa</u>
Beet + Corn + Tomato	<i>Devosiaceae_unclassified</i>
	<i>Opitutaceae_Opitutus</i>
Beet + Corn + Lettuce	(o: Candidatus_Nomurabacteria) <i>unclassified_unclassified</i>
Beet + Tomato + Lettuce	<i>Cyclobacteriaceae_Algoriphagus</i>
	<i>Devosiaceae_Devosia</i>
	<i>Oligoflexaceae_Oligoflexus</i>
	<i>Sphingobacteriaceae_Arcticibacter</i>
Corn + Lettuce + Tomato	<i>Cellvibrionaceae_unclassified</i>
	<i>Fibrobacteraceae_unclassified</i>
	<i>KD3-93_unclassified</i>

Shared by all four crops

<u>Crops</u>	<u>Taxa</u>
Beet + Corn + Lettuce + Tomato	Beijerinckiaceae_ <i>Bosea</i>
	Burkholderiaceae_ <i>Caenimonas</i>
	Caulobacteraceae_ <i>Brevundimonas</i>
	Chitinophagaceae_ <i>Lacibacter</i>
	Rubritaleaceae_ <i>Luteolibacter</i>
	Saccharimonadaceae_ <i>unclassified</i>
	Sphingobacteriaceae_ <i>Pedobacter</i>
	Sphingobacteriaceae_ <i>unclassified</i>
	Sphingomonadaceae_ <i>Sphingoaurantiacus</i>
	Sphingomonadaceae_ <i>Sphingopyxis</i>
	Spirosomaceae_ <i>Dyadobacter</i>
	Spirosomaceae_ <i>Larkinella</i>
	Spirosomaceae_ <i>Rhabdobacter</i>