

Supplementary information for **“Soil amendments with ethylene precursor alleviate negative impacts of salinity on soil microbial properties and productivity”**

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Table S1. Soil bacterial populations (operational taxonomic units (OTUs)) that were influenced by ACC treatments in the saline soils.

OTU_ID	p-values	P-values (corrected)	0: rel. freq. (%)	50: rel. freq. (%)	200: rel. freq. (%)	2000: rel. freq. (%)	Taxonomy
3	0.034	0.376	2.74±0.73	2.02±0.14	1.85±0.04	1.27±0.24	p_Acidobacteria; c_Acidobacteria; o_Acidobacteriales; f_unclassified; g_unclassified
10	0.014	0.150	0.17±0.08	0.43±0.22	0.61±0.05	1.26±0.44	p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Microbacteriaceae; g_Microbacterium
36	0.008	0.093	1.17±0.22	0.39±0.04	0.64±0.15	0.57±0.20	p_SPAM; c_0319-6G9; o_unclassified; f_unclassified; g_unclassified
27	0.028	0.311	0.47±0.08	0.27±0.04	0.30±0.05	0.39±0.04	p_Proteobacteria; c_Betaproteobacteria; o_unclassified; f_unclassified; g_unclassified
OTU_56069	0.012	0.128	0.63±0.05	0.54±0.05	0.37±0.07	0.39±0.09	p_Acidobacteria; c_Acidobacteria; o_Acidobacteriales; f_unclassified; g_unclassified
OTU_63594	0.031	0.341	0.28±0.06	0.41±0.11	0.39±0.07	0.58±0.06	p_Actinobacteria; c_Actinobacteria; o_Solirubrobacteriales; f_Solirubrobacteriaceae; g_unclassified
OTU_76249	0.008	0.083	0.15±0.04	0.28±0.07	0.34±0.08	0.46±0.05	p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Microbacteriaceae; g_Agromyces
OTU_47099	0.023	0.248	0.06±0.03	0.22±0.05	0.20±0.06	0.38±0.13	p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Microbacteriaceae; g_Microbacterium
OTU_72418	0.049	0.541	0.21±0.02	0.25±0.05	0.33±0.04	0.37±0.08	p_Actinobacteria; c_Actinobacteria; o_MC47; f_unclassified; g_unclassified
OTU_62989	0.029	0.321	0.30±0.02	0.35±0.03	0.34±0.04	0.25±0.02	p_Acidobacteria; c_Acidobacteria; o_Acidobacteriales; f_unclassified; g_unclassified
OTU_55567	0.004	0.044	0.06±0.02	0.14±0.08	0.21±0.05	0.32±0.02	p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Microbacteriaceae; g_Microbacterium

The OTUs shown differed between the control and ACC treatments in the saline soils using ANOVA with Tukey-Kramer tests for post-hoc comparisons of means as implemented in STAMP. The values shown in ACC (level 0, 50, 200, 2000 μ M) columns are the mean relative abundances within each treatment \pm SDs. Shown are OTUs present at $\geq 0.3\%$ mean relative abundance in different ACC treatments.

Table S2. Soil prokaryotic populations (operational taxonomic units (OTUs)) that were influenced by ACC treatments in the non-saline soils.

OTU_ID	p-values	p-values	0: rel. freq.	200: rel. freq.	2000: rel. freq.	50: rel. freq.	Taxonomy
		(corrected)	(%)	(%)	(%)	(%)	
1	0.028	0.167	1.00±0.15	1.64±0.02	1.61±0.35	1.00±0.23	k_Archaea; p_Crenarchaeota; c_Thaumarchaeota; o_Nitrososphaerales; f_Nitrososphaeraceae; g_Nitrososphaera
33	0.005	0.030	0.42±0.06	0.70±0.11	0.51±0.08	0.32±0.01	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Chromatiales; f_Sinobacteraceae; g_unclassified
OTU_60523	0.000	0.002	0.47±0.04	0.28±0.01	0.26±0.04	0.24±0.03	k_Bacteria; p_Acidobacteria; c_Acidobacteria; o_Acidobacteriales; f_unclassified; g_unclassified
OTU_8606	0.048	0.287	0.10±0.03	0.24±0.09	0.38±0.01	0.25±0.12	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Microbacteriaceae; g_Microbacterium
OTU_63984	0.021	0.126	0.31±0.04	0.19±0.06	0.14±0.06	0.12±0.03	k_Bacteria; p_Nitrospirae; c_Nitrospira; o_Nitrospirales; f_Nitrospiraceae; g_Nitrospira
OTU_32822	0.010	0.063	0.30±0.04	0.15±0.04	0.17±0.02	0.10±0.06	k_Bacteria; p_Acidobacteria; c_Acidobacteria; o_Acidobacteriales; f_unclassified; g_unclassified

The OTUs shown differed between the control and ACC treatments in the non-saline soils using ANOVA with Tukey-Kramer tests for post-hoc comparisons of means as implemented in STAMP. The values shown in ACC (level 0, 50, 200, 2000 μ M) columns are the mean relative abundances within each treatment \pm SDs. Shown are OTUs present at $\geq 0.3\%$ mean relative abundance in different ACC treatments.

Table S3. Changes in bacterial/fungal communities in response to salinity treatments using phylogenetic molecular ecological networks (pMENs).

Parameters	Bacteria		Fungi	
	Control	Saline	Control	Saline
Clustering coefficient	0.038	0.01	0	0.023
Connected component	17	16	25	18
Network diameter	17	12	4	14
Short paths	4110 (36%)	2214(33%)	160 (3%)	3330 (31%)
Average number of neighbours	1.83	1.71	1.31	1.81
Number of nodes	106	82	70	103
Network heterogeneity	0.584	0.545	0.473	0.578

Table S4. Soil fungal operational taxonomic units (OTUs) that were influenced by salinity treatments.

OTU_ID	P value	P value corrected	Salinity: rel. freq. (%)	non-salinity: rel. freq. (%)	Taxonomy
OTU_8	0.0000	0.0002	4.00±1.24	1.83±0.39	p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Nectriaceae;g_Haematonectria
OTU_9	0.0002	0.0027	3.16±1.18	1.51±0.36	p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Nectriaceae;g_Fusarium
OTU_3	0.0002	0.0030	10.16±1.90	7.16±1.25	p_Basidiomycota;c_Tremellomycetes;o_Trichosporonales;f_Trichosporonaceae;g_Trichosporon
OTU_4	0.0003	0.0035	4.81±1.92	2.20±0.57	p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Nectriaceae;g_Fusarium
OTU_27	0.0006	0.0076	0.77±0.38	0.29±0.12	p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Nectriaceae;g_Fusarium
OTU_1	0.0009	0.0123	9.88±10.87	26.27±9.22	p_Ascomycota;c_Pezizomycetes;o_Pezizales;f_Pezizaceae;g_unidentified
OTU_21	0.0051	0.0660	0.57±0.22	0.96±0.36	p_unidentified;c_unidentified;o_unidentified;f_unidentified;g_unidentified
OTU_2	0.0076	0.0982	21.59±7.61	14.09±3.69	p_Basidiomycota;c_Tremellomycetes;o_Trichosporonales;f_Trichosporonaceae;g_Trichosporon
OTU_25	0.0150	0.1950	0.70±0.27	0.41±0.25	p_Ascomycota;c_unidentified;o_unidentified;f_unidentified;g_unidentified
OTU_12	0.0300	0.3900	1.38±0.39	1.91±0.64	p_Ascomycota;c_Sordariomycetes;o_Sordariales;f_Chaetomiaceae;g_Thielavia
OTU_13	0.0410	0.5330	1.34±0.34	1.64±0.33	p_unidentified;c_unidentified;o_unidentified;f_unidentified;g_unidentified
OTU_30	0.0410	0.5330	0.75±0.76	0.24±0.10	p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Nectriaceae;g_Fusarium
OTU_26	0.0450	0.5850	0.35±0.29	0.72±0.48	p_Zygomycota;c_Incertae_sedis;o_Mortierellales;f_Mortierellaceae;g_Mortierella

The OTUs shown differed between the saline and non-saline conditions using ANOVA with Tukey-Kramer tests for post-hoc comparisons of means as implemented in STAMP. The values shown in saline/non-saline columns are the mean relative abundances within each treatment \pm SDs. Shown are OTUs present at $\geq 0.5\%$ mean relative abundance in different salinity treatments.

Table S5. The correlation between the axes from NMDSs and single taxa tested using the linear correlation test (Pearson).

Bacterial community (OTU>0.05% in relative abundance)						Fungal community (Top 10 OTUs)					
OTU_ID	BACT_1		OTU_ID	BACT_2		OTU_ID	FUNG_1		OTU_ID	FUNG_2	
	r	Sig.		r	Sig.		r	Sig.		r	Sig.
3	0.66	***	1	0.8	***	38	-0.95	***	40	-0.42	*
4	0.64	***	2	0.64	***	39	0.82	***	43	-0.54	**
5	0.53	**	9	-0.44	*	40	0.8	***	45	0.68	***
6	0.64	***	14	-0.47	*	41	0.9	***	46	0.63	**
8	-0.7	***	17	0.57	**	43	0.76	***	47	-0.44	*
9	-0.74	***	18	0.67	***	44	0.7	***			
10	-0.69	***	25	-0.44	*	47	0.42	*			
11	-0.88	***	28	-0.51	*						
12	-0.82	***	33	0.41	*						
13	-0.68	***									
14	-0.77	***									
15	-0.88	***									
16	-0.69	***									
17	0.51	*									
19	-0.54	**									
20	-0.55	**									
21	0.48	*									
22	0.67	***									
24	0.48	*									
26	0.57	**									
27	0.53	**									
29	0.61	***									
30	0.58	**									
31	0.77	***									
32	0.82	***									
36	0.81	***									
37	0.78	***									

Table S6. Soil physicochemical properties.

Soil parameter	Value
Sand (%)	12
Silt (%)	50
Clay (%)	38
pH	7.8
EC (dS m ⁻¹)	1.42
Total carbon content (Wt%)	1.13
Total nitrogen content (Wt%)	0.04
Total Al concentration (Wt%)	4.86
Total As concentration (mg kg ⁻¹)	0.84
Total Ba concentration (mg kg ⁻¹)	251
Total Ca concentration (Wt%)	1.3
Total Cd concentration (mg kg ⁻¹)	0.35
Total Co concentration (mg kg ⁻¹)	2.47
Total Cr concentration (mg kg ⁻¹)	78
Total Cu concentration (mg kg ⁻¹)	52
Total Fe concentration (Wt%)	5.63
Total K concentration (Wt%)	3.6
Total Mg concentration (Wt%)	0.21
Total Mn concentration (mg kg ⁻¹)	1295
Total Mo concentration (mg kg ⁻¹)	1.44
Total Na concentration (Wt%)	2.91
Total Ni concentration (mg kg ⁻¹)	59
Total P concentration (Wt%)	0.31
Total Pb concentration (mg kg ⁻¹)	0.18
Total S concentration (mg kg ⁻¹)	1836
Total Se concentration (mg kg ⁻¹)	0.33
Total Si concentration (Wt%)	32
Total Sr concentration (mg kg ⁻¹)	190
Total Zn concentration (mg kg ⁻¹)	315

Table S7. Primer sequences used for real-time PCR assays designed for the determination of copy numbers of nitrogen and carbon cycling genes in soil.

Genes	Forward sequence	Reverse sequence	Amplicon (bp)	T_m^A (°C)	Reference
<i>Chitinase A</i>	CGTCGACATCGACTGGGARTDBC C	ACGCCGGTCCAGCCNCKNCCR TA	400	63	(Yergeau et al., 2007)
<i>arch-amoA</i>	TTCTTCTTTGTTGCCAGTA	CTGAYTGGGCYTGGACATC	256	63	(Wuchter et al., 2006)
<i>nifH</i>	AAAGGYGGWATCGGYAARTCCAC CAC	TTGTTSGCSCGRTACATSGCC ATCAT	459	60	(Rösch et al., 2002)
<i>amoA</i>	GGGGTTTCTACTGGTGGT	CCCCTCKGSAAAGCCTTCTTC	491	56	(Rotthauwe et al., 1997)
<i>nosZ</i>	CGYTGTTTCMTCGACAGCCAG	CATGTGCAGNGCRTGGCAGAA	700	64	(Rösch et al., 2002)
<i>narG</i>	TAYGTSGGSCARGARAA	TTYTCRTACCABGTBGC	650	59	(Philippot et al., 2002)
<i>acdS</i>	GGCAACAAGMYSCGAAGCT	CTGCACSAGSACGCACTTCA	133	67	(Bouffaud et al., 2018)

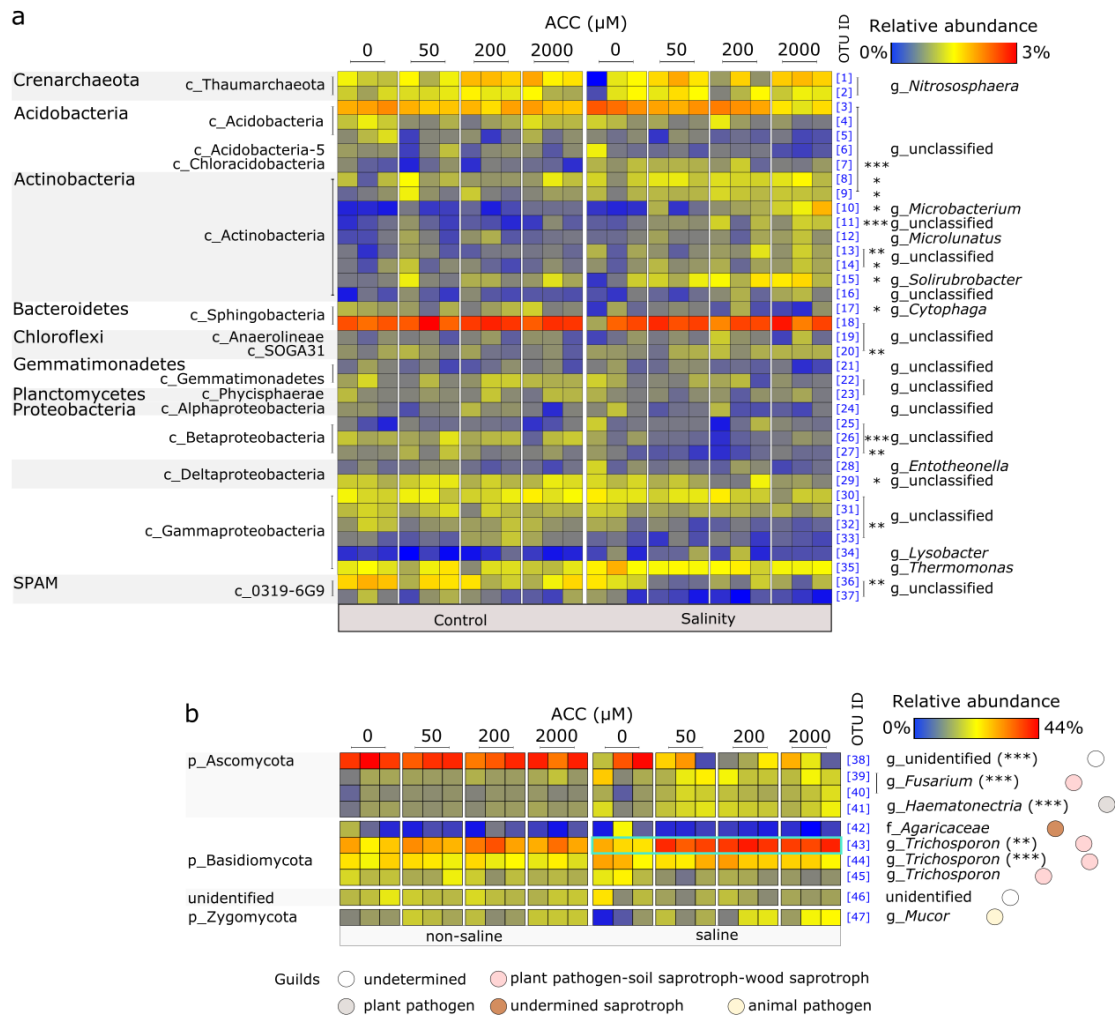


Fig.S1 Heatmap summarizing variations in the microbial taxa based on data obtained from 16S rRNA and ITS amplicon sequencing (shown are taxa $\geq 0.5\%$ in relative abundance). Each Operational Taxonomic Unit (OTU) has a unique bracket that is consistent with those shown in other figures and tables. Stars after each OTU ID represent significant differences between saline and non-saline conditions (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ANOVA, Tukey-Kramer test). The green box inserted in heatmap (b) highlights the differences of OTU [42] between control and ACC treatments in the saline soil.

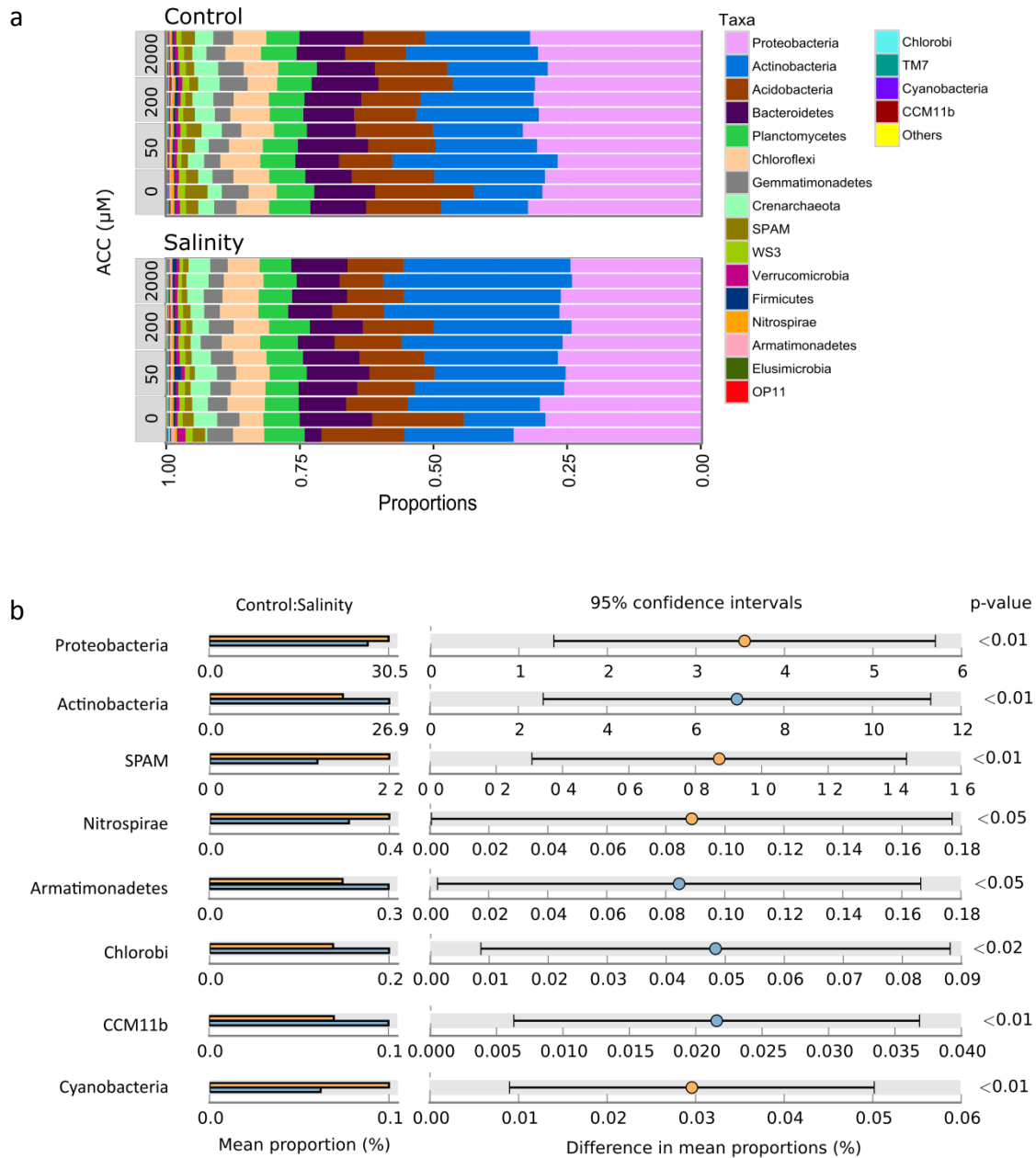


Fig.S2 Barplot summarizing top 20 bacterial/archaeal phyla in the silty clay loam (a) and those bacterial phyla responding to salinity treatments in relative abundances (b). The yellow and blue dots embedded in error bars distinguish decreases and increases in relative abundance of bacterial phyla after salinity treatments, respectively.

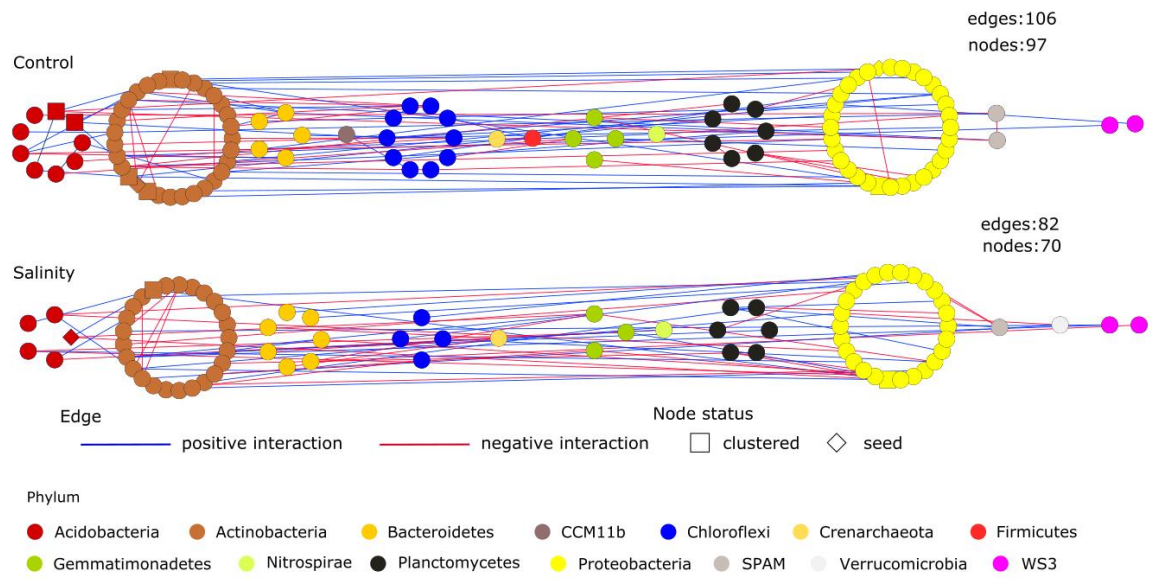


Fig.S3 Changes in soil bacterial/archaeal network structure in response to salinity treatments.

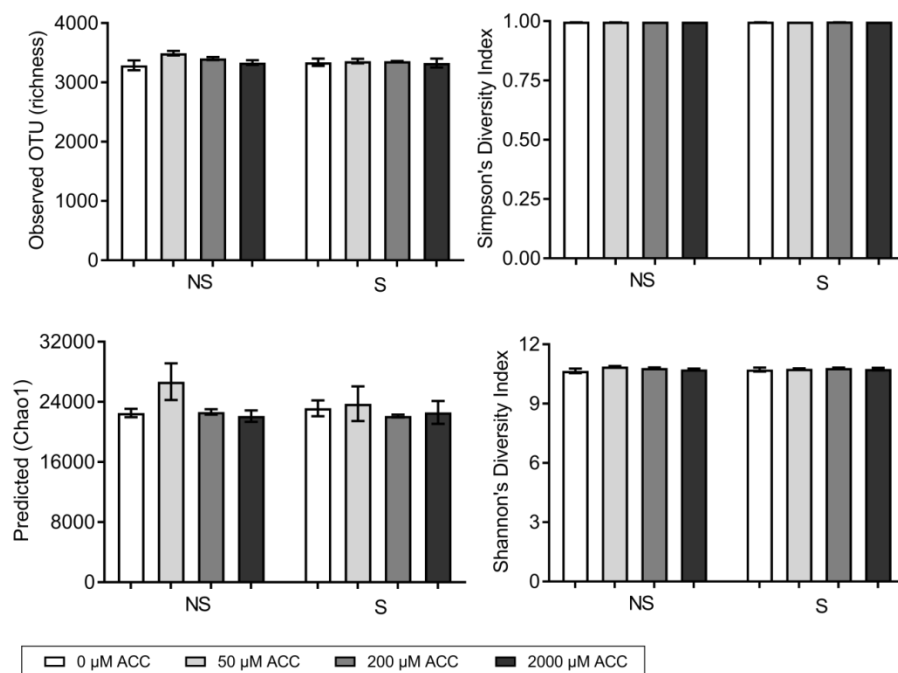


Fig.S4 The effect of ACC and salinity treatments on the observed species, predicted richness and evenness (Simpson's Diversity Index) of bacterial communities associated with soil environments. All values were based on 6,000 rarefied sequences per sample. Error bars denote SEs (n=3).

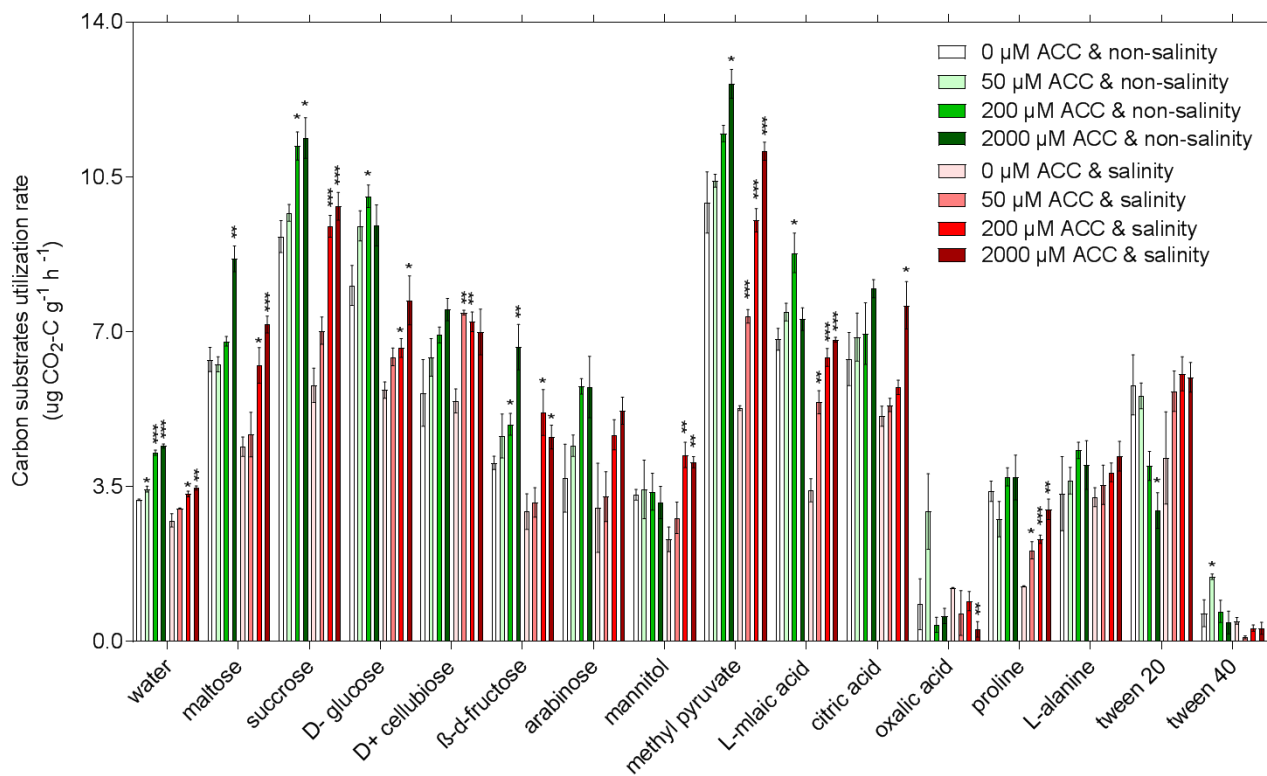


Fig.S5 The effects of ACC amendments on the utilization of 15 carbon substrates in the non-saline and saline soils. Stars above columns represent significant differences between ACC treatments and control (0 μM ACC) ($P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***, ANOVA, Least Significant Difference (LSD)). Error bars denote SDs ($n=3$).

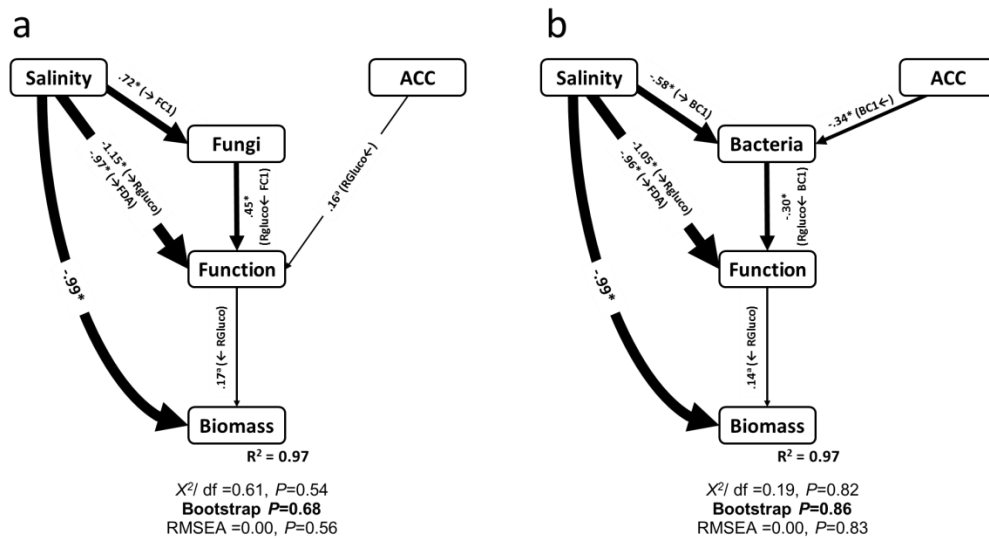


Figure S6. Structural equation models accounting for the direct and indirect effects of salinity and ACC on soil function and plant biomass via changes in microbial community composition (a, b). Numbers adjacent to arrows are path coefficients ($P < 0.05^*$, $P < 0.1^a$), and indicative of the effect size of the relationship. The thickness of the arrow indicates the strength of relationship. R^2 , the proportion of variance explained. Biomass: plant biomass. Function: soil microbial function.

References

- Bouffaud, ML., Renoud, S., Dubost, A., Moëgne-Loccoz, Y., Muller, D. 2018. 1-Aminocyclopropane-1-carboxylate deaminase producers associated to maize and other Poaceae species. *Microbiome* 6, 114.
- Philippot, L., Piutti, S., Martin-Laurent, F., Hallet, S., Germon, J.C., 2002. Molecular analysis of the nitrate-reducing community from unplanted and maize-planted soils. *Appl Environ Microbiol* 68, 6121-6128.
- Rösch, C., Mergel, A., Bothe, H., 2002. Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. *Appl Environ Microbiol* 68, 3818-3829.
- Rothauwe, JH., Witzel, KP., Liesack, W., 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl Environ Microbiol* 63, 4704-4712.
- Wuchter, C., Abbas, B., Coolen, M.J., Herfort, L., van Bleijswijk, J., Timmers, P., Strous, M., Teira, E., Herndl, G.J., Middelburg, J.J., 2006. Archaeal nitrification in the ocean. *PNAS* 103, 12317-12322.
- Yergeau, E., Kang, S., He, Z., Zhou, J., Kowalchuk, G.A., 2007. Functional microarray analysis of nitrogen and carbon cycling genes across an Antarctic latitudinal transect. *ISME J* 1, 163-179.