SUPPLEMENTARY MATERIAL

Combining soil microbial communities and greenhouse gas fluxes along a salinity gradient in temperate Mediterranean coastal wetlands

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Introduction

The supporting information includes tables and figures about the morphological features of the analysed soil profiles, as well as results for microbial diversity indices across the three sampling sites. It also presents statistical analysis results and the bibliography used to identify the ecological functions of the most abundant genus in the soil profiles.

Tab. S1 - Morphological features of soil profiles.

Profile	Horizon		Boundary (D/T)	Matrix Munsell Color (Wet)	Field texture class	Structure (T/G/S)	Fluidity class	Mottles/RMFs (K/Q/S/Sh)	Mottles/RMFs Munsell Color (WET)	Peroxide Color Change (Y/N)	Organic frag/Roots (Q/S)	Odor (K/I)
	Depth (cm)	Master										
PIR	0 - 6/7	Oi/Ase	AS	2.5Y 2.5/1	nd	gr/1/f	VF			N	3/f	S/ST
	6/7 - 15	Ase	CS	10YR 2/2	SaL	gr/1/f	MF			N	2/f	S/ST
	15 - 20	A/Cse	CS	5Y 52.5/2	Sa	sg/0	VF			N	1/m	S/ST
	20 - 31	Cse	CS	5Y 3/1	Sa	sg/0	VF	F3M/c/3/P	5Y 2.5/2	N	1/vf	S/ST
	31 - 50+	Cg	-	Gley1 3/10Y	Sa	sg/0	VF	OSF/c/3/D	Gley1 2.5/10Y	N	1/f	S/SM
CER	0 - 5	Ase	CW	Gley1 2.5/5GY	SiL	pl/1/f	VF			Y		S/ST
	5 - 10	Ag	CW	Gley1 4/10Y	SiL	pl/1/m	SF	F2M/c/2/P	Gley1 3/5GY	Y	1/vf	S/SM
	10 - 23	Cse	AW	Gley1 3/5GY	SiCL	pl/1/m	SF	F2M/m/3/P	Gley2 2.5/5PB	Y	1/vf	S/SM
	23 -35	2Cse	-	Gley1 3/N	LSa	sg/0	MF	F2M/m/4/P	Gley1 2.5/N	Y		S/ST
PA	0 - 4	Ase	CW	5Y 2.5/1	SiL	gr/1/m	VF	OSF/m/1/D	5Y 3/1	Y	2/f	S/SM
	4 - 10	Ag	AW	5Y 3/1	SiL	gr/1/m	MF	OSF/m/1/D	5Y 2.5/1	Y	1/f	S/SL
	10 - 17	Cg1	AW	5Y 4/1	SiCL	sbk/2/f	SF	F3M/c/3/P	5Y 4/2	Y	1/f	S/SL
	17 - 32+	Cg2	-	5Y 5/1	SiCL	sbk/2/f	SF	F3M/m/3/P	5Y 5/3; 2.5Y 5/4	Y		S/SL

Horizon master: se = presence of sulfide; g = gleying. **Horizon boundary:** (D) Distinctness: A = abrupt, C = clear, G = gradual, D = diffuse / (T) Topography: S = smooth, W = wavy, I = irregular, U = unknown; **Field texture class:** Sa = sand, SaL = Sandy Loam, L = Loam, LSa = Loamy Sand, SiL = Silty Loam; SiCL = Silty Clay Loam; **Structure:** (T) Type: gr = granular, abk = angular blocky, sbk = subangular blocky, pl = platy, sg = single grain / (G) Grade: 0 = structureless, 1 = weak, 2 = moderate / (S) Size: vf = very fine, f = fine, m = medium; **Fluidity classes:** SF = Slightly Fluid, MF = Moderately Fluid, VF = Very Fluid; **Mottles/redoximorphic features (RMFs):** (K) Kind: F2M = reduced iron, F3M = oxidated iron, OSF = organic stains / (Q) Quantity: f = few, c = common, m = many / (S) size: 1 = fine, 2 = medium, 3 = coarse, 4 = very coarse, 5 = extremely coarse / (Sh) Shape: D = dendritic, P = platy; **Roots:** (Q) Quantity: 1 = few, 2 = common, 3 = many / (S) Size: vf= very fine, f = fine, m = medium, co = coarse; **Odor:** (K) Kind: N = none, S = sulfurous / (I) Intensity: SL= slight, MD= moderate, ST= strong.

Tab. S2 - Results of ANOVA analysis testing difference in total taxa richness (S) at genus level between sampling sites; Df = Degrees of Freedom; Sum Sq = Sum of Squares; Mean Sq = Mean Square; F value = F statistic; Pr(>F) = p-value.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Site	2	71085	35543	0.701	0.539
Residuals	5	253344	50669		

Tab. S3 - Results of ANOVA analysis testing difference Pielou's index (J) at genus level between sampling sites; Df = Degrees of Freedom; Sum Sq = Sum of Squares; Mean Sq = Mean Square; F = F statistic; Pr(>F) = p-value.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Site	2	0.00102	0.0005098	0.819	0.493
Residuals	5	0.003113	0.0006226		

Tab. S4 – Results of Permanova testing differences on community structures among sampling sites Df = Degrees of Freedom; Sum Sq = Sum of Squares; R2 = Variance explained; F = F statistic; Pr(>F) = p-value. Significant p-values are highlighted in bold.

	Df	Sum Sq	Mean Sq	F	Pr(>F)
Site	2	0.1644	0.5448	2.9916	**0.003**
Residual	5	0.13739	0.4552		
Total	7	0.30179	1.0000		

Tab. S5 – Results of Permdisp testing differences on community structures among sampling sites Df = Degrees of Freedom; Sum Sq = Sum of Squares; Mean Sq = Mean Square; F = F statistic; N.Perm = Number of Permutation; Pr(>F) = p-value.

	Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
Groups	2	0.004092	0.0020	1.3307	9999	0.388
Residuals	5	0.007688	0.0015			

Tab. S6 – Results of anova.cca analysis testing the dbrda() model; Df = Degrees of Freedom; Sum Sq = Sum of Squares; F = F statistic; Pr(>F) = p-value. Significant p-values are highlighted in bold.

	Df	Sum Sq	F	Pr(>F)
Model	2	0.1644	2.9916	**0.0033**
Residual	5	0.13739		

Tab. S7 – Results of anova.cca analysis testing the dbrda() axis of the model; Df = Degrees of Freedom; Sum Sq = Sum of Squares; F = F statistic; Pr(>F) = p-value. Significant p-values are highlighted in bold.

	Df	Sum Sq	F	Pr(>F)
dbRDA1	1	0.098227	3.5748	**0.0042**
dbRDA2	1	0.066177	2.8901	**0.0042**
Residual	5	0.137389		

Tab. S8 – Results of anova.cca analysis testing the dbrda() environmental variables of the model; Df = Degrees of Freedom; Sum Sq = Sum of Squares; F = F statistic; Pr(>F) = p-value. Significant p-values are highlighted in bold.

	Df	Sum Sq	F	Pr(>F)
EC sp 25°C	1	0.095298	3.4682	**0.0018**
ORP	1	0.068945	2.5091	**0.0189**
Residual	5	0.137389		

Tab. S9 – References used to identify ecological functions of most abundant genus.

Genus	**Function**	**Reference**	**DOI**
Thiobacillus	Sulfur oxidation	(Haaijer et al., 2007)	https://doi.org/10.1080/01490450701436489
Sulfovorum	Sulfur oxidation	(Sharma et al., 2020)	https://doi.org/10.1186/s12866-020-01923-3
Sulfuricurvum	Sulfur oxidation	(Cron et al., 2019)	https://doi.org/10.3389/fmicb.2019.02710
Desulfatiglans	Sulfate reduction	(Galushko and Kuever, 2019)	https://doi.org/10.1002/9781118960608.gbm01679
Desulfosarcina	Sulfate reduction	(Watanabe et al., 2020)	https://doi.org/10.1002/9781118960608.gbm01020.pub2
Desulforomonas	Sulfate reduction	(Widdel and Pfennig, 1992)	https://doi.org/10.1007/978-1-4757-2191-1_22
Syntrophus	Syntrophic relationships	(Galushko and Kuever, 2019)	https://doi.org/10.1002/9781118960608.gbm01064.pub2

Tab. S10 - Number of reads assigned to each functional group based on FAPROTAX and bibliographic research (Tab. S5).

FUNCTIONAL GROUP	**PA1**	**PA2**	**CER1**	**CER2**	**CER3**	**PIR1**	**PIR2**	**PIR3**
Sulfur Oxidation	7946	14292	42532	46110	17104	2517	8115	25713
Sulfate Reduction	10482	10215	9963	13911	13761	11620	22751	15436
Methanotrophy	1342	1520	1001	1478	1178	467	1064	1889
Methanol_oxidation	2966	2050	624	344	426	168	58	191
Methylotrophy	4308	3570	1625	1822	1604	635	1122	2080
Dark_hydrogen_oxidation	118	64	186	177	115	106	517	253
Nitrogen_fixation	19	19	23	13	15	12	33	39
Fermentation	2301	1078	1237	942	1287	1017	1478	1097
Aerobic_chemoheterotrophy	5233	2295	4227	2766	2411	1508	4561	3575
Aromatic_compound_degradation	208	68	66	87	63	103	120	101
Hydrocarbon_degradation	1342	1520	1001	1478	1178	467	1064	1889
Chemoheterotrophy	11842	6943	7089	5530	5302	3160	7161	6752
TOTAL	**48107**	**43634**	**69574**	**74658**	**44444**	**21780**	**48044**	**59015**

Tab. S11 – Results of Permanova testing differences on metabolic functions among the three sampling sites; Df= Degrees of Freedom; Sum Sq = Sum of Squares; R2 = Variance explained; F = F statistic; Pr(>F) = p-value. Significant p-values are highlighted in bold.

	Df	Sum Sq	R2	F	Pr(>F)
Site	2	0.054371	0.64283	4.4995	**0.0223**
Residual	5	0.030210	0.35717		
Total	7	0.084581	1.0000		

Tab. S12 - Results of SIMPER analyses (cut-off 70%) based on hellinger transformed data, to identify functional groups that mostly contribute to similarity and dissimilarity between sampling sites. Av.Ab, mean abundance; Av.Sim, mean similarity; Sim/SD, similarity/standard deviation; Av.Dis, mean dissimilarity; Diss/SD, dissimilarity/standard deviation Contrib%, relative contribution to single functional group; Cum%, cumulative contribution.

Group PA

Average similarity: 90,18

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Sulfate reduction	0.48	16.23	SD=0!	18.00	18.00
Sulfur oxidation	0.49	14.13	SD=0!	15.67	33.67
Chemoheterotrophy	0.45	13.87	SD=0!	15.38	49.06
Methylotrophy	0.29	9.95	SD=0!	11.03	60.09
Aerobic chemoheterotrophy	0.28	7.98	SD=0!	8.84	68.93
Methanol oxidation	0.23	7.54	SD=0!	8.36	77.29

Group CER

Average similarity: 90.76

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Sulfur oxidation	0.73	27.09	6.01	29.84	29.84
Sulfate reduction	0.46	15.87	13.78	17.49	47.33
Chemoheterotrophy	0.31	11.52	12.75	12.70	60.03
Aerobic chemoheterotrophy	0.22	8.24	10.34	9.08	69.11
Methylotrophy	0.17	6.17	39.44	6.80	75.91

Group PIR

Average similarity: 87.98

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Sulfate reduction	0.64	21.64	5.63	24.60	24.60
Sulfur oxidation	0.47	13.80	8.75	15.69	40.29
Chemoheterotrophy	0.37	13.38	14.59	15.20	55.49
Aerobic chemoheterotrophy	0.27	9.56	26.53	10.86	66.35
Methylotrophy	0.17	6.03	15.38	6.85	73.20

Groups PA & CER

Average dissimilarity = 17.49

Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Sulfur oxidation	0.49	0.73	4.48	1.93	25.62	25.62
Methanol oxidation	0.23	0.09	2.71	6.27	15.52	41.14
Chemoheterotrophy	0.45	0.31	2.52	2.15	14.41	55.55
Methylotrophy	0.29	0.17	2.36	5.88	13.48	69.03
Sulfate reduction	0.48	0.46	1.37	2.83	7.85	76.88

Groups PA & PIR

Average dissimilarity = 15.68

Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Methanol oxidation	0.23	0.06	3.13	6.07	19.97	19.97
Sulfate reduction	0.48	0.64	3.05	1.61	19.43	39.40
Sulfur oxidation	0.49	0.47	2.44	1.37	15.59	54.99
Methylotrophy	0.29	0.17	2.22	7.35	14.14	69.12

Chemoheterotrophy	0.45	0.37	1.43	1.37	9.10	78.22

Groups CER & PIR

Average dissimilarity = 15.08

Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Sulfur oxidation	0.73	0.47	5.25	1.76	34.77	34.77
Sulfate reduction	0.46	0.64	3.88	1.78	25.70	60.47
Chemoheterotrophy	0.31	0.37	1.14	1.55	7.53	68.00
Aerobic chemoheterotrophy	0.22	0.27	0.95	1.31	6.31	74.30

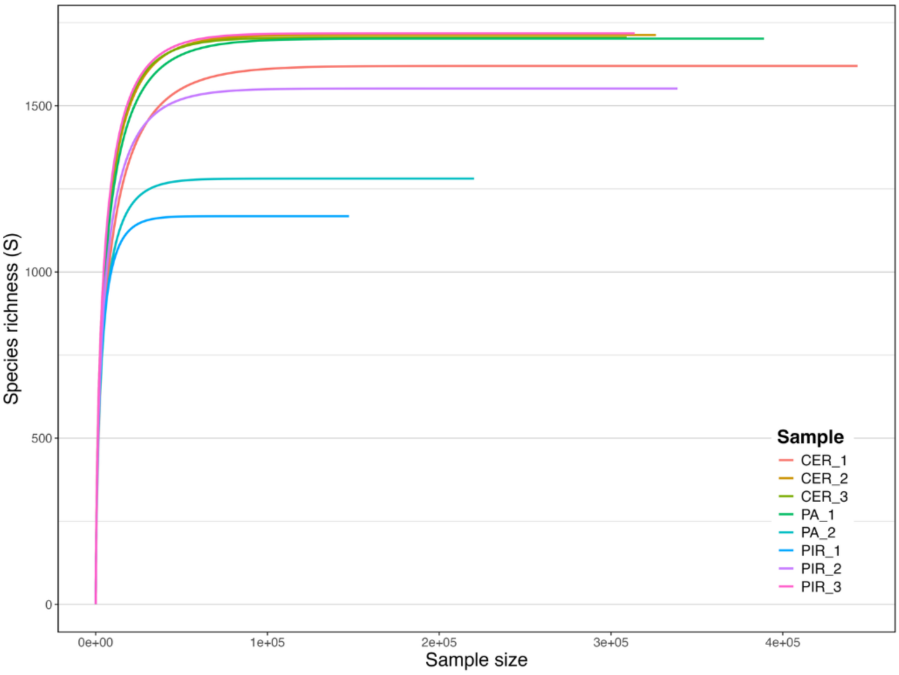

Figure S1 - Species accumulation curve per sample.

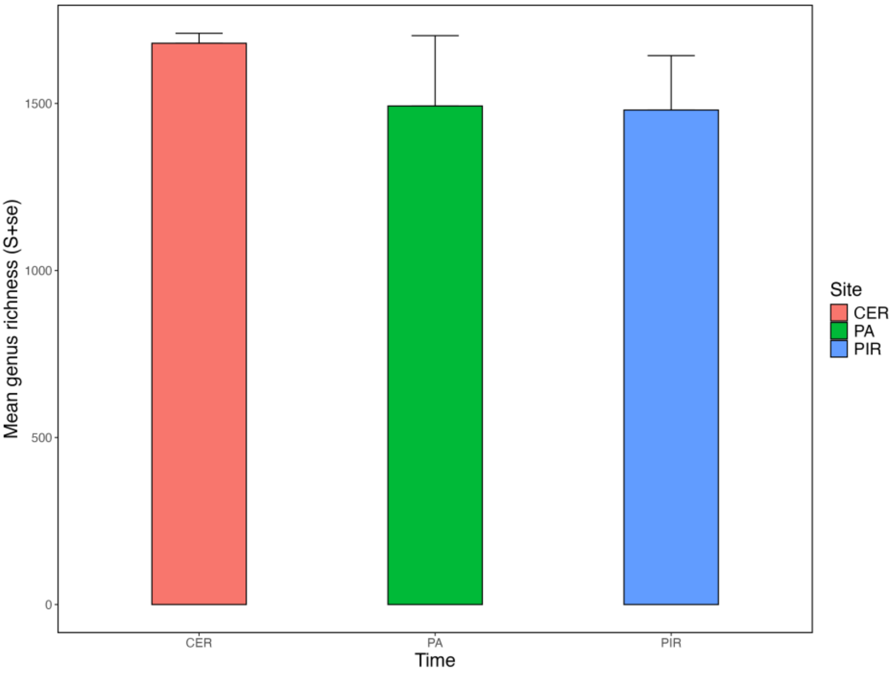

Figure S2 - Mean genus richness (+se) of rarefied data to the minimum number of reads of any sample.

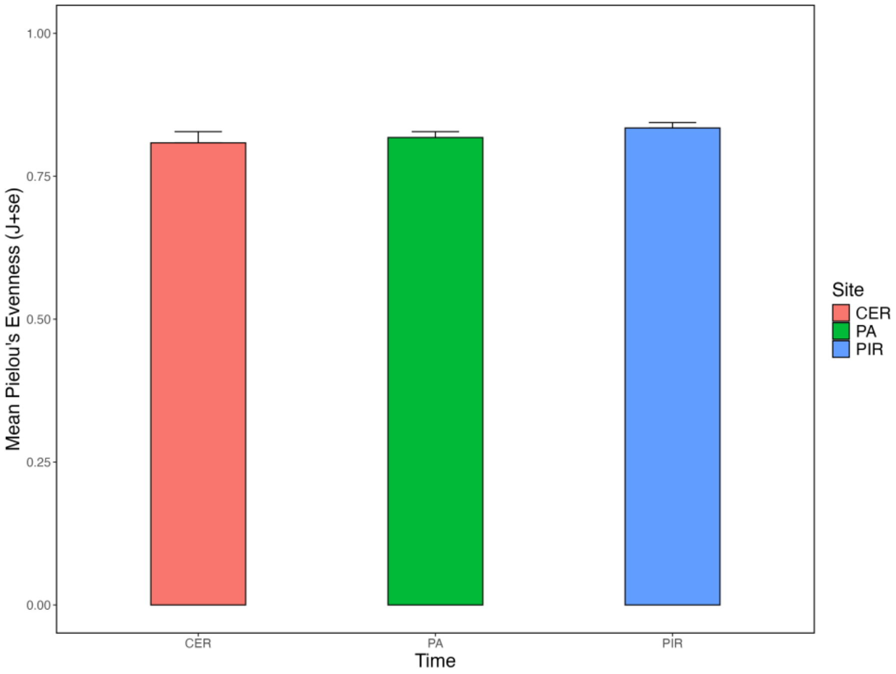

Figure S3 - Mean Pielou's Eveness (+se) of rarefied data to the minimum number of reads of any sample.

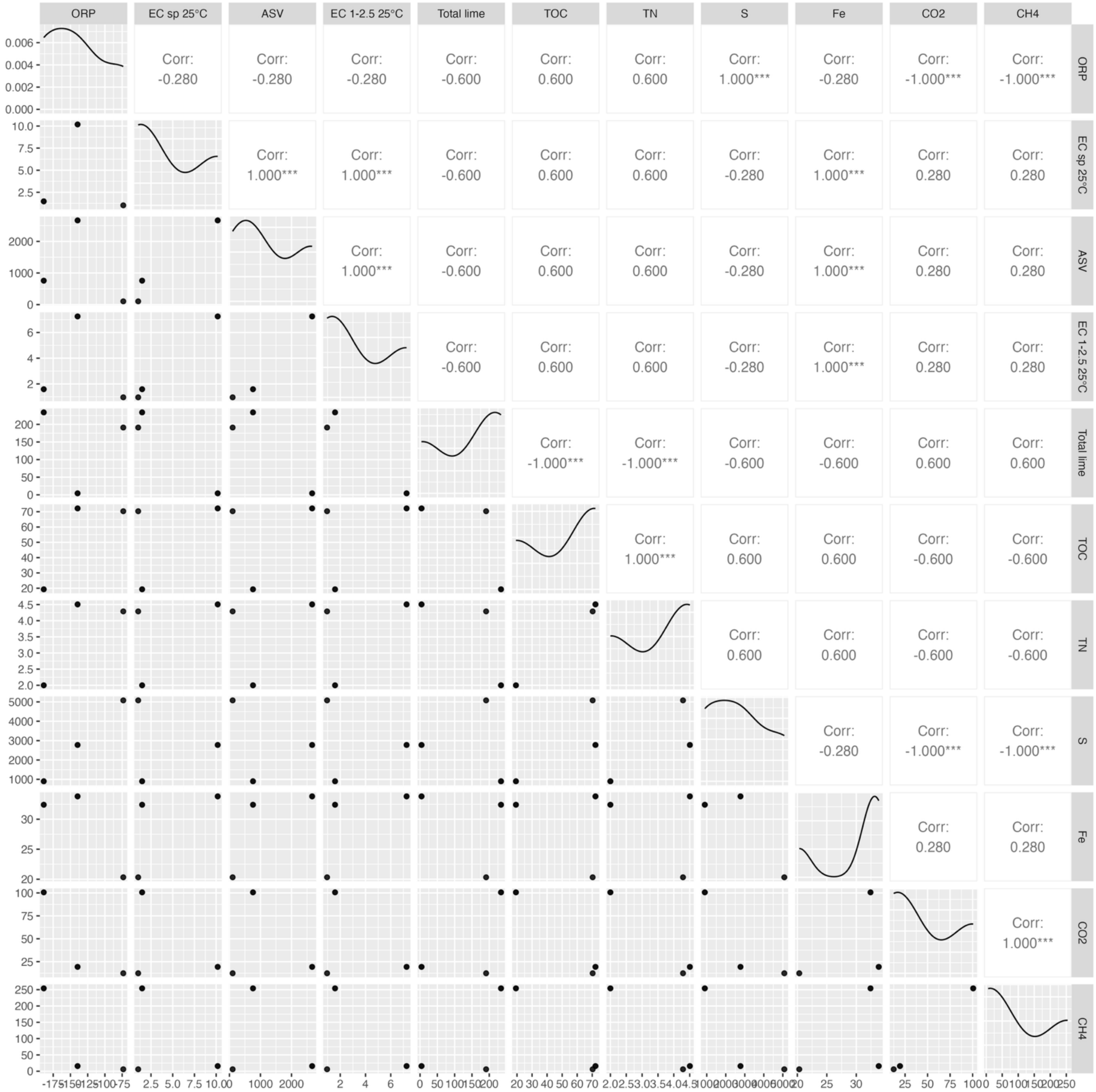

Figure S4 - Spearman correlation matrixes between environmental variables and CO_2 and CH_4 fluxes.

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