APPENDIX S1:

Root endophytes and invasiveness: no difference between native and non-native *Phragmites* in the Great Lakes Region

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Ecosphere

Table S1: Site characteristics and environmental variables

Site	Coordinates	Soil Map Unit	Coastal or Inland	No. Samples	Saturation Levels (# of each)	Soil C (%) mean (range)	Soil N (%) mean (range)	Soil P (mg/kg) mean (range)	Tissue C (%) mean (range)	Tissue N (%) mean (range)	Tissue P (%) mean (range)
Bullard Lake Fen (BL)	42˚38’36”N 83˚42’10”W	Houghton Muck	Inland	12	Unsaturated (6) Saturated (6)	28.37 (18.26 - 38.80)	1.76 (1.03 - 2.73)	16.84 (0.37 - 86.84)	38.00 (12.41 - 43.90)	2.23 (0.78 - 3.24)	0.15 (0.05 - 0.20)
Chelsea Farm (CH)	42˚18’32”N 84˚03’25”W	Houghton Muck	Inland	12	Unsaturated (9) Saturated (3)	10.97 (2.87 - 32.70)	0.60 (0.08 - 2.13)	1.99 (0.34 - 5.64)	41.49 (15.35 - 44.91)	2.32 (0.88 - 3.54)	0.15 (0.03 - 0.24)
Cheboygan Marsh (CM)	45˚39’27”N 84˚28’16”W	Histosols and Aquents	Coastal	6	Saturated (1) High Water (5)	7.17 (1.37 - 12.61)	0.32 (0 - 0.61)	3.73 (0.21 - 11.50)	41.78 (40.20 - 42.90)	2.07 (1.51 - 2.60)	0.11 (0.09 - 0.13)
Cecil Bay (CB)	45˚44’52”N 84˚50’57”W	Stony Lake Beach	Coastal	6	High Water (6)	4.97 (0.88 - 16.54)	0.23 (0 - 0.85)	1.60 (0.70 - 4.35)	42.39 (41.03 - 44.00)	2.01 (1.66 - 2.56)	0.11 (0.09 - 0.15)
Sturgeon Bay (SB)	45˚42’30”N 84˚56’46”W	Sandy Lake Beach	Coastal	2	Saturated (2)	1.32 (0.79 - 1.85)	0.05 (0.02 - 0.07)	1.74 (1.47 - 2.01)	42.79 (42.59 - 43.00)	1.37 (1.30 - 1.44)	0.07 (0.06 - 0.07)
Point Le Barb (PLB)	45˚50’51”N 84˚44’28”W	Histosols and Aquents	Coastal	2	Saturated (1) High Water (1)	8.35 (5.15 - 11.56)	0.38 (0.20 - 0.55)	1.29 (1.09 - 1.50)	41.78 (40.45 - 43.12)	2.05 (1.70 - 2.40)	0.12 (0.11 - 0.13)
Pointe aux Chenes (Rt2)	45˚54’46”N 84˚52’20”W	Leafriver mucky peat	Coastal	6	High Water (6)	0.86 (0.46 - 1.74)	0.02 (0 - 0.07)	2.99 (1.16 - 6.66)	43.57 (42.95 - 44.14)	1.69 (0.69 - 2.24)	0.08 (0.03 - 0.12)
Castle Rock (CR)	45˚54’39”N 84˚44’18”W	Eastport-Leafriver complex	Inland	6	Saturated (6)	12.58 (3.68 - 23.41)	0.63 (0.16 - 1.29)	12.64 (0.07 - 45.83)	32.57 (9.88 - 43.59)	1.59 (0.51 - 2.46)	0.07 (0.01 - 0.12)

Table S2: PCR Conditions and Primer Sequences

Primer Set	Primer	Primer Sequence	Barcode Length	PCR Mastermix	PCR Conditions	Reference
Fungi	ITS1F	5’-CTTGGTCATTTAGAGGAAGTAA-3’	16 bp	2.5 uL 10x Buffer* 0.5 uL BSA 0.5 uL 20uM dNTPs 0.625 uL 20uM ITS1F 0.625 uL 20uM ITS4 0.5 uL Taq* 2 uL Template DNA** 17.75 uL H2O	Initial denaturation: 94℃ for 2 min, 25 cycles, denaturation: 94℃ for 30 s, annealing: 55℃ for 30 s, extension: 72℃ for 45 s (2 min final extension)	White et al. 1990, Gardes and Bruns 1993
	ITS4	5’-TCCTCCGCTTATTGATATGC-3’	16 bp			
Bacteria	27F	5’-AGAGTTTGATCMTGGCTCAG-3’	16 bp	2.5 uL 10x Buffer* 0.5 uL BSA 0.5 uL 20uM dNTPs 0.5 uL 20uM 27F 0.5 uL 20uM 519R 0.5 uL Taq* 2 uL Template DNA** 18 uL H2O	Initial denaturation: 94℃ for 5 min, 25 cycles, denaturation: 94℃ for 30 s, annealing: 55℃ for 60 s, extension: 72℃ for 90 s (10 min final extension)	Lane 1991
	519R	5’-GWATTACCGCGGCKGCTG’3’	16 bp			
Oomycetes (First round)	5.8 SR	5’-TCGATGAAGAACGCAGCG -3’	-	2.5 uL 10x Buffer* 0.5 uL BSA 0.5 uL 20uM dNTPs 0.625 uL 20uM 5.8 SR 0.625 uL 20uM LR7 0.5 uL Taq* 2 uL Template DNA** 17.75 uL H2O	Initial denaturation: 94℃ for 5 min, 30 cycles, denaturation: 94℃ for 30 s, annealing: 47℃ for 90 s, extension: 72℃ for 60 s (10 min final extension)	Vilgalys and Hester 1990
	LR7	5’-TACTACCACCAAGATCT-3’	-			
Oomycetes (Second Round)	Oom1f	5’-GTGCGAGACCGATAGCGAACA-3’	16 bp	2.5 uL 10x Buffer* 0.5 uL 20uM dNTPs 0.625 uL 20uM Oom1f 0.625 uL 20uM Oom1r 0.5 uL Taq* 1 uL Template DNA** 19.25 uL H2O	Initial denaturation: 94℃ for 5 min, 30 cycles, denaturation: 94℃ for 30 s, annealing: 58.4℃ for 30 s, extension: 72℃ for 30 s (10 min final extension)	Arcate et al. 2006
	Oom1r	5’-TCAAAGTCCCGAACAGCAACAA-3’	16 bp			

*Roche Expand High Fidelity PCR System
**DNA was diluted to 5-10 ng per reaction

Table S3: comparisons of environmental characteristics of patches by lineage.

	Native mean	Non-native mean	Coefficient	P-value
Soil N	0.52 %	0.63 %	T = 0.492	0.623
Soil P	3.45 mg / kg	8.98 mg / kg	T = 1.089	0.29
Soil Saturation	--	--	**χ^2 = 11.99**	**0.005**

Table S4: Results of 2-way ANOVA (Site x Lineage) for selected fungal response variables. Alpha diversity, community composition, phylum relative abundance, and genus relative abundance included. Bold indicates significance at the $\alpha<0.05$ level. Italics indicates significance at the $\alpha<0.1$ level.

		Lineage		**Site**		**Site X Lineage**	
	df	**F**	**P**	**F**	**P**	**F**	**P**
Colonization	35	**24.57**	**<0.001**	**6.52**	**<0.001**	1.78	0.122
Shannon	31	0.02	0.894	1.58	0.178	0.80	0.596
Chao	31	0.14	0.708	*2.00*	*0.09*	0.76	0.621
Sobs	31	0.08	0.785	1.49	0.208	1.00	0.450
PCoA Axis 1	31	0.68	0.416	**5.08**	**<0.001**	1.14	0.362
PCoA Axis 2	31	0.18	0.678	**2.56**	**0.033**	0.59	0.761
Asco RA	31	0.34	0.566	*2.16*	*0.066*	1.38	0.250
Basio RA	31	0.19	0.669	1.27	0.295	1.03	0.430
Unclass RA	31	0.00	0.945	*1.95*	*0.09*	0.36	0.919
Zygomycota RA	31	1.13	0.296	0.66	0.704	0.62	0.735
Rozellomycota RA	31	0.00	1.000	0.53	0.801	1.25	0.306
Glomero RA	31	0.84	0.366	1.35	0.262	**2.96**	**0.017**
Gib RA	31	0.58	0.452	**2.64**	**0.029**	0.61	0.746
Micro RA	31	0.03	0.862	0.86	0.548	0.88	0.536
Tetracladium RA	31	0.23	0.638	1.42	0.234	1.25	0.304
Stagonospora RA	31	0.84	0.368	**3.55**	**0.006**	0.38	0.907
Cadophora RA	31	0.45	0.509	0.56	0.781	0.78	0.607
Path RA	31	1.20	0.281	**3.45**	**0.008**	2.10	0.073
Sap RA	31	1.36	0.253	**5.00**	**<0.001**	**2.48**	**0.038**
Path-Sym RA	31	0.13	0.717	0.90	0.516	1.11	0.383
Symbiotroph RA	31	0.02	0.889	1.09	0.391	*2.27*	*0.055*

Table S5: Results of ANCOVA for selected fungal response variables. Variables with a significant site effect in ST3 were included for ANCOVA analysis with environmental variables. Bold indicates significance at the $\alpha<0.05$ level. Italics indicates significance at the $\alpha<0.1$ level.

		r2			Lineage		Saturation		Soil P		Soil N		Sat:Lin	
	Residual df	Multiple r2	Adjusted r2	P	F	P	F	P	F	P	F	P	F	P
Colonization	31	**0.7073**	**0.6601**	**<0.001**	**19.97**	**<0.001**	**23.47**	**<0.001**	29.67	<0.001	1.24	0.274	0.58	0.454
Shannon	31	0.1347	-0.0048	0.454	0.16	0.685	**4.26**	**0.047**	0.07	0.790	0.10	0.756	0.22	0.638
Chao	31	0.2068	0.0789	0.185	2.09	0.158	**4.30**	**0.046**	0.80	0.378	0.22	0.646	0.67	0.418
Sobs	31	0.1319	-0.0081	0.468	1.24	0.275	2.52	0.123	0.42	0.524	0.11	0.741	0.43	0.517
PCoA Axis 1	31	**0.4133**	**0.3186**	**0.004**	0.04	0.840	**21.10**	**<0.001**	0.25	0.620	0.44	0.511	0.01	0.940
PCoA Axis 2	31	0.1315	-0.0085	0.470	2.16	0.152	0.01	0.935	0.10	0.753	2.13	0.155	0.29	0.591
Asco RA	31	0.1477	0.0103	0.393	0.35	0.558	2.72	0.109	1.72	0.200	0.00	0.965	0.58	0.452
Gib RA	31	0.2101	0.0827	0.177	0.91	0.347	**6.96**	**0.013**	0.31	0.583	0.05	0.823	0.02	0.901
Stag RA	31	0.2119	0.0848	0.172	0.06	0.804	**6.83**	**0.014**	0.04	0.837	0.37	0.547	1.02	0.321
Path RA	31	*0.2053`*	*0.1617*	*0.061*	0.53	0.471	**9.13**	**0.005**	1.76	0.194	0.51	0.480	0.01	0.944
Sap RA	31	**0.3817**	**0.2819**	**0.008**	0.74	0.396	**14.63**	**<0.001**	2.02	0.165	1.56	0.221	0.19	0.668

Table S6: Results of 2-way ANOVA (Site x Lineage) for selected bacterial response variables. Alpha diversity, community composition, phylum relative abundance, and genus relative abundance included. Bold indicates significance at the α<0.05 level. Italics indicates significance at the α<0.1 level.

		Lineage		Site		Site X Lineage	
	df	F	P	F	P	F	P
Shannon	33	1.83	0.185	**12.98**	**<0.001**	**2.32**	**0.046**
Chao	33	1.03	0.318	**2.84**	**0.020**	1.62	0.163
Sobs	33	1.72	0.198	**10.30**	**<0.001**	*2.20*	*0.060*
PCoA Axis 1	33	0.01	0.913	**44.76**	**<0.001**	1.19	0.338
PCoA Axis 2	33	**4.29**	**0.046**	1.55	0.185	1.32	0.274
Proteo RA	33	**5.86**	**0.021**	1.36	0.257	1.37	0.250
Firm RA	33	1.44	0.238	1.16	0.352	1.70	0.143
Bacteroidetes RA	33	**8.05**	**0.008**	**9.40**	**<0.001**	1.83	0.114
Actinobacteria RA	33	0.26	0.611	**2.36**	**0.045**	**4.46**	**0.001**
Pseudomonas RA	33	**7.16**	**0.012**	**5.55**	**<0.001**	**2.84**	**0.020**
Flavobacterium RA	33	0.95	0.337	**5.66**	**<0.001**	1.78	0.125
Janthinobacterium RA	33	0.30	0.590	*2.30*	*0.050*	0.35	0.924
Rhizobium RA	33	**5.83**	**0.021**	**7.68**	**<0.001**	1.60	0.70
Duganella RA	33	*3.18*	*0.084*	1.79	0.123	0.15	0.992
Trichococcus RA	33	0.91	0.346	1,72	0.139	1.69	0.147
Aeromonas RA	33	1.73	0.197	*1.99*	*0.086*	1.38	0.245
Aerobe RA	33	0.01	0.917	**3.88**	**0.003**	**3.85**	**0.004**
Facultative Anaerobe RA	33	0.41	0.528	**4.88**	**<0.001**	1.78	0.124
Anaerobe RA	33	0.226	0.616	1.88	0.105	**2.60**	**0.030**

Table S7: Results of ANCOVA for selected bacterial response variables. Variables with a significant site effect in ST5 were included for ANCOVA analysis with environmental variables. Bold indicates significance at the $\alpha<0.05$ level. Italics indicates significance at the $\alpha<0.1$ level.

		r2			Lineage		Saturation		Soil P		Soil N		Sat:Lin	
	Residual df	Multiple r2	Adjusted r2	P	F	P	F	P	F	P	F	P	F	P
Shannon	32	0.189	0.0623	0.220	0.04	0.841	2.57	0.119	1.15	0.290	*3.48*	*0.071*	0.21	0.650
Chao	32	0.1677	0.0376	0.293	2.33	0.137	0.28	0.601	2.19	0.149	1.63	0.211	0.02	0.877
Sobs	32	0.164	0.0334	0.307	0.04	0.840	1.43	0.240	2.03	0.164	2.73	0.108	0.05	0.830
PCoA Axis 1	32	**0.4889**	**0.4091**	**<0.001**	0.01	0.910	**26.37**	**<0.001**	0.53	0.471	*3.22*	*0.082*	0.48	0.494
PCoA Axis 2	32	**0.3694**	**0.2709**	**0.009**	**4.92**	**0.034**	**4.62**	**0.039**	0.32	0.574	**7.47**	**0.010**	1.41	0.244
Proteo RA	32	0.1479	0.0147	0.374	*3.89*	*0.057*	0.36	0.555	0.13	0.722	1.16	0.290	0.02	0.891
Firm RA	32	0.1334	-0.002	0.442	0.85	0.363	0.05	0.823	0.34	0.565	*3.09*	*0.088*	0.60	0.444
Bacteroidetes RA	32	0.1312	-0.005	0.453	*2.99*	*0.094*	1.39	0.247	0.05	0.822	0.26	0.615	0.14	0.706
Actinobacteria RA	32	0.122	-0.015	0.500	0.00	0.987	0.04	0.834	0.03	0.856	0.23	0.631	*4.13*	*0.050*
Pseudomonas RA	32	0.1561	0.0242	0.339	1.74	0.197	*3.48*	*0.071*	0.33	0.570	0.37	0.547	0.00	0.959
Flavobacterium RA	32	0.114	-0.0245	0.542	1.67	0.206	1.90	0.177	0.00	0.957	0.01	0.920	0.53	0.472
Janthinobacterium RA	32	*0.2577*	*0.1418*	*0.076*	0.08	0.773	*3.66*	*0.065*	2.85	0.101	*4.01*	*0.054*	0.50	0.483
Rhizobium RA	32	**0.319**	**0.2126**	**0.025**	2.42	0.130	**9.58**	**0.004**	0.21	0.652	0.46	0.504	2.33	0.137
Duganella RA	32	0.1156	-0.0226	0.534	*3.12*	*0.087*	0.11	0.739	0.05	0.831	0.90	0.350	0.00	0.978
Trichococcus RA	32	0.1866	0.0595	0.228	1.28	0.267	0.58	0.453	0.31	0.584	*3.73*	*0.062*	1.45	0.237
Aeromonas RA	32	0.2406	0.122	0.101	2.06	0.161	1.33	0.257	0.37	0.546	**4.57**	**0.040**	1.80	0.189
Aerobe RA	32	0.1733	0.0413	0.272	0.12	0.729	2.37	0.133	0.37	0.549	*3.83*	*0.059*	0.02	0.884
Facultative Anaerobe RA	32	0.1324	-0.0032	0.447	0.01	0.943	*3.34*	*0.077*	0.08	0.781	0.28	0.603	1.18	0.285
Anaerobe RA	32	0.1558	0.0239	0.340	0.27	0.604	0.58	0.453	0.27	0.608	*3.56*	*0.068*	1.23	0.276

Table S8: Results of 2-way ANOVA (Site x Lineage) for selected oomycete response variables. Alpha diversity, community composition, and genus relative abundance included. Bold indicates significance at the α<0.05 level. Italics indicates significance at the α<0.1 level.

		Lineage		Site		Site X Lineage	
	df	F	P	F	P	F	P
Shannon	32	1.97	0.170	0.09	0.997	0.14	0.990
Chao	32	*3.76*	*0.061*	0.18	0.980	0.27	0.944
Sobs	32	**4.22**	**0.048**	0.16	0.985	0.08	0.998
PCoA Axis 1	32	1.44	0.239	1.47	0.219	**2.65**	**0.033**
PCoA Axis 2	32	0.48	0.492	1.03	0.422	0.72	0.637
Pithium RA	32	0.10	0.750	1.79	0.132	0.82	0.563
Uncultured RA	32	0.09	0.764	1.79	0.133	0.82	0.560
Phytopthora RA	32	0.78	0.384	0.69	0.656	1.11	0.376
Lagena RA	32	0.05	0.820	0.51	0.797	0.70	0.650
Saprolegnia RA	32	0.22	0.646	1.38	0.252	0.80	0.580
Albugo RA	32	0.49	0.491	0.80	0.580	0.80	0.580

Table S9: Results of ANCOVA for selected oomycete response variables. Variables with a significant site effect in ST7 were included for ANCOVA analysis with environmental variables. Bold indicates significance at the $\alpha<0.05$ level. Italics indicates significance at the $\alpha<0.1$ level.

			r2		Lineage		Saturation		Soil P		Soil N		Sat:Lin	
	Residual df	Multiple r2	Adjusted r2	P	F	P	F	P	F	P	F	P	F	P
Shannon	29	0.1662	0.0224	0.354	1.84	0.185	0.86	0.363	2.14	0.154	0.41	0.525	0.53	0.474
Chao	29	0.2521	0.1232	0.116	**4.64**	**0.040**	0.16	0.691	**4.57**	**0.041**	0.40	0.532	0.00	0.949
Sobs	29	0.2545	0.126	0.126	**6.24**	**0.018**	0.25	0.621	2.73	0.109	0.68	0.416	0.00	0.946
PCoA Axis 1	29	*0.2902*	*0.1678*	*0.064*	**4.89**	**0.035**	*4.11*	*0.052*	1.05	0.314	0.93	0.343	0.87	0.358
PCoA Axis 2	29	0.1495	0.0028	0.424	0.01	0.938	1.93	0.176	0.20	0.656	2.62	0.116	0.34	0.565

SUPPLEMENTAL FIGURES

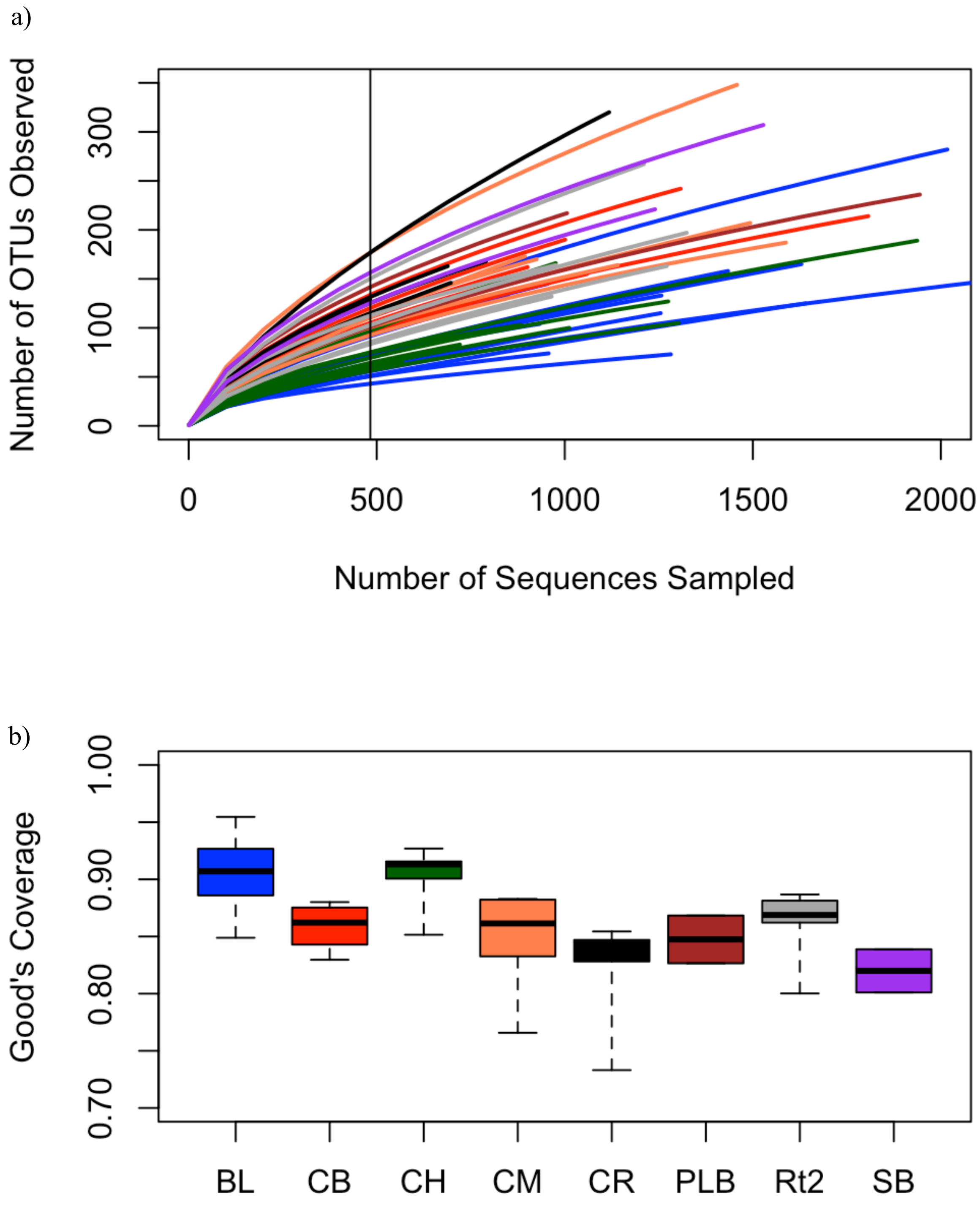
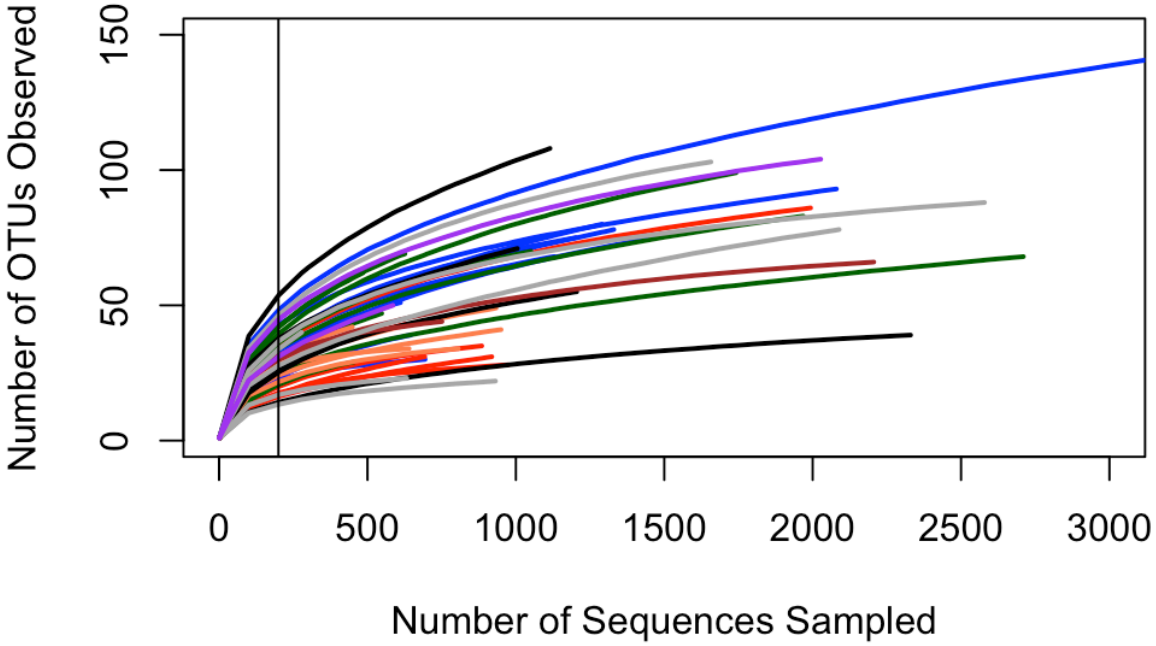


Fig. S1: a) Rarefaction curve and b) Good's Coverage by site for bacteria. Colors represent sites (blue = BL, red = CB, green = CH, coral = CM, black = CR, brown = PLB, gray = Rt2, purple = SB).

a)



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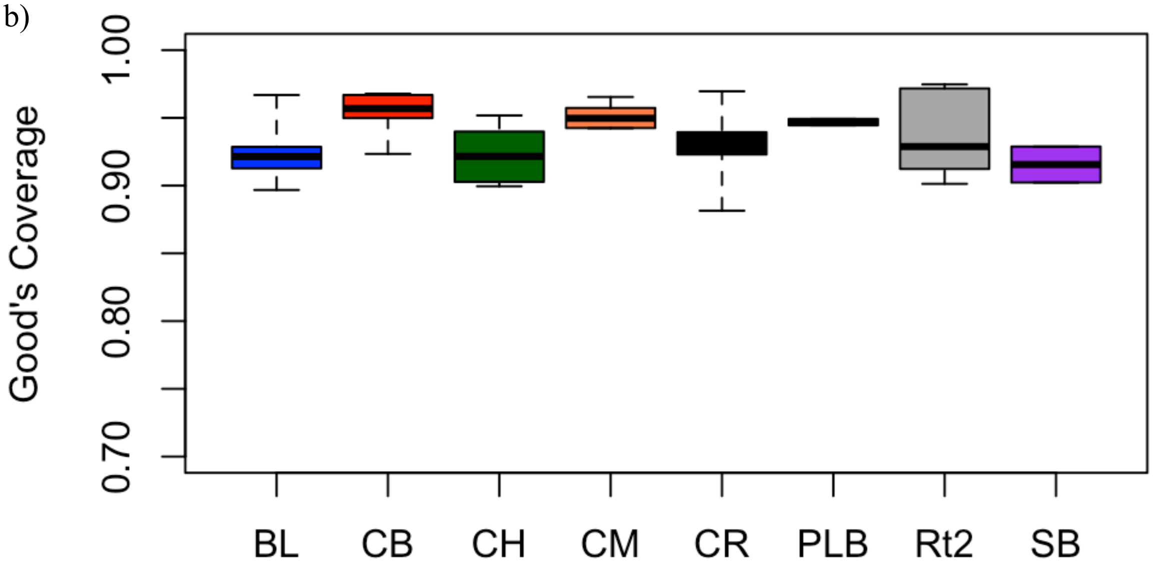
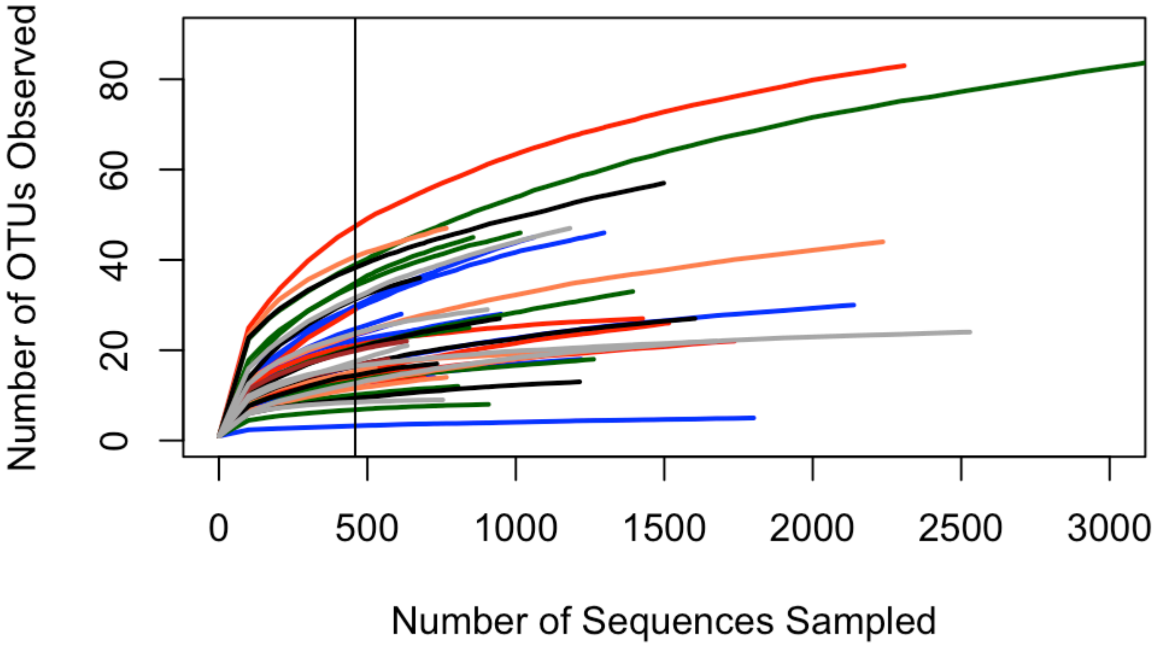


Fig. S2: a) Rarefaction curve and b) Good's Coverage by site for fungi. Colors represent sites (blue = BL, red = CB, green = CH, coral = CM, black = CR, brown = PLB, gray = Rt2, purple = SB).

a)



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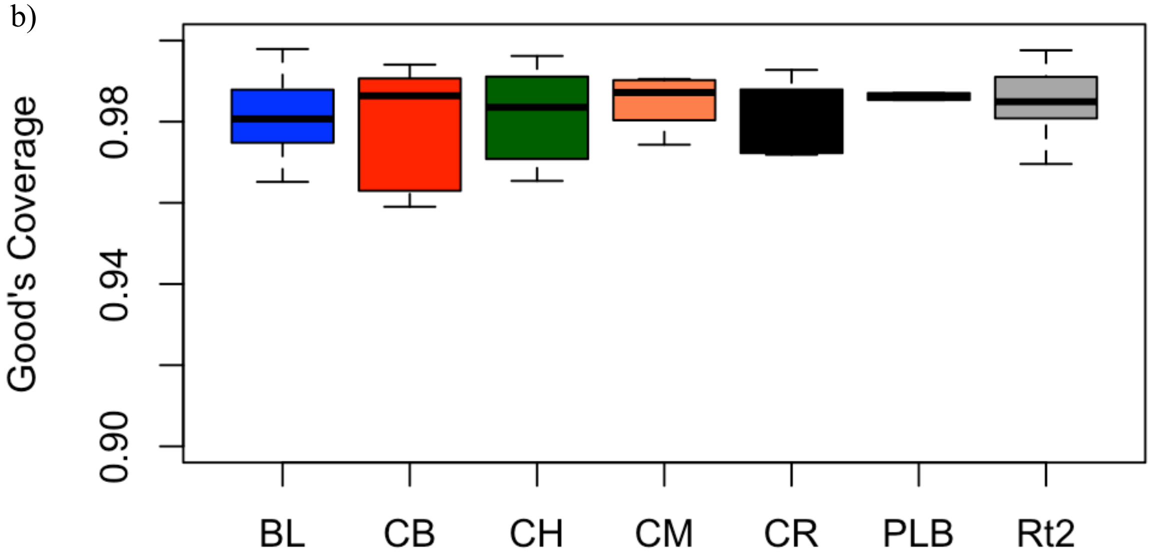


Fig. S3: a) Rarefaction curve and b) Good's Coverage by site for oomycetes. Colors represent sites (blue = BL, red = CB, green = CH, coral = CM, black = CR, brown = PLB, gray = Rt2, purple = SB).

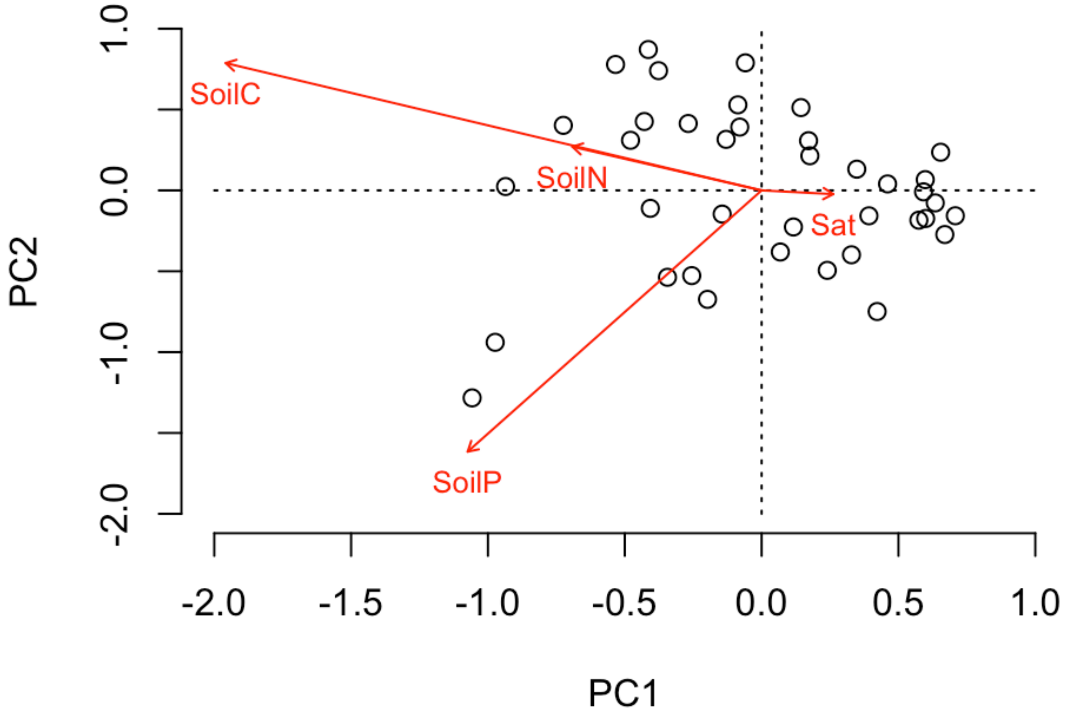


Fig. S4: Principle component analysis for environmental variables at all sampling sites Tissue nutrients not shown because there was low variability by sample (Supplementary Table 1) .

Fig. S5: Linear regressions of environmental variable against all Fungal response variables included in the ANCOVA analysis. Regression lines indicate significant relationship at the α<0.1 level.

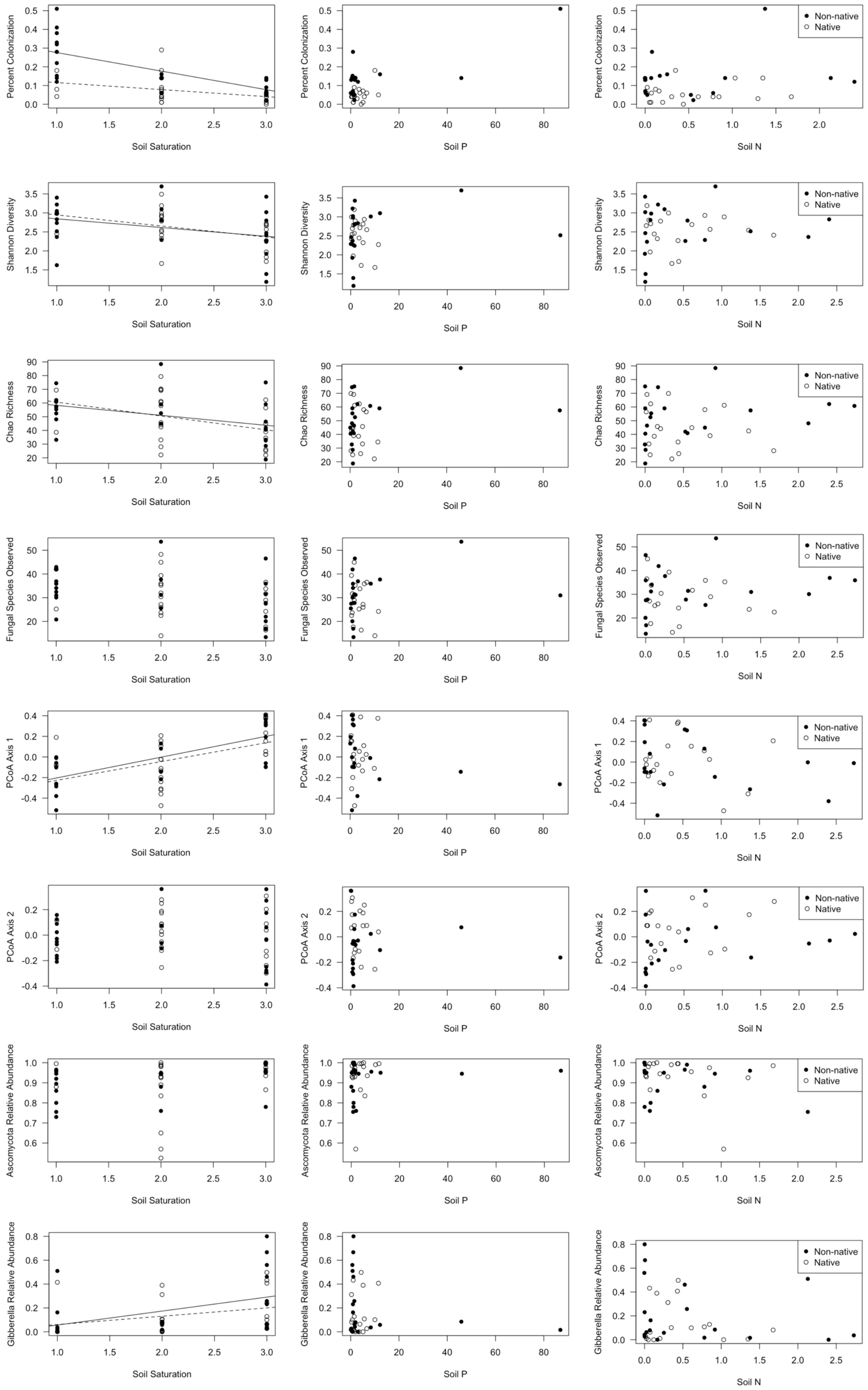


Fig. S5 (cont): Linear regressions of environmental variable against all Fungal response variables included in the ANCOVA analysis. Regression lines indicate significant relationship at the α<0.1 level.

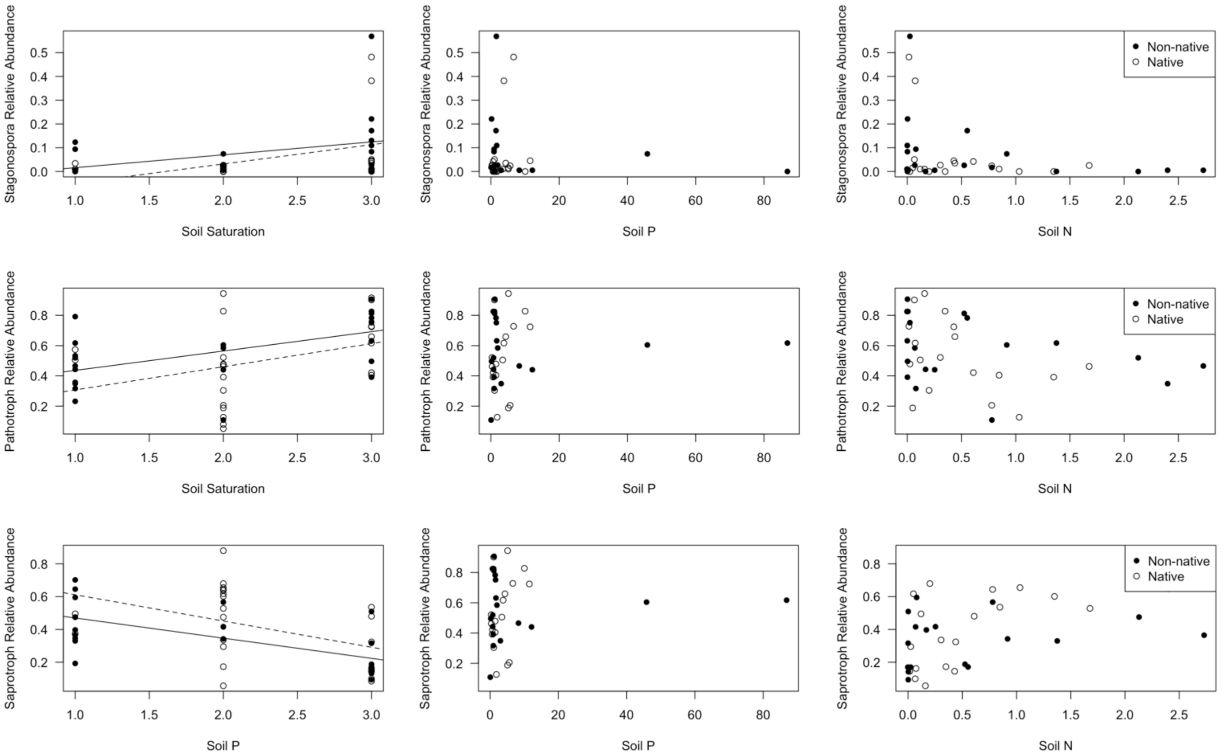


Fig. S6: Linear regressions of environmental variable against all bacterial response variables included in the ANCOVA analysis. Regression lines indicate significant relationship at the α<0.1 level.

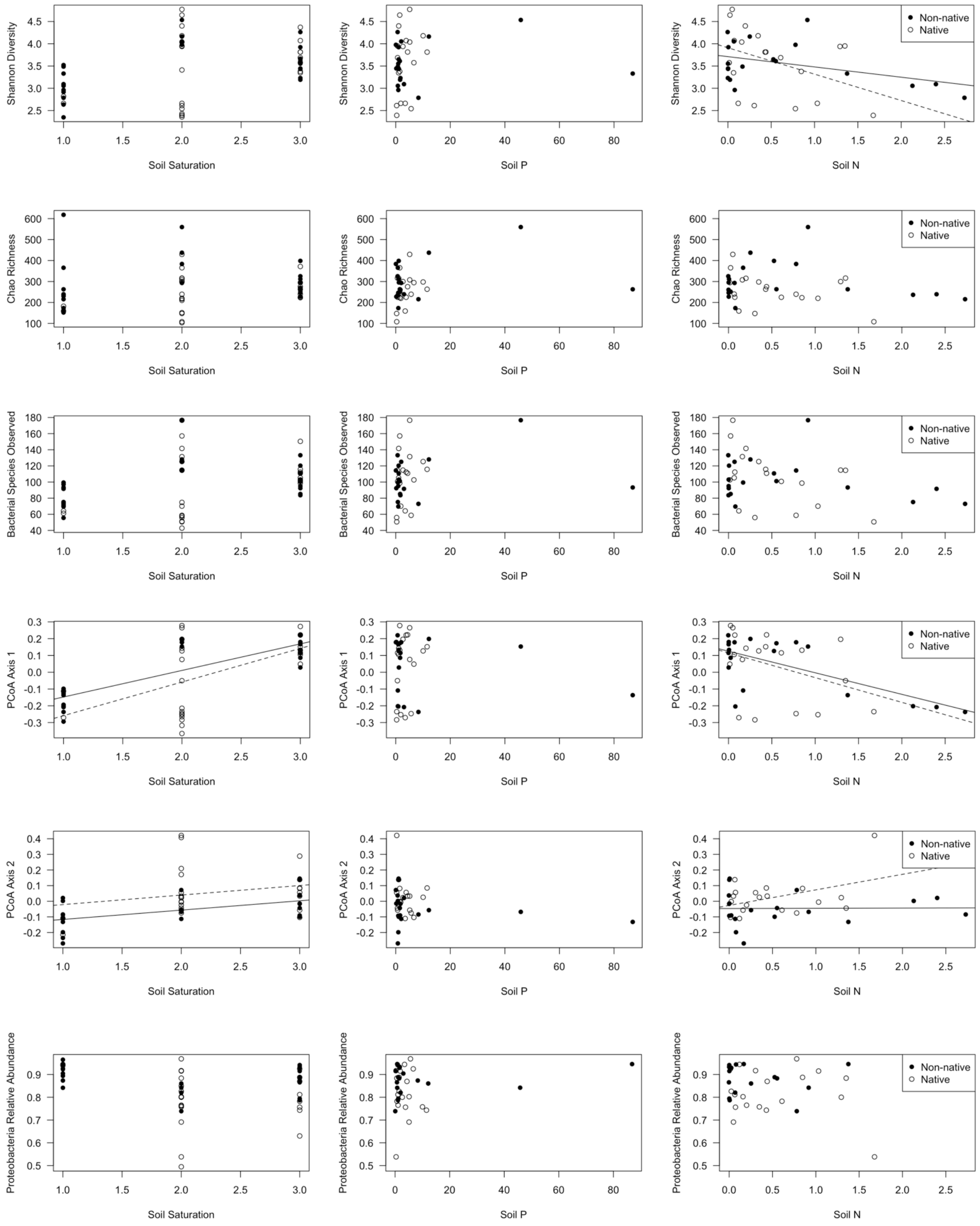


Fig. S6 (cont): Linear regressions of environmental variable against all bacterial response variables included in the ANCOVA analysis. Regression lines indicate significant relationship at the α<0.1 level.

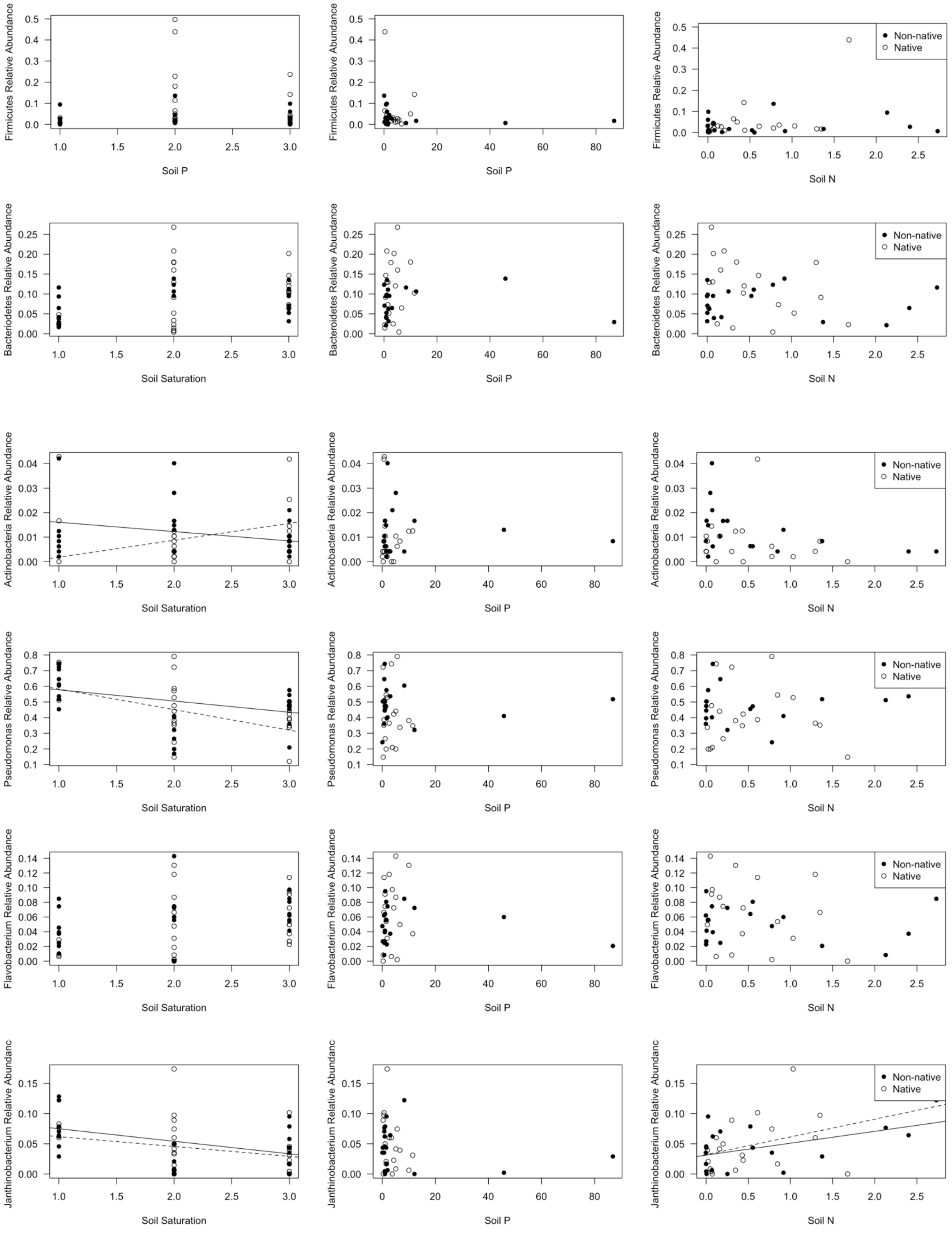


Fig. S6 (cont): Linear regressions of environmental variable against all bacterial response variables included in the ANCOVA analysis. Regression lines indicate significant relationship at the α<0.1 level.

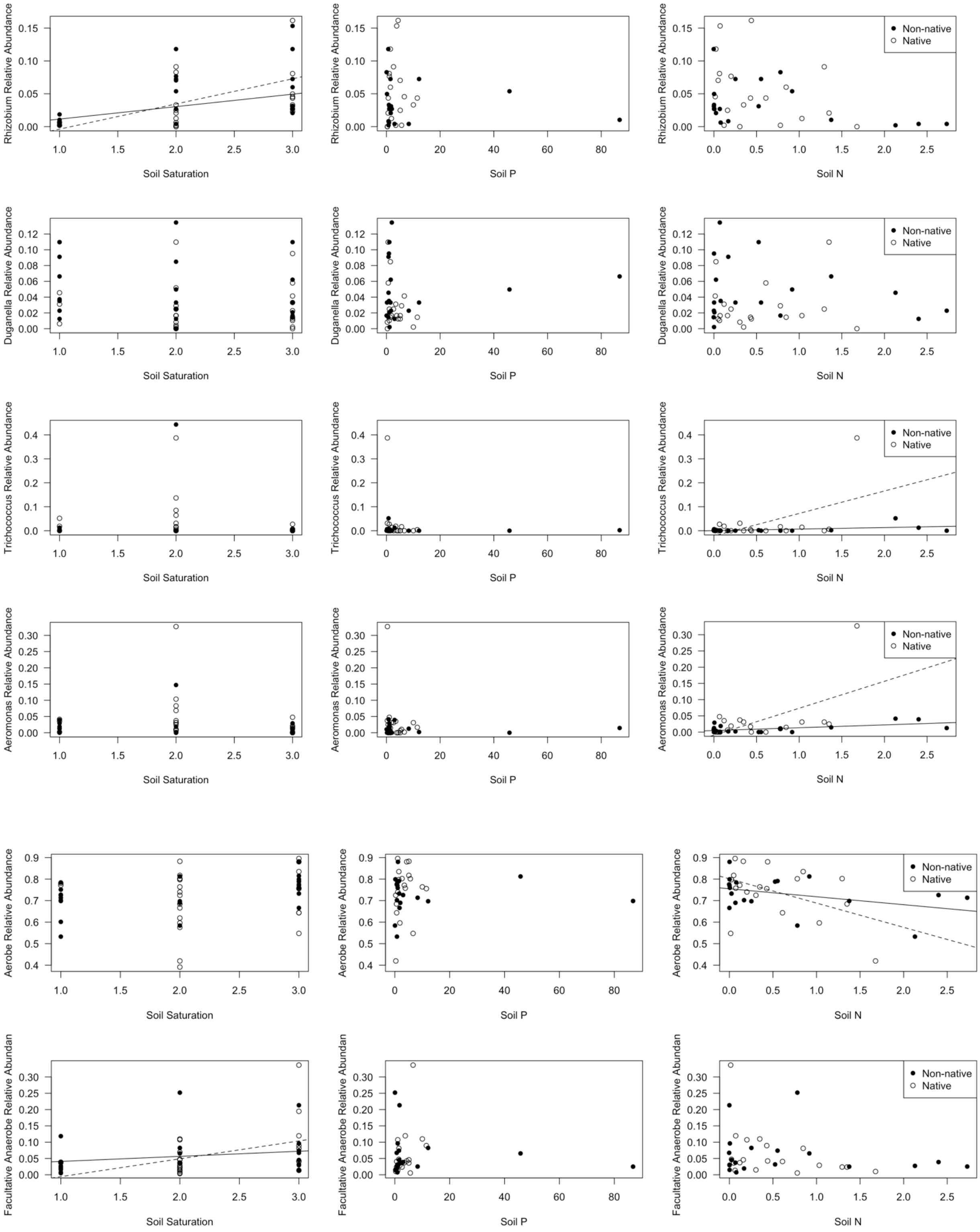


Fig. S6 (cont): Linear regressions of environmental variable against all bacterial response variables included in the ANCOVA analysis. Regression lines indicate significant relationship at the α<0.1 level.

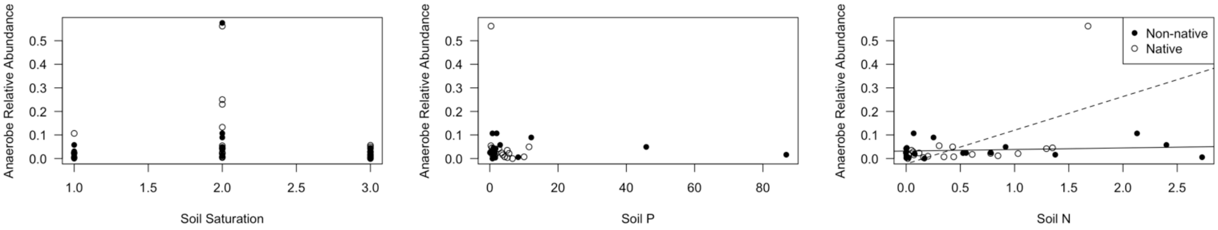


Fig. S7: Linear regressions of environmental variable against all oomycete response variables included in the ANCOVA analysis. Regression lines indicate significant relationship at the α<0.1 level.

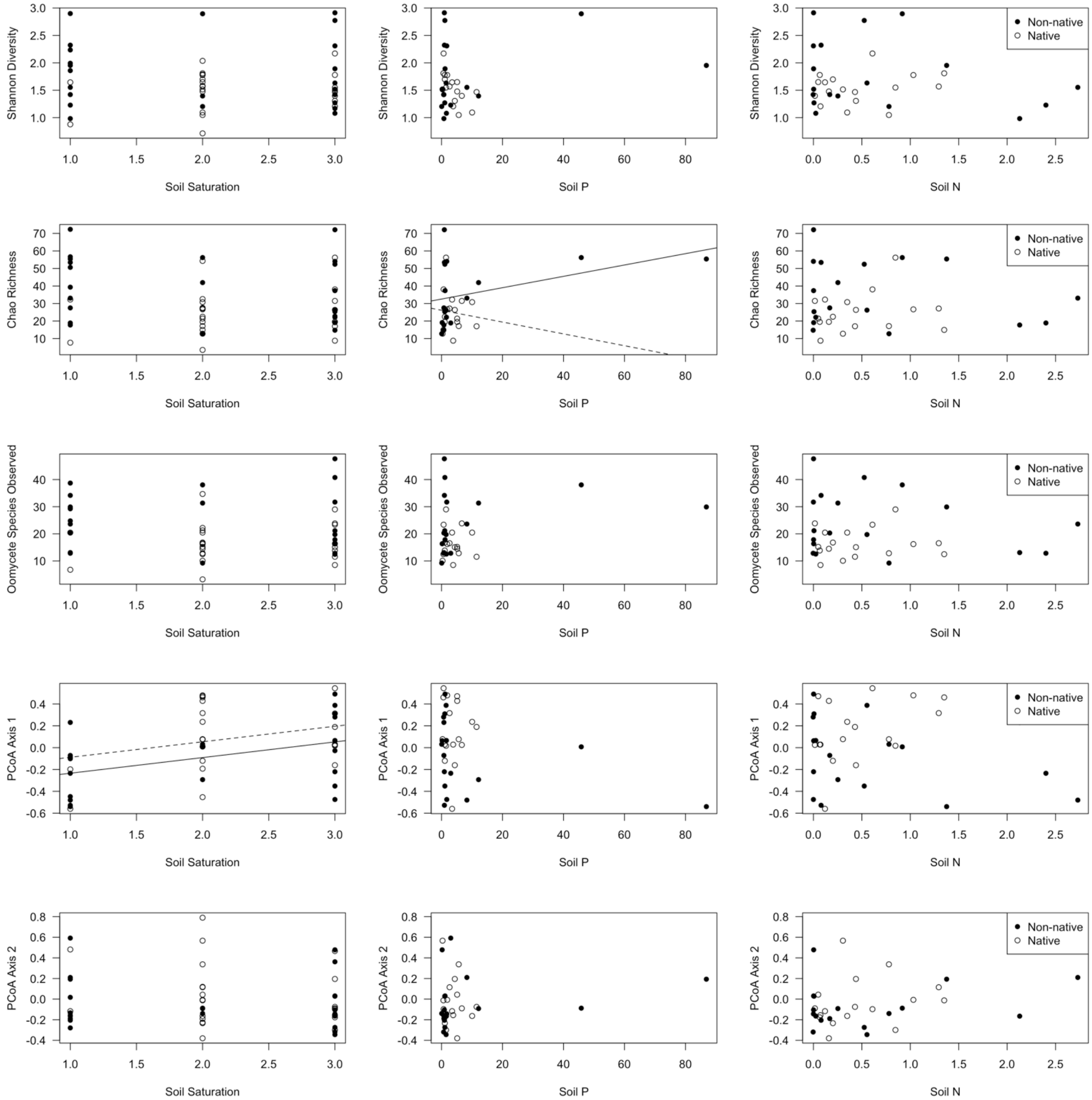
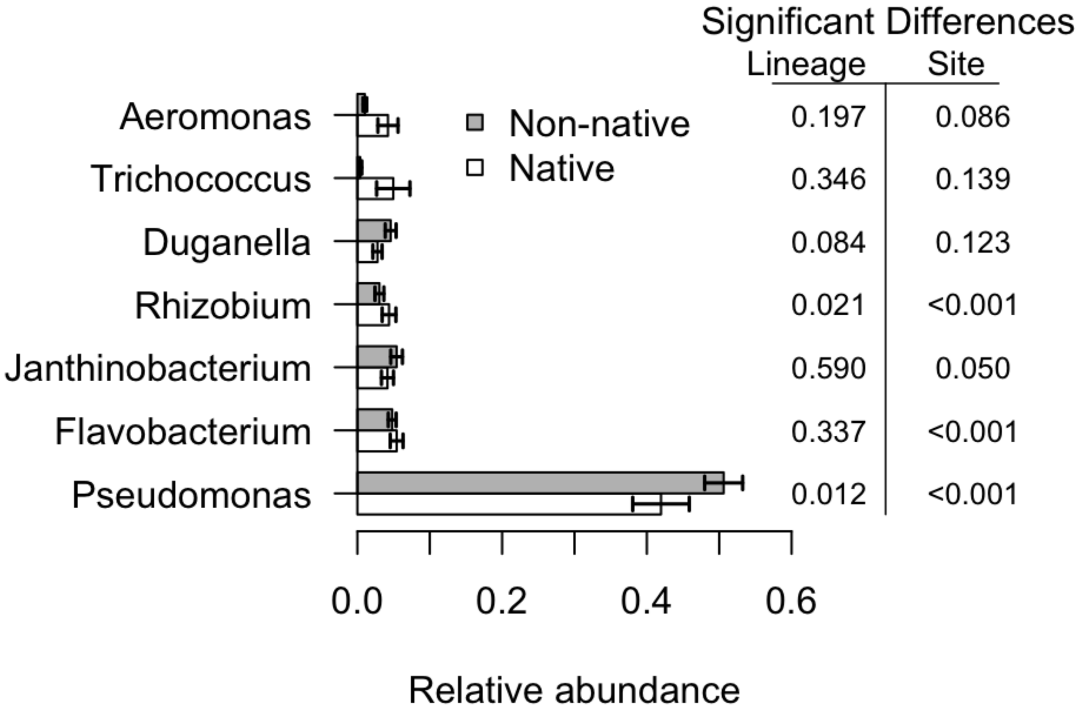


Fig. S8: Relative abundance of bacterial genera by lineage. Significance tested by ANOVA



Significant Differences

	Lineage	Site
Aeromonas	0.197	0.086
Trichococcus	0.346	0.139
Duganella	0.084	0.123
Rhizobium	0.021	<0.001
Janthinobacterium	0.590	0.050
Flavobacterium	0.337	<0.001
Pseudomonas	0.012	<0.001

Fig.S9: Relative abundance of fungal phyla by plant lineage. Significance determined by ANOVA

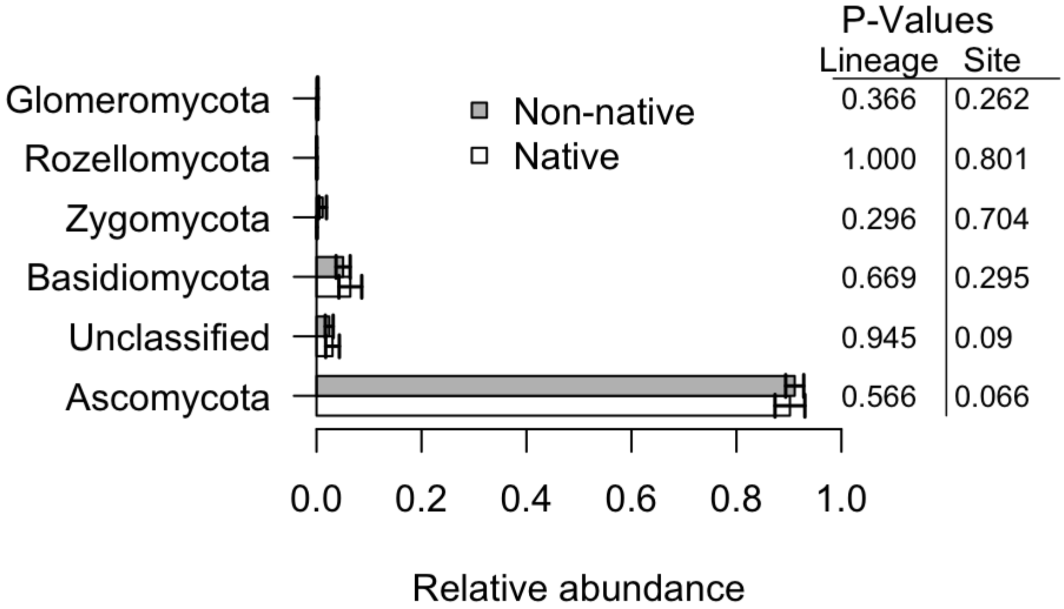


Fig.S10: Relative abundance of Fungal genera by plant lineage. Significance determined by ANOVA

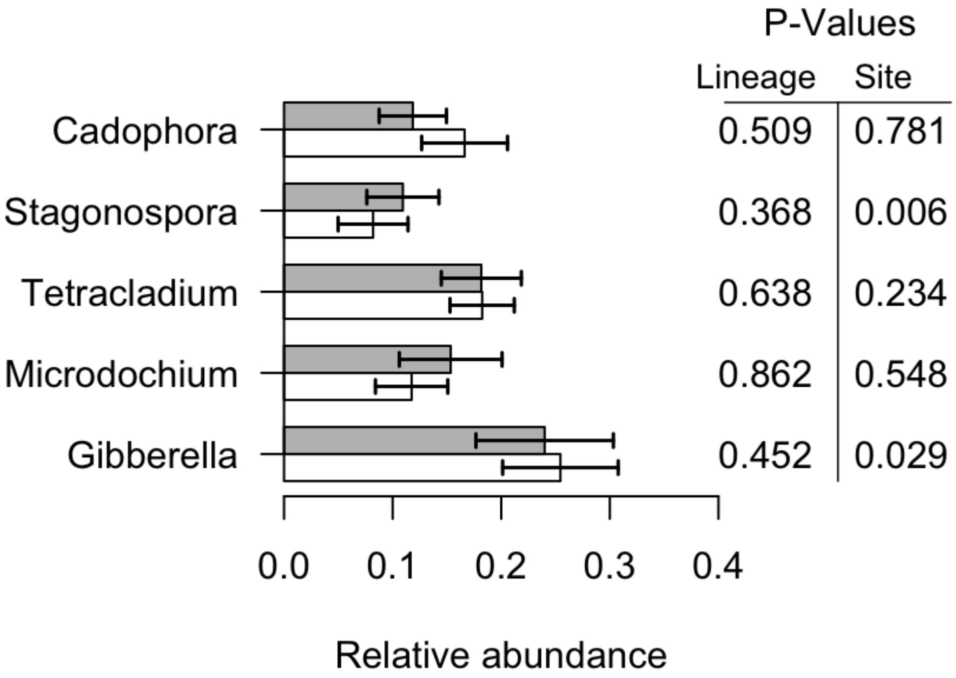
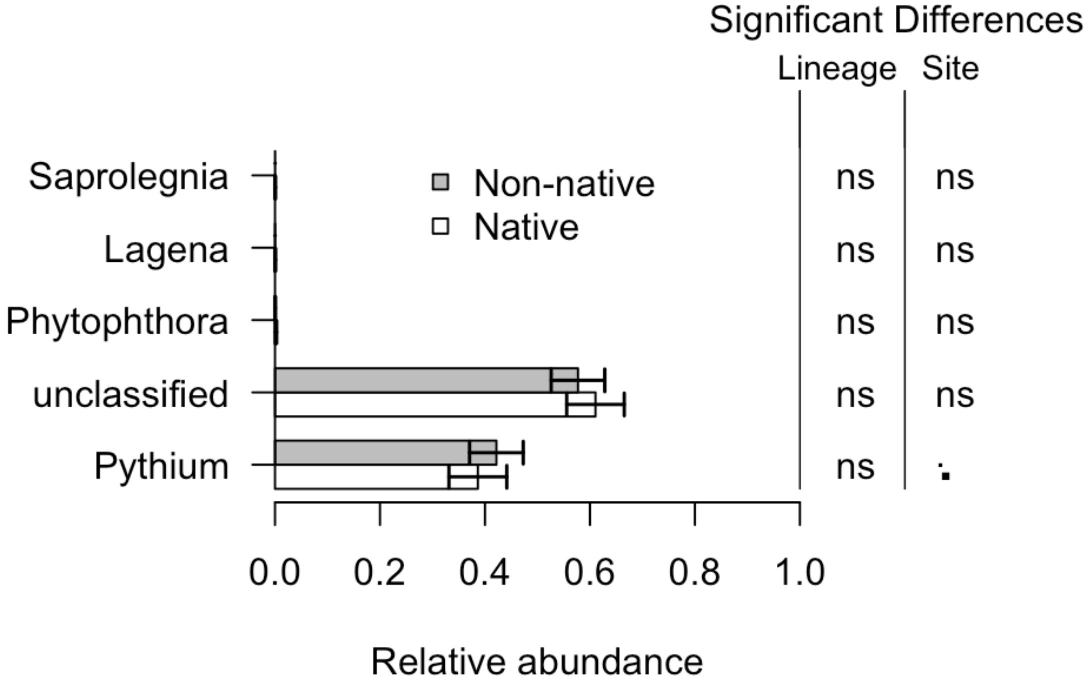


Fig. S11: Relative abundance of Oomycete genera by plant lineage. Significance determined by ANOVA



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