Supporting Information. Bickford WA, Goldberg DE, Zak DR, Snow DS, Kowalski KP. Plant effects on and response to soil microbes in native and non-native *Phragmites australis*. Ecological Applications

Appendix S1.

Table S1: Field collection locations of *Phragmites* rhizomes, seeds, and rhizosphere soils. Corresponding sample IDs are found in Table S2. All samples collected in the fall of 2017.

ID	Material Sampled	Lineage	Location	GPS Coordinates	Approximate Average Plant Height (m)	Approximate Patch Size (m^2)	Monoculture
AA1	Soils, Rhizomes & Seeds	Non-native	North Hydro Park - Crosswalk	42.208587; -83.556201	3	7500	Yes
AA2	Soils, Rhizomes & Seeds	Non-native	North Hydro Park - Trees	42.208265; -83.556149	3	75	Yes
CH1	Soils, Rhizomes & Seeds	Native	Chelsea, MI - Cavanaugh	42.309186; -84.060233	3	100	No
CH2	Soils, Rhizomes & Seeds	Native	Chelsea, MI - Garvey	42.307139; -84.060194	2.5	96	Yes
CH3	Soils, Rhizomes & Seeds	Native	Chelsea, MI - Garvey	42.307139; -84.060194	3	225	Yes
CH4	Soils, Rhizomes & Seeds	Native	Chelsea, MI - JC NE #1	42.337932; -84.000164	2	300	No
OH1	Soils, Rhizomes & Seeds	Native	Cedar Point NWR - Transect 2	41.675778; -83.292382	3	2000	No
OH2	Soils, Rhizomes & Seeds	Non-native	Cedar Point NWR - Transect 1	41.675850; -83.304783	3	1500	Yes
OH3	Soils, Rhizomes & Seeds	Non-native	Cedar Point NWR - Transect 2	41.675693; -83.292545	2.5	900	No
OH4	Soils & Rhizomes	Non-native	Cedar Point NWR - Beach	41.678528; -83.308991	2	375	Yes
OH5	Soils & Rhizomes	Native	Cedar Point NWR - Yondota	41.676160; -83.312036	2	100	No
OH6	Soils, Rhizomes & Seeds	Non-native	Ottawa NWR - Bunkhouse	41.615606; -83.201169	3.5	300	No
OH7	Soils, Rhizomes & Seeds	Native	Ottawa NWR - Bunkhouse	41.615111; -83.199085	2.5	1125	No
OH8	Soils, Rhizomes & Seeds	Non-native	Ottawa NWR - Genome	41.631304; -83.228952	2.5	150	No
OH9	Soils, Rhizomes & Seeds	Native	Ottawa NWR - Ron's Patch	41.632808; -83.234265	2	300	No
OH10	Soils, Rhizomes & Seeds	Non-native	Ottawa NWR - Ron's Patch	41.632616; -83.234190	3.5	1250	Yes
OH11	Soils, Rhizomes & Seeds	Non-native	Turtle Creek, OH	41.604008, -83.155844	2.5	3000	Yes
OH12	Soils, Rhizomes & Seeds	Non-native	Toussaint Creek, OH- Pulloff	41.579143; -83.145310	3	2000	Yes
SH1	Soils, Rhizomes & Seeds	Native	Shiawassee River SGA - West	43.339480; -84.069882	2	3200	No
SH2	Soils, Rhizomes & Seeds	Native	Shiawassee NWR - Confluence	43.372174; -83.998833	2.5	1000	No

Table S2: Quantitative Polymerase Chain Reaction (qPCR) reagents and conditions used to assess abundance of bacteria and fungi in soils.

	Reagent volumes per reaction (uL)						Reaction conditions (40 cycles)				Quality control	
Focal taxon	**Primers**	**ROX reference dye (500x dilution)**	**Bovine serum albumin (20 mg/mL)**	**Molecular biology grade water**	**Brilliant III Ultra-Fast SYBR Green qPCR Master Mix**	**Target DNA**	**Initial denaturation**	**Denaturation**	**Annealing**	**Extension**	**Average efficiency**	**Average R^2**
Bacteria	0.438 (338f/518r)	0.375	2.5	7.75	12.5	1	95°C 03:00	95°C 00:30	53°C 00:20	72°C 00:20	94.8 ± 3.4%	0.99
Fungi	0.625 (ITS1f /5.8s)	0.375	0.5	9.375	12.5	1	95°C 05:00	95°C 00:45	53°C 00:30	72°C 00:30	97.6 ± 3.0%	0.99

Table S3: Polymerase Chain Reaction (PCR) Conditions and Primer Sequences used for amplicon sequencing of bacterial and fungal communities.

Primer Set	Primer	Primer Sequence	Fused Primer Length[†]	PCR Mastermix	PCR Conditions	Reference
Fungi	5.8S_Fun	5'-AACTTTYRRCAAYGGATCWCT-3'	65 bp	5.0 uL 5x Buffer[*] 0.25 uL 20uM dNTPs 0.938 uL 20uM 5.8S 0.938 uL 20uM ITS4 0.25 uL Taq[*] 4 uL Template DNA[**] 13.625 uL H2O	Initial denaturation: 94℃ for 3 min, 27 cycles, denaturation: 94℃ for 30 s, annealing: 57℃ for 45 s, extension: 72℃ for 90 s (10 min final extension)	(Taylor et al. 2016)
	ITS4_Fun	5'-AGCCTCCGCTTATTGATATGCTTAART-3'	76 bp			
Bacteria	515F	5'- GTGCCAGCMGCCGCGGTAA-3'	63 bp	5.0 uL 5x Buffer[*] 0.5 uL 20uM dNTPs 0.5 uL 20uM 515F 0.5 uL 20uM 806R 0.5 uL Taq[*] 2 uL Template DNA[**] 18 uL H2O	Initial denaturation: 95℃ for 2 min, 30 cycles, denaturation: 95℃ for 20 s, annealing: 55℃ for 15 s, extension: 72℃ for 5 min (10 min final extension)	(Kozich et al. 2013)
	806R	5'- GGACTACHVGGGTWTCTAAT'3'	69 bp			

[†] Full fused primer included Illumina adapter (29-bp forward; 24-bp reverse), 8-bp unique barcode, 10-bp pad, 2-bp linker followed by the gene specific primer). Reverse adapter is used with forward primer, Forward adapter with reverse.

[*] Phusion High Fidelity PCR Kit (New England BioLabs)

[**] DNA was diluted to 5-10 ng per reaction

Literature Cited in Table S3:

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. Applied and Environmental Microbiology 79:5112–5120.

Taylor, D. L., W. A. Walters, N. J. Lennon, J. Bochicchio, A. Krohn, J. G. Caporaso, and T. Pennanen. 2016. Accurate Estimation of Fungal Diversity and Abundance through Improved Lineage-Specific Primers Optimized for Illumina Amplicon Sequencing. Applied and Environmental Microbiology 82:7217–7226.

Table S4: Pairwise Permutational Multivariate Analysis of Variance (PERMANOVA) output comparing **pre-treatment bacterial communities** among all possible inhibitor treatment combinations. P-values were adjusted using a Benjamini-Hochberg correction. Bold values indicate significance at $\alpha = 0.05$.

Pairs	F-value	R^2	P-value	Adjusted P-value
BAC vs OOM	1.32	0.034	0.0507	0.089
BAC vs FUN	1.11	0.028	0.2240	0.321
BAC vs NONE	1.04	0.027	0.3448	0.402
BAC vs FUNBAC	1.07	0.027	0.3038	0.386
BAC vs FUNOOM	0.89	0.023	0.7241	0.779
BAC vs BACOOM	0.84	0.022	0.8199	0.850
BAC vs STERILE	**9.78**	**0.205**	**0.0001**	**<0.001**
OOM vs FUN	**1.67**	**0.042**	**0.0046**	**0.014**
OOM vs NONE	**1.46**	**0.037**	**0.0073**	**0.019**
OOM vs FUNBAC	**1.72**	**0.043**	**0.0017**	**0.006**
OOM vs FUNOOM	1.37	0.035	0.0289	0.058
OOM vs BACOOM	**1.52**	**0.038**	**0.0144**	**0.034**
OOM vs STERILE	**11.33**	**0.230**	**0.0001**	**<0.001**
FUN vs NONE	1.03	0.026	0.3591	0.402
FUN vs FUNBAC	1.11	0.028	0.2294	0.321
FUN vs FUNOOM	**1.47**	**0.037**	**0.0214**	**0.046**
FUN vs BACOOM	1.08	0.028	0.2615	0.349
FUN vs STERILE	**7.59**	**0.166**	**0.0001**	**<0.001**
NONE vs FUNBAC	1.18	0.030	0.1310	0.204
NONE vs FUNOOM	1.24	0.032	0.0804	0.132
NONE vs BACOOM	1.04	0.027	0.3317	0.402
NONE vs STERILE	**9.34**	**0.197**	**0.0001**	**<0.001**
FUNBAC vs FUNOOM	**1.53**	**0.039**	**0.0074**	**0.019**
FUNBAC vs BACOOM	0.71	0.018	0.9947	0.994
FUNBAC vs STERILE	**9.32**	**0.197**	**0.0001**	**<0.001**
FUNOOM vs BACOOM	1.34	0.034	0.0511	0.089
FUNOOM vs STERILE	**11.91**	**0.239**	**0.0001**	**<0.001**
BACOOM vs STERILE	**9.45**	**0.199**	**0.0001**	**<0.001**

Table S5: Pairwise Permutational Multivariate Analysis of Variance (Per-MANOVA) output comparing **pre-treatment fungal communities** among all possible inhibitor treatment combinations. P-values were adjusted using a Benjamini-Hochberg correction. Bold values indicate significance at $\alpha = 0.05$.

Pairs	F-value	R^2	P-value	Adjusted P-value
BAC vs OOM	0.99	0.026	0.459	0.984
BAC vs FUN	0.88	0.023	0.664	0.984
BAC vs NONE	0.81	0.023	0.790	0.984
BAC vs FUNBAC	0.91	0.024	0.608	0.984
BAC vs FUNOOM	0.84	0.022	0.757	0.984
BAC vs BACOOM	0.90	0.024	0.642	0.984
BAC vs STERILE	**4.19**	**0.099**	**<0.001**	**<0.001**
OOM vs FUN	1.06	0.028	0.343	0.984
OOM vs NONE	1.01	0.030	0.447	0.984
OOM vs FUNBAC	0.74	0.020	0.898	0.984
OOM vs FUNOOM	0.68	0.019	0.957	0.984
OOM vs BACOOM	0.89	0.024	0.662	0.984
OOM vs STERILE	**5.30**	**0.125**	**<0.001**	**<0.001**
FUN vs NONE	1.32	0.037	0.086	0.302
FUN vs FUNBAC	0.87	0.023	0.693	0.984
FUN vs FUNOOM	1.04	0.027	0.365	0.984
FUN vs BACOOM	0.91	0.024	0.610	0.984
FUN vs STERILE	**5.52**	**0.127**	**<0.001**	**<0.001**
NONE vs FUNBAC	0.95	0.028	0.557	0.984
NONE vs FUNOOM	0.86	0.025	0.752	0.984
NONE vs BACOOM	0.70	0.021	0.934	0.984
NONE vs STERILE	**3.65**	**0.097**	**0.001**	**0.002**
FUNBAC vs FUNOOM	0.63	0.017	0.984	0.984
FUNBAC vs BACOOM	0.69	0.019	0.940	0.984
FUNBAC vs STERILE	**5.01**	**0.119**	**0.000**	**<0.001**
FUNOOM vs BACOOM	0.81	0.022	0.813	0.984
FUNOOM vs STERILE	**4.44**	**0.107**	**<0.001**	**<0.001**
BACOOM vs STERILE	**4.55**	**0.109**	**<0.001**	**<0.001**

Table S6: Pairwise Permutational Multivariate Analysis of Variance (Per-MANOVA) output comparing **post-treatment bacterial communities** among all possible inhibitor treatment combinations. P-values were adjusted using a Benjamini-Hochberg correction. Bold values indicate significance at $\alpha = 0.05$.

Pairs	F-value	R^2	P-value	Adjusted P-value
BAC vs OOM	**5.916**	**0.135**	**<0.001**	**0.003**
BAC vs FUN	**5.012**	**0.117**	**<0.001**	**0.003**
BAC vs NONE	**6.180**	**0.140**	**<0.001**	**0.003**
BAC vs FUNBAC	1.220	0.031	0.093	1.000
BAC vs FUNOOM	**5.154**	**0.119**	**<0.001**	**0.003**
BAC vs BACOOM	0.984	0.025	0.440	1.000
BAC vs STERILE	**5.510**	**0.127**	**<0.001**	**0.003**
OOM vs FUN	**2.977**	**0.073**	**<0.001**	**0.003**
OOM vs NONE	1.172	0.030	0.119	1.000
OOM vs FUNBAC	**6.912**	**0.154**	**<0.001**	**0.003**
OOM vs FUNOOM	**2.431**	**0.060**	**<0.001**	**0.003**
OOM vs BACOOM	**6.294**	**0.142**	**<0.001**	**0.003**
OOM vs STERILE	**4.833**	**0.113**	**<0.001**	**0.003**
FUN vs NONE	**2.838**	**0.069**	**<0.001**	**0.003**
FUN vs FUNBAC	**4.793**	**0.112**	**<0.001**	**0.003**
FUN vs FUNOOM	0.986	0.025	0.432	1.000
FUN vs BACOOM	**5.015**	**0.117**	**<0.001**	**0.003**
FUN vs STERILE	**4.024**	**0.096**	**<0.001**	**0.003**
NONE vs FUNBAC	**7.272**	**0.161**	**<0.001**	**0.003**
NONE vs FUNOOM	**2.224**	**0.055**	**<0.001**	**0.006**
NONE vs BACOOM	**6.485**	**0.146**	**<0.001**	**0.003**
NONE vs STERILE	**4.902**	**0.114**	**<0.001**	**0.003**
FUNBAC vs FUNOOM	**5.356**	**0.124**	**<0.001**	**0.003**
FUNBAC vs BACOOM	1.471	0.037	0.030	0.829
FUNBAC vs STERILE	**5.921**	**0.135**	**<0.001**	**0.003**
FUNOOM vs BACOOM	**5.157**	**0.119**	**<0.001**	**0.003**
FUNOOM vs STERILE	**3.964**	**0.094**	**<0.001**	**0.003**

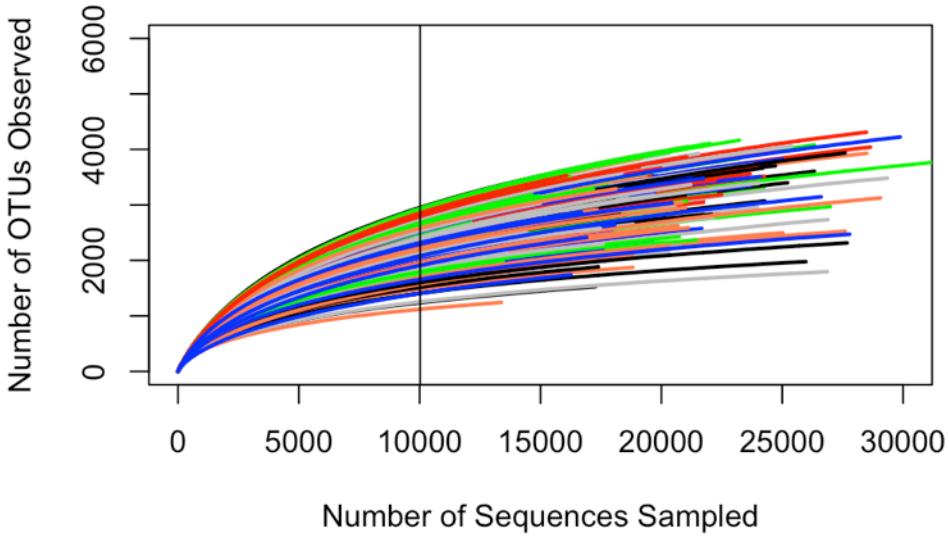
Table S7: Pairwise Permutational Multivariate Analysis of Variance (Per-MANOVA) output comparing **post-treatment fungal communities** among all possible inhibitor treatment combinations. P-values were adjusted using a Benjamini-Hochberg correction. Bold values indicate significance at $\alpha = 0.05$.

Pairs	F-value	R^2	P-value	Adjusted P-value
BAC vs OOM	**1.98**	**0.051**	**<0.001**	**<0.001**
BAC vs FUN	**2.21**	**0.059**	**<0.001**	**<0.001**
BAC vs NONE	**2.71**	**0.070**	**<0.001**	**<0.001**
BAC vs FUNBAC	1.16	0.030	0.212	0.220
BAC vs FUNOOM	**2.96**	**0.074**	**<0.001**	**<0.001**
BAC vs BACOOM	**1.44**	**0.038**	**0.041**	**0.047**
BAC vs STERILE	**1.66**	**0.043**	**0.002**	**0.002**
OOM vs FUN	**2.42**	**0.063**	**<0.001**	**<0.001**
OOM vs NONE	1.15	0.030	0.206	0.220
OOM vs FUNBAC	**2.65**	**0.065**	**<0.001**	**<0.001**
OOM vs FUNOOM	**2.46**	**0.061**	**<0.001**	**<0.001**
OOM vs BACOOM	**2.04**	**0.051**	**0.001**	**0.002**
OOM vs STERILE	**2.26**	**0.056**	**<0.001**	**<0.001**
FUN vs NONE	**2.44**	**0.065**	**<0.001**	**<0.001**
FUN vs FUNBAC	**2.12**	**0.056**	**<0.001**	**<0.001**
FUN vs FUNOOM	1.16	0.031	0.225	0.225
FUN vs BACOOM	**2.53**	**0.066**	**<0.001**	**<0.001**
FUN vs STERILE	**1.88**	**0.050**	**<0.001**	**<0.001**
NONE vs FUNBAC	**3.25**	**0.081**	**<0.001**	**<0.001**
NONE vs FUNOOM	**2.37**	**0.060**	**<0.001**	**<0.001**
NONE vs BACOOM	**2.94**	**0.074**	**<0.001**	**<0.001**
NONE vs STERILE	**2.55**	**0.064**	**<0.001**	**<0.001**
FUNBAC vs FUNOOM	**2.59**	**0.064**	**<0.001**	**<0.001**
FUNBAC vs BACOOM	1.35	0.034	0.089	0.100
FUNBAC vs STERILE	**2.18**	**0.054**	**<0.001**	**<0.001**
FUNOOM vs BACOOM	**3.27**	**0.079**	**<0.001**	**<0.001**
FUNOOM vs STERILE	**2.82**	**0.069**	**<0.001**	**<0.001**
BACOOM vs STERILE	**2.07**	**0.052**	**<0.001**	**<0.001**

Table S8: Analysis of Variance (ANOVA) output (Type III Sum of Squares) comparing relative abundance of Glomeromycota sequences among treatment groups. Bold values indicate significance at $\alpha = 0.05$.

Factor	Sum Sq	Df	F-Value	P-Value
Inhibitor	**0.029**	**7**	**3.61**	**0.001**
Conditioning Lineage	**0.016**	**1**	**13.94**	**<0.001**
Seedling Lineage	**0.009**	**1**	**7.88**	**0.006**
Inhibitor * Conditioning Lineage	**0.020**	**7**	**2.46**	**0.021**
Inhibitor * Seedling Lineage	0.017	7	2.03	0.056
Conditioning Lineage * Seedling Lineage	0.000	1	0.00	0.999
Inhibitor * Conditioning * Seedling	0.012	7	1.44	0.193

a) Bacteria



b) Fungi

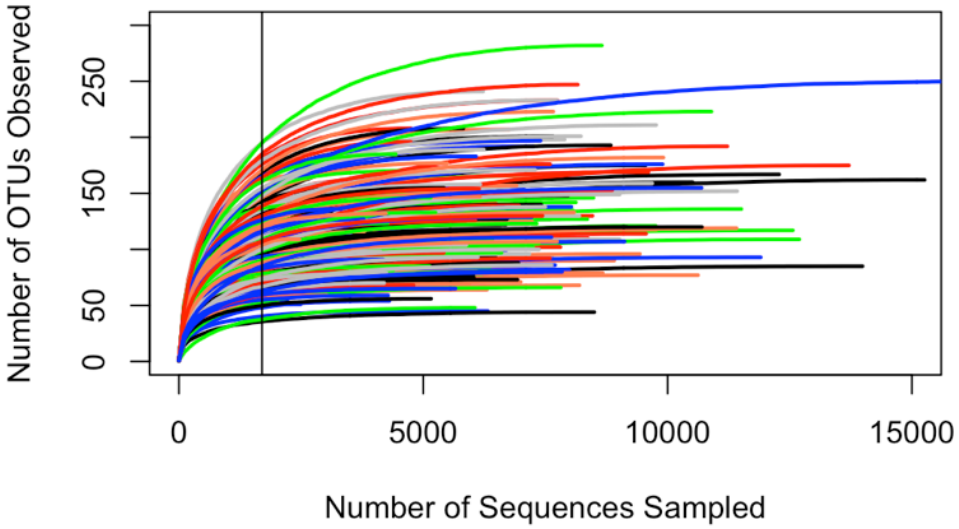


Fig. S1: Rarefaction curves for a) bacterial and b) fungal sequences. Colors indicate treatment groups. Vertical lines indicate sequence subsample threshold.

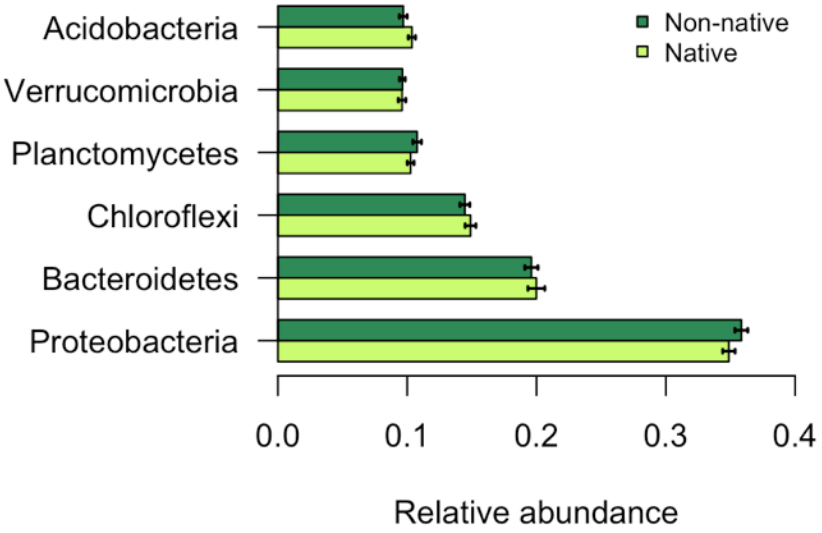
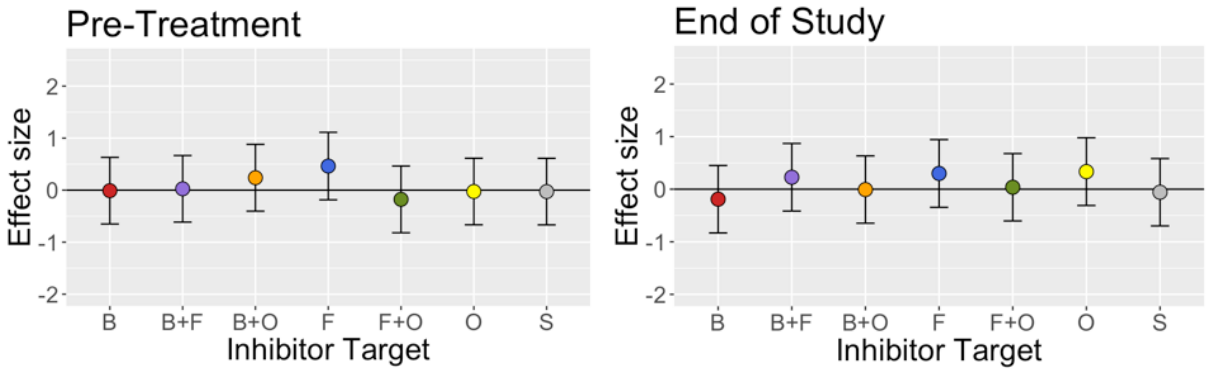


Fig. S2: Relative abundance of major bacterial phyla recovered from soils conditioned by each *Phragmites* lineage at the end of the conditioning phase. Error bars represent standard error of the mean. No lineage comparisons were significant at $\alpha = 0.05$.

a) Bacterial Gene Copy number



b) Fungal Gene Copy Number

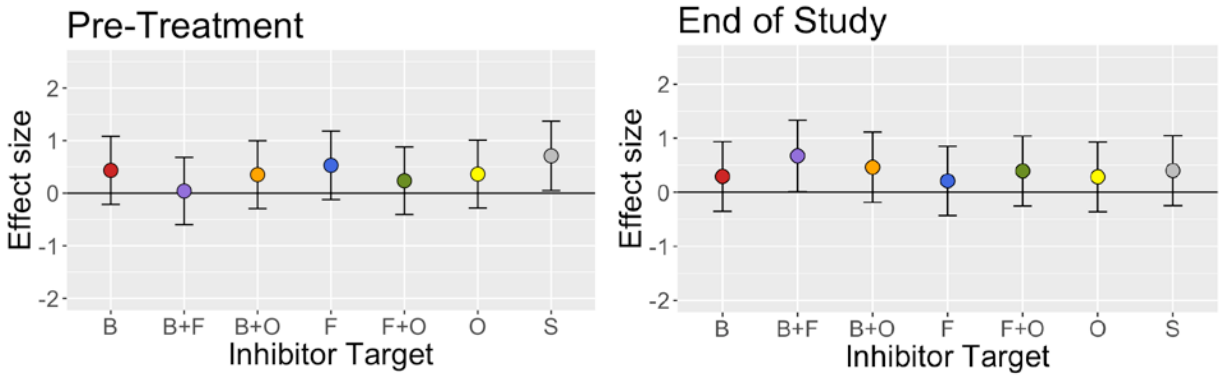


Fig S3: a) Bacterial and b) fungal gene copy number changes by inhibitor treatment. Plots show treatments relative to control both before application of the inhibitor treatments and at the end of the seedling phase. Neither microbial group differed in abundance before treatments or changed in response to any of the treatments. All values are relative to controls with no inhibitors and calculated using Cohen's d. Error bars represent 95% confidence intervals. Values with error bars not overlapping zero are considered statistically significantly different from the control. Inhibitor treatments are abbreviated B+F: Antibacterial/ Antifungal, B: Antibacterial, B+O: Antibacterial/ Anti-oomycete, O: Anti-oomycete, F+O: Antifungal/ Anti-oomycete, F: Antifungal, S: Sterile.

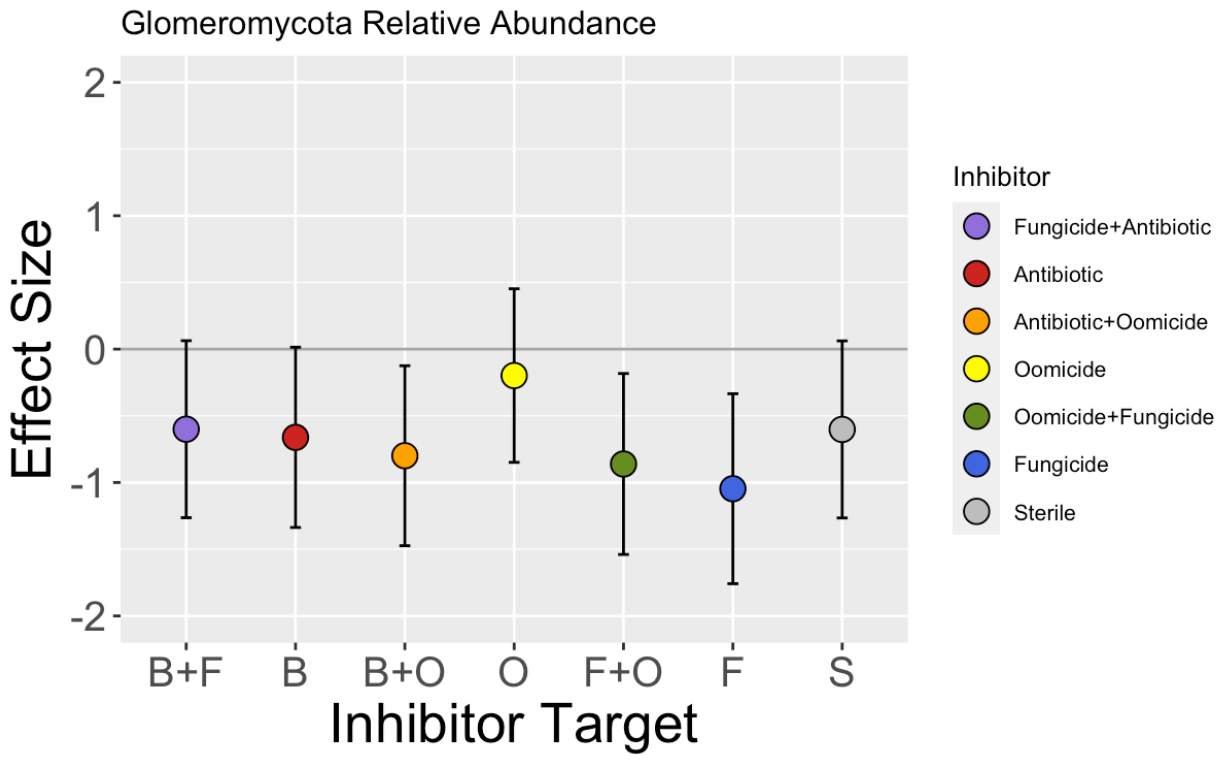


Fig. S4: Effect of inhibitor treatments on Glomeromycota relative abundance in fungal communities of soils conditioned by each lineage. All values are relative to controls with no inhibitors and calculated using Cohen's d. Error bars represent 95% confidence intervals. Values with error bars not overlapping zero are considered statistically significantly different from the control. Inhibitor treatments are abbreviated B+F: Antibacterial/ Antifungal, B: Antibacterial, B+O: Antibacterial/ Anti-oomycete, O: Anti-oomycete, F+O: Antifungal/ Anti-oomycete, F: Antifungal, S: Sterile.