

Supplementary materials

Experimentally induced dieback conditions limit *Phragmites australis* growth

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Note: Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. All data used to support this study are available at [1].

Table S1: Plant biomass data from a preliminary study measuring biomass response to various short-chain fatty acid treatments. Biomass measures were from total (above- and belowground) dry weights from each mesocosm. All treatment groups contained eight replicate mesocosms. All acids were applied at the 0.05 M application rate. Significance at $\alpha = 0.05$ was assigned following an ANOVA with post-hoc Tukey HSD adjustment. Full dataset can be found at [1].

| Treatment | Mean Biomass (g) | Standard Error | Significance |
|------------------|-------------------------|-----------------------|---------------------|
| Control | 43.83 | ± 3.56 | b |
| Calcium Acetate | 15.30 | ± 2.31 | c |
| Calcium Butyrate | 2.05 | ± 0.43 | a |
| Calcium Cocktail | 1.40 | ± 0.30 | a |
| Sodium Acetate | 7.40 | ± 1.92 | ac |
| Sodium Butyrate | 6.32 | ± 1.75 | ac |
| Sodium Cocktail | 3.09 | ± 0.95 | a |
| Novyrate | 15.89 | ± 4.25 | c |
| Tributylin | 1.24 | ± 0.20 | a |

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

| Primers | Reagent volumes per reaction (µL) | | | | | Reaction conditions (40 cycles) | | | Quality control | |
|-------------------|-----------------------------------|---------------------------------|-------------------------------|---|------------|---------------------------------|---------------|-----------------------|--------------------|------------------------|
| | 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 |
| 0.438 (338f/518r) | 0.375 | 2.5 | 7.75 | 12.5 | 1 | 95°C 03:00 | 95°C 00:05 | 60°C 00:10 | 94.8 ± 3.4% | 0.99 |

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

| Primer Name | Primer Sequence | Fused Primer Length [†] | PCR Mastermix | PCR Conditions | Reference |
|-------------|-----------------------------|----------------------------------|---|--|-----------|
| 515f | 5'- GTGCCAGCMGCCGCGTAA-3' | 63 bp | 17 µL Accuprime pfx Supermix 1.0 µL 20 µM 515F | Initial denaturation: 95°C for 2 min, 30 cycles, denaturation: 95°C for 20 s, annealing: 55°C for 15 s, extension: 72°C for 5 min (10 min final extension) | [2] |
| 806r | 5'- GGACTACHVGGGTWTCTAAT'3' | 69 bp | 1.0 µL 20 µM 806R 1.0 µL Template DNA | | |

[†] 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.



Table S4: Proportion of mesocosms with white vs. non-white and rigid vs non-rigid roots following SCFA treatments in a) low concentration *Phragmites* mesocosms, b) high concentration *Phragmites* mesocosms, and c) high concentration mesocosms of *Phragmites* or native species. *P.aus* = *Phragmites australis*; *S.acu* = *Schoenoplectus acutus*; *S.pec* = *Spartina pectinate*.

| Experiment 1: 0.01 M | | | | | | | | | | | | |
|----------------------|--------------|--------------|--------------|--------------|---------------|--------------|---------------|--------------|--------------|--------------|--------------|--------------|
| | Control | | Ca Butyrate | | Ca Propionate | | Cocktail | | | | | |
| | Monthly | Weekly | Monthly | Weekly | Monthly | Weekly | Monthly | Weekly | Monthly | Weekly | | |
| Non-White Roots | 0 | 0 | 0 | 0 | 0 | 0.17 | 0 | 0.17 | 0 | 0 | 0.17 | |
| White Roots | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 0.83 | 1.0 | 0.83 | 1.0 | 1.0 | 0.83 | |
| Non-Rigid | 0 | 0 | 0.17 | 0 | 0 | 0.17 | 0 | 0.17 | 0 | 0 | 0.17 | |
| Rigid | 1.0 | 1.0 | 0.83 | 1.0 | 1.0 | 0.83 | 1.0 | 0.83 | 1.0 | 1.0 | 0.83 | |
| Experiment 2: 0.05 M | | | | | | | | | | | | |
| | Control | | Ca Butyrate | | Ca Propionate | | Cocktail | | | | | |
| | Monthly | Weekly | Monthly | Weekly | Monthly | Weekly | Monthly | Weekly | Monthly | Weekly | | |
| Non-White Roots | 0 | 0 | 0 | 0.67 | 0.17 | 0.67 | 0.33 | 0.67 | 0.33 | 0.67 | 0.67 | |
| White Roots | 1.0 | 1.0 | 1.0 | 0.33 | 0.83 | 0.33 | 0.67 | 0.33 | 0.67 | 0.33 | 0.33 | |
| Non-Rigid | 0 | 0 | 0 | 0.17 | 0.17 | 0.67 | 0.17 | 0.67 | 0.17 | 0.4 | 0.4 | |
| Rigid | 1.0 | 1.0 | 1.0 | 0.83 | 0.83 | 0.33 | 0.83 | 0.33 | 0.83 | 0.6 | 0.6 | |
| Experiment 3: 0.05 M | | | | | | | | | | | | |
| | Control | | | Ca Butyrate | | | Ca Propionate | | | Cocktail | | |
| | <i>P.aus</i> | <i>S.acu</i> | <i>S.pec</i> | <i>P.aus</i> | <i>S.acu</i> | <i>S.pec</i> | <i>P.aus</i> | <i>S.acu</i> | <i>S.pec</i> | <i>P.aus</i> | <i>S.acu</i> | <i>S.pec</i> |
| Non-White Roots | 0 | 1.0 | 0.17 | 0.67 | 1.0 | 0.5 | 0.67 | 1.0 | 0.33 | 0.67 | 1.0 | 0.83 |
| White Roots | 1.0 | 0 | 0.83 | 0.33 | 0 | 0.5 | 0.33 | 0 | 0.67 | 0.33 | 0 | 0.17 |
| Non-Rigid | 0 | 0.17 | 0.17 | 0.17 | 0.5 | 0.33 | 0.67 | 0.33 | 0.17 | 0.4 | 0.33 | 0.17 |
| Rigid | 1.0 | 0.83 | 0.83 | 0.83 | 0.5 | 0.67 | 0.33 | 0.67 | 0.83 | 0.6 | 0.67 | 0.83 |

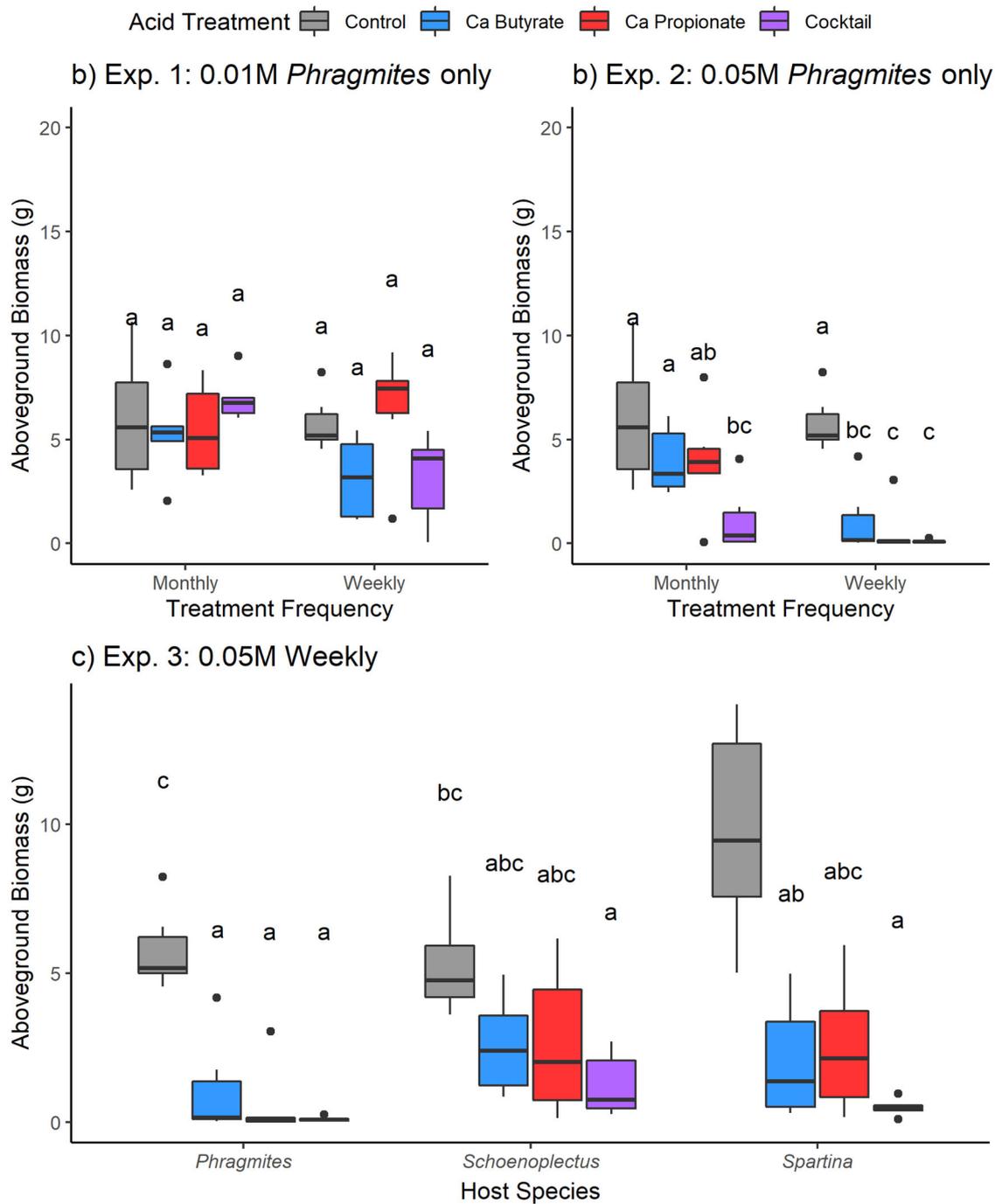


Figure S1: Aboveground plant biomass response to SCFA treatments in **a)** low concentration *Phragmites* mesocosms, **b)** high concentration *Phragmites* mesocosms, and **c)** high concentration mesocosms of *Phragmites* or native species. Colors indicate SCFA type. Letters indicate significant differences among all treatments within an experiment following ANOVA with post-hoc Tukey HSD adjustment.

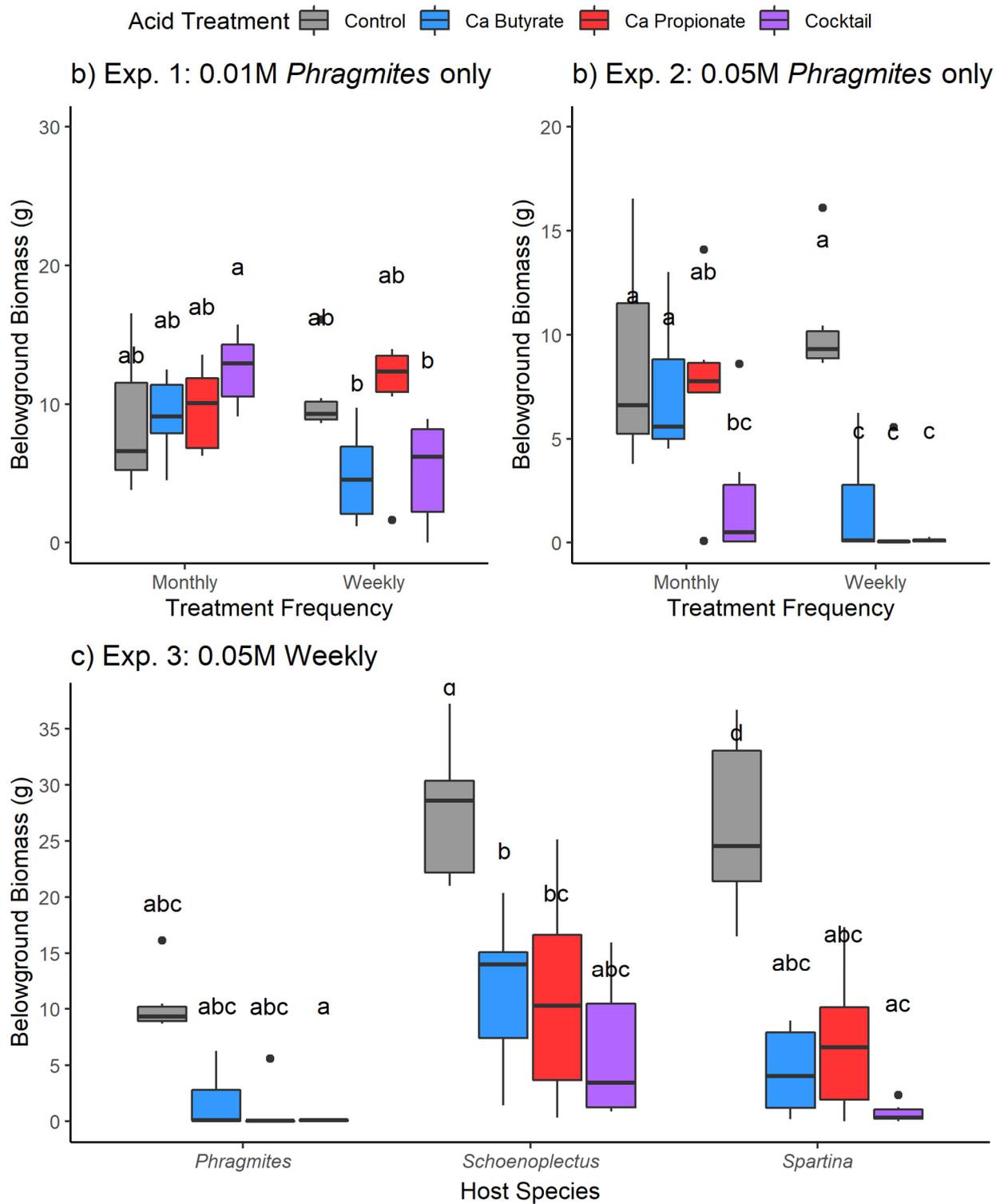


Figure S2: Belowground plant biomass response to SCFA treatments in **a)** low concentration *Phragmites* mesocosms, **b)** high concentration *Phragmites* mesocosms, and **c)** high concentration mesocosms of *Phragmites* or native species. Colors indicate SCFA type. Letters indicate significant differences among all treatments within an experiment following ANOVA with post-hoc Tukey HSD adjustment.

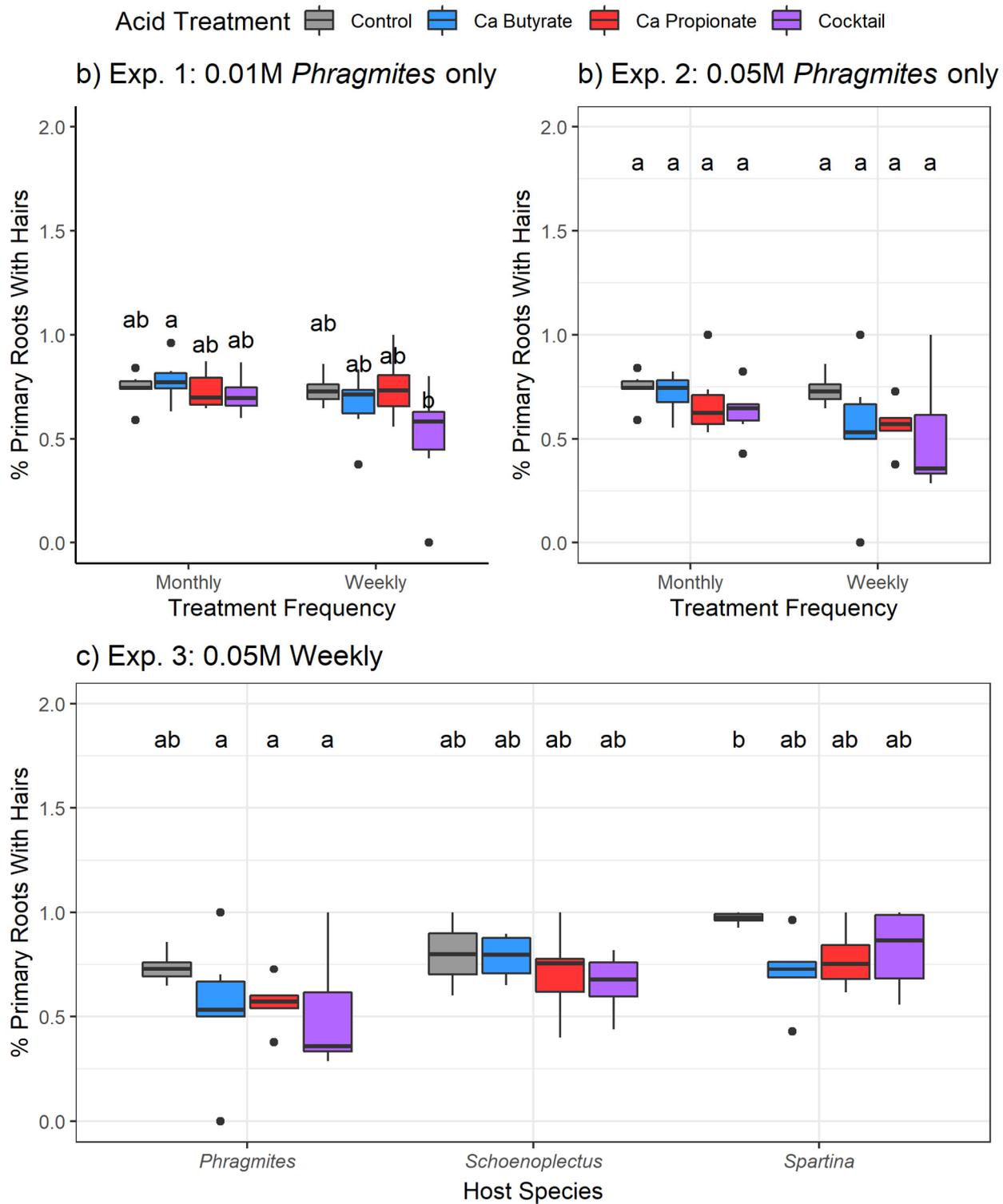


Figure S3: Root Hair response to SCFA treatments in **a)** low concentration *Phragmites* mesocosms, **b)** high concentration *Phragmites* mesocosms, and **c)** high concentration mesocosms of *Phragmites* or native species. Colors indicate SCFA type. Letters indicate significant differences among all treatments within an experiment following ANOVA with post-hoc Tukey HSD adjustment.

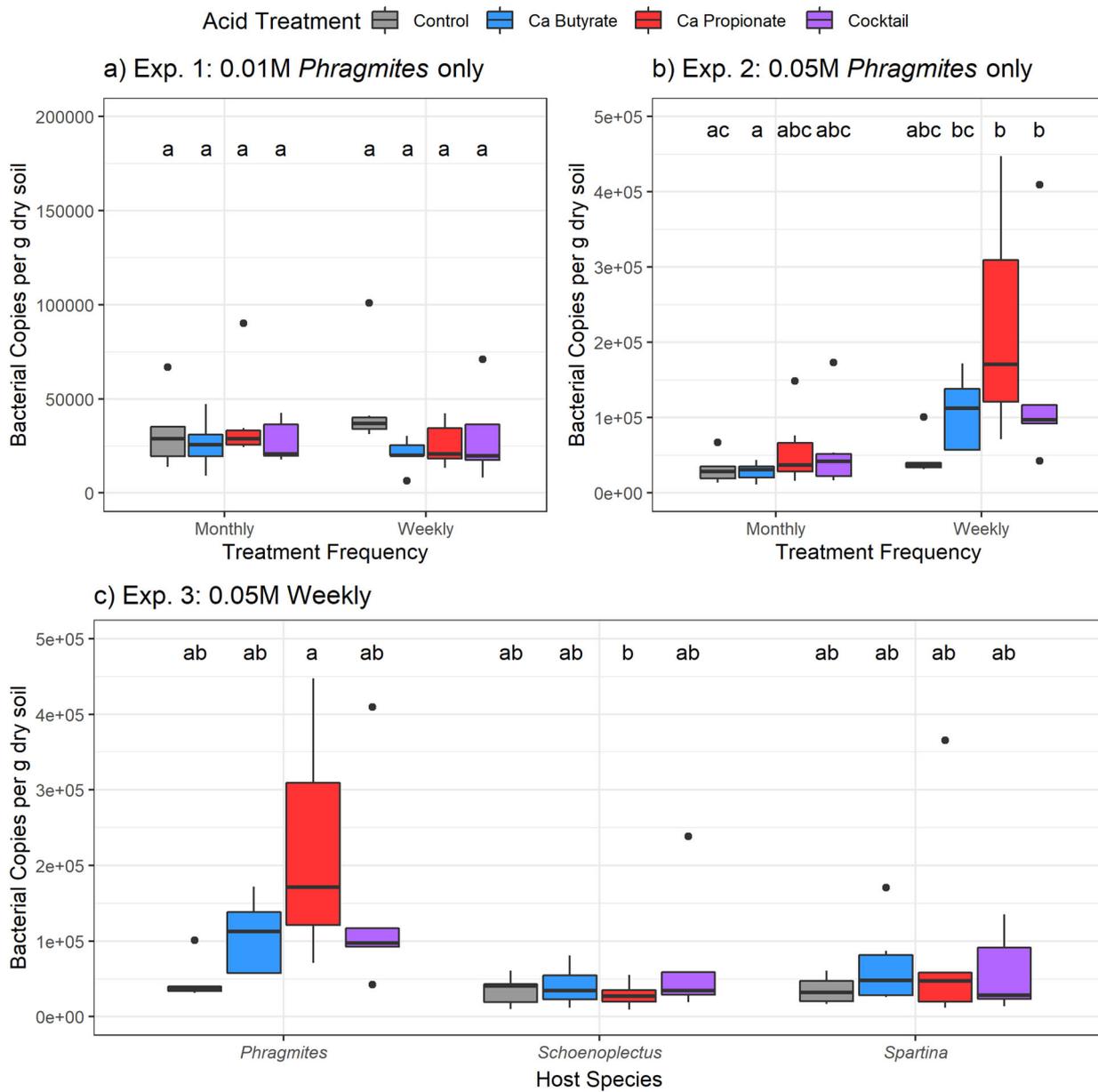


Figure S4: Rhizosphere bacterial abundance in response to SCFA treatments in **a)** low concentration *Phragmites* mesocosms, **b)** high concentration *Phragmites* mesocosms, and **c)** high concentration mesocosms of *Phragmites* or native species. Colors indicate SCFA type. Letters indicate significant differences among all treatments within an experiment following ANOVA with post-hoc Tukey HSD adjustment.

Exp. 1: 0.01M Phragmites only

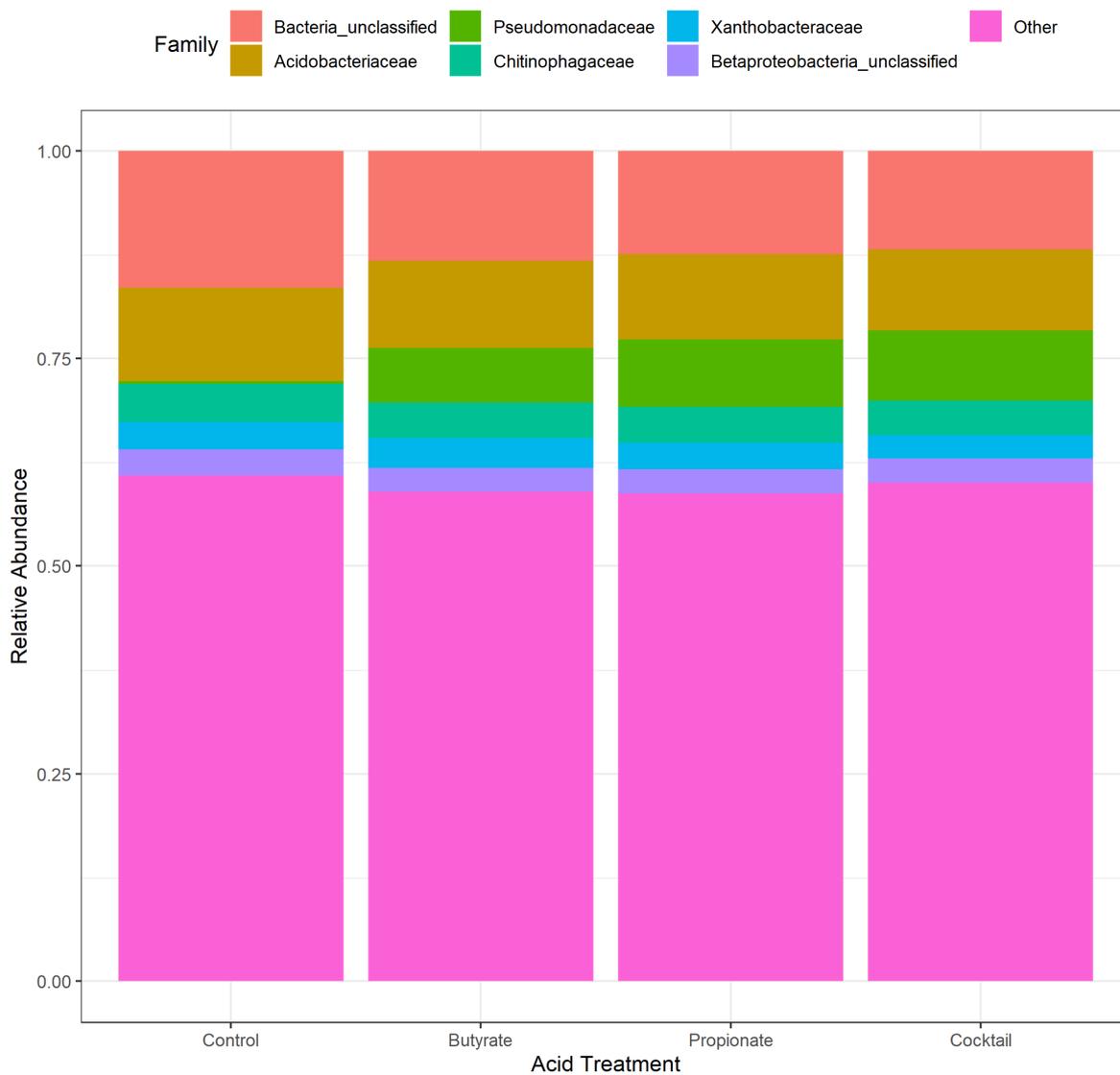


Figure S5: Relative abundance of bacterial families midway through Experiment 1 in response to each SCFA treatment. Colors indicate bacterial families. Only the top six families by relative abundance are represented. The “Other” category encompasses minor families outside of the top six.

Exp. 2: 0.05M Phragmites only

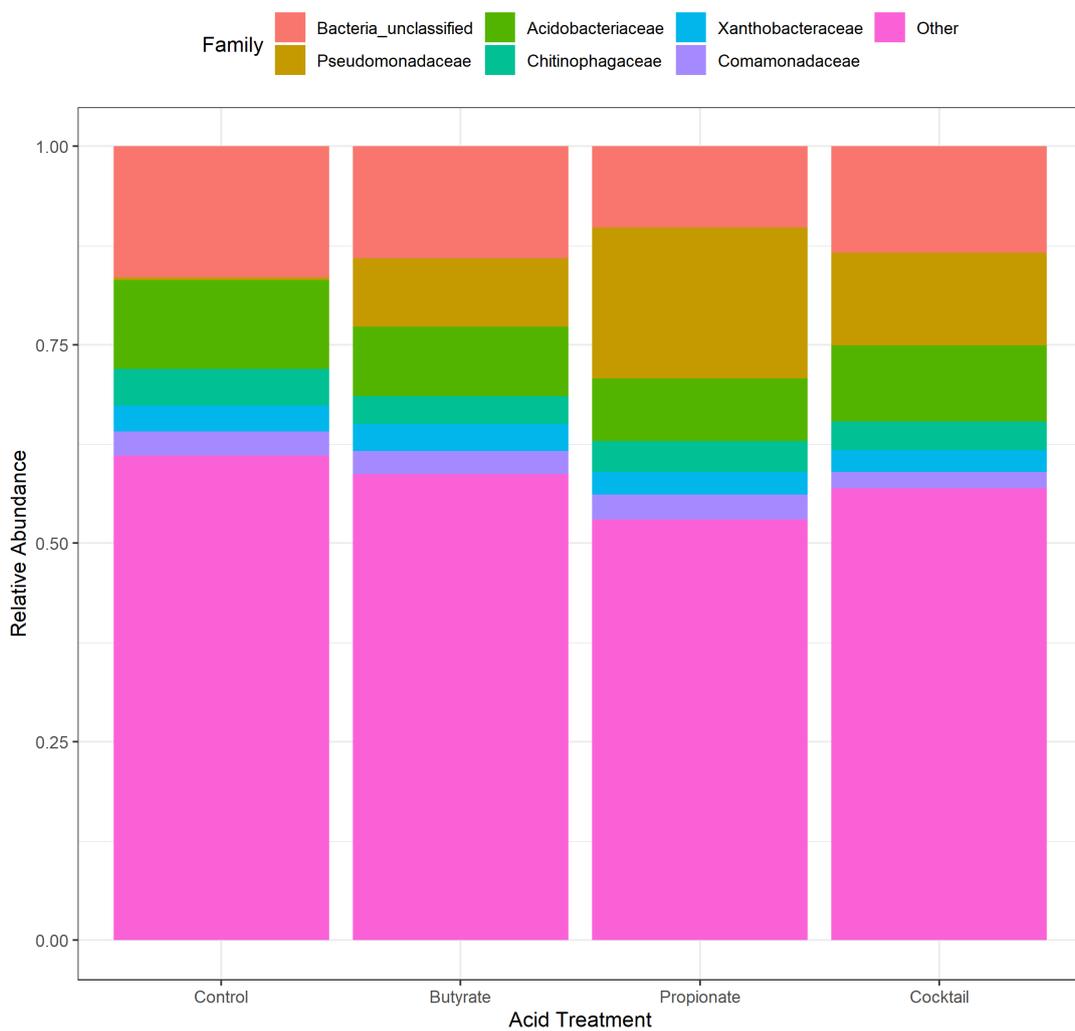


Figure S6: Relative abundance of bacterial families midway through Experiment 2 in response to each SCFA treatment. Colors indicate bacterial families. Only the top six families by relative abundance are represented. The “Other” category encompasses minor families outside of the top six.

Exp. 3: 0.05M Weekly

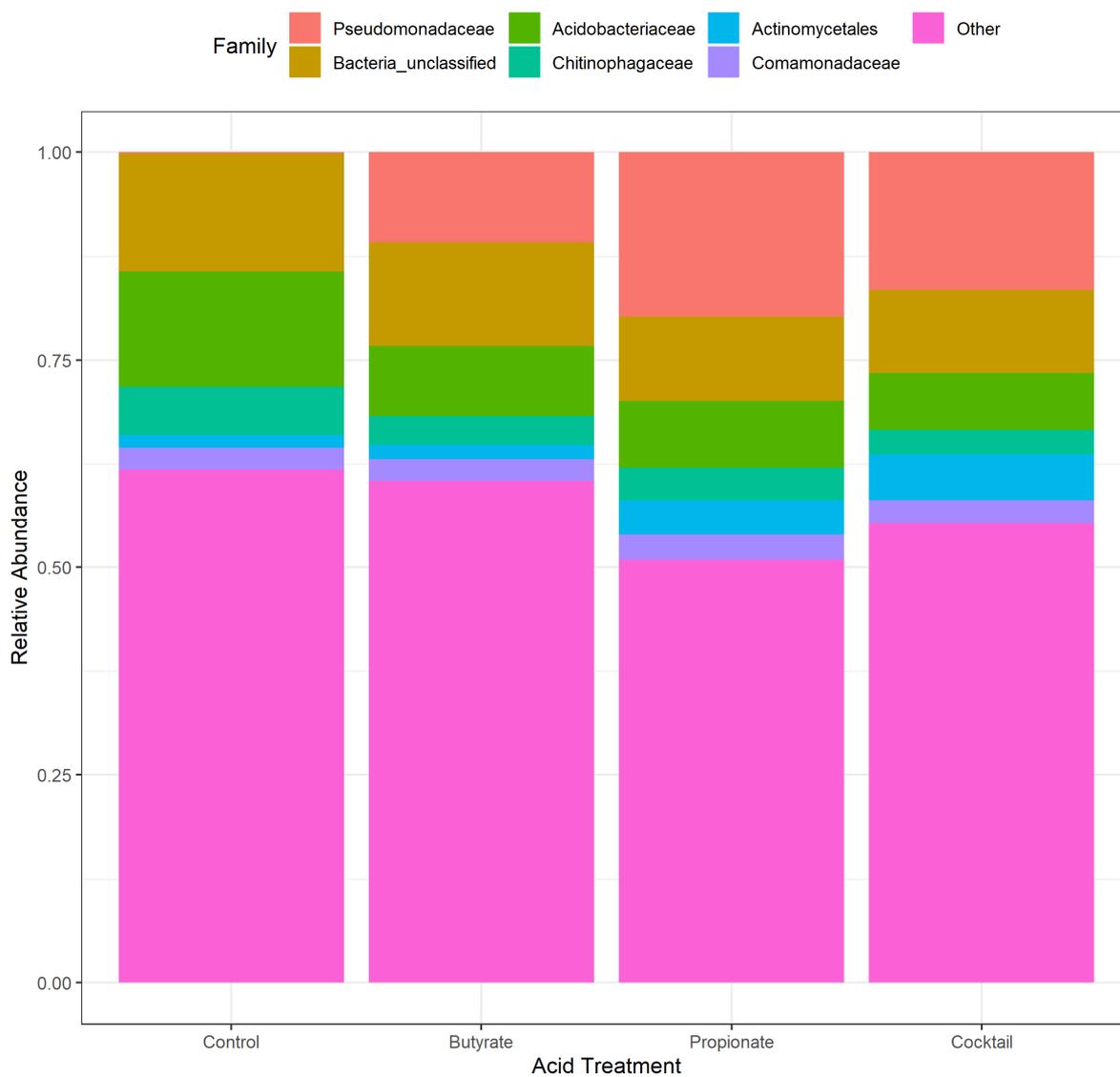


Figure S7: Relative abundance of bacterial families midway through Experiment 3 in response to each SCFA treatment. Colors indicate bacterial families. Only the top six families by relative abundance are represented. The “Other” category encompasses minor families outside of the top six.

References:

1. Bickford, W.A.; Snow, D.S.; Smith, M.K.H.; Kingsley, K.I.; White, J.F.; Kowalski, K.P. Plant and Microbial Response to Organic Acid Applications (2018-19 Experiment) **2023**. U.S. Geological Survey Data Release.
2. Kozich, J.J.; Westcott, S.L.; Baxter, N.T.; Highlander, S.K.; Schloss, P.D. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq. **2013**, *79*, 5112–5120, doi:10.1128/AEM.01043-13.