

Attachment 1: Supporting Information to Chapter 2 (Figure S1, Figure S2, and Appendix S1)

Pfennigwerth, AA. *Plasticity and biotic interactions mediate plant persistence in a changing world*. Master's thesis.

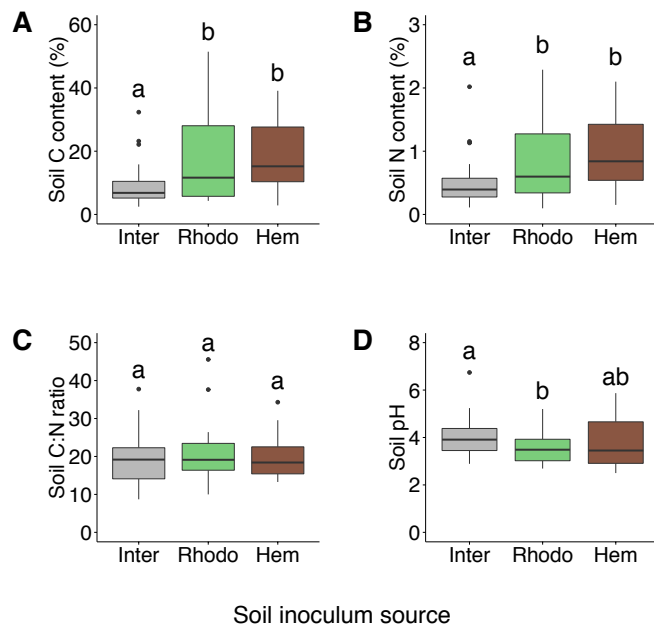


Figure S1. Field abiotic soil characteristics of interspace (Inter, white bars), *Rhododendron maximum*-conditioned (Rhodo, light grey bars), and *Tsuga canadensis*-conditioned (Hem, dark grey bars) soils. Soil carbon (C) content (panel A) and nitrogen (N) content (panel B) are significantly higher in *R. maximum* and *T. canadensis* soils than in interspace soils ($p = 0.0001$, 0.006 , respectively; evidence for soil conditioning by these plant species). Soil carbon:nitrogen (C:N) ratio (panel C) does not differ by soil source ($p = 0.797$). Soil pH (panel D) is highest in interspace, medial in *T. canadensis*-conditioned, and lowest in *R. maximum*-conditioned soils ($p = 0.032$; evidence for soil conditioning by *R. maximum*). Within each panel, bars sharing the same letter are not significantly different from each other ($p > 0.05$).

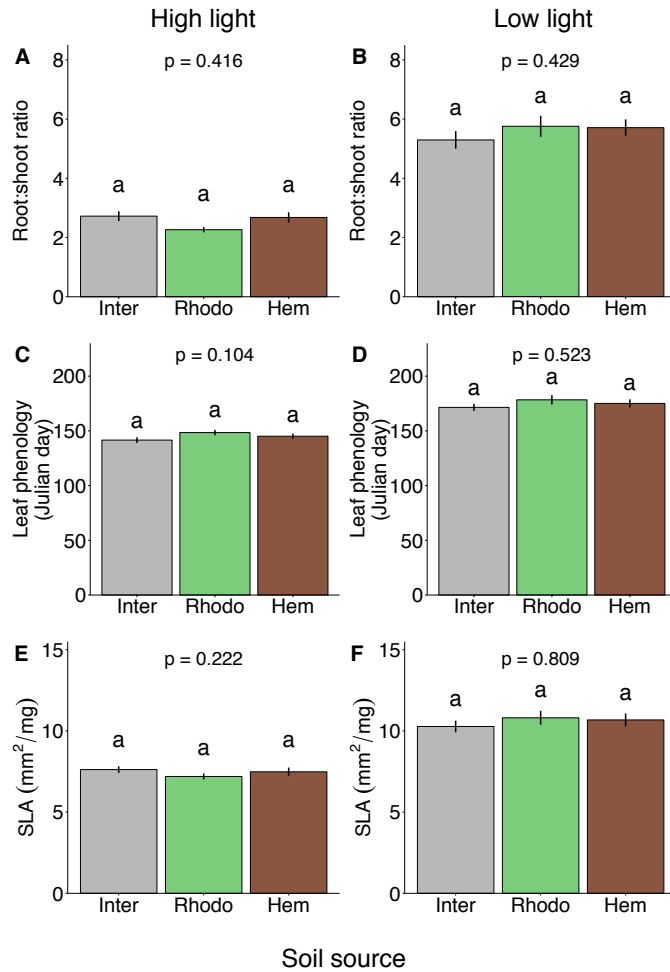


Figure S2. Mean ($\pm$ SE) root-to-shoot ratio (A, B), leaf bud break phenology (C, D), and specific leaf area (SLA; E, F) of *Rhododendron maximum* seedlings grown in soils inoculated with soil communities collected from three paired locations: interspace areas outside the influence of *Tsuga canadensis* or *R. maximum* individuals (i.e., Inter, white bars), beneath *R. maximum* individuals (i.e., Rhodo, light grey bars), or beneath declining *T. canadensis* trees (i.e., Hem, dark grey bars) and subjected to either high or low light environments. Bars that do not share letters are significantly different from each other (Tukey test $p < 0.05$); within each panel, the p-value representing the significance of the soil source effect is shown.

Appendix S1. ITS2 sequencing methods.

Amplicons were produced in a two-step protocol as suggested by Berry *et al.* (2011). Briefly, samples were amplified in triplicate PCR reactions for the ITS2 region with 5.8S_Fun/ITS4_Fun primers. First round reactions were performed in triplicate in 384 well plates. The 8 μ L volumes contained the following: 1 μ M each primer (Eurofins MWG Operon, LLC, Louisville, KY), 200 μ M each dNTP (Phenix Research, Candler, NC), 0.01 U/ μ L Phusion Hot Start II DNA Polymerase (Life Technologies, Carlsbad, CA), 1X HF Phusion Buffer (Life Technologies), 3 mM MgCl₂, 6% glycerol, and 1 μ L normalized template DNA. Cycling conditions were: 2 minutes at 95°C followed by 25 cycles of 30 seconds at 95°C, 30 seconds at 55°C, 4 minutes at 60°C. Triplicate reactions for each sample were pooled by combining 4 μ L from each, and 2 μ L was used to check for results on a 1% agarose gel. The remainder was diluted 10-fold and used as template in a second PCR reaction in which 12 base Golay indexed sequencing tails (Caporaso *et al.*, 2012) were added. Second round reaction conditions were identical to the first round except only one reaction was conducted per sample and only 15 total cycles were performed. Indexed PCR products were purified using a 1:1 ratio of 18% PEG and carboxylated magnetic beads as described in Rohland & Reich (2012), quantified by PicoGreen fluorescence, and an equal mass of each sample was combined into a final sample pool. The pool was purified and concentrated, and subsequently quantified by quantitative PCR against Illumina DNA Standards (Kapa Biosystems, Wilmington, MA). Sequencing was carried out on a MiSeq Desktop Sequencer (Illumina Inc, San Diego, CA) running in paired end 2x250 mode.

References

- Berry, D., Mahfoudh, K.B., Wagner, M. & Loy, A. (2011) Barcoded Primers Used in Multiplex Amplicon Pyrosequencing Bias Amplification. *Applied and Environmental Microbiology*, **77**, 612.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., & Knight, R. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, **6**, 1621–1624.
- Rohland, N. & Reich, D. (2012) Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Research*, **22**, 939–46.