

DeAngelis et al., 2014

Supplemental Materials

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Table S1. Summary of OTU and observation counts after each successive application of data curation.

| Run | OTUs (count) | Observations (count) | Notes |
|------------------------------|-------------------------|---------------------------------|--|
| Total reads | n.a. | 11,355,423 | Total reads after sequencing |
| Initial QC | n.a. | 10,192,548 | Quality filtering and de-multiplexing |
| Post-assembly | n.a. | 5,096,274 | Paired-end assembly using FLASH aligner |
| De-multiplexed | n.a. | 3,487,689 | Qiime demultiplexed and quality filtered |
| Open, 99%, raw | 108,432 | 3,145,351 | Open referenced OTU calling, no singletons |
| Open, 99% | 86,646 | 3,020,293 | No singletons, chimeras excluded |
| Open, 99%, no2s | 45,875 | 2,938,751 | No singletons or doubletons, chimeras excluded |
| Open, 99%, no2s, rarefied | 43,909 | 1,561,200 | No singletons or doubletons, chimeras excluded, samples rarefied to observations in smallest sample |
| Open, 99%, >0.1% | 155 | 1,537,364 | Dominants (>0.1% relative abundance(>2,938)) |

Table S2. Community differences by groups for all samples (n=48)

Full Community (N=45,875)

| | Organic soil | | | | Mineral soil | | | |
|--------|--------------|----------|-----------|----------|--------------|----------|-----------|----------|
| | site | <i>P</i> | treatment | <i>P</i> | site | <i>P</i> | treatment | <i>P</i> |
| MRPP | 0.089 | *** | 0.037 | * | 0.161 | *** | 0.0194 | 0.07 |
| Adonis | 2.89 | *** | 1.97 | * | 4.26 | *** | 1.55 | 0.08 |
| Anosim | 0.316 | *** | 0.119 | * | 0.567 | *** | 0.080 | 0.09 |

Dominant Subset Community (N=155)

| | Organic soil | | | | Mineral soil | | | |
|--------|--------------|----------|-----------|----------|--------------|----------|-----------|----------|
| | site | <i>P</i> | treatment | <i>P</i> | site | <i>P</i> | treatment | <i>P</i> |
| MRPP | 0.091 | *** | 0.041 | ** | 0.175 | *** | 0.023 | 0.06 |
| Adonis | 3.43 | *** | 2.17 | * | 6.97 | *** | 1.65 | 0.11 |
| Anosim | 0.260 | *** | 0.985 | * | 0.577 | *** | 0.753 | 0.10 |

NB: These results are from tests that were performed on the 99% identity OTUs from the open-reference OTU picking in QIIME, with singletons and doubletons removed and rarefied community. Statistical significance is indicated by * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), and *n.s.* (not significant).

Table S3a. Measures of diversity by warming treatment for each site separately for the total community (N=43,909)

| | SP (5 yrs) | | | BW (8 yrs) | | | PH (20 yrs) | | |
|-----------------------|------------|--------|----------|------------|--------|----------|-------------|--------|----------|
| | Control | Heated | <i>P</i> | Control | Heated | <i>P</i> | Control | Heated | <i>P</i> |
| Shannon's H | 6.22 | 6.14 | n.s. | 6.68 | 6.56 | n.s. | 6.34 | 6.56 | ** |
| Simpson's D | 0.990 | 0.990 | n.s. | 0.995 | 0.994 | n.s. | 0.991 | 0.993 | 0.07 |
| Inverse Simpsons | 113.0 | 112.0 | n.s. | 206.9 | 171.8 | n.s. | 126.6 | 148.4 | 0.08 |
| Alpha diversity | 1373.4 | 1267.3 | n.s. | 1667.8 | 1555.1 | n.s. | 1454.2 | 1599.4 | 0.06 |
| Richness (S) | 4384.0 | 4149.1 | n.s. | 5028.1 | 4795.5 | n.s. | 4574.5 | 4893.0 | 0.06 |
| Evenness (Pielou's J) | 0.742 | 0.738 | n.s. | 0.784 | 0.774 | n.s. | 0.752 | 0.772 | ** |

NB: Statistical significance is indicated by * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), and *n.s.* (not significant).

Table S3b. Measures of diversity by site for the total community (N=43,909)

| | SWaN Plots (5 years) | Barre Woods (8 years) | Prospect Hill (20 years) | R²_{adj} | F | <i>P</i> |
|-----------------------|-------------------------------------|--------------------------------------|---|------------------------------------|----------|-----------------|
| Shannon's H | 6.24 a | 6.68 b | 6.51 b | 0.335 | 12.8 | *** |
| Simpson's D | 0.9898 a | 0.9942 b | 0.9924 b | 0.265 | 9.46 | *** |
| Inverse Simpsons | 112.37 a | 189.86 b | 136.68 a | 0.392 | 16.1 | *** |
| Alpha diversity | 1706 a | 2015 b | 2012 b | 0.172 | 5.89 | ** |
| Richness (S) | 6101 | 6835 | 6992 | n.s. | | |
| Evenness (Pielou's J) | 0.717 a | 0.757 b | 0.737 ab | 0.27 | 9.7 | *** |

Table S4. Quantitative PCR of bacteria and fungi, reported mean copies of small subunit ribosomal RNA gene (copies) per gram soil (dry weight) plus or minus standard error. Significance is denoted as * $P < 0.05$, *n.s.* not significant. Bayesian inference (*BI*) is also used to compare group sizes, with mean effect size reported and boldface denoting credible differences in control versus heated group sizes. Values for bacteria and fungi are means across all three sites (N=48), while values for the Acidobacteria, Actinobacteria and Alphaproteobacteria are for the Prospect Hill organic soil horizon sites only (N=8).

| | Organic | | | | <i>P</i> | <i>BI</i> |
|----------------------|---------|----------|--------|----------|-------------|--------------|
| | Control | | Heated | | | |
| Bacteria | 3.88e9 | ± 9.15e8 | 4.18e9 | ± 6.71e8 | <i>n.s.</i> | 0.166 |
| Fungi | 1.39e9 | ± 7.03e8 | 5.64e8 | ± 2.12e8 | 0.07 | 0.744 |
| Acidobacteria | 1.32e8 | ± 1.25e8 | 3.10e8 | ± 1.13e8 | 0.08 | 1.31 |
| Actinobacteria | 3.43e8 | ± 8.81e7 | 3.94e8 | ± 8.09e7 | <i>n.s.</i> | 0.691 |
| Alpha-proteobacteria | 9.89e8 | ± 4.45e8 | 1.84e9 | ± 1.99e8 | * | 2.79 |
| | Mineral | | | | <i>P</i> | |
| | Control | | Heated | | | |
| Bacteria | 3.57e9 | ± 7.35e8 | 3.48e9 | ± 4.28e8 | <i>n.s.</i> | 0.063 |
| Fungi | 4.62e8 | ± 8.84e7 | 3.79e8 | ± 7.83e7 | <i>n.s.</i> | 0.475 |

Table S5. OTUs of changing abundance in warmed (“W”) compared to control (“C”) soils from Prospect Hill site. Tests performed were T1, IndVal; T2, volcano; T3, NSC; T4, pls-da; T5, Bayesian groups; T6, Rank abundance T7, Student T-test uncorrected; see Methods for further details about the tests performed.

| Phylum | Subphylum Taxonomy | OTU | Rel Abund Control (%) | Rel Abund Warmed (%) | Ratio C:W | T1 | T2 | T3 | T4 | T5 | T6 | T7 | Sum of Significance Tests |
|-----------------|--------------------------------------|--------------|-----------------------|----------------------|-----------|----|----|----|----|----|----|----|---------------------------|
| Acidobacteria | Acidobacteria; Acidobacteriales | NewOTU232430 | 0.073 | 0.388 | 0.188 | W | W | | | | W | | WWW |
| | Acidobacteria; Acidobacteriales | NewOTU321566 | 0.523 | 1.138 | 0.460 | W | W | | | | | | WW |
| | Acidobacteria; Acidobacteriales | NewOTU402783 | 0.061 | 0.125 | 0.488 | W | | | | | | | W |
| | Acidobacteria-2 | NewOTU216724 | 0.075 | 0.363 | 0.208 | W | W | | | | W | | WWW |
| | Acidobacteria-2 | NewOTU90931 | 0.053 | 0.247 | 0.215 | W | W | | | | | W | WWW |
| | Acidobacteria-2 | NewOTU17822 | 0.069 | 0.281 | 0.246 | W | W | | | | | | WW |
| | Acidobacteria-2 | NewOTU373042 | 0.403 | 1.132 | 0.356 | W | W | | | | | | WW |
| | Acidobacteria-2 | NewOTU216750 | 0.018 | 0.179 | 0.103 | | | | | | W | | W |
| | Acidobacteria-2 | NewOTU85375 | 0.017 | 0.074 | 0.229 | | W | | | | | | W |
| | Acidobacteria-2 | NewOTU331926 | 0.014 | 0.059 | 0.234 | W | | | | | | | W |
| Acidobacteria-2 | NewOTU337407 | 0.042 | 0.127 | 0.333 | | W | | | | | | W | |
| Actinobacteria | Actinobacteria; Actinomycetales | NewOTU241326 | 0.026 | 0.127 | 0.206 | W | W | | | | | | WW |
| | Actinobacteria; Actinomycetales | 1075732 | 0.245 | 0.871 | 0.282 | W | W | | | | | | WW |
| | Actinobacteria; Actinomycetales | NewOTU72804 | 0.062 | 0.155 | 0.401 | | W | | | | | | W |
| | Thermoleophilia; Solirubrobacterales | NewOTU379583 | 0.119 | 0.249 | 0.478 | | W | | | | | | W |
| Planctomycetes | Planctomycetia; Gemmatales | NewOTU285592 | 0.021 | 0.174 | 0.119 | W | | | | | | | W |
| | Planctomycetia; Planctomycetales | 938844 | 0.025 | 0.123 | 0.200 | | W | | | | | | W |
| Proteobacteria | Alpha-; Rhizobiales | NewOTU362740 | 0.019 | 0.105 | 0.184 | W | W | | | | W | | WWW |
| | Alpha-; Rhizobiales | NewOTU393214 | 0.142 | 0.753 | 0.189 | W | W | | | W | | | WWW |
| | Alpha-; Rhizobiales | NewOTU196341 | 0.025 | 0.142 | 0.173 | W | W | | | | | | WW |
| | Alpha-; Rhizobiales | 545247 | 0.126 | 0.445 | 0.283 | W | W | | | | | | WW |
| | Alpha-; Rhizobiales | NewOTU431587 | 1.782 | 2.760 | 0.646 | W | | W | | | | | WW |
| | Alpha-; Rhizobiales | NewOTU393499 | 0.080 | 0.259 | 0.309 | W | | | | | | | W |
| | Alpha-; Rhodospirillales | NewOTU211289 | 0.029 | 0.193 | 0.151 | W | | | | | | | W |
| | Delta-; Syntrophobacterales | NewOTU390196 | 0.045 | 0.178 | 0.251 | W | W | | | | | | WW |
| | Delta-; Syntrophobacterales | NewOTU307821 | 0.021 | 0.059 | 0.351 | | W | | | | | | W |
| | Gamma-; Xanthomonadales | NewOTU196148 | 0.045 | 0.228 | 0.195 | W | W | | | | | | WW |
| Acidobacteria | Acidobacteria; Acidobacteriales | NewOTU197652 | 0.668 | 0.435 | 1.535 | C | | | | | | | C |
| Actinobacteria | Actinobacteria; Actinomycetales | NewOTU5624 | 4.493 | 1.018 | 4.412 | C | C | C | C | | | C | CCCC |
| | Actinobacteria; Actinomycetales | NewOTU151494 | 0.667 | 0.062 | 10.716 | C | | | | | C | | CC |
| | Actinobacteria; Actinomycetales | NewOTU88918 | 0.154 | 0.044 | 3.509 | | | | | | C | | C |
| Proteobacteria | Alpha-; Rhizobiales | NewOTU162828 | 0.337 | 0.788 | 0.428 | | C | | | | | | C |
| | Alpha-; Rhizobiales | NewOTU126606 | 0.028 | 0.058 | 0.493 | | | | | | C | | C |
| | Alpha-; Rhizobiales | NewOTU323079 | 0.367 | 0.152 | 2.409 | C | | | | | | | C |
| | Alpha-; Rhodospirillales | NewOTU164307 | 0.380 | 0.051 | 7.485 | C | | | | C | C | | CCC |
| | Alpha-; Rhodospirillales | NewOTU357934 | 0.430 | 0.201 | 2.137 | C | C | | | | | | CC |
| | Alpha-; Rhodospirillales | NewOTU33857 | 0.313 | 0.099 | 3.155 | C | | | | | | | C |
| | Gamma-; Xanthomonadales | NewOTU327286 | 1.041 | 0.707 | 1.472 | C | | | | | | | C |
| | Gamma-; Xanthomonadales | NewOTU230354 | 0.425 | 0.209 | 2.033 | C | | | | | | | C |

Figure S1. Rarefaction curves for each of the 48 samples from the Harvard Forest long-term warming experiment.

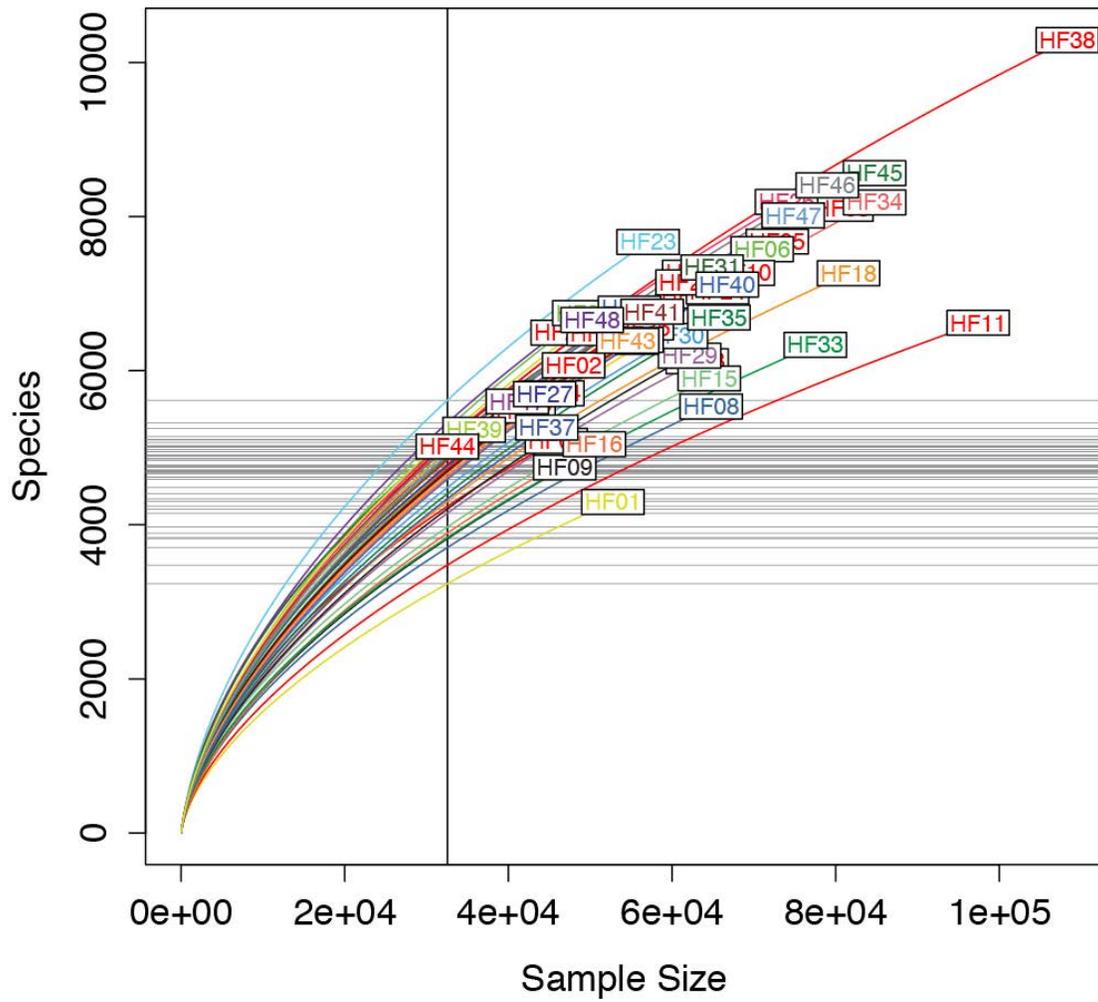
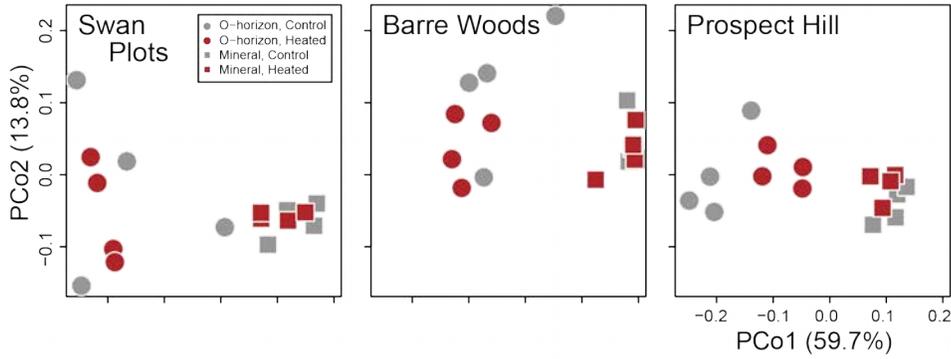
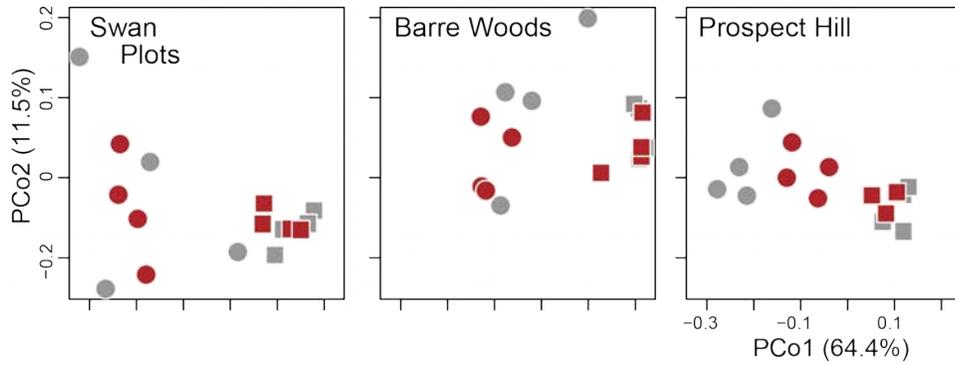


Figure S2. Ordination for (A) whole community, (B) dominant subset of taxa, and (C) copy number corrected dominant subset of taxa.

A. All samples, no singletons or doubletons (N=43,909)



B. Dominant OTUs only, >0.1% relative abundance (N=155)



C. Dominant OTUs only with copy number corrected (N=155)

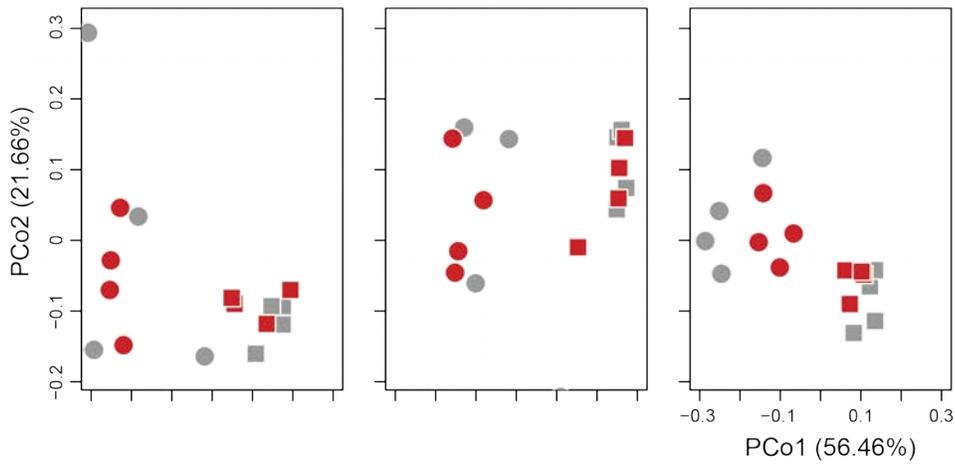


Figure S3. Plot of estimated 16S ribosomal RNA copy number by relative abundance for the dominant subset community (n=155) in the Prospect Hill (20-year warmed) organic horizon soil microbial communities. Estimated 16S ribosomal RNA copy number is calculated based on phylogenetic inference; see methods for details. While the most abundant taxa (the top 15) in these forest soils tend to have only a few copies of the 16S rRNA operon, taxa that ranked in the top 155 (but not the top 15) tended to have more copies than average and would have been overestimated in their abundance

