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Supplemental Information

Environmental drivers and cryptic biodiversity hotspots define endophytes in Earth's largest terrestrial biome

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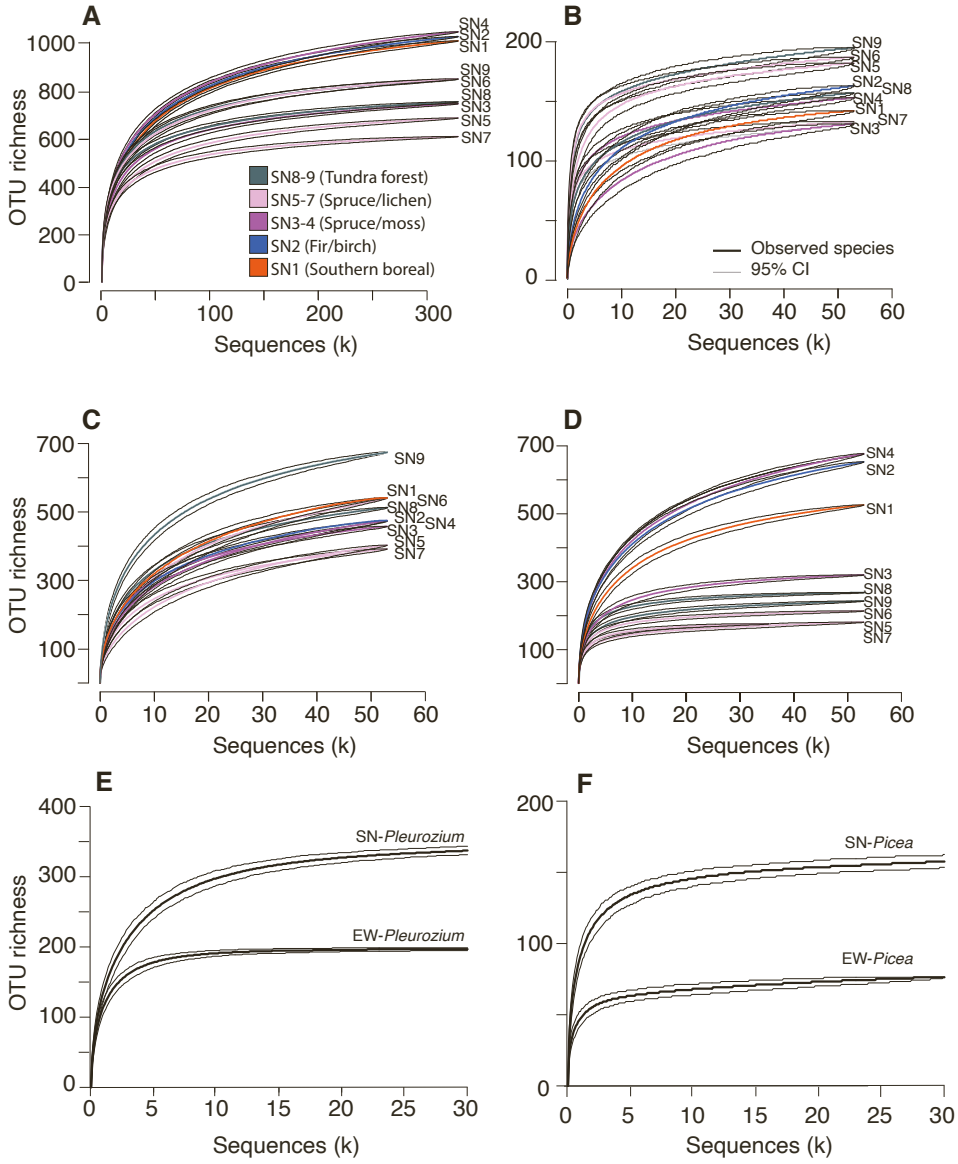

Figure S1. Rarefaction of endophytic fungal communities associated with boreal hosts, Related to STAR Methods. **(A)** All sites across the transboreal SN gradient, and each focal host shown separately on that gradient: **(B)** *Picea*, **(C)** *Pleurozium,* and **(D)** *Cladonia*; **(E)** comparison of transboreal (SN) and longitudinal (EW) gradient for *Pleurozium*, and **(F)** for *Picea*. For panels **A-D**, lines are colored according to Figure 1A, and each sample was subsampled to 17,672 reads (after removal of samples with < 5000 reads). For panels **E-F**, data from the SN transect were sub-setted to match the same number of samples as the EW transect, and OTU with < 25 reads were removed from the analyses (see Methods; results are similar when all OTU were included). Thin grey lines represent 95% confidence intervals around the means.

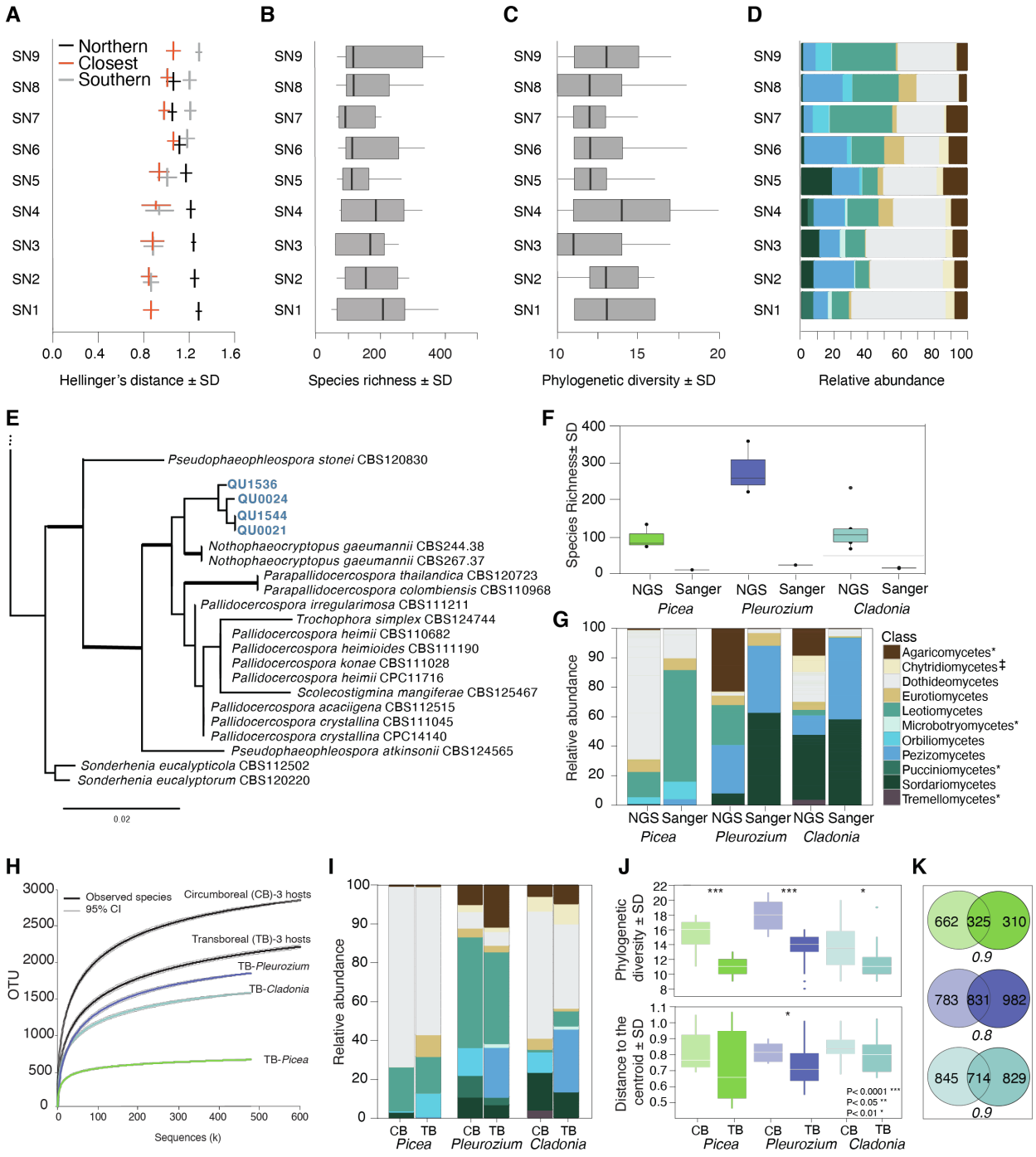

Figure S2. Summary of transboreal data for all host species from culture-free Illumina analyses and culture-based collections, and comparison of transboreal and circumboreal data sets, Related to Figure 2, Figure 3, and STAR Methods. (**A**) Beta diversity (mean ± SD), (**B**) species richness, (**C**) phylogenetic diversity, and (**D**) phylogenetic composition of endophytes from three hosts at a transboreal scale. Phylogenetic diversity was calculated as the number of fungal classes per sample. Colors for fungal classes follow Figure 2B. Beta diversity was calculated as Hellinger's distance between endophyte communities in the closest site vs. the southernmost (SN1) and northernmost (SN9) site. For example, the endophyte community in the southernmost site, SN1 was more similar to that in SN2 (lower Hellinger's

distance) than in the northernmost site (higher Hellinger's distance). **(E)** Phylogenetic analysis of four cultured representatives of the dominant Capnodiales OTU in *Picea* from the transboreal transect (shown in blue) illustrates a sister relationship to the pathogen of Douglas fir, *Nothophaeocryptopus gaeumannii*. The tree represents a subset of the phylogenetic analysis of LSU nrDNA (see Methods). Thickened branches indicate bootstrap support > 70%. **(F-G)** Comparison of culture-free NGS and culturing on the richness (**F**) and taxonomic composition (**G**) of endophytes of *Picea, Pleurozium,* and *Cladonia* at SN5. All sequences and OTU were included in analyses. Taxonomic comparisons were limited to the most abundant classes for visualization purposes. Symbols denote non-Ascomycota classes (i.e., Basidiomycota: asterisk; Chytridiomycota: double dagger). Comparison with cultures revealed seventeen-fold greater richness obtained by NGS compared to culturing. When Illumina data were subsampled to match the total number of sequenced cultures per sample, NGS provided an approximately twofold increase in richness relative to culturing. **(H)** Richness from transboreal sampling (TB) in Québec (SN1-SN9) vs. circumboreal sampling (CB) of the same host genera (*Picea, Pleurozium, Cladonia*) across Eurasia and North America, with the same methods for culture-free analyses[4]. CB methods and details are in ref. 4. **(I)** Composition of endophyte communities obtained by barcode amplicon sequencing for TB vs. CB (symbols denote non-Ascomycota). **(J)** Phylogenetic and beta dispersion (asterisks indicate significant differences, CB vs TB). **(K)** For CB (lighter) and TB (darker), distinctive and shared species and Hellinger community distance (below circle).