Supplemental Information for:

Bark coverage shifts assembly processes of microbial decomposer communities in dead wood

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Proceedings B

https://doi.org/10.1098/rspb.2019.1744

Appendix S1 Primers and PCR protocol.

Each PCR was carried out in triplicate. The 25-µl reactions each contained 12.5 µl of GoTaq Green Mastermix (Promega, Madison, USA), 1 µl of *forward* primer mix (10 µM), 1 µl of *reverse* primer mix (10 µM), 9.5 µl of nuclease-free water, and 1 µl of template DNA. Fungal ITS2-rRNA was amplified as follows: denaturation for 5 min at 95 °C followed by 32 cycles at 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min 15 s, and a final elongation step at 72 °C for 10 min. Bacterial 16S rRNA genes were amplified as follows: denaturation for 3 min at 94 °C, followed by 29 cycles at 94 °C for 45 s, 50 °C for 1 min, 72 °C for 1 min 30 s, and a final elongation step at 72 °C for 10 min. PCR products were quality checked by separation on a 1.5% agarose gel. The products of each triplicate reaction were recovered from the agarose gel, pooled and then purified using the innuPREP gel extraction kit (Analytik Jena, Jena, Germany) by following the manufacturer's protocol. The PCR yield was quantified spectrophotometrically using Quant-iTTM PicoGreen® dsDNA reagent (Thermo Fisher Scientific Inc., Waltham, MA, USA) according to Ahn et al. [1].

Table S1.1: Primers used in this study.

Primer name	Primer sequence 5′–3′
P5-5N-ITS4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNTCCTCCGCTTATTGATATGC
P5-6N-ITS4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNTCCTCCGCTTATTGATATGC
P7-3N-fITS7	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNGTGARTCATCGAATCTTTG
P7-4N-fITS7	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNGTGARTCATCGAATCTTTG
P5-8N-515F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGTGCCAGCMGCCGCGGTAA
P5-7N-515F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNGTGCCAGCMGCCGCGGTAA
P7-2N-806r	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNGGACTACHVGGGTWTCTAAT
P7-1N-806r	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNGGACTACHVGGGTWTCTAAT
P5-index	AATGATACGGCGACCACCGAGATCTACACiiiiiiii ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
P7-index	CAAGCAGAAGACGGCATACGAGATiiiiiiii GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T

https://doi.org/10.1098/rspb.2019.1744

Appendix S2 Diversity of the microbial phyla.

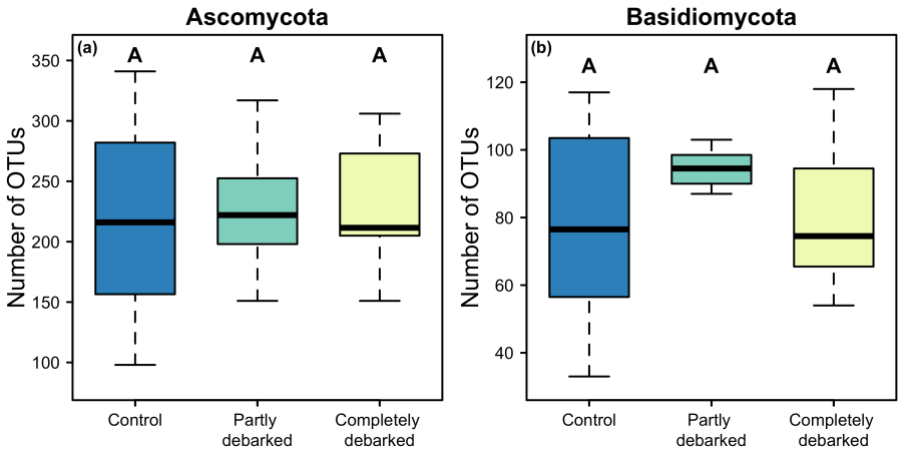

Figure S2.1: Fungal species richness as defined by the number of OTUs assigned to the phyla (a) Ascomycota and (b) Basidiomycota.

https://doi.org/10.1098/rspb.2019.1744

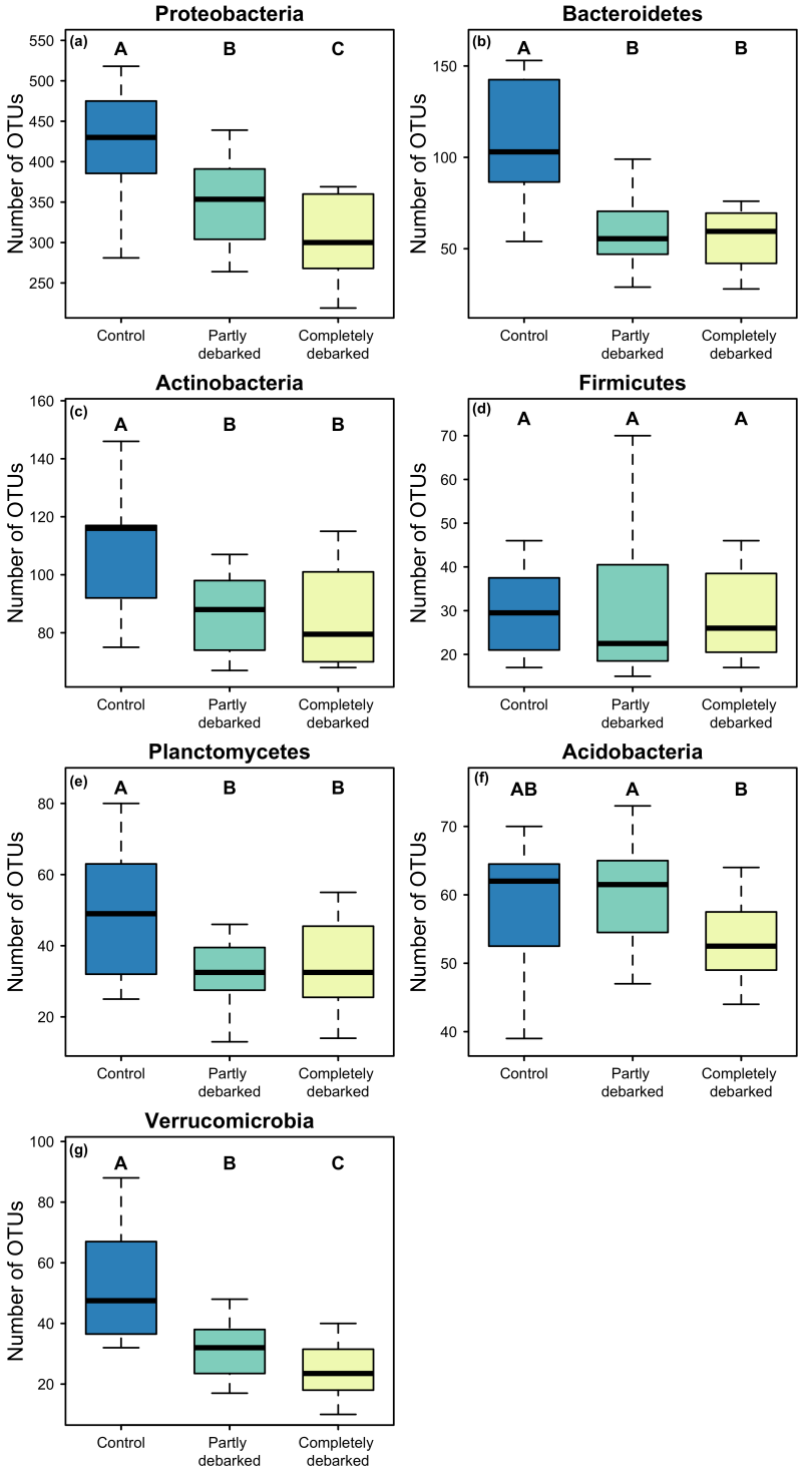

Figure S2.2: Bacterial species richness as defined by the number of OTUs assigned to the phyla (a) Proteobacteria, (b) Bacteroidetes, (c) Actinobacteria, (d) Firmicutes, (e) Planctomycetes, (f) Acidobacteria and (g) Verrucomicrobia.

https://doi.org/10.1098/rspb.2019.1744

Appendix S3 Phylogenetic trees of bacteria and fungi.

For the bacterial phylogeny, the 16S rRNA gene region was directly used for the phylogenetic inferences. The sequences were aligned using MAFFT [2] and the multiple sequence alignment was then subjected to maximum likelihood tree inference using FastTree [3], with parameters as in Kembel et al. [4]. Fungal phylogeny inferences were performed used the BLASTn best hit for fungal 5.8S rRNA. The sequences were aligned using MAFFT and the topology and branch lengths were estimated using RAxML [5] on the CIPRES Science Gateway [6]. However, because fungal 5.8S rRNA can be uninformative, averaged taxonomic distances were also computed (function *taxa2dist*, R package *vegan* [7]) based on taxonomic information, followed by calculation of the beta mean nearest taxon distance (βMNTD) based on a patristic phylogenetic (function *cophenetic*, R package *stats*) and taxonomic distance matrix. Given the strong correlation between the two βMNTD matrices in the Procrustes analysis (R^2=0.90, p=0.001); only the results based on the phylogeny are presented.

https://doi.org/10.1098/rspb.2019.1744

Appendix S4 List of fungal and bacterial OTUs and their corresponding taxonomic classifications.

The lists are given in an Excel file. For fungi, the traits extracted from FUNGuild [8] and the resulting classification of the OTUs of wood-decaying fungi are presented in the list.

Appendix S5 Frequency distribution based on the sequence data.

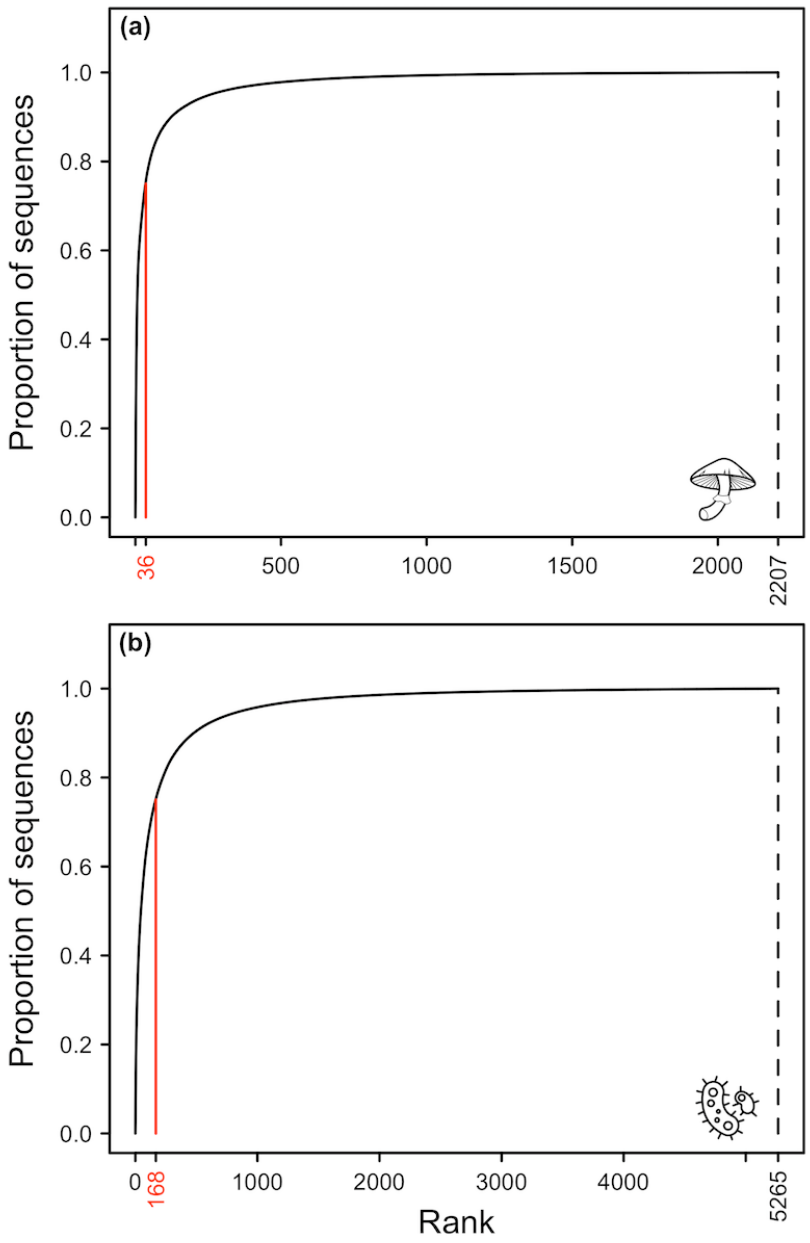

Figure S5.1: Accumulation curves in decreasing order of (a) fungal and (b) bacterial OTU abundances (rank). The vertical red lines indicate the number of OTUs comprising 75% of all sequences and therefore the most abundant sequences. Vertical dashed lines indicate the total number of OTUs corrected for the number of singletons.

Appendix S6 Microbial species richness.

Table S6.1: Results of the generalized linear mixed-effects models for the species richness of fungi, the subset of wood-decaying fungi and bacteria. The effects of bark coverage (i.e., control, partly debarked and completely debarked) and canopy openness (sun-exposed and shaded sites) were tested. The interaction between bark coverage and canopy openness was tested to investigate whether the effects of bark coverage treatment differed between sun-exposed and shaded sites. Values in bold indicate significant effects.

	Estimate	**Std. error**	**z-value**	**p-value**
Fungi				
Control - Partly debarked	0.056	0.109	0.516	0.606
Control - Completely debarked	0.083	0.109	0.767	0.443
Partly debarked - Completely debarked	0.027	0.107	0.251	0.802
Sun-exposed - Shaded	−0.003	0.088	−0.029	0.977
Control : Sun-exposed - Shaded	−0.218	0.151	−1.448	0.148
Partly debarked : Sun-exposed - Shaded	0.166	0.146	1.137	0.256
Completely debarked : Sun-exposed - Shaded	0.046	0.144	0.320	0.749
Subset of wood-decaying fungi				
Control - Partly debarked	**0.301**	**0.070**	**4.298**	**<0.001**
Control - Completely debarked	**0.364**	**0.069**	**5.257**	**<0.001**
Partly debarked - Completely debarked	0.062	0.064	0.982	0.326
Sun-exposed - Shaded	−0.006	0.055	−0.116	0.908
Control : Sun-exposed - Shaded	0.078	0.105	0.744	0.457
Partly debarked : Sun-exposed - Shaded	−0.009	0.090	−0.099	0.922
Completely debarked : Sun-exposed - Shaded	−0.029	0.087	−0.334	0.738
Bacteria				
Control - Partly debarked	**−0.263**	**0.067**	**−3.951**	**<0.001**
Control - Completely debarked	**−0.352**	**0.068**	**−5.151**	**<0.001**
Partly debarked - Completely debarked	−0.089	0.073	−1.226	0.220
Sun-exposed - Shaded	−0.063	0.062	−1.023	0.306
Control : Sun-exposed - Shaded	−0.035	0.090	−0.386	0.699
Partly debarked : Sun-exposed - Shaded	0.129	0.102	1.271	0.204
Completely debarked : Sun-exposed - Shaded	0.130	0.106	1.227	0.220

Appendix S7 Interpolation and extrapolation of Hill numbers.

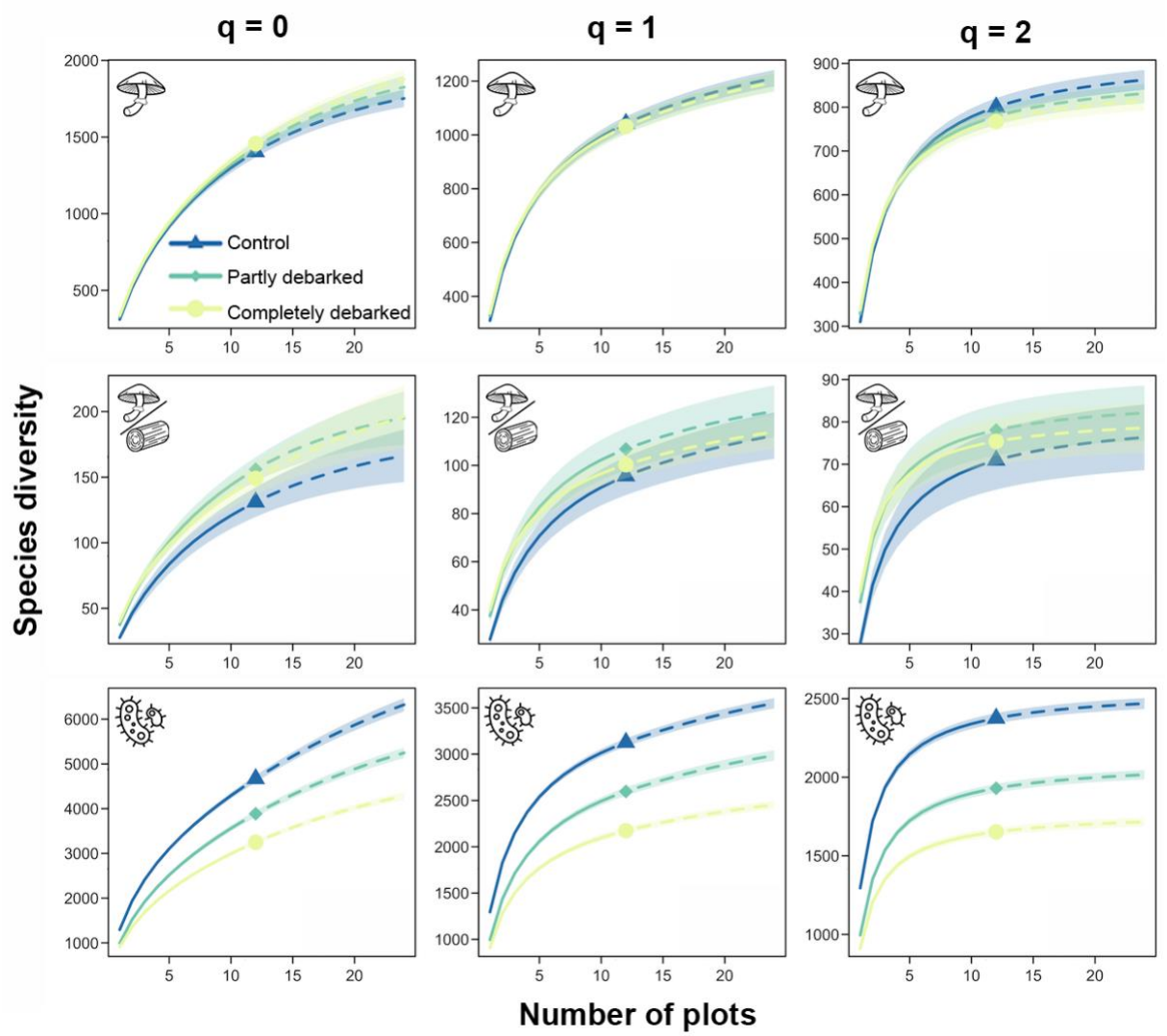

Figure S7.1: Sample-based rarefaction (solid lines) and extrapolation (dotted lines, up to twice the actual sample size) of the data for fungi, wood-decaying fungi and bacteria for control, partly debarked and completely debarked felled Norway spruce trees. The 95% unconditional confidence intervals (transparent shading) are also shown. Species diversity was estimated for Hill numbers: q = 0 (species richness, left panel), q = 1 (exponential of Shannon's entropy index, middle panel) and q = 2 (inverse of Simpson's concentration index, right panel). Solid symbols represent the total number of reference samples.

https://doi.org/10.1098/rspb.2019.1744

Appendix S8 Correlation of incidence and abundance.

In nature, species abundance and the size of the area over which those species are recorded (species incidence) are not independent [9]. To test whether the performance of the number of sequences representing one OTU was similar to that of species abundance, quasi-Poisson linear models were separately applied for the number of sequences and OTU incidences indicated in the fungal and bacterial OTU datasets. The incidence ranged from 1 (present only on one sampled tree) to 36 (present on all 36 sampled trees).

The number of colonized trees (incidence) correlated strongly with the number of OTU sequences (Fig. S8.1) and therefore with the species abundance.

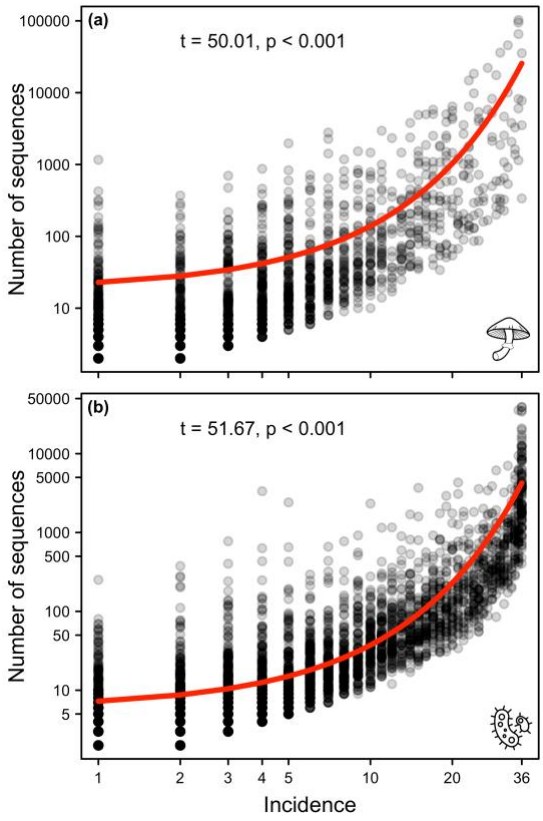

Figure S8.1: Correlation of OTU incidence (number of trees [maximum 36] with an OTU,) and OTU abundance (number of sequences) for (a) fungi and (b) bacteria. Note the log scale of the axes.

Appendix S9 Microbial community compositions.

Table S9.1: Effects of bark removal and canopy openness (sun-exposed and shaded sites) on the community composition of fungi, the subset of wood-decaying fungi, and bacteria (Bray-Curtis dissimilarity), both overall and for pairwise comparisons between trees of different bark coverage.

	Comparison	Variable	F statistic	Partial R^2	Adj. p value	
Fungi	Overall	Bark coverage	2.688	0.135	0.008	**
	Overall	Canopy openness	2.384	0.060	0.008	**
	Control - Partly debarked	Bark coverage	2.142	0.086	0.008	**
	Control - Partly debarked	Canopy openness	1.867	0.075	0.008	**
	Control - Completely debarked	Bark coverage	3.377	0.129	0.008	**
	Control - Completely debarked	Canopy openness	1.898	0.072	0.008	**
	Partly debarked - Completely debarked	Bark coverage	2.569	0.100	0.024	*
	Partly debarked - Completely debarked	Canopy openness	2.107	0.082	0.024	*
Subset of wood-decaying fungi	Overall	Bark coverage	2.968	0.151	0.008	**
	Overall	Canopy openness	1.463	0.037	0.008	**
	Control - Partly debarked	Bark coverage	2.685	0.108	0.016	*
	Control - Partly debarked	Canopy openness	1.242	0.050	0.016	*
	Control - Completely debarked	Bark coverage	3.516	0.137	0.016	*
	Control - Completely debarked	Canopy openness	1.200	0.046	0.016	*
	Partly debarked - Completely debarked	Bark coverage	2.658	0.106	0.008	**
	Partly debarked - Completely debarked	Canopy openness	1.428	0.057	0.008	**
Bacteria	Overall	Bark coverage	2.910	0.146	0.008	**
	Overall	Canopy openness	2.097	0.053	0.008	**
	Control - Partly debarked	Bark coverage	2.799	0.110	0.008	**
	Control - Partly debarked	Canopy openness	1.564	0.062	0.008	**
	Control - Completely debarked	Bark coverage	3.983	0.148	0.008	**
	Control - Completely debarked	Canopy openness	1.925	0.072	0.008	**
	Partly debarked - Completely debarked	Bark coverage	2.058	0.082	0.040	*
	Partly debarked - Completely debarked	Canopy openness	1.951	0.078	0.040	*

https://doi.org/10.1098/rspb.2019.1744

Appendix S10 Rank abundance distribution.

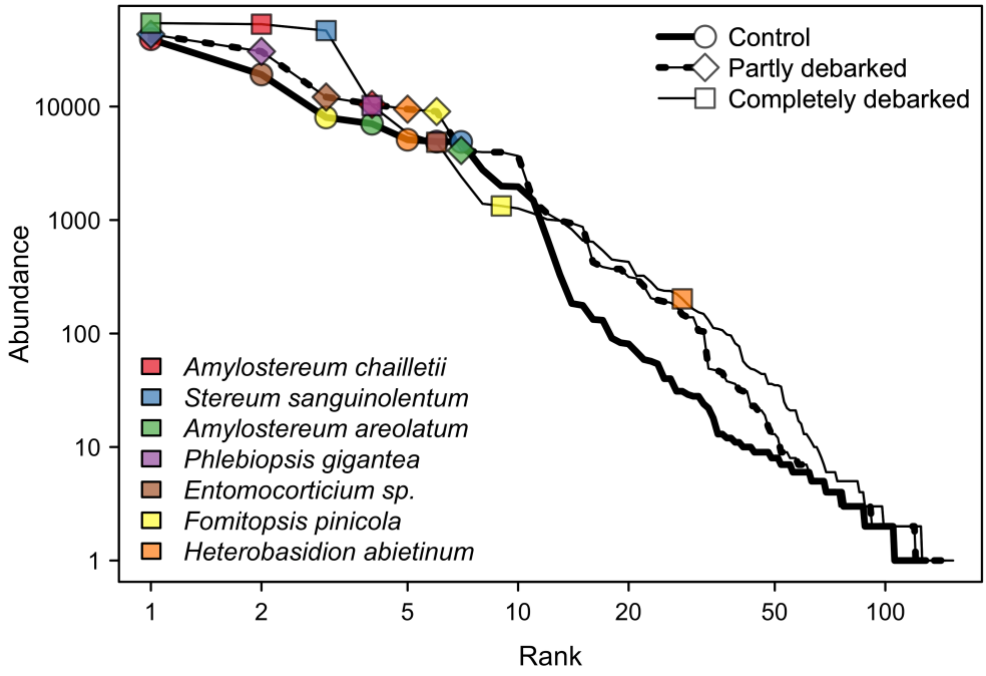

Figure S10.1: Rank abundance distribution of wood-decaying fungi (subset of all fungal OTUs) belonging to the communities on trees differing in their bark coverage (i.e., control with bark, partly debarked, completely debarked). The positions of the seven over all most abundant (highest number of sequences) wood-decaying fungi are highlighted by color-coded symbols for trees of different bark coverage. Note the log scale of the axes.

Appendix S11 Spatial dissimilarity of the microbial communities.

Mantel statistics (*mantel* function of R package *vegan* [7]) were used to test for the presence of a spatial pattern of community dissimilarly. The Euclidean distance between sampled trees was calculated (*pointDistance* function of R package *raster* [10]) as was the Bray-Curtis dissimilarly between communities of fungi and of bacteria (*vegdist* function of R package *vegan* [7]).

There was no spatial pattern of community dissimilarity for either fungi or bacteria for a local landscape scale of up to 14 km distance between experimental trees (Fig. S11.1). This confirmed a previous report on wood-inhabiting fungi, in which a spatial scale of > 600 km between dead-wood objects was determined [11]. Thus, wood-inhabiting fungi and bacteria had no dispersal limitations on a landscape scale [12]. This finding was consistent with the dominant relevancies of the properties of a single dead-wood object (e.g., bark coverage) and the microclimate for microbial communities.

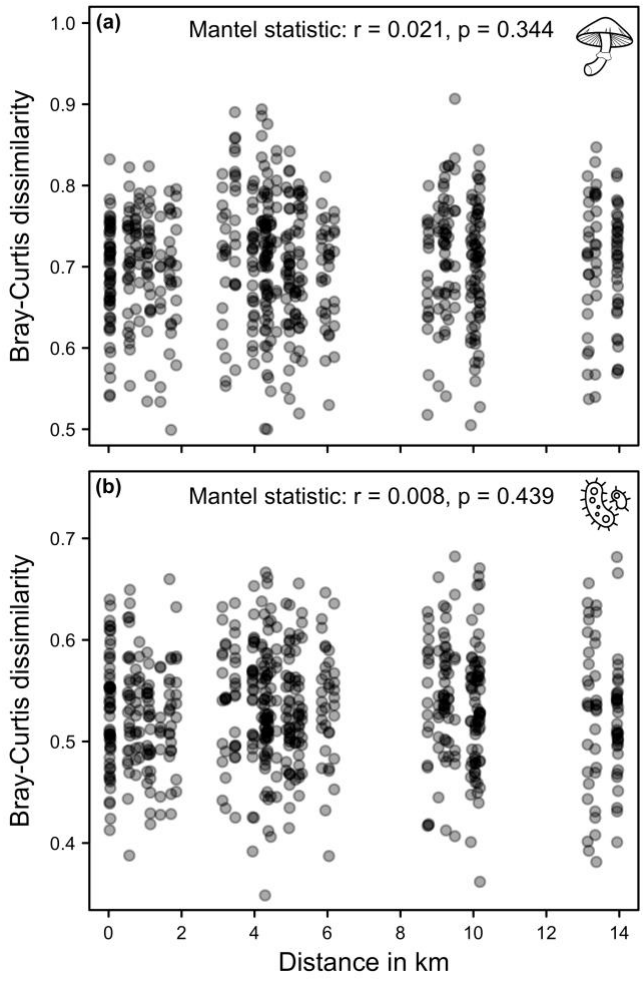

Figure S11.1: Spatial dissimilarity in the (a) fungal and (b) bacterial communities as measured by the Bray-Curtis dissimilarity.

References

1. Ahn SJ, Costa J, Emanuel JR. 1996 PicoGreen quantitation of DNA: effective evaluation of samples pre- or post-PCR. *Nucleic Acids Res.* **24**, 2623–2625. (doi:10.1093/nar/24.13.2623)
2. Katoh K, Standley DM. 2013 MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780. (doi:10.1093/molbev/mst010)
3. Price MN, Dehal PS, Arkin AP. 2009 Fasttree: computing large minimum evolution trees with profiles instead of a distance matrix. *Mol. Biol. Evol.* **26**, 1641–1650. (doi:10.1093/molbev/msp077)
4. Kembel SW, O'Connor TK, Arnold HK, Hubbell SP, Wright SJ, Green JL. 2014 Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proc. Natl. Acad. Sci.* **111**, 13715–13720. (doi:10.1073/pnas.1216057111)
5. Stamatakis A. 2014 RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313. (doi:10.1093/bioinformatics/btu033)
6. Miller MA, Pfeiffer W, Schwartz T. 2011 The CIPRES science gateway: a community resource for phylogenetic analyses. *Proc. 2011 TeraGrid Conf. Extrem. Digit. Discov.* , Article No. 41. (doi:10.1145/2016741.2016785)
7. Oksanen J *et al.* 2016 vegan: community ecology package.
8. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016 FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **20**, 241–248. (doi:10.1016/j.funeco.2015.06.006)
9. Gaston KJ, Lawton JH. 1990 Effects of scale and habitat on the relationship between regional distribution and local abundanc. *Oikos* **58**, 329–335. (doi:10.2307/3545224)
10. Hijmans RJ. 2016 raster: geographic data analysis and modeling.
11. Purahong W, Arnstadt T, Kahl T, Bauhus J, Kellner H, Hofrichter M, Krüger D, Buscot F, Hoppe B. 2016 Are correlations between deadwood fungal community structure, wood physico-chemical properties and lignin-modifying enzymes stable across different geographical regions? *Fungal Ecol.* **22**, 98–105. (doi:10.1016/j.funeco.2016.01.002)
12. Komonen A, Müller J. 2018 Dispersal ecology of dead wood organisms: implications for connectivity conservation. *Conserv. Biol.* **32**, 535–545. (doi:10.1111/cobi.13087)