# **Supporting Information**

### Zimmerman and Vitousek 10.1073/pnas.1209872109



Fig. S1. Rarefaction curves of OTU richness using the Chao1 richness estimator. Each gray line represents the mean of five rarefaction runs. OTUs were designated based on 95% similarity of the ITS1 rDNA region.

	Sequences	Percentage	OTUs
Fungi			
Ascomycota			
Dothideomycetes			
Dothideomycetidae	239,769	36.0	428
Pleosporomycetidae	26,422	4.0	130
Dothideomycetes incertae sedis	6,241	0.9	96
Sordariomycetes			
Xylariomycetidae	19,199	2.9	131
Sordariomycetidae	10,054	1.5	79
Hypocreomycetidae	3,785	0.6	69
Sordariomycetes incertae sedis	43	<0.1	11
Unknown	109	<0.1	16
Leotiomycetes	5,347	0.8	41
Eurotiomycetidae	2,449	0.4	49
Chaetothyriomycetidae	1,531	0.2	52
Mycocaliciomycetidae	1,054	0.2	42
Lecanoromycetidae	88	<0.1	14
Mitosporic Saccharomycetales	203	<0.1	12
Saccharomycetaceae	93	<0.1	4
Unknown	6,662	1.0	132
Basidiomycota	2,639	0.4	187
Chytridiomycetes	374	0.1	9
Streptophyta	2,200	0.3	16
No hits	68,546	10.3	1,221
Not assigned/environmental sample	268,347	40.3	1,514
Total	665,155	100	4,253

## Table S1. Summary of common taxa determined via a BLASTn search of GenBank

Summary includes the number of sequences, the percentage of the total number of sequences that each represented, and the number (based on 95% rDNA ITS1 similarity) of OTUs attributed to each taxon. "Not assigned/environmental sample" refers to those sequences that had a match in GenBank but the best match was to an environmental sample of unidentified taxonomic identity. "No hits" refers to sequences that had no matches to GenBank with an acceptable e-value, either because no similar sequences were found or because sequences were of low complexity or too short to produce good matches. Also note that the majority of Chytridiomycetes identified were in the genus *Rhyzophidium*, members of which have previously been found in canopy debris (1).

1. Longcore JE (2005) Zoosporic fungi from Australian and New Zealand tree-canopy detritus. Aust J Bot 53:259–272.

#### Table S2. Per-tree diversity summary

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	Samples	Seque	ences	Sobs	;	Nonsing	gles	Chao	01	Shannor	n (H)	Fisher's	α
	(n)	Mean (±SD)	Range	Mean (±SD)	Range	Mean (±SD)	Range	Mean ( $\pm$ SD)	Range	Mean (±SD)	Range	Mean (±SD)	Range
Total	130	5,117 ± 1,575	739–7,897	105 ± 42	40–257	68 ± 30	16–190	157 ± 68	62–359	1.87 ± 0.85	0.1–3.8	19 ± 9	6–58
W100Y	10	5,918 ± 957	4,388–7,665	162 ± 51	45–228	$106 \pm 43$	16–169	233 ± 47	180–352	1.9 ± 1.1	0.1–3.6	31 ± 11	6–47
W100O	10	5,716 ± 1,170	3,289–7,092	$160 \pm 73$	40–257	$103 \pm 57$	18–190	232 ± 87	69–329	2.1 ± 1.2	0.1–3.8	32 ± 17	6–58
W700Y	10	5,909 ± 1,161	4,094–7,897	95 ± 28	65–154	60 ± 27	37–116	146 ± 32	90–199	$1.3 \pm 0.6$	0.5–2.6	$16 \pm 6$	10–29
W700O	10	6,196 ± 955	4,474–7,805	79 ± 23	53–113	48 ± 20	28–78	136 ± 44	86–215	$1.1 \pm 0.6$	0.3–1.8	13 ± 5	8–20
W1100Y	10	2,509 ± 1,312	739–5,092	93 ± 22	55–118	80 ± 20	44–103	$103 \pm 23$	62–133	$3.2 \pm 0.4$	2.2–3.6	$20 \pm 3$	14–24
W1100O	10	3,019 ± 1,016	1,508–4,470	86 ± 11	67–97	$70 \pm 9$	54–80	98 ± 16	73–126	$2.2 \pm 0.8$	1.2–3.5	17 ± 3	12–21
W1800Y	10	5,932 ± 1,159	3,681–7,536	123 ± 22	100–157	68 ± 8	59–88	$210~\pm~79$	105–347	$1.8 \pm 0.6$	1.0-3.0	22 ± 4	18–29
W1800O	10	4,973 ± 1,450	2,015–6,553	112 ± 15	82–131	68 ± 9	57–89	175 ± 42	94–245	$1.8 \pm 0.4$	0.9–2.4	21 ± 3	15–24
W2400Y	10	5,020 ± 1,254	3,319–6,795	84 ± 11	67–96	$51 \pm 10$	34–67	129 ± 35	78–184	$1.2 \pm 0.5$	0.8–2.3	14 ± 2	11–16
W2400O	10	4,512 ± 1,393	2,208–6,552	92 ± 21	59–118	55 ± 12	31–73	146 $\pm$ 43	72–196	$1.2 \pm 0.5$	0.3–2.0	$16 \pm 4$	10–22
D700Y	10	5,670 ± 1,102	3,853–7,019	83 ± 18	63–126	$51 \pm 13$	32–73	$130~\pm~49$	74–218	$1.8 \pm 0.3$	1.2–2.1	$14 \pm 4$	10–23
D700O	10	5,533 ± 1,115	3,816–7,499	82 ± 23	45–110	58 ± 22	24–84	$108\pm19$	88–150	$2.0\pm0.6$	1.0–2.8	$14 \pm 5$	6–19
D1700Y	10	5,610 ± 1,144	3,698–7,109	121 ± 43	74–213	73 ± 21	47–119	$194 \pm 95$	109–359	$2.5 \pm 0.5$	1.7–3.1	22 ± 9	13–42

Includes means, SDs, and ranges for the number of sequences, OTUs observed ( $S_{obs}$ ), nonsingleton OTUs, the Chao1 richness estimator, the Shannon diversity index, and Fisher's  $\alpha$  index for trees. D, dry; O, old; W, wet; Y, young.

#### Table S3. Per-site diversity summary

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	Samples									Inverse β
	(n)	Sequences	Sobs	Nonsingles	Chao1	Bootstrap	Shannon (H)	Fisher's (α)	$\beta$ (site/tree)	(tree/site)
All sites, mean $\pm$ SD	13	51,170 ± 11,465	551 ± 134	341 ± 94	872 ± 231	692 ± 164	$2.4\pm0.8$	87 ± 24	5.35 ± 0.27	0.19 ± 0.01
(range)		(25,092–61,955)	(401–829)	(255–550)	(570–1,260)	(500–1,032)	(1.4–3.7)	(59–137)	(4.76–5.65)	(0.18–0.21)
W100Y	1	59,175	771	516	1,077	953	2.7	125	4.76	0.21
W100O	1	57,159	829	550	1,260	1,032	2.9	137	5.18	0.19
W700Y	1	59,090	519	323	818	653	1.8	78	5.46	0.18
W700O	1	61,955	418	260	648	524	1.5	60	5.29	0.19
W1100Y	1	25,092	463	365	579	580	3.7	81	4.98	0.20
W1100O	1	30,191	454	350	570	572	2.7	76	5.28	0.19
W1800Y	1	59,322	652	344	1,160	817	2.1	102	5.30	0.19
W1800O	1	49,728	586	326	982	739	2.3	93	5.23	0.19
W2400Y	1	50,195	471	267	759	597	1.5	72	5.61	0.18
W2400O	1	45,124	510	283	899	646	1.4	81	5.54	0.18
D700Y	1	56,696	469	260	814	594	2.5	70	5.65	0.18
D700O	1	55,326	401	255	653	500	2.8	59	4.89	0.20
D1700Y	1	56,102	621	333	1,113	783	3.6	98	5.13	0.19

Includes means, SDs, and ranges for the total number of sequences, OTUs observed ( $S_{obs}$ ), nonsingleton OTUs, the Chao1 richness estimator, the bootstrap richness estimator, the Shannon diversity index, and Fisher's  $\alpha$  index for sites.  $\beta$ -diversity using the given site or location as a base is also included as Whittaker's multiplicative  $\beta$ , as well as the inverse of that value. The inverse value represents the proportion of  $\gamma$  (total) richness captured by the mean value of  $\alpha$  (local) richness. D, dry; O, old; W, wet; Y, young.

#### Table S4. Per-location diversity summary

	Samples (n)	Sequences	S <sub>obs</sub>	Nonsingles	Chao1	Bootstrap	Shannon (H)	Fisher's $\alpha$	$\beta$ (location/site)	Inverse β (site/location)
All locations, mean ± SD (range)	7	95,020 ± 28,021 (56,102–121,045)	848 ± 205 (708–1,229)	519 ± 155 (417–817)	1,340 ± 295 (999–1,793)	1,066 ± 249 (783–1,521)	2.7 ± 0.8 (1.5–3.6)	129 ± 33 (98–192)	1.63 ± 0.05 (1.54–1.68)	0.61 ± 0.02 (0.6–0.65)
W100	1	116,334	1,229	817	1,793	1,521	2.9	192	1.54	0.65
W700	1	121,045	782	480	1,246	989	1.8	112	1.67	0.60
W1100	1	55,283	769	584	999	971	3.6	126	1.68	0.60
W1800	1	109,050	1,005	547	1,655	1,262	2.3	153	1.62	0.62
W2400	1	95,319	822	453	1,418	1,044	1.5	124	1.68	0.60
D700	1	112,022	708	417	1,157	891	2.8	101	1.63	0.61
D1700	1	56,102	621	333	1,113	783	3.6	98	NA	NA

Includes means, SDs, and ranges for the total number of sequences, OTUs observed ( $S_{obs}$ ), nonsingleton OTUs, the Chao1 richness estimator, the bootstrap richness estimator, the Shannon diversity index, and Fisher's  $\alpha$  index for locations.  $\beta$ -diversity using the given location or site as a base is also included as Whittaker's multiplicative  $\beta$ , as well as the inverse of that value. The inverse value represents the proportion of  $\gamma$  (total) richness captured by the mean value of  $\alpha$  (local) richness. D, dry; O, old; W, wet; Y, young.

#### Table S5. Landscape diversity summary

	Samples (n)	Sequences	S <sub>obs</sub>	Nonsingles	Chao1	Bootstrap	Shannon (H)	Fisher's $\alpha$	$\beta$ (landscape/location)	Inverse β (location/landscape)
Landscape	1	665,155	4,253	2,552	6,812	5,320	3.6	608	5.01	0.20

Includes sequences, OTUs observed ( $S_{obs}$ ), nonsingleton OTUs, the Chao1 richness estimator, the bootstrap richness estimator, the Shannon diversity index, and Fisher's  $\alpha$  index for the entire landscape.  $\beta$ -diversity using the given landscape or location as a base is also included as Whittaker's multiplicative  $\beta$ , as well as the inverse of that value. The inverse value represents the proportion of  $\gamma$  (total) richness captured by the mean value of  $\alpha$  (local) richness.

Table	S6.	Sensitivity	of	analyses	to	community	distance	metrics:
PERM		/A						

	PERMANOVA using distance matrices				
Community	R <sup>2</sup>	Р			
Chao					
Rainfall	0.35986	0.001			
Elevation	0.17612	0.001			
Flow Age	0.02318	0.001			
Residuals	0.44084				
Total	1				
Jaccard					
Rainfall	0.13896	0.001			
Elevation	0.1202	0.001			
Flow Age	0.01456	0.008			
Residuals	0.72628				
Total	1				
Horn					
Rainfall	0.23329	0.001			
Elevation	0.20399	0.001			
Flow Age	0.02169	0.002			
Residuals	0.54102				
Total	1				
Bray					
Rainfall	0.19121	0.001			
Elevation	0.16265	0.001			
Flow Age	0.01724	0.01			
Residuals	0.6289				
Total	1				

This table includes the variance and *P* values for the PERMANOVA analyses.

Table S7.	Sensitivity of ana	alyses to	community	distance	metrics:
ANOSIM					

	ANOSIM				
Distance metric	R	Р			
Chao	0.659	0.001			
Jaccard	0.738	0.001			
Horn	0.678	0.001			
Bray	0.738	0.001			
Rarefied Bray	0.733	0.001			
Raup–Crick	0.873	0.001			

This table includes the sample statistic (R) and  $\ensuremath{\textit{P}}$  values for the ANOSIM test.

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	dbRDA constrained with PCNM (P)					
Distance metric	Rainfall	Elevation	Flow age			
Chao	0.01	0.01	0.01			
Jaccard	0.01	0.01	0.02			
Horn	0.01	0.01	0.01			
Bray	0.01	0.01	0.02			
Rarefied Bray	0.01	0.01	0.02			

This table includes the *P* values for the dbRDA analyses conditioned using principal components of neighbor matrices (PCNM).

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	Rainfall	Elevation	Flow age
Mantel R	0.174	0.379	NA
Р	0.001	0.001	NA

### Table S10. Comparison of the OTU richness found in this study to other research, when rarefied to the same number of sequences

Paper	Amend et al. (1)	Jumpponen and Jones (2)	Redford et al. (3)
Organisms of interest	Indoor fungi	Phyllosphere fungi	Phyllosphere bacteria
Scope	Global	Two locations (one host)	Local (many hosts) + global (one host)
No. of sequences	97,557	18,020	115,394
97% 165 OTUs			5,476
ITS1 95% OTUs	3,500*	689	
Rarefied richness from this study	1,895	846	2,042

Amend et al. (1) describe the diversity of indoor fungi from a set of global samples, Jumpponen and Jones (2) describe the phyllosphere fungal communities of *Quercus macrocarpa* in urban and rural sites, and Redford et al. (3) describe phyllosphere bacteria communities from a large number of different plants hosts at one site and then for one host at many sites around the world. \*Approximate from figure.

1. Amend AS, Seifert KA, Samson R, Bruns TD (2010) Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. Proc Natl Acad Sci USA 107:13748–13753.

Jumpponen A, Jones KL (2009) Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. *New Phytol* 184:438–448.
Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N (2010) The ecology of the phyllosphere: Geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environ Microbiol* 12:2885–2893.

#### Table S11. Comparison of surface-sterilized and non-surfacesterilized leaves

	Surface-sterilized			Not surface-sterilized		
	Sobs	Rarefied	Chao1	Sobs	Rarefied	Chao1
W100O: tree 6	186	180	212	223	199	333
W100Y: tree 8	162	156	209	258	258	548

The "Rarefied" column represents richness rarefied to 5,333 sequences, the lowest number in any of the four samples in the table. O, old; W, wet; Y, young.

Site code	Mean annual temperature (°C)	Mean rainfall (mm/y)	Flow age (y bp)	Elevation (m)	Easting (m)	Northing (m)
W100Y	22.2	3,900	Young	90	280,609	2,179,988
W100O	22.2	3,900	Old	97	280,589	2,179,777
W700Y	18.9	5,500	Young	728	269,321	2,179,147
W700O	18.9	5,500	Old	726	269,440	2,178,343
W1100Y	17.2	4,000	Young	1,142	262,292	2,178,451
W1100O	17.2	4,000	Old	1,144	262,049	2,179,796
W1800Y	13.9	2,200	Young	1,767	251,003	2,174,911
W1800O	13.9	2,200	Old	1,788	250,406	2,175,256
W2400Y	10.6	1,000	Young	2,440	243,061	2,168,284
W2400O	10.6	1,000	Old	2,485	243,429	2,167,539
D700Y	18.9	500	Young	691	204,302	2,194,604
D700O	18.9	500	Old	707	205,136	2,194,867
D1700Y	14.4	550	Young	1,697	214,927	2,174,342

#### Table S12. Site characteristics

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Site codes represent the rainfall (D, dry; W, wet), elevation (in meters), and substrate age (O, old; Y, young) of each location. For flow age, "Young" refers to substrate derived from flows that occurred between 100 and 150 y before present, and "Old" refers to substrate from flows that occurred ca. 3,000 y before present. Global positioning system coordinates are presented in meters with Easting and Northing values from the Universal Transverse Mercator projection (NAD 1983 Zone 5).