

1 **Supplementary Materials**

2 List of Supplementary Materials

3 **Materials and Methods**

4 **References**

5 **Table S1.** Counts of denitrification traits and their co-occurrences in fungal genomes.

6 **Table S2.** Results from approximately unbiased tests for the monophyly of fungal classes within
7 *napA*, *nirK*, and *p450nor* gene trees. Where indicated, the monophyly of two lineages was also
8 assessed. Bold font data indicate that the AU test rejected the monophyly of the taxa. Test
9 significance was evaluated at $p \leq 0.05$.

10 **Table S3.** Results from species-tree gene-tree reconciliation using NOTUNG software for *napA*,
11 *nirK*, and *p450nor* genes in fungi. Values are averages of solutions with standard deviations
12 reported in parentheses.

13 **Table S4.** Predicted horizontal gene transfers of fungal *p450nor*, *napA*, and *nirK* genes based on
14 alien index algorithm.

15 **Table S5.** List of genera containing species with and without *p450nor*.

16 **Figure S1.** Gene abundances of *narG*, *napA*, *nirK*, *p450nor*, and flavohemoglobins (colored
17 bars) mapped on to fungal families (cladogram, left). Relationships among fungal families in the
18 cladogram were derived from the NCBI taxonomy using the online tool phyloT
19 (<http://phylot.biobyte.de/index.html>).

20 **Figure S2.** Maximum-Likelihood phylogenies connecting fungal species with their respective
21 NO reductase (*p450nor*) gene sequence(s). On the left, an amino acid phylogeny of 238
22 concatenated single copy orthologues from fungal species in which one or more *p450nor* gene(s)
23 were detected. The *p450nor* nucleotide phylogeny (right) demonstrates many instances of

24 incongruence with the fungal species phylogeny. Black dots in each phylogeny represent
25 bootstrap percentages greater than or equal to 90%. Scale bars represent amino acid (left tree)
26 and nucleotide (right tree) substitutions per site. A high-resolution file of the tree is available at
27 <https://doi.org/10.6084/m9.figshare.c.3845692>.

28 **Figure S3.** Cophylogenetic plot of *napA*-containing fungal species (left, N = 75) and the *napA*
29 nucleotide tree (right, N = 78). Both are midpoint rooted Maximum-Likelihood trees where black
30 dots represent bootstrap percentages $\geq 90\%$. Scale bars indicate substitutions per site for the
31 concatenated amino acid species phylogeny and nucleotide phylogeny, respectively. A high-
32 resolution file of the tree is available at <https://doi.org/10.6084/m9.figshare.c.3845692>.

33 **Figure S4.** Cophylogenetic plot of *nirK*-containing fungal species (left, N = 82) and the *nirK*
34 nucleotide tree (right, N = 83). Both are midpoint rooted Maximum-Likelihood trees where black
35 dots represent bootstrap percentages $\geq 90\%$. Scale bars indicate substitutions per site for the
36 concatenated amino acid species phylogeny and nucleotide phylogeny, respectively. A high-
37 resolution file of the tree is available at <https://doi.org/10.6084/m9.figshare.c.3845692>.

38 **Figure S5.** Plot of alien index values observed for *p450nor* genes (N = 178). Points above the
39 hashed line at the origin are indicative of HGT. Names of fungal species with alien index values
40 above zero are ordered as their points appear on the graph. Thick horizontal lines represent the
41 median alien index value. See Materials and Methods in the Supplementary Materials for details
42 on alien index calculations.

43 **Figure S6.** Bayesian tree reconstruction of actinobacterial and proteobacterial 16S rRNA genes
44 (left, N = 55) and cytochrome P450 family 105 amino acid sequences (right, N = 57). Both
45 phylogenies represent 50% majority-rule consensus trees. The tree on the left is rooted with
46 proteobacterial sequences as outgroup to the *Actinobacteria*. The tree on the right is midpoint

47 rooted. Nodes with posterior probabilities ≥ 0.95 are indicated by black circles on an adjacent
48 branch.

49 **Figure S7.** Midpoint rooted Bayesian (left) and Maximum-Likelihood phylogenies (right) of
50 cytochrome P450 sequences (N = 408) demonstrating the affiliation of P450nor with other
51 sequences belonging to members of the bacterial phyla Actinobacteria and Proteobacteria.
52 Cyanobacterial cytochrome P450 sequences were included as outgroups. Black squares on
53 branches (left tree) indicate ≥ 0.95 posterior probability or ≥ 90 % bootstrap replication (right
54 tree). The colored legend indicates the cytochrome P450 family specified by shared amino acid
55 identity of ≥ 40 % (D.R. Nelson, Hum Genomics 4:59-65, 2009).

56 **Figure S8.** Bayesian and Maximum-likelihood phylogenies of NapA, NirK, and P450nor amino
57 acid sequence homologs extracted from the RefSeq protein database. A high-resolution file of
58 these trees are available at <https://doi.org/10.6084/m9.figshare.c.3845692>.

59 **Figure S9.** Genome regions chosen for in depth presentation of protein coding genes
60 surrounding *p450nor* in predicted BGC regions. Labels above genes are functional annotations
61 from alignments to the eggNOG database. NCBI gene loci accessions are labeled below each
62 gene.

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64 **Materials and Methods**

65 *Phylogenetic reconstructions*

66 Selection of the optimal evolutionary model for ML tree reconstruction was performed using
67 protest (Abascal *et al.*, 2005) (amino acid alignment) and jmodeltest (Posada, 2008) (nucleotide
68 alignment) software prior to ML tree reconstruction. Please refer to SI for additional details
69 about evolutionary models used in phylogenetic analysis. Phylogenetic analysis with RAxML

70 was performed by sampling 20 starting trees and performing 1,000 replicate bootstrap analyses.
71 The tree with the maximal negative log likelihood score was compared to 1,000 replicates in
72 RAxML to generate the final tree. Bayesian tree construction was performed using 3 independent
73 runs with 6 chains for 5,000,000 generations. Output from MrBayes was evaluated with the
74 sump and sumt commands within the software to ensure Markov Chain Monte Carlo chain
75 mixing and convergence (potential scale reduction factor of 1.0) and standard deviation of split
76 frequencies ~ 0.01 or lower. MrBayes output was further visualized in the program Tracer
77 (<http://tree.bio.ed.ac.uk/software/tracer/>) to ensure convergence was reached.

78 Optimal evolutionary models for Bayesian analysis were estimated from the alignments
79 using MrBayes software with a mixed amino acid model with 4 chains. The analysis continued
80 for 1,000,000 generations, with sampling performed every 1,000th generation and a default
81 burn-in of 25 %. Optimal amino acid models inferred by prottest ML analyses were LG (Le and
82 Gascuel, 2008) (NapA, P450nor) or JTT (Jones *et al.*, 1992) models (NirK), whereas for
83 nucleotide sequences, the GTR (Rodriguez *et al.*, 1990) model with variation in rate
84 heterogeneity among sites was selected by jmodeltest as the optimal evolutionary model for each
85 gene. Optimal amino acid models inferred with MrBayes were the WAG (Whelan and Goldman,
86 2001) (NapA and P450nor) or the JTT model (NirK). The GTR model with rate heterogeneity
87 among sites was also the optimally inferred evolutionary model for nucleotide alignments used
88 for Bayesian tree reconstruction. For phylogenetic analysis of fungal NapA, NirK, and P450nor
89 with additional RefSeq protein sequences, the LG (ML) or WAG (Bayesian) models were
90 selected in the respective phylogenetic software. All amino acid tree reconstruction utilized
91 gamma distributed rate heterogeneity among sites, and additional tree reconstruction parameters
92 were estimated from the alignment.

93 BayesTraits and NOTUNG analyses

94 For trait correlations, the concatenated 238 BUSCO gene alignment (see main text Materials and
95 Methods) of 709 fungal taxa was bootstrapped into 800 replicate alignments using the PHYLIP
96 software function seqboot (6) and 800 ML trees created as described in the main text using
97 FastTree2 software. These alignments were paired with presence/absence data regarding
98 denitrification traits and provided as input to BayesTraits software. BayesTraits was first
99 operated in ML mode (100 ML tries setting) to generate parameter estimates for dependent (trait
100 correlation) and independent (no trait correlation) models to be compared. These parameter
101 estimates were then entered into BayesTraits, and three independent runs of the software in
102 Bayesian mode using the dependent and independent model of trait correlation between the two
103 traits being compared were performed. The analysis was run for 1,000,000 generations with
104 samples taken every 1,000th generation and a burn-in of 50,000 generations. A stepping stone
105 analysis (100 stones, 10,000 samples) was performed to generate log marginal likelihood values
106 for Bayes Factor (BF) calculations to test which model (correlation or no correlation) best fit the
107 data. Bayes Factors are comparable to a likelihood ratio test for model selection, and the larger
108 the Bayes Factor the more certainty there is in the more complex, dependent model (indicating
109 trait correlation). Hence, a BF of 1 is indicative of weak or no trait correlation, but a BF of 10 or
110 larger indicates strong selection of the dependent model and trait correlation (Pagel *et al.*, 2004).
111 A similar analysis is performed for ancestral state reconstruction, except that trees from a
112 Bayesian analysis were used as input to the MultiState method of the software. Multistate was
113 run for 5,500,000 generations with sampling every 2,000th generation and a burn-in of 500,000
114 generations. The probability of a given character state at a node within the tree was averaged

115 over all generations after the burn-in period and was used to determine support for the state of a
116 node within the tree.

117 NOTUNG performs reconciliation by matching nodes between species and gene trees to
118 infer numbers of GD, GL, and GT events. These reconciliations are used to calculate a weighted
119 sum, termed the event score, by multiplying user supplied event costs for GD, GL, and GTs.
120 When inferring GTs, multiple solutions may be reached, and NOTUNG reports all
121 reconciliations reached to obtain a minimized event score. NOTUNG analyses were
122 implemented with a duplication cost of 2, loss cost of 1, and a variable transfer cost from 3 to 15.
123 Ratcheting the transfer costs assumes GD is prevalent, which is likely the case for fungi, in
124 which GT events are assumed to be less frequent than for *Bacteria* and *Archaea*. All other
125 settings were default. NOTUNG ignores incomplete lineage sorting as an evolutionary
126 mechanism when both a rooted species and gene tree are used as input, as was the case for the
127 present study.

128 *Alien index calculations*

129 The alien index (AI) was calculated as previously described and modified for use with a single
130 gene (Wisecaver *et al.*, 2016). Briefly, pairwise amino acid sequence alignments were performed
131 using blastp for fungal NapA, NirK, and P450nor sequences. The in group was defined as the
132 aligned sequence with the highest bitscore (excluding the query) belonging to the same
133 taxonomic class as the query sequence. Accordingly, the out group was defined as the aligned
134 sequence with the highest bitscore not belonging to the same taxonomic class as the query. The
135 maximum bitscore was the bitscore derived from the alignment of the query to itself. Therefore,
136 AI is calculated as follows:

$$137 \quad AI = (out\ group\ bitscore / max\ bitscore) - (in\ group\ bitscore / max\ bitscore)$$

138 AI values range from 1 to -1. Values greater than zero are indicative of HGT or contamination of
139 foreign DNA within the genome sequence being queried.

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141 **References**

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159 **Table S1.** Counts of denitrification traits and their co-occurrences in fungal genomes.

Fungal lineage	<i>napA</i>	<i>nirK</i>	<i>p450nor</i>	flavoHb*	<i>napA+nirK</i>	<i>napA+</i> <i>p450nor</i>	<i>nirK+</i> <i>p450nor</i>	<i>napA+</i> <i>nirK+</i> <i>p450nor</i>	<i>p450nor+</i> flavoHb	(<i>p450nor+</i> flavoHb)/ <i>p450nor</i> †
Sordariomycetes	23	20	63	155	7	11	15	6	62	0.98
Leotiomycetes	2	9	36	25	1	2	8	1	19	0.53
Eurotiomycetes	34	52	35	80	19	15	24	11	16	0.46
Dothideomycetes	7	1	28	75	0	2	1	0	27	0.96
Tremellomycetes	1	0	3	12	0	0	0	0	1	0.33
Atractiellomycetes	0	0	1	0	0	0	0	0	0	0
Pezizomycetes	0	0	1	0	0	0	0	0	0	0
Mixiomycetes	0	0	0	0	0	0	0	0	0	n/a
Agaricomycetes	1	0	0	17	0	0	0	0	0	n/a
Saccharomycetes	0	0	0	63	0	0	0	0	0	n/a
Pucciniomycetes	2	0	0	1	0	0	0	0	0	n/a
Monoblepharidomycetes	0	0	0	0	0	0	0	0	0	n/a
Chytridiomycetes	0	0	0	0	0	0	0	0	0	n/a
Wallemiomycetes	0	0	0	1	0	0	0	0	0	n/a
Ustilaginomycetes	0	0	0	1	0	0	0	0	0	n/a
Orbiliomycetes	0	0	0	2	0	0	0	0	0	n/a
Basidiobolomycetes	0	0	0	0	0	0	0	0	0	n/a
Dacrymycetes	0	0	0	0	0	0	0	0	0	n/a
Geminibasidiomycetes	0	0	0	0	0	0	0	0	0	n/a
Zoopagomycota	0	0	0	1	0	0	0	0	0	n/a
Schizosaccharomycetes	0	0	0	3	0	0	0	0	0	n/a
Pneumocystidomycetes	0	0	0	0	0	0	0	0	0	n/a
Blastocladiomycetes	0	0	0	0	0	0	0	0	0	n/a
Lecanoromycetes	1	0	0	0	0	0	0	0	0	n/a
Malasseziomycetes	0	0	0	12	0	0	0	0	0	n/a
Taphrinomycetes	0	0	0	1	0	0	0	0	0	n/a
Microbotryomycetes	4	0	0	0	0	0	0	0	0	n/a
Exobasidiomycetes	0	0	0	0	0	0	0	0	0	n/a
Entomophthoromycetes	0	0	0	1	0	0	0	0	0	n/a
Neocallimastigomycetes	0	0	0	0	0	0	0	0	0	n/a
Glomeromycetes	0	0	0	0	0	0	0	0	0	n/a
Total	75	82	167	450	27	30	48	18	125	n/a

A “+” indicates that each gene had to be present in each genome evaluated in order to add to the overall count for that lineage.

*FlavoHb = flavohemoglobin

†n/a = Not applicable since *p450nor* genes were not detected

161 **Table S2.** Results from approximately unbiased tests for the monophyly of fungal classes within *napA*, *nirK*, and *p450nor* gene trees.
 162 Where indicated, the monophyly of two lineages was also assessed. Bold font data indicate that the AU test rejected the monophyly of
 163 the taxa. Test significance was evaluated at $p \leq 0.05$.

Gene	Lineage	No. Genes	No. Taxa	No. Genera	Approximately unbiased test					
					Monophyletic		Amino acid		Nucleotide	
					<i>Amino acid</i>	<i>Nucleotide</i>	<i>Diff -lnL*</i>	<i>P value</i>	<i>Diff -lnL</i>	<i>P value</i>
<i>napA</i>	Agaricomycetes	2	1	1	Yes	Yes	-	-	-	-
	Dothideomycetes	7	7	6	No	No	40	0.015	40	0.001
	Eurotiomycetes	36	34	14	No	No	753	2.00E-06	550	4.00E-04
	Lecanoromycetes (Lec)	1	1	1	Yes	Yes	-	-	-	-
	Leotiomycetes (L)	2	2	2	Yes	Yes	-	-	-	-
	Microbotryomycetes	4	4	1	Yes	Yes	-	-	-	-
	Pucciniomycetes	2	2	2	No	No	1303	9.00E-05	1088	2.00E-08
	Sordariomycetes (S)	23	23	18	No	No	806	7.00E-61	770	9.00E-56
	Tremellomycetes	1	1	1	Yes	Yes	-	-	-	-
	L+S share MRCA				No	No	908	2.00E-71	973	6.00E-74
	Lec+E share MRCA				No	No	75	3.00E-05	257	9.00E-06
<i>nirK</i>	Dothideomycetes	1	1	1	Yes	Yes	-	-	-	-
	Eurotiomycetes	52	52	17	No	No	19	0.142	33	0.003
	Leotiomycetes	10	9	1	Yes	Yes	-	-	-	-
	Sordariomycetes	20	20	8	Yes	Yes	-	-	-	-
	D+E share MRCA				No	No	15	0.042	30.1	0.004
	L+S share MRCA				No	No	18	0.142	35.3	0.001

164 *Diff -lnL = difference in negative log-likelihood of the observed tree to the constraint tree in which the taxa were constrained to be monophyletic.

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168 **Table S2.** (continued)

Gene	Lineage	No. Genes	No. Taxa	No. Genera	Approximately unbiased test					
					Monophyletic		Amino acid		Nucleotide	
					<i>Amino acid</i>	<i>Nucleotide</i>	<i>Diff -lnL*</i>	<i>P value</i>	<i>Diff -lnL</i>	<i>P value</i>
<i>p450nor</i>	Dothideomycetes (D)	28	28	26	No	No	1153	4.00E-05	1294	8.00E-51
	Eurotiomycetes (E)	36	35	17	No	No	891	1.00E-32	917	2.00E-37
	Leotiomycetes (L)	37	36	16	No	No	465	2.00E-39	694	4.00E-40
	Sordariomycetes (S)	72	63	32	No	No	1159	5.00E-10	1206	5.00E-15
	Tremellomycetes	3	3	2	No	No	125	3.00E-04	125	3.00E-08
	Atractiellomycetes	1	1	1	Yes	Yes	-	-	-	-
	Pezizomycetes	1	1	1	Yes	Yes	-	-	-	-
	L+S share MRCA				No	No	1386	3.00E-43	1420	5.00E-72
	D+E share MRCA				No	No	1481	0.001	1481	2.00E-63
	Ascomycota (A)				No	No	139	5.00E-05	140	6.00E-06
	Basidiomycota (B)				No	No	139	4.00E-56	139	1.00E-39
	A+B share MRCA				No	No	139	3.00E-11	140	3.00E-04

*Diff -lnL = difference in negative log-likelihood of the observed tree to the constraint tree in which the taxa were constrained to be monophyletic.

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176 **Table S3.** Results from species-tree gene-tree reconciliation using NOTUNG software for *napA*, *nirK*, and *p450nor* genes in fungi.
 177 Values are averages of solutions with standard deviations reported in parentheses.

Gene	Phylogeny	Duplications	Codivergences	Transfers	Losses	Duplication cost	Transfer cost	Loss cost	Solutions
<i>p450nor</i>	amino acid	-	-	-	-	2	3	1	0
		-	-	-	-	2	5	1	0
		-	-	-	-	2	7	1	0
		-	-	-	-	2	9	1	0
		49.0 (0.0)	0.0 (0.0)	15.0 (0.0)	253.0 (0.0)	2	11	1	1000
		61.0 (0.0)	0.0 (0.0)	6.0 (0.0)	333.0 (0.0)	2	13	1	180
		62.1 (1.0)	0.0 (0.0)	5.4 (0.5)	339.3 (5.4)	2	15	1	420
<i>p450nor</i>	nucleotide	-	-	-	-	2	3	1	0
		-	-	-	-	2	5	1	0
		-	-	-	-	2	7	1	0
		45.0 (0.0)	0.0 (0.0)	16.0 (0.0)	215.0 (0.0)	2	9	1	1000
		53.6 (0.8)	0.0 (0.0)	8.2 (0.4)	277.6 (2.8)	2	11	1	1000
		56.0 (0.0)	0.0 (0.0)	6.0 (0.0)	299.0 (0.0)	2	13	1	100
		60.0 (0.0)	0.0 (0.0)	4.0 (0.0)	319.0 (0.0)	2	15	1	60
<i>napA</i>	amino acid	1.4 (0.5)	0.0 (0.0)	30.8 (0.8)	14.9 (1.7)	2	3	1	1000
		9.2 (1.4)	0.0 (0.0)	19.8 (1.4)	43.7 (4.3)	2	5	1	1000
		15.0 (0.0)	0.0 (0.0)	14.0 (0.0)	64.0 (0.0)	2	7	1	36
		22.0 (1.3)	0.0 (0.0)	7.8 (1.0)	100.8 (6.4)	2	9	1	20
		28.0 (0.0)	0.0 (0.0)	3.0 (0.0)	135.0 (0.0)	2	11	1	1
		28.0 (0.0)	0.0 (0.0)	3.0 (0.0)	135.0 (0.0)	2	13	1	1
		31.0 (1.0)	0.0 (0.0)	0.5 (0.5)	165.5 (5.5)	2	15	1	2
<i>napA</i>	nucleotide	-	-	-	-	2	3	1	0
		-	-	-	-	2	5	1	0
		-	-	-	-	2	7	1	0
		-	-	-	-	2	9	1	0
		-	-	-	-	2	11	1	0
		28.0 (1.0)	0.0 (0.0)	2.5 (0.5)	142.5 (4.5)	2	13	1	2
		30.0 (0.0)	0.0 (0.0)	1.0 (0.0)	159.0 (0.0)	2	15	1	1

179 **Table S4.** Predicted horizontal gene transfers of fungal *p450nor*, *napA*, and *nirK* genes based on alien index algorithm.

Gene	Query assembly ID*	Query name	IG† bitscore	OG bitscore	Max bitscore	Alien index value	IG taxon	OG taxon	IG name	OG name	IG assembly ID	OG assembly ID
<i>p450nor</i>	Apimo1	<i>Apiospora montagnei</i> NRRL 25634 v1.0	418	423	851	0.006	Sordariomycetes	Dothideomycetes	<i>Valettoniel lopsis laxa</i> CBS 191.97 v1.0	<i>Peltaster fructicola</i>	Valla1	GCA_0015 92805.1
	GCA_0000 02855.2	<i>Aspergillus niger</i> CBS 513 88	353	437	859	0.098	Eurotiomycetes	Dothideomycetes	<i>Uncinocarpus reesii</i> 1704	<i>Macroventuria anomochaeta</i> CBS 525.71 v1.0	GCA_0000 03515.2	Macan1
	GCA_0001 51355.1__3	<i>Nectria haematococca</i> mpVI 77-13-4	469	583	881	0.129	Sordariomycetes	Eurotiomycetes	<i>Sporothrix pallida</i>	<i>Aspergillus parasiticus</i> SU-1	GCA_0007 10705.2	GCA_0009 56085.1
	GCA_0002 93215.1	<i>Trichosporon asahii</i> var <i>asahii</i> CBS 2479	400	572	813	0.212	Tremellomycetes	Sordariomycetes	<i>Mrakia blollopis</i>	<i>Plectosphaerella cucumerina</i> DS2psM2a 2 v1.0	GCA_0009 50635.1	Plecu1
	GCA_0004 97085.1	<i>Byssochlamys spectabilis</i>	528	659	827	0.158	Eurotiomycetes	Sordariomycetes	<i>Exophiala xenobiotica</i>	<i>Trichoderma virens</i> Gv29-8	GCA_0008 35505.1	GCA_0001 70995.2
	GCA_0007 10705.2	<i>Sporothrix pallida</i>	577	656	833	0.095	Sordariomycetes	Dothideomycetes	<i>Colletotrichum gloeosporioides</i> Nara gc5	<i>Rhytidhysterium rufulum</i>	GCA_0003 19635.1	Rhyru1_1
	GCA_0007 43665.1	<i>Geotrichum candidum</i>	586	650	854	0.075	Leotiomycetes	Dothideomycetes	<i>Meliniomyces variabilis</i> F v1.0	<i>Acidomyces richmondensis</i>	Melva1	GCA_0015 72075.1
	GCA_0008 15965.1	<i>Mrakia frigida</i>	279	421	745	0.191	Tremellomycetes	Eurotiomycetes	<i>Trichosporon asahii</i> var <i>asahii</i>	<i>Monascus purpureus</i>	GCA_0009 50635.1	Monpu1
	GCA_0008 35505.1	<i>Exophiala xenobiotica</i>	573	637	887	0.072	Eurotiomycetes	Dothideomycetes	<i>Microsporium gypseum</i> CBS 118893	<i>Acidomyces richmondensis</i>	GCA_0001 50975.2	GCA_0015 72075.1
	GCA_0009 50635.1	<i>Mrakia blollopis</i>	371	531	852	0.188	Tremellomycetes	Eurotiomycetes	<i>Trichosporon asahii</i> var <i>asahii</i>	<i>Monascus purpureus</i>	GCA_0002 93215.1	Monpu1

180 *Assembly IDs with two underscores followed by a number indicate the query gene is multi copy within the genome.

181 †IG = Ingroup, OG = Outgroup.

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183 **Table S4.** (continued)

Gene	Query assembly ID*	Query name	IG† bitscore	OG bitscore	Max bitscore	Alien index value	IG taxon	OG taxon	IG name	OG name	IG assembly ID	OG assembly ID
<i>p450nor</i>	GCA_001572075.1	<i>Acidomyces richmondensis</i>	584	637	831	0.064	Dothideomycetes	Eurotiomycetes	<i>Rhizidhysterium rufulum</i>	<i>Exophiala xenobiotica</i>	Rhyru1_1	GCA_000835505.1
	GCA_001592805.1	<i>Peltaster fruticola</i>	451	483	840	0.038	Dothideomycetes	Leotiomycetes	<i>Myriangiururiai</i> CBS 260.36 v1.0	<i>Sclerotinia sclerotiorum</i> 1980 UF-70	Myrdu1	GCA_000146945.1
	Macan1	<i>Macroventuria anomochaeta</i> CBS 525.71	393	437	846	0.052	Dothideomycetes	Eurotiomycetes	<i>Coniosporium apollinis</i> CBS 100218	<i>Aspergillus niger</i> CBS 513 88	GCA_000281105.1	GCA_000002855.2
	Myrdu1	<i>Myriangiururiai</i> CBS 260.36	451	508	840	0.068	Dothideomycetes	Sordariomycetes	<i>Peltaster fruticola</i>	<i>Valetoniella opsis laxa</i> CBS 191.97 v1.0	GCA_001592805.1	Valla1
	Valla1	<i>Valetoniella opsis laxa</i> CBS 191.97 v1.0	418	508	838	0.107	Sordariomycetes	Dothideomycetes	<i>Apiosporamontagnei</i> NRRL 25634 v1.0	<i>Myriangiururiai</i> CBS 260.36 v1.0	Apimo1	Myrdu1
<i>napA</i>	GCA_000225285.2	<i>Epichloe glyceriae</i> E277	270	326	1367	0.041	Sordariomycetes	Agaricomycetes	<i>Balansia oblecta</i> B249	<i>Clavaria fumosa</i>	GCA_000709145.1	GCA_001179745.1__2
	GCA_000281105.1	<i>Coniosporium apollinis</i> CBS 100218	1416	1436	2040	0.010	Dothideomycetes	Leotiomycetes	Fungal sp. No 11243	<i>Pseudogymnoascus</i> sp. VKM F-4513 FW-928	GCA_000836255.1	GCA_000750755.1
	GCA_000315175.1	<i>Herpotrichiella laceae</i> sp. UM238	1265	1286	1989	0.011	Eurotiomycetes	Sordariomycetes	<i>Aspergillus ustus</i>	<i>Colletotrichum higginsianum</i>	GCA_000812125.1	GCA_000313795.2
	GCA_000464645.1	<i>Melampsora pinitorqua</i> Mpini7	437	872	1645	0.264	Pucciniomycetes	Eurotiomycetes	<i>Cronartium ribicola</i> 11-2	<i>Amauroascus niger</i>	GCA_000500245.1	GCA_001430945.1__2

184 *Assembly IDs with two underscores followed by a number indicate the query gene is multi copy within the genome.

185 †IG = Ingroup, OG = Outgroup.

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188 **Table S4.** (continued)

Gene	Query assembly ID*	Query name	IG [†] bitscore	OG bitscore	Max bitscore	Alien index value	IG taxon	OG taxon	IG name	OG name	IG assembly ID	OG assembly ID
<i>napA</i>	GCA_0005_00245.1	<i>Cronartium ribicola</i> 11-2	431	905	1626	0.292	Pucciniomycetes	Sordariomycetes	<i>Melampsora pinitorqua</i> Mpini7	<i>Balansia obtecta</i> B249	GCA_0004_64645.1	GCA_0007_09145.1
	GCA_0006_11775.1	<i>Umbilicaria muehlenbergii</i>	n/a	1421	2035	0.698	Lecanoromycetes	Leotiomycetes	<i>Umbilicaria muehlenbergii</i>	<i>Pseudogymnoascus</i> sp. VKM F-4513 FW-928	GCA_0006_11775.1	GCA_0007_50755.1
	GCA_0007_09145.1	<i>Balansia obtecta</i> B249	278	925	1854	0.349	Sordariomycetes	Pucciniomycetes	<i>Epichloe glyceriae</i> E277	<i>Cronartium ribicola</i> 11-2	GCA_0002_25285.2	GCA_0005_00245.1
	GCA_0007_50755.1	<i>Pseudogymnoascus</i> sp. VKM F-4513 FW-928	1415	1436	2037	0.010	Leotiomycetes	Dothideomycetes	<i>Geotrichum candidum</i>	<i>Coniosporium apollinis</i> CBS 100218	GCA_0007_43665.1	GCA_0002_81105.1
	GCA_0014_68955.1	<i>Cryptococcus albidus</i>	n/a	1033	2058	0.502	Tremellomycetes	Sordariomycetes	<i>Cryptococcus albidus</i>	<i>Colletotrichum orbiculare</i> MAFF 240422	GCA_0014_68955.1	GCA_0003_50065.1
<i>nirK</i>	GCA_0015_72075.1	<i>Acidomyces richmondensis</i>	n/a	559	888	0.630	Dothideomycetes	Eurotiomycetes	<i>Acidomyces richmondensis</i>	<i>Arthroderma otae</i> CBS 113480	GCA_0015_72075.1	GCA_0001_51145.1

189 *Assembly IDs with two underscores followed by a number indicate the query gene is multi copy within the genome.

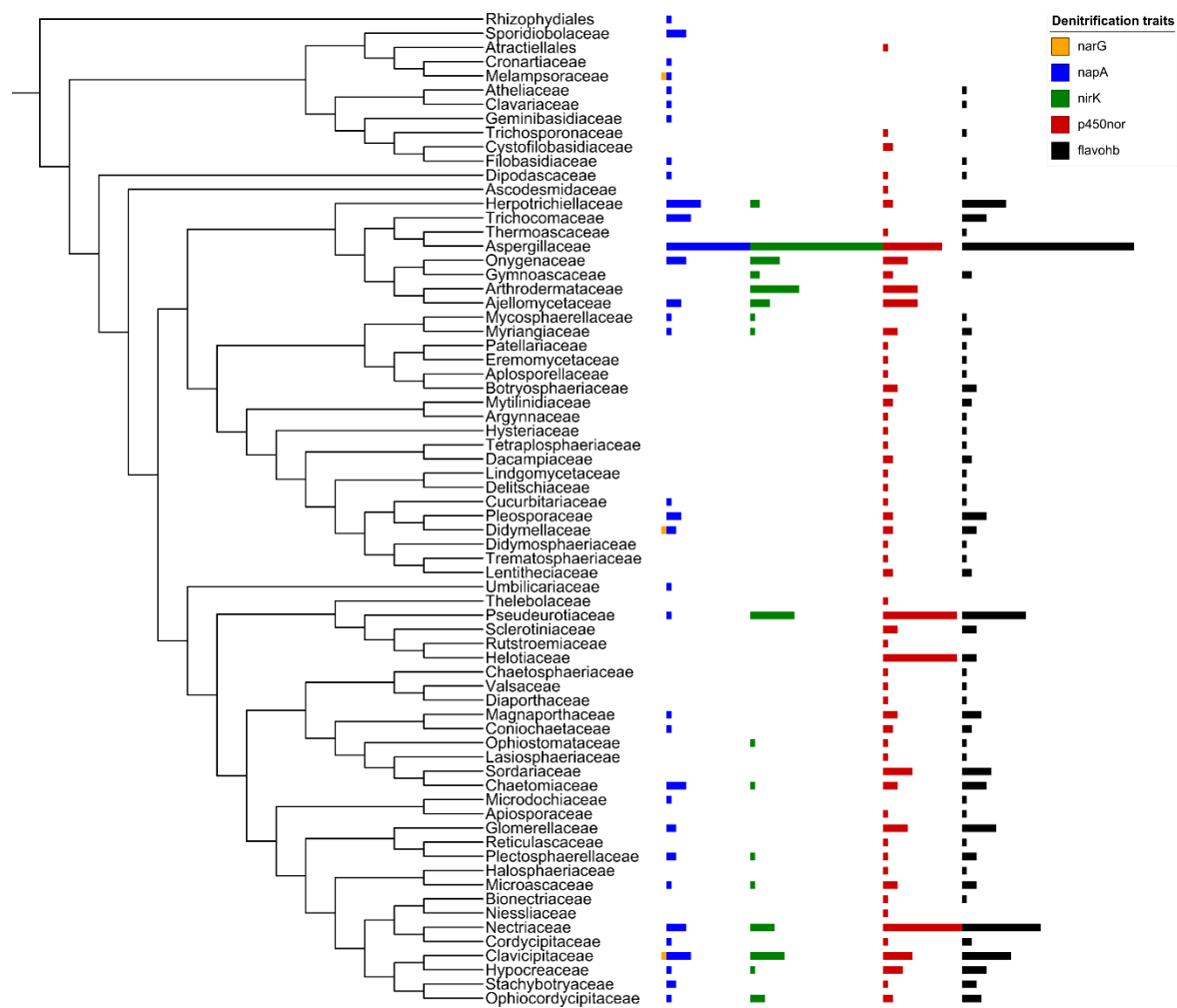
190 †IG = Ingroup, OG = Outgroup.

191 **Table S5.** List of genera containing species with and without *p450nor*.

Genus	Total species	Species with <i>p450nor</i>	Percentage with <i>p450nor</i>
<i>Arthroderma</i>	2	1	50.0
<i>Aspergillus</i>	20	9	45.0
<i>Bipolaris</i>	6	2	33.3
<i>Colletotrichum</i>	10	5	50.0
<i>Diaporthe</i>	3	1	33.3
<i>Diplodia</i>	3	2	66.7
<i>Exophiala</i>	7	1	14.3
<i>Fusarium</i>	16	13	81.3
<i>Hirsutella</i>	2	1	50.0
<i>Hymenoscyphus</i>	7	5	71.4
<i>Metarhizium</i>	7	6	85.7
<i>Neosartorya</i>	2	1	50.0
<i>Neurospora</i>	6	4	66.7
<i>Pseudogymnoascus</i>	16	15	93.8
<i>Pyrenochaeta</i>	3	1	33.3
<i>Rhytidhysterion</i>	2	1	50.0
<i>Rutstroemia</i>	2	1	50.0
<i>Sclerotinia</i>	3	2	66.7
<i>Sporothrix</i>	3	1	33.3
<i>Trichoderma</i>	8	4	50.0
<i>Trichophyton</i>	6	5	83.3
<i>Trichosporon</i>	2	1	50.0

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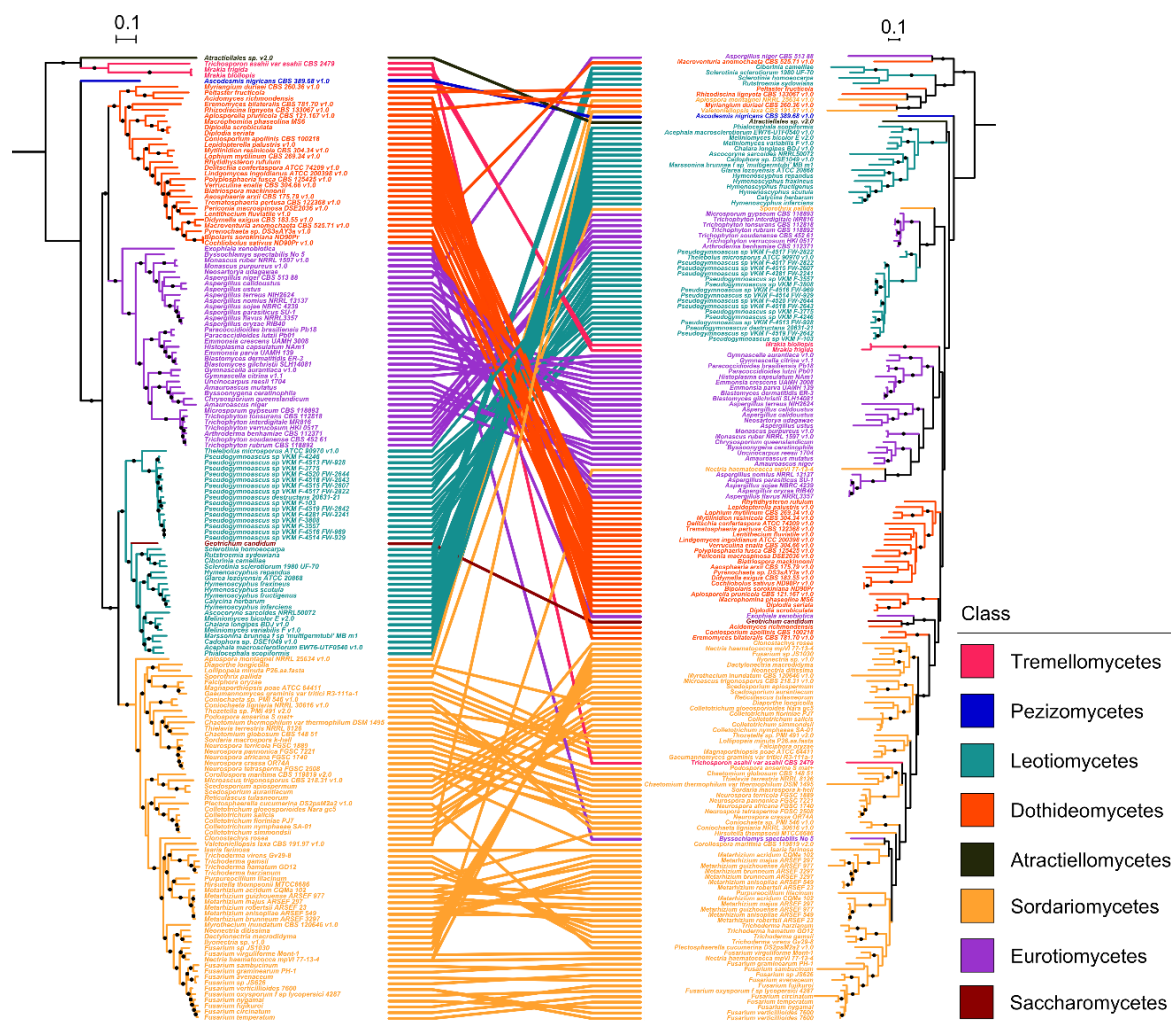


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196 **Figure S1.** Gene abundances of *narG*, *napA*, *nirK*, *p450nor*, and flavohemoglobins (colored
 197 bars) mapped on to fungal families (cladogram, left). Relationships among fungal families in the
 198 cladogram were derived from the NCBI taxonomy using the online tool phyloT
 199 (<http://phylo.t.biobyte.de/index.html>).

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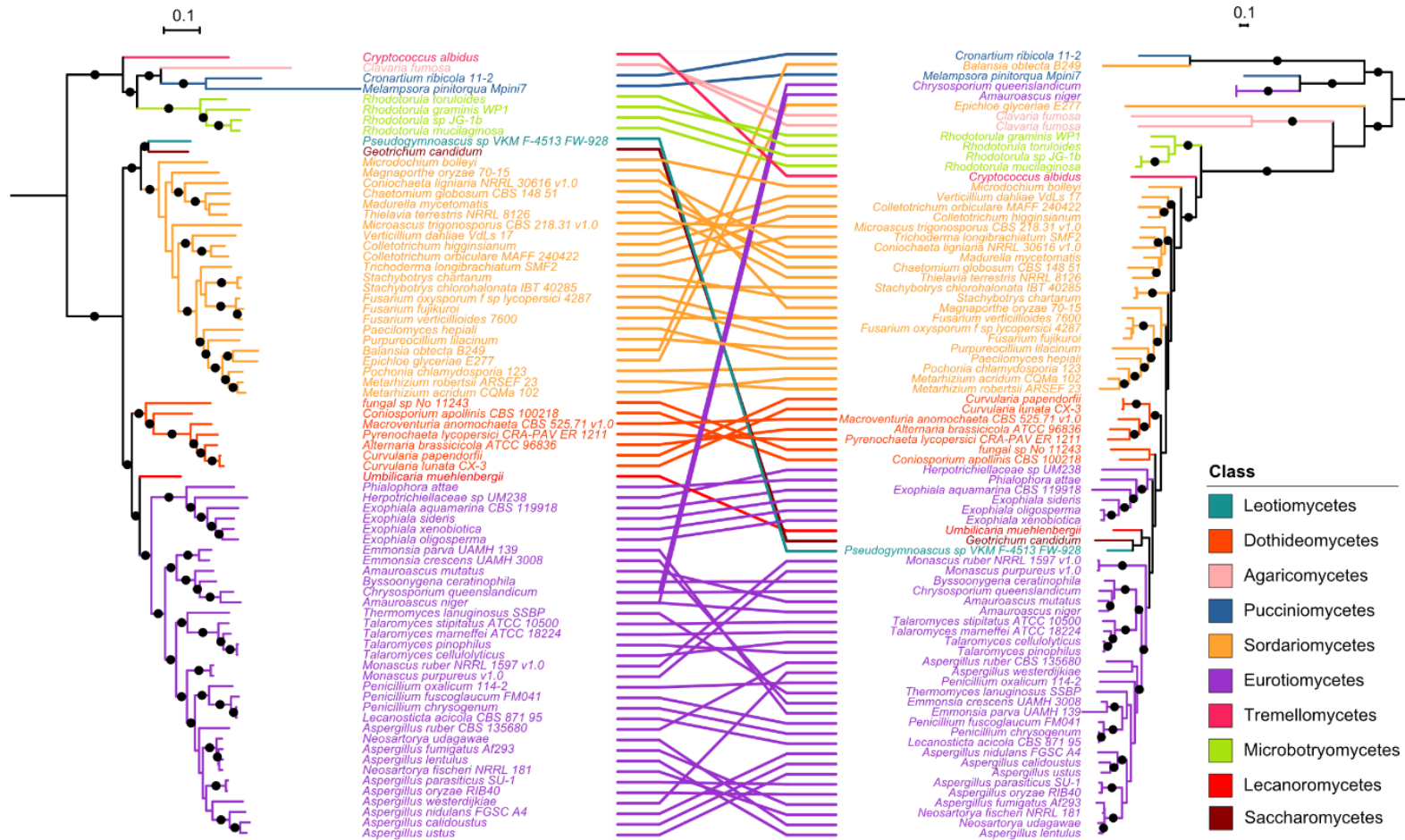
203 **Figure S2.** Maximum-Likelihood phylogenies connecting fungal species with their respective
 204 NO reductase (*p450nor*) gene sequence(s). On the left, an amino acid phylogeny of 238
 205 concatenated single copy orthologues from fungal species in which one or more *p450nor* gene(s)
 206 were detected. The *p450nor* nucleotide phylogeny (right) demonstrates many instances of
 207 incongruence with the fungal species phylogeny. Black dots in each phylogeny represent
 208 bootstrap percentages greater than or equal to 90%. Scale bars represent amino acid (left tree)
 209 and nucleotide (right tree) substitutions per site. A high-resolution file of the tree is available at
 210 <https://doi.org/10.6084/m9.figshare.c.3845692>.

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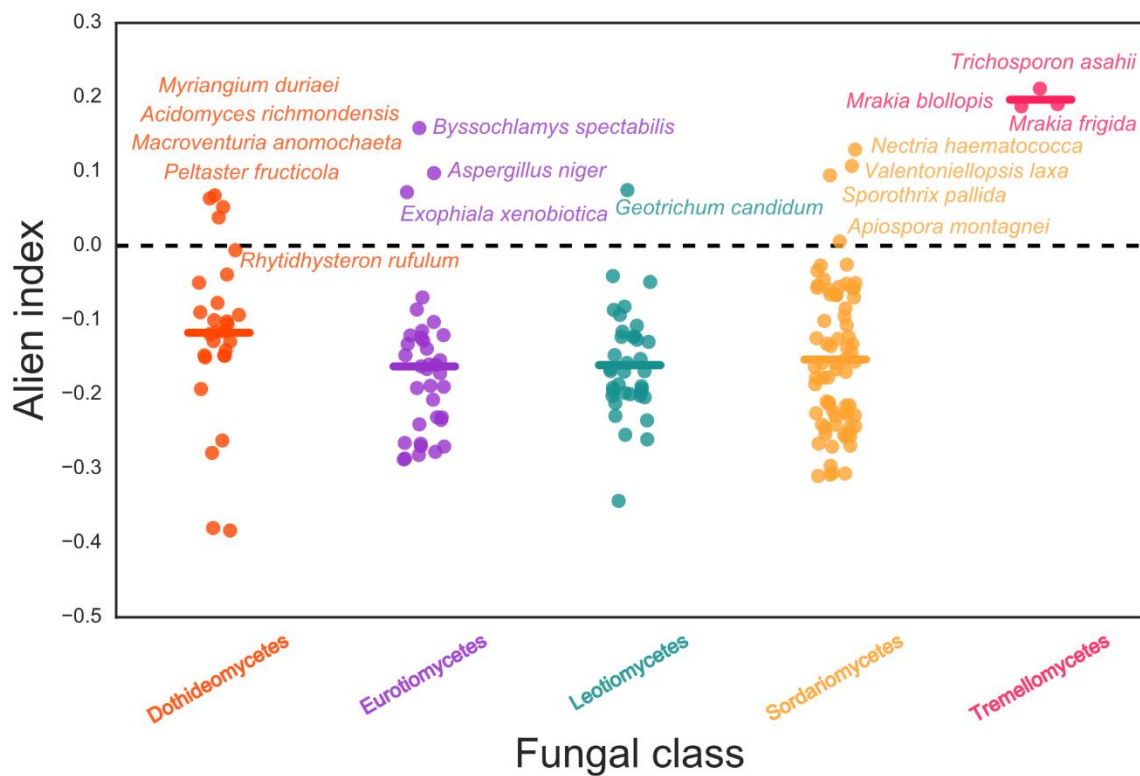
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217 **Figure S3.** Cophylogenetic plot of *napA*-containing fungal species (left, N = 75) and the *napA* nucleotide tree (right, N = 78). Both are
 218 midpoint rooted Maximum-Likelihood trees where black dots represent bootstrap percentages ≥ 90 %. Scale bars indicate substitutions
 219 per site for the concatenated amino acid species phylogeny and nucleotide phylogeny, respectively. A high-resolution file of the tree is
 220 available at <https://doi.org/10.6084/m9.figshare.c.3845692>.



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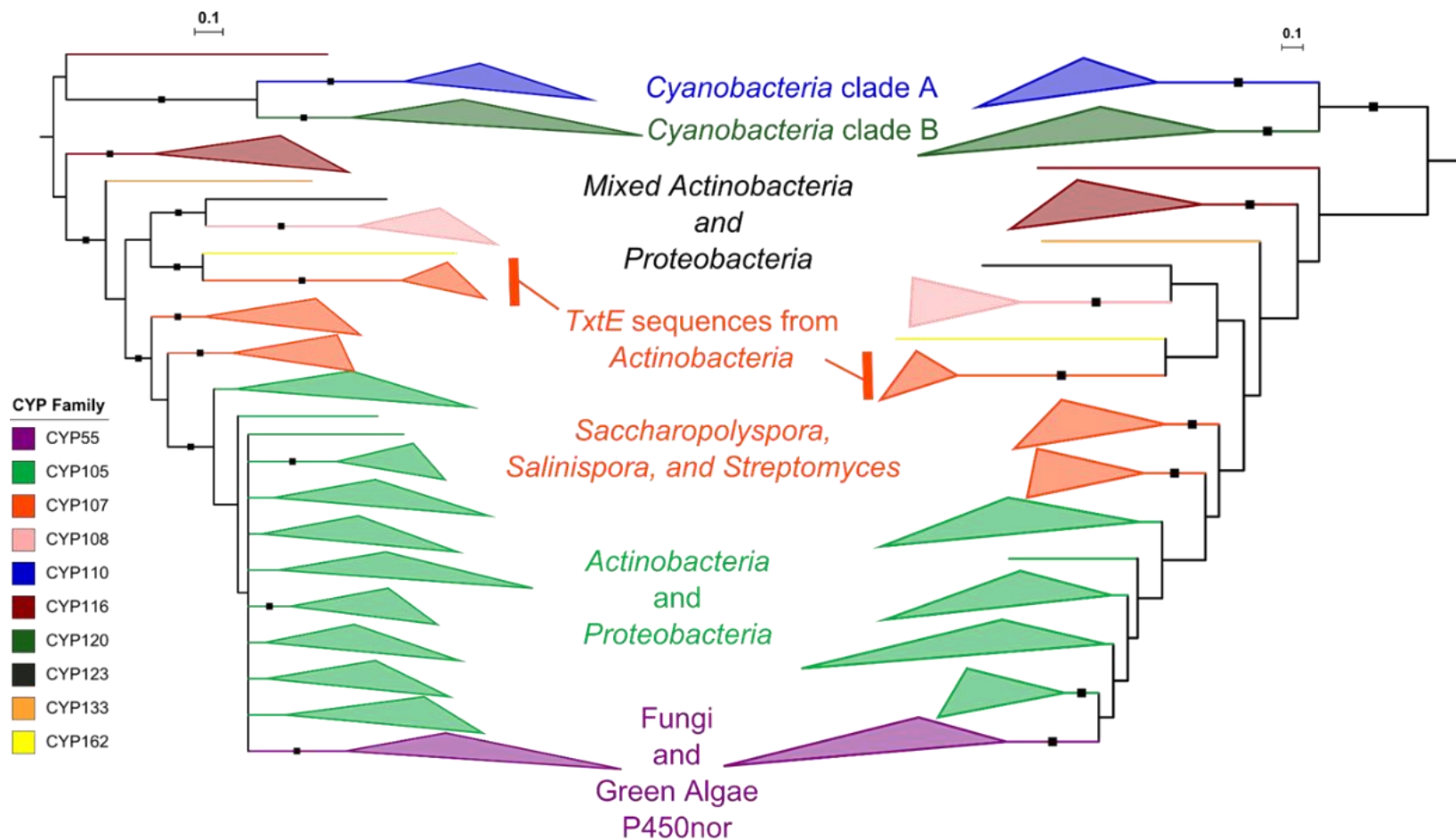
228 **Figure S5.** Plot of alien index values observed for *p450nor* genes (N = 178). Points above the
 229 hashed line at the origin are indicative of HGT. Names of fungal species with alien index values
 230 above zero are ordered as their points appear on the graph. Thick horizontal lines represent the
 231 median alien index value. See Materials and Methods in Supplementary Materials for details on
 232 alien index calculations.

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235 **Figure S6.** Bayesian tree reconstruction of actinobacterial and proteobacterial 16S rRNA genes (left, N = 55) and cytochrome P450
 236 family 105 amino acid sequences (right, N = 57). Both phylogenies represent 50% majority-rule consensus trees. The tree on the left is
 237 rooted with proteobacterial sequences as outgroup to the *Actinobacteria*. The tree on the right is midpoint rooted. Nodes with posterior
 238 probabilities ≥ 0.95 are indicated by black circles on an adjacent branch.

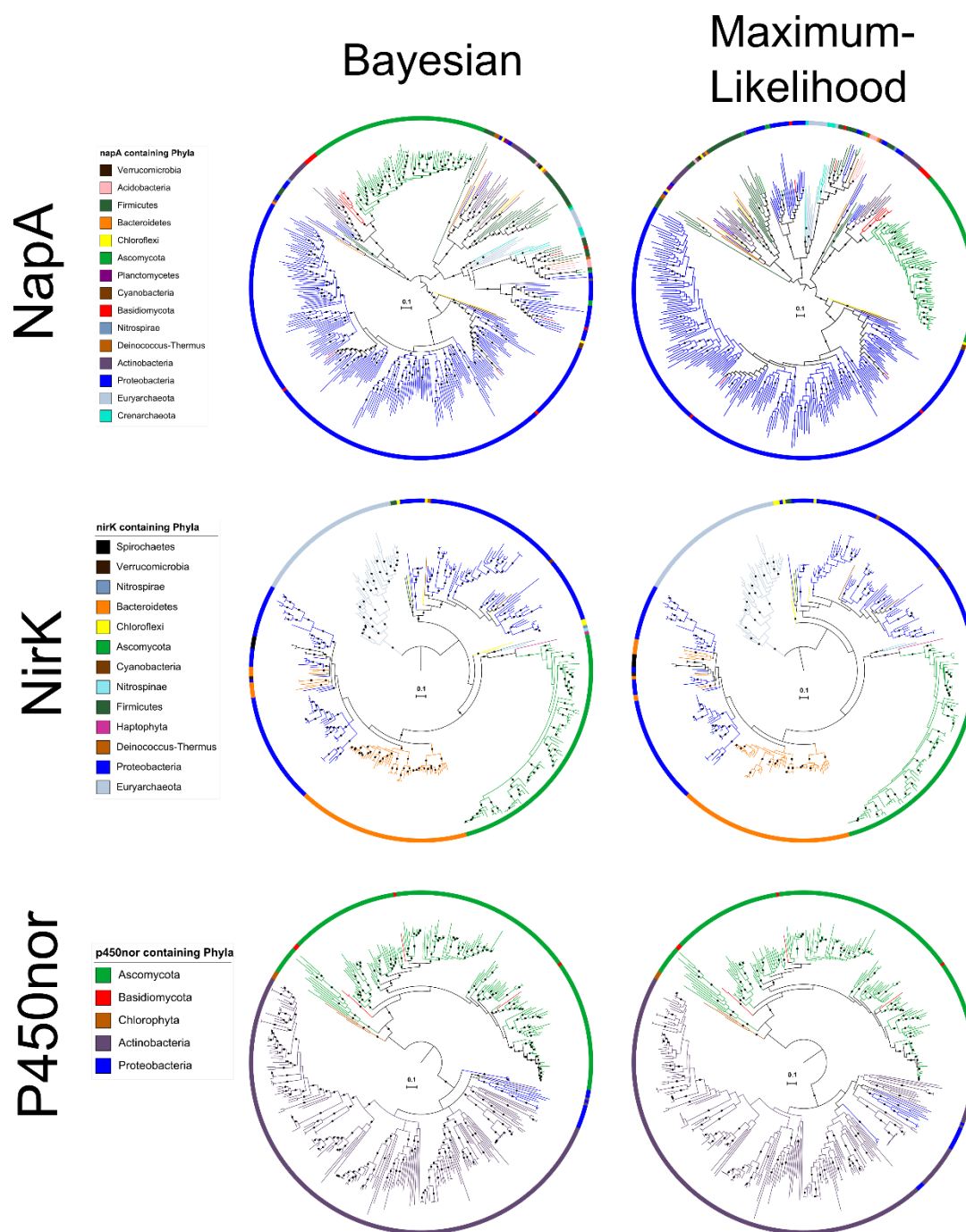


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241 **Figure S7.** Midpoint rooted Bayesian (left) and Maximum-Likelihood phylogenies (right) of cytochrome P450 sequences (N = 408)
 242 demonstrating the affiliation of P450nor with other sequences belonging to members of the bacterial phyla *Actinobacteria* and
 243 *Proteobacteria*. Cyanobacterial cytochrome P450 sequences were included as outgroups. Black squares on branches (left tree) indicate
 244 ≥ 0.95 posterior probability or $\geq 90\%$ bootstrap replication (right tree). The colored legend indicates the cytochrome P450 family
 245 specified by shared amino acid identity of $\geq 40\%$ (39).

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248 **Figure S8.** Bayesian and Maximum-likelihood phylogenies of NapA, NirK, and P450nor amino
 249 acid sequence homologs extracted from the RefSeq protein database. A high-resolution file of
 250 these trees are available at <https://doi.org/10.6084/m9.figshare.c.3845692>.

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