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Table S3. Results from species-tree gene-tree reconciliation using NOTUNG software for \( \text{napA} \), \( \text{nirK} \), and \( \text{p450nor} \) genes in fungi. Values are averages of solutions with standard deviations reported in parentheses.

Table S4. Predicted horizontal gene transfers of fungal \( \text{p450nor} \), \( \text{napA} \), and \( \text{nirK} \) genes based on alien index algorithm.

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

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

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

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

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

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

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

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

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

**Materials and Methods**

**Phylogenetic reconstructions**

Selection of the optimal evolutionary model for ML tree reconstruction was performed using prottest (Abascal et al., 2005) (amino acid alignment) and jmodeltest (Posada, 2008) (nucleotide alignment) software prior to ML tree reconstruction. Please refer to SI for additional details about evolutionary models used in phylogenetic analysis. Phylogenetic analysis with RAxML
was performed by sampling 20 starting trees and performing 1,000 replicate bootstrap analyses.
The tree with the maximal negative log likelihood score was compared to 1,000 replicates in RAxML to generate the final tree. Bayesian tree construction was performed using 3 independent runs with 6 chains for 5,000,000 generations. Output from MrBayes was evaluated with the sump and sumt commands within the software to ensure Markov Chain Monte Carlo chain mixing and convergence (potential scale reduction factor of 1.0) and standard deviation of split frequencies ~ 0.01 or lower. MrBayes output was further visualized in the program Tracer (http://tree.bio.ed.ac.uk/software/tracer/) to ensure convergence was reached.

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

For trait correlations, the concatenated 238 BUSCO gene alignment (see main text Materials and Methods) of 709 fungal taxa was bootstrapped into 800 replicate alignments using the PHYLIP software function seqboot (6) and 800 ML trees created as described in the main text using FastTree2 software. These alignments were paired with presence/absence data regarding denitrification traits and provided as input to BayesTraits software. BayesTraits was first operated in ML mode (100 ML tries setting) to generate parameter estimates for dependent (trait correlation) and independent (no trait correlation) models to be compared. These parameter estimates were then entered into BayesTraits, and three independent runs of the software in Bayesian mode using the dependent and independent model of trait correlation between the two traits being compared were performed. The analysis was run for 1,000,000 generations with samples taken every 1,000th generation and a burn-in of 50,000 generations. A stepping stone analysis (100 stones, 10,000 samples) was performed to generate log marginal likelihood values for Bayes Factor (BF) calculations to test which model (correlation or no correlation) best fit the data. Bayes Factors are comparable to a likelihood ratio test for model selection, and the larger the Bayes Factor the more certainty there is in the more complex, dependent model (indicating trait correlation). Hence, a BF of 1 is indicative of weak or no trait correlation, but a BF of 10 or larger indicates strong selection of the dependent model and trait correlation (Pagel et al., 2004).

A similar analysis is performed for ancestral state reconstruction, except that trees from a Bayesian analysis were used as input to the MultiState method of the software. Multistate was run for 5,500,000 generations with sampling every 2,000th generation and a burn-in of 500,000 generations. The probability of a given character state at a node within the tree was averaged
over all generations after the burn-in period and was used to determine support for the state of a node within the tree.

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

Alien index calculations

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

\[ AI = \frac{\text{out group bitscore}}{\text{max bitscore}} - \frac{\text{in group bitscore}}{\text{max bitscore}} \]
AI values range from 1 to -1. Values greater than zero are indicative of HGT or contamination of foreign DNA within the genome sequence being queried.

References

Abascal F, Zardoya R, Posada D. (2005). ProtTest: selection of best-fit models of protein evolution. *Bioinforma* **21**: 2104–2105.

Jones D, Taylor W, Thornton J. (1992). The rapid generation of mutation data matrices from protein sequences. *Comput Appl Biosci* **8**.

Le SQ, Gascuel O. (2008). An improved general amino acid replacement matrix. *Mol Biol Evol* **25**: 1307–20.

Pagel M, Meade A, Barker D. (2004). Bayesian estimation of ancestral character states on phylogenies. *Syst Biol* **53**: 673–684.

Posada D. (2008). jModelTest: Phylogenetic model averaging. *Mol Biol Evol* **25**: 1253–1256.

Rodriguez R, Oliver JL, Marin A, Medina JR. (1990). The general stochastic model of nucleotide substitution. *J Theor Biol* **142**. doi:10.1016/S0022-5193(05)80104-3.

Whelan S, Goldman N. (2001). A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. *Mol Biol Evol* **18**: 691–699.

Wisecaver JH, Alexander WG, King SB, Todd Hittinger C, Rokas A. (2016). Dynamic evolution of nitric oxide detoxifying flavohemoglobins, a family of single-protein metabolic modules in *Bacteria* and Eukaryotes. *Mol Biol Evol*. doi:10.1093/molbev/msw073.
Table S1. Counts of denitrification traits and their co-occurrences in fungal genomes.

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

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

| Gene | Lineage         | No. Genes | No. Taxa | No. Genera | Monophyletic | Amino acid | Nucleotide | Diff –lnL | P value | Diff –lnL | P value |
|------|-----------------|-----------|----------|------------|--------------|------------|------------|-----------|---------|-----------|---------|
| napA | 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-01  | 770     | 9.00E-56  |
|      | Tremellomycetes | 1         | 1        | 1          | Yes          | Yes        | -          | -         | -       | -         |
|      | L+S share MRCA | No        | No       |            | No           | No         | 908        | 2.00E-07  | 973     | 6.00E-74  |
|      | Lec+E share MRCA| No        | No       |            | No           | No         | 75         | 3.00E-05  | 257     | 9.00E-06  |
|      | *Diff –lnL = difference in negative log-likelihood of the observed tree to the constraint tree in which the taxa were constrained to be monophyletic.

nirK

| Lineage         | No. Genes | No. Taxa | No. Genera | Monophyletic | Amino acid | Nucleotide | Diff –lnL | P value | Diff –lnL | P value |
|-----------------|-----------|----------|------------|--------------|------------|------------|-----------|---------|-----------|---------|
| Dothideomycetes | 1         | 1        | 1          | Yes          | Yes        | -          | -         | -       | -         | -       |
| Eurotiomycetes  | 52        | 52       | 17         | No           | No         | 19         | 0.142     | 33      | 0.003     |
| Leotiomycetes   | 10        | 10       | 1          | Yes          | Yes        | -          | -         | -       | -         |
| Sordariomycetes | 20        | 20       | 8          | Yes          | Yes        | -          | -         | -       | -         |
| D+E share MRCA  | No        | No       |            | No           | No         | 15         | 0.042     | 30.1    | 0.004     |
| L+S share MRCA  | No        | No       |            | No           | No         | 18         | 0.142     | 35.3    | 0.001     |
| Gene          | Lineage            | No. Genes | No. Taxa | No. Genera | Monophyletic | Amino acid | Nucleotide | Diff \(-\text{lnL}\) | P value | Diff \(-\text{lnL}\) | P value |
|--------------|--------------------|-----------|----------|------------|--------------|------------|------------|----------------|---------|----------------|---------|
| p450nor      | 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 |             | 1386      | 1420     | 5.00E-72   |             |            |            |         |         |         |
| D+E share MRCA |             | 1481      | 1481     | 2.00E-63   |             |            |            |         |         |         |
| Ascomycota (A)  |             | 139       | 140      | 6.00E-06   |             |            |            |         |         |         |
| Basidiomycota (B)|             | 139       | 139      | 1.00E-39   |             |            |            |         |         |         |
| A+B share MRCA |             | 139       | 140      | 3.00E-04   |             |            |            |         |         |         |

*Diff \(-\text{lnL}\) = difference in negative log-likelihood of the observed tree to the constraint tree in which the taxa were constrained to be monophyletic.
Table S3. Results from species-tree gene-tree reconciliation using NOTUNG software for napA, nirK, and p450nor genes in fungi. Values are averages of solutions with standard deviations reported in parentheses.

| Gene   | Phylogeny | Duplications | Codivergences | Transfers | Losses   | Duplication cost | Transfer cost | Loss cost | Solutions |
|--------|-----------|--------------|---------------|-----------|----------|------------------|---------------|-----------|-----------|
| p450nor amino acid | -     | -            | -             | -         | 2        | 3                | 1             | 0         |           |
|        | 49.0 (0.0)| 0.0 (0.0)    | 15.0 (0.0)    | 253.0 (0.0)| 2        | 11               | 1             | 0         | 1000      |
|        | 61.0 (0.0)| 0.0 (0.0)    | 6.0 (0.0)     | 333.0 (0.0)| 2        | 13               | 1             | 0         | 180       |
|        | 62.1 (1.0)| 0.0 (0.0)    | 5.4 (0.5)     | 339.3 (5.4)| 2        | 15               | 1             | 0         | 420       |
| p450nor nucleotide | -     | -            | -             | -         | 2        | 3                | 1             | 0         |           |
|        | 45.0 (0.0)| 0.0 (0.0)    | 16.0 (0.0)    | 215.0 (0.0)| 2        | 9                | 1             | 0         | 1000      |
|        | 53.6 (0.8)| 0.0 (0.0)    | 8.2 (0.4)     | 277.6 (2.8)| 2        | 11               | 1             | 0         | 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        |
| napA 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         |
| napA 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         |           |
### 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 |
|------------|-------------------|------------|--------------|-------------|--------------|------------------|----------------|----------------|--------------------------------|--------------------------------|----------------|----------------|
| *p450nor* | Apimol            | Apiospora  | 418          | 242         | 851          | 0.006           | Sordariomycetes | Dothideomycetes | V. lopsis laxa CBS 191.97 v1.0 | P. fracticola                | GCA_0015      | 92805.1       |
|            |                   | montagnei  |              |             |              |                  |                 |                |                                |                               |                |                |
|            |                   | NRRL 25634 |              |             |              |                  |                 |                |                                |                               |                |                |
| GCA_0000  | Aspergillus      | niger     | 353          | 437         | 859          | 0.098           | Eurotiomycetes  | Dothideomycetes | U. r. CBS 1704                      | S. parasiticus SU-1           | GCA_0000      | 03515.2       |
| 02855.2    |                   | 513 88     |              |             |              |                  |                 |                |                                |                               |                |                |
| GCA_0001  | Nectria           | haematococc | 469          | 583         | 881          | 0.129           | Sordariomycetes | Eurotiomycetes | S. pallida               | T. virens Gv29-8                | GCA_0007      | 10705.2       |
| 51355.1_3 |                   | a mpVI 77- |              |             |              |                  |                 |                |                                |                               | GCA_0009      | 56085.1       |
| 13-4       |                   | 2479       |              |             |              |                  |                 |                |                                |                               |                |                |
| GCA_0002  | Trichosporon     | asahii var | 400          | 572         | 813          | 0.212           | Tremellomycetes | Sordariomycetes | M. blollopis          | T. rufum                      | GCA_0009      | 50635.1       |
| 93215.1    |                   | asahii CBS |              |             |              |                  |                 |                |                                |                               |                | Plecu1         |
|            |                   | 2479       |              |             |              |                  |                 |                |                                |                               |                |                |
| GCA_0004  | Byssoschlamy     | s spectabilis | 528         | 659         | 827          | 0.158           | Eurotiomycetes  | Sordariomycetes | E. xenobiotic a colletotria chum | T. rufum                      | GCA_0008      | 35505.1       |
| 97085.1    |                   |            |              |             |              |                  |                 |                |                                |                               | GCA_0001      | 70995.2       |
| GCA_0007  | Sporothrix       | paludula  | 577          | 656         | 833          | 0.095           | Sordariomycetes | Dothideomycetes | R. rufum               | A. richmonden sis                  | GCA_0003      | 19635.1       |
| 10705.2    |                   |            |              |             |              |                  |                 |                |                                |                               |                | Rhyru1_1       |
| GCA_0007  | Geotrichum       | candidum  | 586          | 650         | 854          | 0.075           | Leotiomycetes   | Dothideomycetes | T. r. CBS 18893                     | A. richmonden sis                  | GCA_0015      | 72075.1       |
| 43665.1    |                   |            |              |             |              |                  |                 |                |                                |                               |                |                |
| GCA_0008  | Mrakia           | frigida   | 279          | 421         | 745          | 0.191           | Tremellomycetes | Eurotiomycetes | T. r. CBS 18893                     | A. richmonden sis                  | GCA_0009      | 50635.1       |
| 15965.1    |                   |            |              |             |              |                  |                 |                |                                |                               |                | Monpu1         |
| GCA_0008  | Exophiala        | xenobiotic a | 573         | 637         | 887          | 0.072           | Eurotiomycetes  | Dothideomycetes | T. r. CBS 18893                     | A. richmonden sis                  | GCA_0001      | 50975.2       |
| 35505.1    |                   |            |              |             |              |                  |                 |                |                                |                               | GCA_0015      | 72075.1       |
| GCA_0009  | Mrakia           | blollopis | 371          | 531         | 852          | 0.188           | Tremellomycetes | Eurotiomycetes | T. r. CBS 18893                     | A. richmonden sis                  | GCA_0002      | 93215.1       |
| 50635.1    |                   |            |              |             |              |                  |                 |                |                                |                               |                | Monpu1         |

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

†IG = Ingroup, OG = Outgroup.
**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 |
|------------|-------------------|-----------------------------------------|----------------------|-------------|--------------|-------------------|---------------------|---------------------|-----------------------------|-----------------------------|----------------|----------------|
| p450nor    | GCA_0015 72075.1  | Acidomyces richmondensis                | 584                  | 637         | 831          | 0.064             | Dothideomycetes    | Eurotiomycetes     | Rhytidhys teron rufalum     | Myriangi um duriae CBS     | GCA_0008 35505.1 | Myru1_1         |
|            |                   |                                         |                      |             |              |                   |                     |                     | Sclerotinia sclerotiorum m  | 1980 UF-70                  | GCA_0001 46945.1 |                |
| GCA_0015   | 92805.1           | Peltaster fructicola                    | 451                  | 483         | 840          | 0.038             | Dothideomycetes    | Leotiomyctetes      | Aspergillus niger CBS      | 513 88                      | GCA_0002 81105.1 | GCA_0000 02855.2 |
| Macan1     |                   | Macrophom a anomochlaeta CBS 525.71    | 393                  | 437         | 846          | 0.052             | Dothideomycetes    | Eurotiomycetes     | Valetiell opinis laxa CBS  | 191.97 v1.0                 | GCA_0015 92805.1 | Valla1          |
| Myrdu1     |                   | Myriangium duriae CBS 260.36            | 451                  | 508         | 840          | 0.068             | Dothideomycetes    | Sordariomyctetes   | Apiospora montagne i NRRL  | 25634 v1.0                 | GCA_0002 81105.1 | GCA_0000 02855.2 |
| Valla1     |                   | Valetoniellopsis laxa CBS 191.97 v1.0  | 418                  | 508         | 838          | 0.107             | Sordariomyctetes   | Dothideomycetes    | Clavaria famosa           |                             | Apimo1 | Myrdu1         |
| napA       | GCA_0002 25285.2  | Epichloe glyceriae E277                 | 270                  | 326         | 1367         | 0.041             | Sordariomyctetes   | Agaricomycetes     | Pseudogym noascues sp. VKM F- | 4513 FW-928                 | GCA_0007 09145.1 | GCA_0011 79745.1  |
| GCA_0002   | 81105.1           | Coniosporia m apollinis CBS 100218      | 1416                 | 1436        | 2040         | 0.010             | Dothideomycetes    | Leotiomyctetes     | Fungal sp. No 11243       |                             | GCA_0008 36255.1 | GCA_0007 50755.1 |
| GCA_0003   | 15175.1           | Herpotrich laceae sp. UM238             | 1265                 | 1286        | 1989         | 0.011             | Eurotiomycetes     | Sordariomyctetes   | Aspergillum ustus          |                             | GCA_0008 12125.1 | GCA_0003 13795.2 |
| GCA_0004   | 64645.1           | Melampsora pinitorqua Mpin7            | 437                  | 872         | 1645         | 0.264             | Pucciniomyctetes   | Eurotiomycetes     | Cronarti um ribicola 11-2  |                             | GCA_0005 00245.1 | GCA_0014 30945.1  |

\(^{+}\)Assembly IDs with two underscores followed by a number indicate the query gene is multi copy within the genome.

\(^{1}\)IG = Ingroup, OG = Outgroup.
Table S4. (continued)

| Gene | Query assembly ID | Query name | IG' bitscore | OG bitscore | Max bitscore | Alien index value | IG taxon | OG taxon | IG assembly ID | OG assembly ID |
|------|-------------------|------------|--------------|-------------|--------------|-----------------|----------|----------|---------------|----------------|
| napA | GCA_0005 00245.1  | Cronartium ribicola 11-2 | 431 | 905 | 1626 | 0.292 | Pucciniomycetes | Sordariomycetes | Melampyssara pinitorquera Mpin7 | Balansia obtecta B249 | GCA_0004 64645.1 | GCA_0007 09145.1 |
|      | GCA_0006 11775.1  | Umbilicaria muehlenbergii | n/a | 1421 | 2035 | 0.698 | Lecanoromycetes | Leotiomyces | Umbilicaria muehlenbergii | Pseudogymnoascus sp. VKM F-4513 FW-928 | GCA_0006 11775.1 | GCA_0007 50755.1 |
|      | GCA_0007 09145.1  | Balansia obtecta B249 | 278 | 925 | 1854 | 0.349 | Sordariomycetes | Pucciniomycetes | Epichloe glyceriae E277 | Cronartium ribicola 11-2 | GCA_0002 25285.2 | GCA_0005 00245.1 |
|      | GCA_0007 50755.1  | Pseudogymnoascus sp. VKM F-4513 FW-928 | 1415 | 1436 | 2037 | 0.010 | Leotiomyces | Dothideomycetes | Geotrichum candidum | Coniosporum apollinis CBS 100218 Colletotrichum orbiculare MAFF 240422 | GCA_0007 43665.1 | GCA_0002 81105.1 |
|      | GCA_0014 68955.1  | Cryptococcus albidus | n/a | 1033 | 2058 | 0.502 | Tremellomycetes | Sordariomycetes | Cryptococcus albidus | Cryptococcus orbiculare MAFF 240422 | GCA_0014 68955.1 | GCA_0003 50065.1 |
|      | GCA_0015 72075.1  | Acidomyces richmondensis | n/a | 559 | 888 | 0.630 | Dothideomycetes | Eurotiomyces | Acidomyces richmondensis | Arthrobotrya otae CBS 113480 | GCA_0015 72075.1 | GCA_0001 51145.1 |

*Assembly IDs with two underscores followed by a number indicate the query gene is multi copy within the genome.
†IG = Ingroup, OG = Outgroup.
Table S5. List of genera containing species with and without $p450$nor.

| Genus              | Total species | Species with $p450$nor | Percentage with $p450$nor |
|--------------------|---------------|------------------------|---------------------------|
| Arthroderma        | 2             | 1                      | 50.0                      |
| Aspergillus        | 20            | 9                      | 45.0                      |
| Bipolaris          | 6             | 2                      | 33.3                      |
| Colletotrichum     | 10            | 5                      | 50.0                      |
| Diaporthe          | 3             | 1                      | 33.3                      |
| Diploida           | 3             | 2                      | 66.7                      |
| Exophiala          | 7             | 1                      | 14.3                      |
| Fusarium           | 16            | 13                     | 81.3                      |
| Hirsutella         | 2             | 1                      | 50.0                      |
| Hymenoscyphus      | 7             | 5                      | 71.4                      |
| Metarhizium        | 7             | 6                      | 85.7                      |
| Neosartorya        | 2             | 1                      | 50.0                      |
| Neurospora         | 6             | 4                      | 66.7                      |
| Pseudogymnoascus   | 16            | 15                     | 93.8                      |
| Pyrenochaeta       | 3             | 1                      | 33.3                      |
| Rhytidhysterion    | 2             | 1                      | 50.0                      |
| Rutstroemia        | 2             | 1                      | 50.0                      |
| Sclerotinia        | 3             | 2                      | 66.7                      |
| Sporothrix         | 3             | 1                      | 33.3                      |
| Trichoderma        | 8             | 4                      | 50.0                      |
| Trichophyton       | 6             | 5                      | 83.3                      |
| Trichosporon       | 2             | 1                      | 50.0                      |
Figure S1. Gene abundances of *narG*, *napA*, *nirK*, *p450nor*, and flavohemoglobins (colored bars) mapped on to fungal families (cladogram, left). Relationships among fungal families in the cladogram were derived from the NCBI taxonomy using the online tool phyloT (http://phylot.biobyte.de/index.html).
Figure S2. Maximum-Likelihood phylogenies connecting fungal species with their respective NO reductase (p450nor) gene sequence(s). On the left, an amino acid phylogeny of 238 concatenated single copy orthologues from fungal species in which one or more p450nor gene(s) were detected. The p450nor nucleotide phylogeny (right) demonstrates many instances of incongruence with the fungal species phylogeny. Black dots in each phylogeny represent bootstrap percentages greater than or equal to 90%. Scale bars represent amino acid (left tree) and nucleotide (right tree) substitutions per site. A high-resolution file of the tree is available at https://doi.org/10.6084/m9.figshare.c.3845692.
Figure S3. Cophylogenetic plot of napA-containing fungal species (left, N = 75) and the napA nucleotide tree (right, N = 78). Both are midpoint rooted Maximum-Likelihood trees where black dots represent bootstrap percentages ≥90%. Scale bars indicate substitutions per site for the concatenated amino acid species phylogeny and nucleotide phylogeny, respectively. A high-resolution file of the tree is available at https://doi.org/10.6084/m9.figshare.c.3845692.
Figure S4. Cophylogenetic plot of nirK-containing fungal species (left, N = 82) and the nirK nucleotide tree (right, N = 83). Both are midpoint rooted Maximum-Likelihood trees where black dots represent bootstrap percentages ≥90 %. Scale bars indicate substitutions per site for the concatenated amino acid species phylogeny and nucleotide phylogeny, respectively. A high-resolution file of the tree is available at https://doi.org/10.6084/m9.figshare.c.3845692.
**Figure S5.** Plot of alien index values observed for *p450nor* genes (N = 178). Points above the hashed line at the origin are indicative of HGT. Names of fungal species with alien index values above zero are ordered as their points appear on the graph. Thick horizontal lines represent the median alien index value. See Materials and Methods in Supplementary Materials for details on alien index calculations.
Figure S6. Bayesian tree reconstruction of actinobacterial and proteobacterial 16S rRNA genes (left, N = 55) and cytochrome P450 family 105 amino acid sequences (right, N = 57). Both phylogenies represent 50% majority-rule consensus trees. The tree on the left is rooted with proteobacterial sequences as outgroup to the *Actinobacteria*. The tree on the right is midpoint rooted. Nodes with posterior probabilities ≥ 0.95 are indicated by black circles on an adjacent branch.
Figure S7. Midpoint rooted Bayesian (left) and Maximum-Likelihood phylogenies (right) of cytochrome P450 sequences (N = 408) demonstrating the affiliation of P450nor with other sequences belonging to members of the bacterial phyla Actinobacteria and Proteobacteria. Cyanobacterial cytochrome P450 sequences were included as outgroups. Black squares on branches (left tree) indicate ≥0.95 posterior probability or ≥90 % bootstrap replication (right tree). The colored legend indicates the cytochrome P450 family specified by shared amino acid identity of ≥40 % (39).
**Figure S8.** Bayesian and Maximum-likelihood phylogenies of NapA, NirK, and P450nor amino acid sequence homologs extracted from the RefSeq protein database. A high-resolution file of these trees are available at https://doi.org/10.6084/m9.figshare.c.3845692.
Figure S9. Genome regions chosen for in depth presentation of protein coding genes surrounding p450nor in predicted BGC regions. Labels above genes are functional annotations from alignments to the eggNOG database. NCBI gene loci accessions are labeled below each gene. Numbers in parentheses represent the proportion of these genes shown that are also found within closely related genomes where available.