

1 **SUPPLEMENTAL MATERIAL**

2 **DNA extraction.** Fungal biomass (0.3 g wet weight) was placed in a 2-mL screw cap plastic
3 tube containing approximately 200 mg of 0.5 mm silica and zirconium beads (Fisher Scientific,
4 USA). Then, 1 mL of Carlson lysis buffer (1) and 1.5 µL β-mercaptoethanol (Fisher Scientific,
5 USA) were added to each tube, followed by two rounds of bead-beating at room temperature for
6 5 minutes in an Omni bead raptor 24 (Omni International, Kennesaw, GA) at 5.65 m/s. After
7 sample incubation at 74 °C for 30 minutes, an initial chloroform:isoamyl alcohol (24:1, [v/v])
8 wash step was followed by two additional wash steps with 750 µL phenol:chloroform:isoamyl
9 alcohol (25:24:1 [v/v]) before 750 µL chloroform:isoamyl alcohol (24:1, [v/v]) was added. The
10 upper aqueous phase was transferred to a fresh tube, to which 0.6 volumes of ice-cold isopropyl
11 alcohol was added, followed by cooling at -20 °C for at least 2 hours. Samples were then
12 centrifuged at 4 °C for 15 minutes at 10,000 x g, the supernatants discarded, and the pellet
13 washed with ice-cold 70 % ([v/v]) molecular grade ethanol. Following a final centrifugation step
14 (10,000 x g, 5 minutes), the supernatants were discarded, and the pellets air-dried at room
15 temperature. The pellets were suspended in 100 µL DNase-free H₂O and stored at -20 °C until
16 analysis. DNA extraction from soil samples was performed using the FastDNA spin kit for soil
17 (MP Biomedicals, Santa Ana, CA) following the manufacturer's protocol. DNA concentration
18 and purity were analyzed with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA)
19 and a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA) using the dsDNA BR Assay Kit
20 following manufacturer's recommendations (Life Technologies, Carlsbad, CA).

21

22 **Primer design.** To design primers targeting *p450nor* gene sequences, primary literature
23 resources were queried to identify available *p450nor* gene and protein sequences. These

24 reference P450nor sequences were assessed using multiple sequence alignment (2) to identify
25 conserved residues, followed by selection of additional sequences by querying public sequence
26 databases (GenBank, UniProt) using BLASTx (3). Overall, 38 *p450nor* reference gene sequences
27 were obtained and aligned using the program T-Coffee (2) followed by manual inspection.
28 PAL2NAL (4) was used to generate a codon-aware nucleotide alignment to assist in selecting
29 sites for primer design. In order to design primers specific to fungal *p450nor* sequences and
30 prevent binding to other genes present within the diverse cytochrome P450 protein family,
31 features unique to the 38 P450nor reference sequences were identified (5–7). Specifically,
32 mutations in a secondary structural element of the P450nor protein, referred to as the B helix,
33 abolished the reducing capacity of the enzyme and implicated specific amino acid residues in
34 NADH or NADPH co-substrate binding (5). Additionally, the B helix has been proposed as a
35 unique and variable structural element of the CYP55 family of P450 cytochromes (8); therefore,
36 an adjacent, conserved site was selected for forward primer binding (*p450nor394F*, Table 1).
37 Further inspection of the 38 reference protein sequences revealed conserved secondary structural
38 elements 650–750 nucleotides downstream of the forward priming site suitable for reverse primer
39 design (Table 1).

40

41 REFERENCES

- 42 1. **Carlson JE, Tulsieram LK, Glaubitz JC, Luk VWK, Kauffeldt C, Rutledge R.** 1991.
43 Segregation of random amplified DNA markers in F1 progeny of conifers. Theor. Appl.
44 Genet. **83**:194–200.
- 45 2. **Notredame C, Higgins DG, Heringa J.** 2000. T-coffee: a novel method for fast and
46 accurate multiple sequence alignment. J. Mol. Biol. **302**:205–217.

- 47 3. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** 1990. Basic local alignment
48 search tool. *J. Mol. Biol.* **215**:403–410.
- 49 4. **Suyama M, Torrents D, Bork P.** 2006. PAL2NAL: robust conversion of protein
50 sequence alignments into the corresponding codon alignments. *Nucleic Acids Res.* **34**
51 :W609–W612.
- 52 5. **Zhang L, Kudo T, Takaya N, Shoun H.** 2002. Distribution, structure and function of
53 fungal nitric oxide reductase P450nor—recent advances. *Int. Congr. Ser.* **1233**:197–202.
- 54 6. **Oshima R, Fushinobu S, Su F, Zhang L, Takaya N, Shoun H.** 2004. Structural
55 evidence for direct hydride transfer from NADH to cytochrome P450nor. *J. Mol. Biol.*
56 **342**:207–217.
- 57 7. **Kudo T, Takaya N, Park S-Y, Shiro Y, Shoun H.** 2001. A positively charged cluster
58 formed in the heme-distal pocket of cytochrome P450nor is essential for interaction with
59 NADH. *J. Biol. Chem.* **276**:5020–5026.
- 60 8. **Nelson DR.** 2009. The cytochrome P450 homepage. *Hum. Genomics* **4**:59–65.
- 61

62 **SUPPLEMENTAL FIGURE LEGENDS**

63 **Figure S1.** Stereomicroscopic images of representative members of isolated fungal groups (A)
64 and singletons (B) identified by sequencing of the cloned ITS region from each fungal isolate.
65 The group identifier and putative taxonomic designation are listed in the white boxes to the left
66 of the images. Scale bars and fungal isolate ID number are located in the upper left and lower left
67 corner of each image, respectively.

68

69 **Figure S2.** Alignment of P450nor sequences from select organisms displaying primer binding
70 sites (pink), and key residues involved in cofactor (NAD(P)H) binding and P450nor activity. The
71 N-terminal region of the alignment has been removed for clarity. Stars below the alignment
72 (blue, red, black) indicate positive, negative and charged amino acids and key residues involved
73 in NAD(P)H binding, respectively. Sites outlined in black with all bold amino acid characters
74 indicate 100% amino acid identity. Columns highlighted yellow and outlined in black indicate \geq
75 80% amino acid similarity. Stars with a white "S" and "H" indicate involvement in a salt bridge
76 and proton channel, respectively. PP stands for a residue believed to be involved in binding
77 pyrophosphate on the NAD(P)H molecule. The B' helix (helix 3 α above) is a hypervariable
78 region of P450 proteins and represents an area of NAD(P)H recognition by P450nor. P450nor
79 secondary structure is provided by the PDB accession 1JFB and was added using the ESPript 3.0
80 web server. F_oxysorum = *Fusarium oxysporum*, F_lichenicola = *Fusarium lichenicola*,
81 C_globosum = *Chaetomium globosum*, T_virens = *Trichoderma virens*, S_macrospora =
82 *Sordaria macrospora*, G_graminis = *Gaeumannomyces graminis*, A_terreus = *Aspergillus*
83 *terreus* NRRL 255, A_flavus = *Aspergilus flavus* NRRL 3357, U_reesii = *Uncinocarpus reesii*,
84 C_apollinis = *Coniosporium apollinis*, M_gypseum = *Microsporum gypseum*, M_brunnea =
85 *Marssonina brunnea*, M_phaseolina = *Macrophomina phaseolina*, T_cutaneum = *Trichosporon*
86 *cutaneum*, C_reinhardtii = *Chlamydomonas reinhardtii*, C_variabilis = *Chlorella variabilis*.
87
88 **Figure S3.** N₂O production in soil microcosms amended with chloramphenicol and streptomycin
89 to inhibit bacterial activity. NO₃⁻ (2 mM) or NO₂⁻ (1 mM) were added as the sole nitrogen
90 source. Acetate (●), formate (□), pyruvate (▲), or plant residue (◊, dashed line), were added as
91 carbon source and electron donor for NO₃⁻ or NO₂⁻ reduction, respectively, in Havana (left

92 column) or Urbana (right column) microcosms. Note the use of different scales on the y-axes.

93 Carbon sources were not monitored during the enrichment.

94

95 **Figure S4.** NO_3^- (\blacktriangle), NO_2^- (\blacksquare), and N_2O (\circ) dynamics in transfer cultures from Havana and
96 Urbana microcosms amended with ampicillin and kanamycin to prevent bacterial activity. NO_3^-
97 (2 mM) or NO_2^- (1 mM) were provided as the sole nitrogen source. Acetate (A, B, C, D), formate
98 (E, F, G, H), plant residue (I, J, K, L), or pyruvate (M, N, O, P) were added as carbon source and
99 electron donor. Note the differences in scale on the y-axes.

100

101 **Figure S5.** Intron structure of the region amplified by *p450nor* primer set
102 *p450nor394F/p450nor809R* in 47 *p450nor* sequences. Only one to four introns are detected in
103 the amplified region. Thick black lines indicate the gene, and black rectangles above this line
104 indicate the coding sequences (CDS). Thin lines between CDS rectangles indicate an intron.
105 Green triangles indicate *p450nor394F/p450nor809R* binding sites. Some genes have been
106 reversed for clarity (indicated by red REV next to sequence name).

107

108 SUPPLEMENTAL DATASET LEGENDS

109 **Dataset S1.** NCBI protein GI and Accession numbers of P450nor sequences used for primer
110 design.

111 **Dataset S2.** Accessory metadata for the fungal isolates from Havana and Urbana soils.

112 **Dataset S3.** The SILVA 18S rRNA gene classification of 37 fungal isolates in Figure 1.

113 **Dataset S4.** *p450nor* amplicon lengths and number of introns in amplified region using primer
114 set *p450nor394F/p450nor809R*.

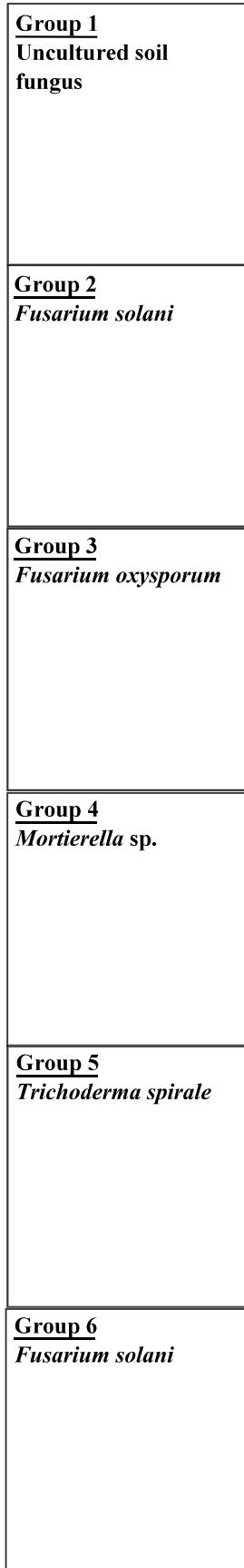
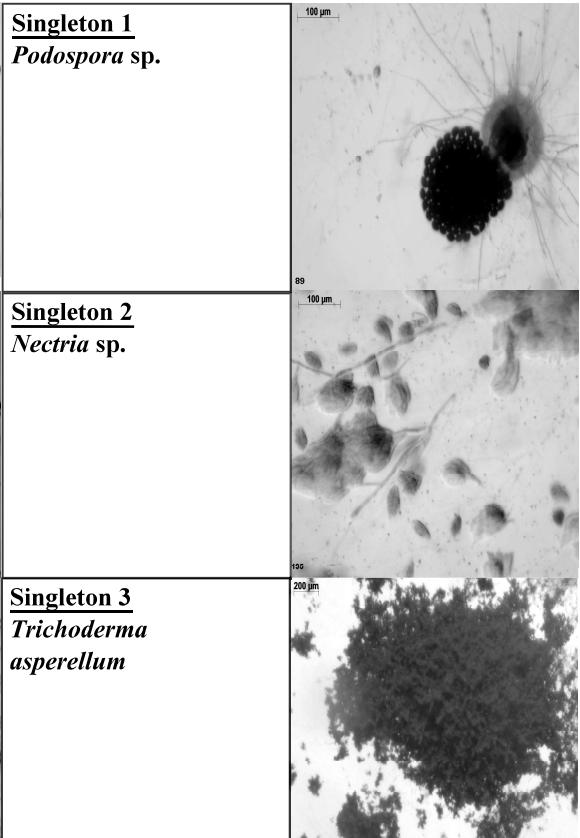
A**B**

Figure S1. Stereomicroscopic images of representative members of isolated fungal groups (A) and singletons (B) identified by sequencing of the cloned ITS region from each fungal isolate. The group identifier and putative taxonomic designation are listed in the white boxes to the left of the images. Scale bars and fungal isolate ID number are located in the upper left and lower left corner of each image, respectively.

The figure shows a sequence alignment of F. oxysporum and other fungal species. The alignment highlights conserved regions with yellow shading. Above the alignment, positions 10, 20, 30, 40, 50, and 60 are indicated. Below the alignment, five blue arrows labeled α_1 , β_1 , β_2 , α_2 , and β_3 point to specific regions. The species listed are: *F. oxysporum*, *F. lichenicola*, *C. globosum*, *T. virens*, *S. macrospora*, *G. graminis*, *A. terreus*, *A. flavus*, *U. reesii*, *C. apollinis*, *M. gypseum*, *M. brunnea*, *M. phaseolina*, *T. cutaneum*, *C. reinhardtii*, and *C. variabilis*.

B' Helix

<i>F. oxysporum</i>	►	7 0	8 0	9 0	1 0 0	1 1 0
<i>F. oxysporum</i>		S K V R T R Q G F P E L S A S G K Q A A K . . . A K P T F V D M D P P F E H M H Q R S M V E P T F T P A V K				
<i>F. lichenicola</i>		S K V R T R P G F P E L N A G G K Q A A K . . . A K P T F V D M D P P A D H M N Q R G M V E S L F T L I E H V K				
<i>C. globosum</i>		S K E R S R P G F P E F S D G G K A A G K . . . Q R P T F V D M D P P P E H M R H R S M V E S W F T P E K V K				
<i>T. virens</i>		S K V R T R P G F P E L G A G G K E A A K . . . A K P T F V D M D P P P D H M Q R S M V E S L F S A E H I K				
<i>S. macrospora</i>		S K E R T R P G F P E L S A G G K E A A K . . . N R A T F V D M D P P P K H M Q Y R G M V Q P I F N I E H I Q				
<i>G. graminis</i>		S K Q R T R P G F P E L S A G G K A A A K . . . N K A T F V D M D P P A P D H M R Q R G M V E P L F V L E H I K				
<i>A. terreus</i>		S K E R S R A G F P E M N P G G K A A A K . . . N R P T F V D M D P P P N H M R Q R Y W				
<i>A. flavus</i>		S K V R Q R D G F P E M S P G G K A A A R . . . N R P T F V D M D P P A P D H M H Q R S M V S A F F N D A Y V E				
<i>U. reesii</i>		S K Q R Q R D G F P E M S A G G K A A A K . . . N R P T F V D M D P P P D H M R Q R G M V T S S F T P E Y T E				
<i>C. apollinis</i>		S K I R T T P G F P E L S A G G K A A A K . . . N K P T F V D M D P P P E H M K Q R S M V E P F F T R Q H V E				
<i>M. gypseum</i>		S K Q R N R P G F P E L D A G G K E A A K . . . R K P T F V D M D P P P E H M Q Q R S M V E P L F T R H V E				
<i>M. brunnea</i>		S K Q R N R P G F P E L S A G G K E A A K . . . N K P T F V D M D P P P Q H M Q Q R S M V E S V F T K Q H I D				
<i>M. phaseolina</i>		S K E R S R P G F P E M S P G G K E A A K . . . N R P T F V D M D P P P A H G H Q R G M V A D L F S Y E H I E				
<i>T. cutaneum</i>		S K V R T R P G F P E L S A G G K A A A Q . . . A Q P T F V D M D P P A D H M K Q R G M V L I V E A F F T P E Y V E				
<i>C. reinhardtii</i>		S K V R T L P G F P E L S P G G K A A A Q S G N A A T F V D M D P P P E H T K Y R G M V W P Y L T P E A V E				
<i>C. variabilis</i>		S K V R T H P G F P E L A G K A A V E . . . G R E A S Y V D M D P P P E H T R Y R A I F E P F F D E A A . . .				

PP

	NAD(P)H recognition region											
<i>F. oxysporum</i>	$\alpha 5$					$\beta 4$	$\alpha 6$	$\alpha 7$				
	120	130	140	150	160							
<i>F. oxysporum</i>	NLQPYTQRTVDDLL	LEQMKQKG	C.	CANG	PVDLV	KEF	ALP	VPS	YI	TYT	LLG	VPF
<i>F. lichenicola</i>	KLQPYI	QKTVDDLLAAMKKKG	.	CANG	PVDLV	KEF	ALP	VPS	YI	IYT	ILG	VPFN
<i>C. globosum</i>	SMEPYI	DKTVNDLLDRMVAKG	G.	CTNG	PVDLV	EEF	ALP	VPS	YI	IYT	LLG	VPF
<i>T. virens</i>	DMQPYI	QKTVDNLLAALKAKG	CANG	PVDLV	KEF	ALP	VPS	YI	IYT	ILG	VPFH	
<i>S. macrospora</i>	ELKPYI	QKTVDDLEEMKSKG	GCEGG	PVDLV	QNF	ALP	VPS	YI	IYT	ILG	VPF	
<i>G. graminis</i>	TLPQYI	QKTVDDLLDKLAAGG	. GKE	PVDLV	ESF	ALP	VPS	YI	VIYT	LLG	VPF	
<i>A. terreus</i>	...	QCAIFQQL	ISFE	QEY	GVG	FLYSR	IYH	IIG	VPT
<i>A. flavus</i>	SRLPFI	RDTVQY	YLGRLLIRAG	EDGK	PVDLV	KHF	ALP	IPS	HI	IYD	ILG	IPIE
<i>U. reesii</i>	SIKEPOQI	QKTVDRYLEEMIKGG	CE.	KPVDLV	EKF	ALP	IPSC	II	IYD	ILG	IP	FE
<i>C. apollinis</i>	SLRPYI	QKTVDELIDAMIAG	CEK.	PVDLV	EKF	ALP	VPS	II	YI	ILG	VPF	
<i>M. gypseum</i>	SMRPHI	QKTVDTLDIMIKGG	GK.	PAVDIVE	KALP	..	TASYI	YI	YG	ILG	VPF	
<i>M. brunnea</i>	SMRPOQI	QKTVNSLLDKVIALG	GA.	E.PFD	FVEKF	ALP	VPS	YI	TS	ILG	VPLA	
<i>M. phaseolina</i>	ELRLIKI	RKTVNELLDKMLERY	..	DGNP	PLDLV	ENFSLP	VPS	YI	TYE	ILG	VPS	
<i>T. cutaneum</i>	GLKPYI	QSVIDEALDKMAAKT	..	QPADLV	SEF	ALI	VPS	YI	VIY	ILG	VPLK	
<i>C. reinhardtii</i>	QLRPSI	QAKADKLVDAMIARG	..	G	P	L	DLNEAFSMP	LPFRV	IVYD	FIG	PEA	
<i>C. variabilis</i>	...	AQVEELIDGMQRRG	PGADG	GVDLA	EAF	SLP	LAFRV	VIYE	MLG	IP	FE	

<i>F. oxysporum</i>	α8					α9					α10																
	170		180		190		200		210																		
<i>F. oxysporum</i>	D	L	E	YLT	QQN	AIRTN	GSS	T	ARE	A	SAA	ANQ	ELL	D	YLA	I	LV	E	QRL	V	P	K	DD	IIS	KL		
<i>F. lichenicola</i>	D	L	D	HTL	NTQNN	AIRTN	GSS	T	ARE	A	SAA	ANQ	ELL	D	YLA	I	LV	E	QRL	V	P	K	DD	DLI	SKL		
<i>C. globosum</i>	D	L	E	FLT	EQQN	SIR	RTNGS	S	T	ARE	A	SAA	ANQ	ELL	D	YLT	T	VL	T	NK	LE	E	P	K	ND	IVS	QL
<i>T. virens</i>	D	L	E	YLT	THQN	AIRTN	GSS	T	ARE	A	SAA	ANQ	GLL	D	YLT	TK	LV	D	QR	IQE	P	K	DD	DLI	SKL		
<i>S. macrospora</i>	D	L	E	FLT	TQQN	SIR	RTNGS	S	T	ARE	A	SAA	ANQ	ELL	D	GYL	RRA	EDR	LKE	P	K	DD	DLI	SKL			
<i>G. graminis</i>	D	L	A	FLT	TTQNK	AVR	RTNGS	S	T	AQE	AS	ANQ	TLL	D	GYL	ADL	LVD	KRI	IAE	P	K	DD	DLI	SKL			
<i>A. terreus</i>	D	M	D	YLT	QNK	AVR	SSG	S	T	AAA	QAQN	ELL	D	AYL	GSL	LDK	R	IA	DPK	KD	IST						
<i>A. flavus</i>	D	F	E	YL	LSGCD	A	RTNGS	S	T	AAA	QAQAN	KEI	D	LEY	LER	VL	DKK	TTN	PS	H	DV	IST					
<i>U. reesii</i>	D	S	D	SL	TQWN	AIR	RTNGS	S	T	AAA	AA	TAN	TELL	D	GYL	DKL	LV	DK	K	IAN	P	Q	ND	DLI	STL		
<i>C. apollinis</i>	D	L	G	FLT	EQN	AVR	RTSGS	S	T	ASE	AQI	ASNS	LL	D	VYI	GKM	VDQ	R	MED	PK	DD	DLI	SKL				
<i>M. gypseum</i>	D	L	K	YLT	QQQA	AIR	RSNGS	S	T	AAA	ASA	ANQ	ELL	D	YIG	GLV	DQ	TR	VE	P	Q	ND	DLI	SKL			
<i>M. brunneaa</i>	D	L	P	KL	TNQF	AIR	RSNGS	S	T	ATE	AS	AN	ALL	D	SYM	DGL	LVT	TH	LAE	P	Q	SD	DLI	SKL			
<i>M. phaseolina</i>	D	L	K	FLT	KQAF	AVR	RSNGS	S	T	AAE	AS	AN	QELL	D	YM	MAS	LVD	KRV	PQ	DD	DLI	SHL					
<i>T. cutaneum</i>	D	L	E	FLT	LQN	AIR	RTNGS	S	T	ARE	A	SAA	SAG	LL	D	EY	LGK	LV	DAR	MDE	PK	DD	DLV	STL			
<i>C. reinhardtii</i>	D	F	A	YL	SANV	AVR	SSG	S	T	AKD	AAA	ADD	LVI	D	KYMD	DNLV	AEK	ERNP	TGKD	LISE							
<i>C. variabilis</i>	D	Y	E	FLS	SNV	AVR	ASGS	S	T	ARD	AAA	Q	QLO	D	YE	MEG	GLV	AAK	EAK	PT	DD	VLS	KV				

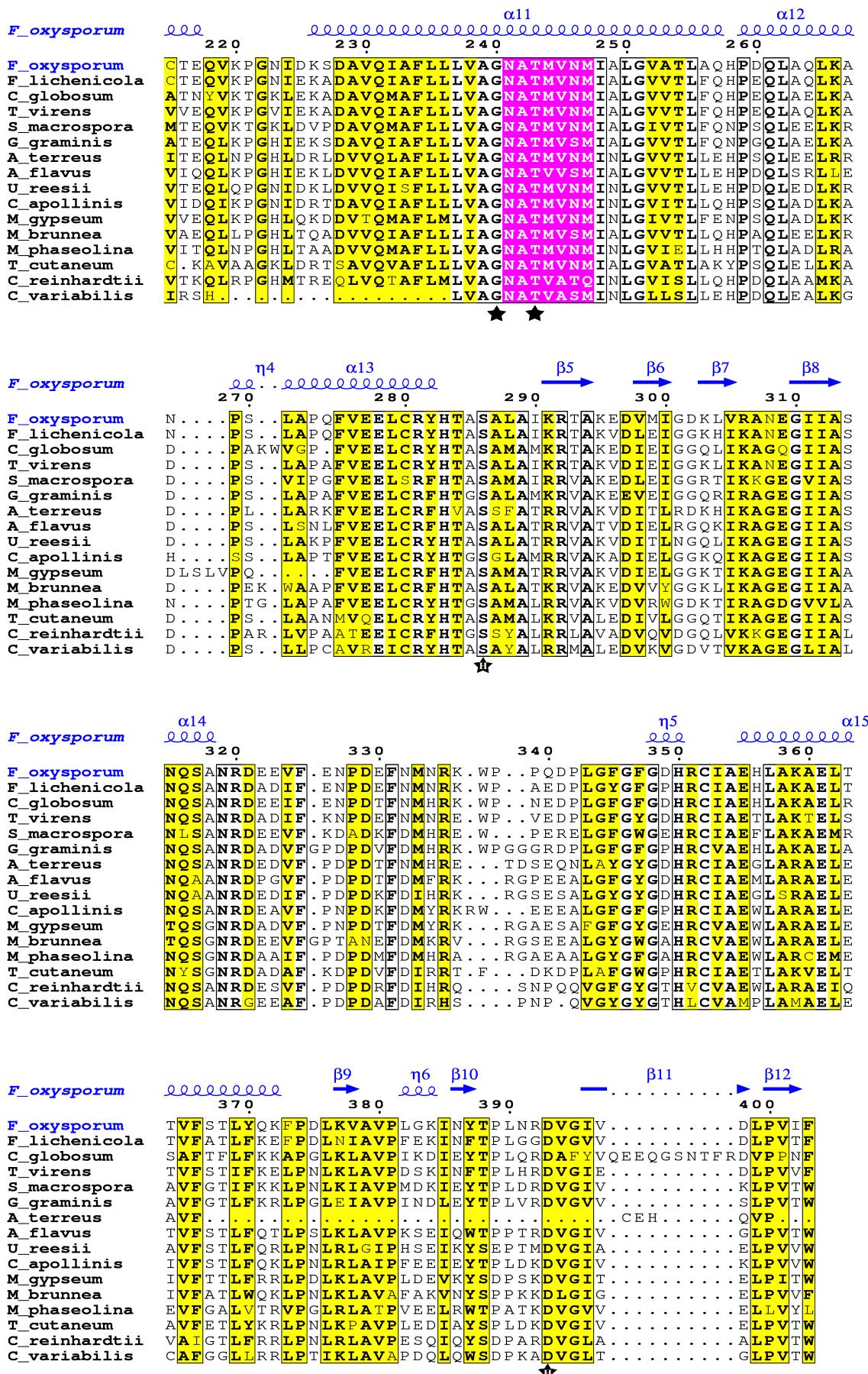


Figure S2. Alignment of P450nor sequences from select organisms displaying primer binding sites (pink), and key residues involved in cofactor (NAD(P)H) binding. The N-terminal region of the alignment has been removed for clarity. Stars below the alignment (blue, red, black) indicate positive, negative and charged amino acids and key residues involved in NAD(P)H binding, respectively. Sites outlined in black with all bold amino acid characters indicate 100% amino acid identity. Columns highlighted yellow and outlined in black indicate ≥ 80% amino acid similarity. Stars with a white "S" and "H" indicate involvement in a salt bridge and proton channel, respectively. PP stands for a residue believed to be involved in binding pyrophosphate on the NAD(P)H molecule. The B' helix (helix 3 α above) is a hypervariable region of P450 proteins and represents an area of NAD(P)H recognition by P450nor. P450nor secondary structure is provided by the PDB accession 1JFB and was added using the ESPript 3.0 web server. F_oxysporum = *Fusarium oxysporum*, F_lichenicola = *Fusarium lichenicola*, C_globosum = *Chaetomium globosum*, T_virens = *Trichoderma virens*, S_macrospora = *Sordaria macrospora*, G_graminis = *Gaeumannomyces graminis*, A_terreus = *Aspergillus terreus* NRRL 255, A_flavus = *Aspergillus flavus* NRRL 3357, U_reesii = *Uncinocarpus reesii*, C_apollinis = *Coniosporium apollinis*, M_gypseum = *Microsporum gypseum*, M_brunnea = *Marssonina brunnea*, M_phaseolina = *Macrophomina phaseolina*, T_cutaneum = *Trichosporon cutaneum*, C_reinhardtii = *Chlamydomonas reinhardtii*, C_variabilis = *Chlorella variabilis*.

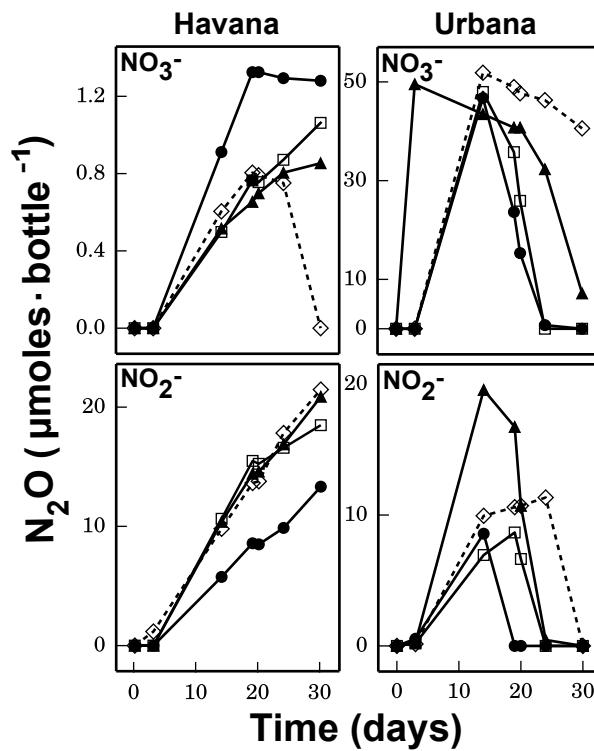


Figure S3. N_2O production in soil microcosms amended with chloramphenicol and streptomycin to inhibit bacterial activity. NO_3^- (2 mM) or NO_2^- (1 mM) were added as the sole nitrogen source. Acetate (●), formate (□), pyruvate (▲), or plant residue (◊, dashed line) were added as carbon source and electron donor for NO_3^- or NO_2^- reduction, respectively, in Havana (left column) or Urbana (right column) microcosms. Note the use of different scales on the y-axes. Carbon sources were not monitored during the enrichment.

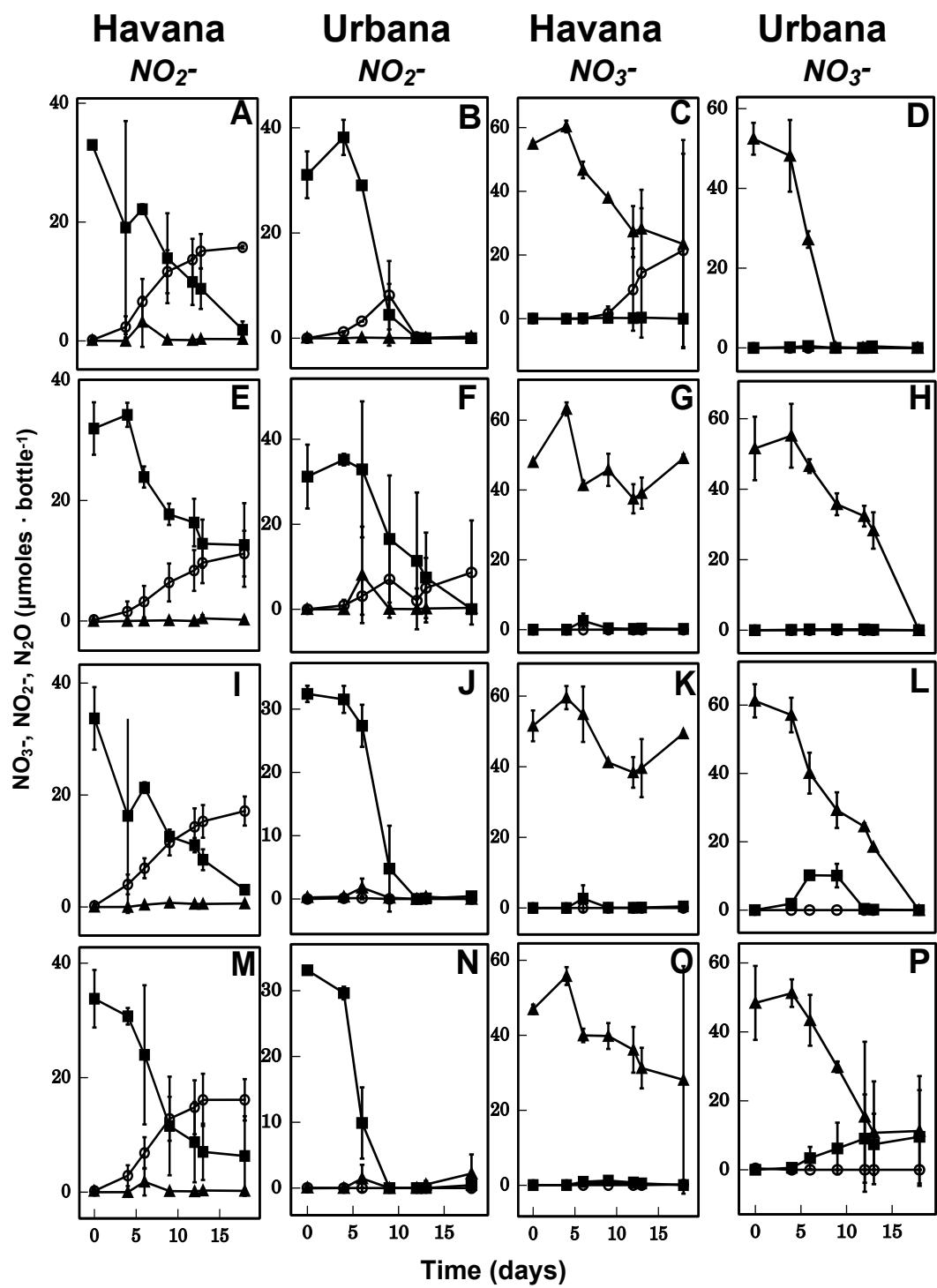


Figure S4. NO_3^- (\blacktriangle), NO_2^- (\blacksquare), and N_2O (\circ) dynamics in transfer cultures from Havana and Urbana microcosms amended with ampicillin and kanamycin to prevent bacterial activity. NO_2^- (1 mM) or NO_3^- (2 mM) were provided as the sole nitrogen source. Acetate (A, B, C, D), formate (E, F, G, H), plant residue (I, J, K, L), or pyruvate (M, N, O, P) were added as carbon and electron donor source. Note the differences in scale on the y-axes.

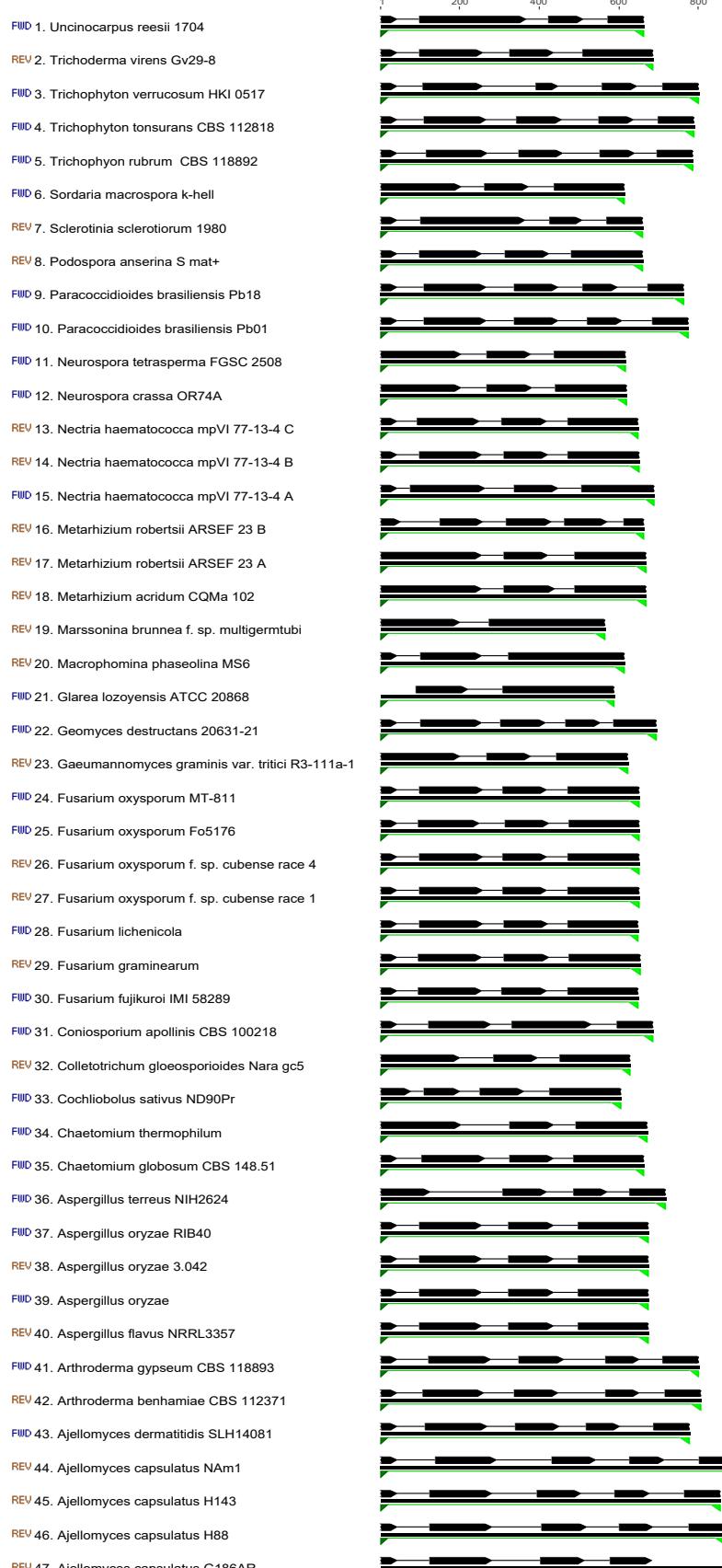


Figure S5. Intron structure of the region amplified by *p450nor* primer set *p450nor394F/p450nor809R* in 47 *p450nor* sequences. Only one to four introns are detected in the amplified region. Thick black lines indicate the gene, and black rectangles above this line indicate the coding sequences (CDS). Thin lines between CDS rectangles indicate an intron. Green triangles indicate *p450nor394F/p450nor809R* binding sites. Some genes have been reversed for clarity (indicated by red REV next to sequence name).