1 SUPPLEMENTAL MATERIAL

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

21

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

40

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62	SUPF	PLEMENTAL FIGURE LEGENDS
63	Figur	re S1. Stereomicroscopic images of representative members of isolated fungal groups (A)
64	and si	ngletons (B) identified by sequencing of the cloned ITS region from each fungal isolate.
65	The g	roup 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	corne	r 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 3α 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 = <i>Chaetomium globosum</i> , T_virens = <i>Trichoderma virens</i> , 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	<i>Marssonina brunnea</i> , M_phaseolina = <i>Macrophomina phaseolina</i> , T_cutaneum = <i>Trichosporon</i>
86	cutaneum, C_reinhardtii = Chlamydomonas rheinhardtii, C_variabilis = Chlorella variabilis.
87	

Figure S3. N₂O production in soil microcosms amended with chloramphenicol and streptomycin
to inhibit bacterial activity. NO₃⁻ (2 mM) or NO₂⁻ (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 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

Figure S4. NO_3^{-1} (\blacktriangle), NO_2^{-1} (\blacksquare), and N_2O (\circ) dynamics in transfer cultures from Havana and 95 Urbana microcosms amended with ampicillin and kanamycin to prevent bacterial activity. NO₃⁻ 96 97 (2 mM) or NO₂⁻ (1 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 source and 98 electron donor. Note the differences in scale on the y-axes. 99 100 Figure S5. Intron structure of the region amplified by *p450nor* primer set 101 p450nor394F/p450nor809R in 47 p450nor sequences. Only one to four introns are detected in 102 the amplified region. Thick black lines indicate the gene, and black rectangles above this line 103 indicate the coding sequences (CDS). Thin lines between CDS rectangles indicate an intron. 104 Green triangles indicate p450nor394F/p450nor809R binding sites. Some genes have been 105 106 reversed for clarity (indicated by red REV next to sequence name). 107 108 SUPPLEMENTAL DATASET LEGENDS Dataset S1. NCBI protein GI and Accession numbers of P450nor sequences used for primer 109 design. 110 111 **Dataset S2.** Accessory metadata for the fungal isolates from Havana and Urbana soils. Dataset S3. The SILVA 18S rRNA gene classification of 37 fungal isolates in Figure 1. 112

Dataset S4. *p450nor* amplicon lengths and number of introns in amplified region using primer

114 set *p450nor*394F/*p450nor*809R.

Α		В	
Group 1 Uncultured soil fungus	4 2 3	Singleton 1 Podospora sp.	190 pm
<u>Group 2</u> Fusarium solani		Singleton 2 Nectria sp.	100 µm.
<u>Group 3</u> Fusarium oxysporum		<u>Singleton 3</u> Trichoderma asperellum	20 jm
<u>Group 4</u> Mortierella sp.		<u>Singleton 4</u> Uncultured Coniochaetaceae	
<u>Group 5</u> Trichoderma spirale			
<u>Group 6</u> Fusarium solani			

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.

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F_lichenicola	SKVRTR	GFPE <mark>ln</mark> ž	A <mark>GG</mark> KQAAK	AKPTFVDMD	APDHMNQRG <mark>N</mark>	IV ESL FT LEH <mark>V</mark> K								
C_globosum	SKERSR	GFPE <mark>FS</mark> I	O <mark>GG</mark> KAAGK	QRPTFVDMD	P P E HM RH R S <mark>M</mark>	IV ESW <mark>FT</mark> PEK <mark>V</mark> K								
T_virens	SKVRTRP	GFPE <mark>L</mark> G	A <mark>gg</mark> keaak	AKPTFVDMD	P P D HM HQ R S <mark>M</mark>	IV ESL <mark>FS</mark> AEH <mark>I</mark> K								
S_macrospora	SKERTR	GFPE <mark>LS</mark> ž	A <mark>GG</mark> KEAAK	NRATEVDMD	P P K HM QY R G <mark>M</mark>	IV QPI <mark>FN</mark> IEH <mark>I</mark> Q								
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F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis A_terreus	PP 0000000 1 NLQPYIQ KLQPYIQ SMEPYID DMQPYID ELKPYIQ TLQPYIQ QCQ	NAD(P)H α5 200000 20 RTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDD11 KTVD011 KTVD011 KTVD011 KTVD011	recognition 130 EQMKQKG AAMKKKG DRMVAKG AALKAKG ALKAKG DKLAAKG	region β4 α6 → 200 140 . CANGPVDLVKI . CANGPVDLVKI . CANGPVDLVKI . CANGPVDLIKI . CANGPVDLIKI . CASGPVDLIKI . CASGPVDLIKI . CESGPVDLVQI ISFEQI	Q Q Q E F A L P A L P A L P V P S F A L P V P S F A L P V P S F A L P V P S F A L P V P S F A L P V S F A L P S S F A L P S S S S S S S S S S S S S S S S S S	7 η3 160 10 11YTLLGVPFN 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE								
F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis A_terreus A_flavus	PP	NAD(P)H α5 0000000 20 RTV DDL KTV DDL KTV DDL KTV DDL KTV DDL XTV DDL XTV DDL XTV DDL XTV DDL XTV DDL XTV DDL XTV DDL	recognition	region β4 α6 000 140 .CANGPVDLVKI	Q Q Q E F A L P S F A L P S F A L P S F A L P S F A L P S F A L P S F A L P S F A L P S F A L P S S F A L P S S F A L P S S S F A L P S S S S S S S S S S S S S S S S S S	7 93 000000 160 11YTLLGVPFN 11YTLLGVPFN 11YTLLGVPFE 11YTLLGVPFE VIYTLLGVPFE VIYTLLGVPTE 11YDILGIPIE								
F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis A_terreus A_flavus U_reesii	PP QQQQQQQQ I NIQPYIQ SMEPYID DMQPYIQ ELKPYIQ TLQPYIQ QCQ SRLPFIR SIKPQIQ	NAD(P)H α5 0000000 20 xTVDDL KTVDDL KTVDCL KTVDCL KTVDCL KTVDCL KTVDCL KTVDC KTVDC KTVDC KTVDC	recognition	region β4 α6 0 000 140 . CANGPVDLVKI . CEGGPVDLVIII	Q Q Q Q Q Q Q Q Q Q Q Q Q Q	7 93 160 11YTLLGVPFN 11YTLLGVPFN 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YDILGIPFE 11YDILGIPFE								
F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis A_terreus A_flavus U_reesii C_apollinis	PP QQQQQQQ 1 NLQPYIQ SMEPYID DMQPYIQ ELKPYIQ TLQPYIQ S.LPFIR SIKPQIQ SLRPYIQ	NAD(P)H α5 0000000 20 KTVDDL1 KTVDQUU KTVDQUU KTVDQUU KTVDR11 KTVDR11 KTVDUU	recognition	region β4 α6 0 140 .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CEGGPVDLVEI	Q Q Q E F A L P V P S F A L P V P S F A L P V P S F A L P V P S F A L P V P S F A L P V P S F A L P V P S F A L P V P S S F A L P V P S S S F A L P V P S S S F A L P V P S S S F A L P V P S S S S C F A L P V P S S S S S C F A L P V P S S S S S C F A L P V P S S S S S S S S S S S S S S S S S	7 160 160 11YTLLGVPFN 11YTLLGVPFN 11YTLLGVPFE 11YTLLGVPFE VIYTLLGVPFE VIYTLLGVPFE 11YTLLGVPTE 11YDILGIPFE 11YDILGIPFE 11YTLGVPFE								
F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis A_terreus A_flavus U_reesii C_apollinis M_gypseum	PP	NAD(P)H α5 0000000 20 RTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDL1 KTVDDL1 KTVDE1 QTVDT1	recognition	region β4 α6 000 140 .CANGPVDLVKI .CANGPVDLVEI .CANGPVDLVEI .CANGPVDLVEI .CANGPVDLVEI .CANGPVDLVEI .CANGPVDLVEI .CEGGPVDLVQI	Q Q Q Q Q Q Q Q Q Q Q Q Q Q	7 160 160 160 11YTLLGVPFN 11YTLLGVPFE 11YTILGVPFE VIYTLLGVPFE VIYTLLGVPFE VIYTLLGVPFE 11YDILGIPFE 11YDILGIPFE 11YTILGVPFE 11YGILGVPFE								
F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis A_terreus A_flavus U_reesii C_apollinis M_gypseum M_brunnea	PP	NAD(P)H α5 20 20 RTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDL1 KTVDL1 KTVDL1 KTVDL1 KTVDL1 KTVDL1 KTVDR	recognition	region β4 α6 000 140 .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVEI .CANGPVDLVEI .CEGGPVDLVQI	Q Q Q Q Q Q Q Q Q Q Q Q Q Q	7 160 160 160 11YTLLGVPFN 1YTLLGVPFE 1YTLLGVPFE 1YTLLGVPFE VIYTLLGVPFE VIYTLLGVPFE 1YDILGIPFE 1YDILGIPFE 1YGILGVPFE 1YGILGVPFK TYSILGVPKA								
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	PP	NAD(P)H a5 20 20 RTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDGL1 KTVDGL1 KTVDGL1 KTVDR1 KTVDR1 KTVDR1 KTVDR1 KTVDR1 KTVNSL1 KTVNSL1 KTVNSL1	recognition	region β4 α6 0 140 .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVEI .CANGPVDLVEI .CEGGPVDLVQI	Q Q Q Q Q Q Q Q Q Q Q Q Q Q	7 160 160 160 11YTLLGVPFN 1YTLLGVPFH 1YTLLGVPFH 1YTLLGVPFH 1YTLLGVPFE 1YTLLGVPFE 1YTLLGVPFE 1YDILGIPFE 1YGLLGVPFE 1YSLLGVPFK TIYSLLGVPSE								
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	PP QQQQQQQQ I NLQPYIQ SMEPYID DMQPYIQ ELKPYIQ C.LQPYIQ C.LQPYQQ SRLPFIR SIKPQIQ SMRPHIQ SMRPHIQ SMRPQIQ SMRPQIQ SMRPYIQ SMRPYIQ	NAD(P)H α5 0000000 20 xTVDDL KTVDDL KTVDEL QTVQTL KTVNSL KTVNSL KTVEL SVIDEA	recognition	region β4 α6 000 140 .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CEGGPVDLVQI	Q Q	7 ¶3 160 160 11YTLLGVPFN 11YTLLGVPFN 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLLGVPFE 11YTLGVPFE 11YTLGVPFE 11YDILGIPFE 11YGILGVPFE 11YGILGVPFE 11YGILGVPFE 11YGILGVPEA TIYELGVPSE 11YTLGVPEA YTILGVPEA 11YGILGVPEA								
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	PP	NAD(P)H α5 0000000 20 xTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDDL1 KTVDL1 KTVDL1 KTVDC11 KTVDC11 KTVDC11 KTVDC11 KTVDC11 KTVD211 KTVD211 KTVD211 KTVD211 KTVD221 SV1024 AK AK	recognition	region β4 α6 → QQ0 140 .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CANGPVDLVKI .CEGGPVDLV[1]	Q Q	7 93 160 11YTLLGVPFN 11YTLLGVPFN 11YTLLGVPFE 11YTLGVPFE 11YDILGIPFE 11YGLGVPFE 11YGLGVPFE 11YSLLGVPFE 11YSLLGVPE VIYTLGVPE VIYTLGVPE VIYTLGVPE VIYTLGVPE VIYTLGVPE VIYTLGVPE VIYTLGVPE								

		α8			α9		α10
F_oxysporum	elle	مععععه	222	lllll	000000000	2222222	llll
		170	18	•	190	200	210
F_oxysporum	DLE <mark>Y</mark>	LTQQN <mark>A</mark>]	RTNGSS	TAREASAA	NQELLDYLA	I LV EQ R LVE P	K.D DIIS K <mark>l</mark>
F_lichenicola	DLDH	L <mark>T</mark> NQN <mark>A</mark>]	RTNGSS	TAREASAA	NQE <mark>LL</mark> DYLA	.S <mark>LV</mark> EK <mark>R</mark> LEE P	K.D dlis K <mark>l</mark>
C_globosum	DLEF	L <mark>T</mark> QQN <mark>S</mark>	RTNGSA	TARDASAA	ADE <mark>LI</mark> R YL T	V <mark>LT</mark> NK <mark>R</mark> LEE P	K.N DIVS QL
T_virens	DLE <mark>Y</mark>	LTHQNA]	RTNGSS	TAREASAA	NQG <mark>LL</mark> DYLT	K <mark>lv</mark> dq <mark>r</mark> iqe p	K.D dlis K <mark>l</mark>
S_macrospora	DLEF	L <mark>T</mark> QQNSI	RTNGSS	TAREASAA	NQELLGYLR	R <mark>LA</mark> ED <mark>R</mark> LKE P	K.D dlis k <mark>l</mark>
G_graminis	DLAF	L <mark>T</mark> TQN <mark>AN</mark>	<mark>7 r t n</mark> g s a	TAQEAASA	NQT <mark>LL</mark> G YL A	.D <mark>lv</mark> dk <mark>r</mark> iae p	K.D dlis k <mark>l</mark>
A terreus	DMD Y	L T Q K N <mark>A 1</mark>	7 R S S G S S	TAAAAQNAI	NEELLAYLG	S <mark>LV</mark> DK <mark>R</mark> IAD P	K.K dlis t <mark>l</mark>
A_flavus	DFE <mark>Y</mark>	L <mark>S</mark> GCD <mark>A</mark>	RTNGSS	TAAAAQAA	NKE <mark>IL</mark> E YL E	R <mark>LV</mark> DK <mark>K</mark> TTN P	S.H DVIS T <mark>L</mark>
U_reesii	DSDS	L <mark>T</mark> QWN <mark>A</mark>]	RTNGSS	TAAAAATA	NTELLGYLD	K <mark>LV</mark> DK <mark>K</mark> IAN P	Q.NDLISTL
C_apollinis	DLGF	LTEQNAL	7 R T S G S S	TASEAQIA	SNS <mark>LL</mark> V YI G	K <mark>MV</mark> DQ <mark>R</mark> MED P	K.D dlis K <mark>l</mark>
Mgypseum	DLK <mark>Y</mark> I	LTQQAA	RSNGSA	TAAAASAA	NQELLDYIG	G <mark>LV</mark> DQ <mark>R</mark> TVE P	Q.NDLISKL
M_brunnea	DLPK	LTNFA <mark>A</mark>]	RSNGSG	TATEASNA	NAALLSYMD	G <mark>LV</mark> TT H LAE P	Q.S dlis l
M phaseolina	DLKF	L <mark>T</mark> KQA <mark>A</mark> N	7 R S N G S A	TAAEASNA	NQE <mark>LL</mark> NY <mark>M</mark> A	.S <mark>lv</mark> dk <mark>r</mark> vrk p	Q.DDLISHL
T_cutaneum	DLEF	LTLQNA	RTNGSS	TAREASAA	SAGLLEYLG	K <mark>LV</mark> DA <mark>R</mark> MDE P	K.DDLVSTL
C_reinhardtii	DFAY	L <mark>S</mark> ANV <mark>A</mark>	7 R S S G S S	NAKDAAAA.	add lv k ym d	N LVAEKERNP	TGK DLIS EL
C variabilis	DYE <mark>F</mark> I	L <mark>S</mark> SNV <mark>A</mark> N	7 R A S G S S	TARDAAAA	QQQ <mark>LQ</mark> EY <mark>M</mark> E	G <mark>LV</mark> AA <mark>K</mark> EAK P	T.DDVLSKV
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			α11		α12
F_oxysporum	ll	0000000000	200000000	0000000000	000 00000000
	220	230	240	250	260
F_oxysporum	CTE QV KP G N	DKS <mark>DAVQIAFLI</mark>	LVAGNATM	VNMIALGVAT	LAQHPDQLAQLKA
F_lichenicola	CTE <mark>QV</mark> KP G N <mark>J</mark>	EKA <mark>DAVQIAFLI</mark>	L <mark>V</mark> AG <mark>NATM</mark>	VNMIALG <mark>VVT</mark> :	LFQHPEQLAQLKA
C_globosum	ATNYVKTGKI	LEKA <mark>DAVQMAFLI</mark>	L <mark>V</mark> AG <mark>NATM</mark>	VNMIALG <mark>VVT</mark> :	LFQH P E QL AE <mark>LK</mark> A
T_virens	VEQVKPGV3	EKA <mark>DAVQIAFLI</mark>	L <mark>V</mark> AG <mark>NATM</mark>	VNMIALG <mark>VVT</mark> :	LFQH P E QL AQ <mark>LK</mark> A
S_macrospora	MTEQVKTGKI	DVP <mark>DAVQMAFLI</mark>	L <mark>V</mark> AG <mark>NATM</mark>	VNMIALGIVT:	LFQN P S QL EE <mark>LK</mark> R
G_graminis	ATEQLKPGH]	EKS <mark>DAVQIAFLI</mark>	L <mark>VAGNATM</mark>	VSMIALG <mark>VVT</mark> :	LFQNPG QL AE <mark>LK</mark> A
A_terreus	ITEQLNPGH	DRL <mark>DVVQLAFLI</mark>	L <mark>V</mark> AG <mark>NATM</mark>	VNMINLG <mark>VVT</mark> :	LLEH P S QL EE <mark>LR</mark> R
A_flavus	V IQ <mark>QL</mark> KP <mark>G</mark> H]	EKL <mark>DVVQIAFLI</mark>	L <mark>VAG</mark> NATV	VSMIALG <mark>VVT</mark> :	LLEH P D QL SR <mark>LL</mark> E
U_reesii	VTEQLQPGN]	DKL <mark>DVVQISFLI</mark>	L <mark>VAG</mark> NATM	VNMINLG <mark>VVT</mark> :	LLEH P D QL ED <mark>LK</mark> R
C_apollinis	V ID <mark>QI</mark> KP <mark>G</mark> N <mark>J</mark>	DRT <mark>DAVQIAFLI</mark>	L <mark>V</mark> AG <mark>NATM</mark>	VNMINLG <mark>VIT</mark> :	LLQH P S QL AD <mark>LK</mark> A
M_gypseum	VEQLKPGH	QKD <mark>DVTQMAFLN</mark>	<mark>1 L V</mark> AG <mark>NATM</mark>	VNMINLGIVT:	LFEN P S QL AD <mark>LK</mark> K
M_brunnea	V AE <mark>QL</mark> LP <mark>G</mark> H <mark>I</mark>	TQA <mark>DVVQIAFLI</mark>	LIAG <mark>NATM</mark>	VSMIALG <mark>VVT</mark> :	LLQH P A QL EE <mark>LK</mark> R
M_phaseolina	V IT QL NP G H <mark>I</mark>	TAA <mark>dvvqmafli</mark>	L <mark>VAGNATM</mark>	VNMINLG <mark>VIE</mark> :	LLHH P T QL AD LR A
T_cutaneum	C.KA V AA <mark>G</mark> KI	DRT <mark>SAVQVAFLI</mark>	L <mark>V</mark> AG <mark>NATM</mark>	VNMIALG <mark>VAT</mark> :	LAKY P S QL EL <mark>LK</mark> A
C_reinhardtii	∨ TK <mark>QL</mark> RP <mark>G</mark> H <mark></mark>	<mark>(</mark> tre <mark>qlvqtafl</mark>	<mark>1 L</mark> VAG <mark>NATV.</mark>	ATQINLG <mark>VIS</mark> :	L LQH P D QL AA <mark>MK</mark> A
C variabilis	TRSH		T T A C N A U M	ACMINIT CTTC	
		• • • • • • • • • • • • • • • • • • •	LVAGNALV		nng ued fa gy <mark>ny</mark> g
_		····			DIFUED A DE V <mark>HK</mark> G
_					UTRUED E TE A UN G
_		<u></u>			UTETED ENERG
_	<u>1</u>	α13		<u>в</u> 5 в6	B7 B8
F oxysporum	η4	α13			
- F_oxysporum	η4 270	α13 2000000000 280	★ ★ 290		
F_oxysporum F oxysporum	η4 <u>9</u> 0 270 ΝΡς	α13 20000000000 280 .APOFVEELCRYE	290 TASALAIK	β5 β6 3 ο ο RTAKE DV MIG	$\frac{\beta7}{310}$
F_oxysporum F_oxysporum F_lichenicola	η4 <u>270</u> ΝΡςΙ	α13 20000000000 280 ΑΡΟ ΓΥΕΕLCRYF ΑΡΟ ΓΥΕΕLCRYF	290 TASALAIK	β5 β6 3 ο ο RTAKEDVMIGI RTAKVDIEIG	β7 β7 β8 β7 β8 β8 β7 β8 β8 β8 β7 β8 β8 β7 β8 β8 β7 β8 β8 β7 β8 β8 β7 β8 β8 β7 β8 β8 β8 β7 β8 β8 β8 β7 β8 β8 β8 β8 β8 β8 β8 β8 β8 β8 β8 β8 β8
F_oxysporum F_oxysporum F_lichenicola C_globosum	η4 <u>270</u> ΝΡςΙ ΝΡςΙ ΟΡςΙ	α13 200000000000 APQFVEELCRYF GP.FVEELCRYF	290 ITASALAIK ITASALAIK ITASALAIK	β5 β5 β5 β6 300 RTAKEDVMIGI RTAKEDVMIGI RTAKEDIEIG	β7 β7 β8 310 DKLVRANEGIIAS GKHIKANEGIIAS GQLIKAGQGIIAS
F_oxysporum F_oxysporum F_lichenicola C_globosum T_virens	η4 270 NPsI DPsI DPsI DPsI	α13 20000000000 280 ΑΡQFVEELCRYE GP.FVEELCRYE GP.FVEELCRYE APAFVEELCRYE	290 ITASALAIK ITASALAIK ITASALAIK ITASALAIK	β5 β5 β5 β6 300 RTAKEDVMIG RTAKVDIEIG RTAKVDIEIG RTAKVDIEIG	β7 β7 β8 310 DKLVRANEGIIAS GKLIKANEGIIAS GKLIKANEGIIAS
F_oxysporum F_oxysporum F_lichenicola C_globosum T_virens S_macrospora	η4 270 ΝΡς ΝΡς ΔΡς ΔΡς ΔΡς ΔΡς	α13 280 280 ΑΡΟFVEELCRYE ΑΡΟFVEELCRYE ΑΡΑFVEELCRYE ΑΡΑFVEELCRYE ΑΡΑFVEELCRYE	290 ITASALAIKI ITASALAIKI ITASAMAMKI ITASAMAIKI ITASAMAIKI	β5 β6 300 RTAKEDVMIG RTAKEDIEIG RTAKEDIEIG RTAKEDIEIG RTAKEDIEIG	β7 β7 β8 310 DKLVRANEGIIAS GKHIKANEGIIAS GKLIKAGGGIIAS GKLIKKGEGVIAS
F_oxysporum F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis	η4 <u>2</u> ,	α13 20000000000 280 ΑΡQFVEELCRYF ΑΡQFVEELCRYF ΑΡΑFVEELCRYF ΙΡGFVEELCRYF ΑΡΑFVEELCRFF	290 TASALAIK TASALAIK TASALAIK TASALAIK TASALAIK TASALAIK TASALAIK	β5 β6 3 ο ο RTAKEDVMIGI RTAKEDIEIG RTAKEDIEIG RVAKEDIEIG RVAKEVEIG	β7 β7 β8 310 DKLVRANEGIIAS GKHIKANEGIIAS GKLIKANEGIIAS GRTIKKGEGVIAS GQRIRAGEGIIAS
F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis A_terreus	η4 270 ΝΡς DΡς DΡς DΡς DΡς D Pς D Pς D Pς D Pς	α13 2000000000 280 APQFVEELCRYE APQFVEELCRYE APAFVEELCRYE APAFVEELCRYE APAFVEELCRFE APKFVEELCRFE	290 ITASALAIK ITASALAIK ITASALAIK ITASAMAMKI ITASAMAIR ITGSALAMKI IVASSFATR	β5 β5 β6 300 RTAKEDVMIG RTAKEDIEIG RTAKEDIEIG RTAKEDIEIG RVAKEVEIG RVAKEVII	β7 β7 β8 310 DKLVRANEGIIAS GKHIKANEGIIAS GQLIKAGQGIIAS GRTIKAGEGVIAS GQRIRAGEGIIAS DKHIKAGEGIIAS
F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis A_terreus A_flavus	η4 270 NPs DPs DPs DPs DPs DPs DPs DPs DPs DPs DPs	α13 280 280 ΑΡΟΓΥΕΕΙΟΩΥΑ ΑΡΟΓΥΕΕΙΟΩΥΑ ΑΡΟΓΥΕΕΙΟΩΥΑ ΑΡΑΓΥΕΕΙΟΩΥΑ ΙΡΑΓΥΕΕΙΟΩΥΑ ΑΡΑΓΥΕΕΙΟΩΥΑ ΑΡΑΓΥΕΕΙΟΩΓΑ ΑΓΥΕΕΙΟΩΓΑ	290 ITASALAIK ITASALAIK ITASALAIK ITASAMAKI ITASAMAIR ITASAMAIR ITASALAIK ITASALAIK ITASALAIR	β5 β5 β5 β5 β6 300 300 300 800 800 800 800 800	β7 β7 β8 310 DKLVRANEGIIAS GKHIKANEGIIAS GKLIKACQGIIAS GRTIKKGEGVIAS GQRIRAGEGIIAS DKHIKAGEGIIAS
F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis A_terreus A_flavus U_reesii	η4 270 NPSΙ NPSΙ DPSΙ DPSΙ DPSΙ DPSΙ DPSΙ DPSΙ DPSΙ DPSΙ	α13 280 280 ΑΡΟΓΥΕΕΙCRΥΕ ΑΡΟΓΥΕΕΙCRΥΕ ΑΡΑΓΥΕΕΙCRΥΕ ΑΡΑΓΥΕΕΙCRΥΕ ΑΡΑΓΥΕΕΙCRΥΕ ΑΡΑΓΥΕΕΙCRΓΕ ΑΡΑΓΥΕΕΙCRΓΕ ΑΡΑΓΥΕΕΙCRΓΕ ΑΓΓΕΕΙCRΓΕ ΑΓΓΕΓΥΕΕΙCRΓΕ	290 ITASALAIK ITASALAIK ITASALAIK ITASALAIK ITASAMAKI ITASALAIK ITASALAIK IVASSFATRI ITASALATRI ITASALATRI	β5 β5 β6 300 RTAKEDVMIGI RTAKEDIEIG RTAKEDIEIG RVAKEDIEIG RVAKEDIEIG RVAKEVEIG RVAKEUIEIG	β7 β7 β7 β8 β7 β8 β7 β8 β7 β8 β7 β8 β7 β8 β7 β8 β8 β7 β8 β8 β7 β8 β8 β7 β8 β8 β7 β8 β8 β7 β8 β8 β8 β8 β8 β8 β7 β8 β8 β8 β8 β8 β7 β8 β8 β7 β8 β8 β7 β8 β8 β7 β8 β7 β8 β7 β8 β8 β7 β8 β7 β8 β7 β8 β7 β8 β7 β8 β8 β7 β8 β8 β8 β8 β8 β8 β8 β8 β8 β8
F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis A_terreus A_flavus U_reesii C_apollinis	η4 <u>2</u> 70 ΝΡς ΔΡς ΔΡς ΔΡς ΔΡς ΔΡς ΔΡς ΔΡς ΔΡς ΔΡς Δ Ρς Δ Γς	α13 200000000000 280 APQFVEELCRYF APQFVEELCRYF APAFVEELCRYF APAFVEELCRFF APAFVEELCRFF ARKFVEELCRFF ARKFVEELCRFF AKFVEELCRFF AKFVEELCRFF AKFVEELCRFF AKFVEELCRYF	290 TASALAIK TASALAIK TASALAIK TASALAIK TASALAIK TASALAIK TGSALAMK VASSFATR TASALATR TASALATR TASALATR	β5 β5 β5 β6 β7 β7 β β β β β β β β β β β β β	β7 β7 β8 β7 β8 β7 β8 β8 β8 β7 β8 β8 β8 β8 β8 β8 β8 β8 β8 β8
F_oxysporum F_lichenicola C_globosum T_virens S_macrospora G_graminis A_terreus A_flavus U_reesii C_apollinis M_gypseum	η4 <u>2</u> 70 ΝΡςι DΡς.ι	α13 2000000000 280 APQFVEELCRYF APQFVEELCRYF APAFVEELCRYF APAFVEELCRFF APAFVEELCRFF SNLFVEELCRFF SNLFVEELCRFF APTFVEELCRFF APTFVEELCRFF	290 TASALAIK TASALAIK TASALAIK TASALAIK TASALAIK TASALAIK TGSALAMK TGSALAMK TASALATR TASALATR TASALATR TASALATR	β5 β5 β5 β5 β6 β7 β7 β7 β7 β7 β7 β7 β7 β7 β7	β7 β7 β8 310 DKLVRANEGIIAS GKHIKANEGIIAS GKLIKANEGIIAS GQRIRAGEGIIAS GQRIRAGEGIIAS GQKIRAGEGIIAS GQKIRAGEGIIAS GKQIKAGEGIIAS GKQIKAGEGIIAS GKTIKAGEGIIAS

	270	280	290	зоо	310
F_oxysporum	NPSLA	Q FVEELCRYHTA	SALAI <mark>K</mark> RTAKE <mark>I</mark>	V M <mark>I</mark> GDKL	VRANE<mark>GIIA</mark>S
F_lichenicola	N P S LA B	Q F V E E L C R Y H T A S	S <mark>al</mark> ai <mark>k</mark> rtakv <mark>i</mark>) L E I GGKH	ikane<mark>giia</mark>s
C_qlobosum	D P AKW <mark>V</mark> G	P. FVEELCRYHTAS	S <mark>amamk</mark> rtake <mark>i</mark>	J E I GGQL	ikagqgiias
T_virens	DPSLA	PAFVEELCRYHTAS	S <mark>alaik</mark> rtakv <mark>i</mark>	IEI GGKL	IKANEGIIA
S_macrospora	DPSVI	G F V E E L S R F H T A S	S <mark>am</mark> ai <mark>r</mark> rvake <mark>i</mark>	L E I GGRT	ikkgegvia s
G_graminis	DPSLA	PAFVEELCRFHTGS	S <mark>al</mark> am <mark>k</mark> rvake <mark>e</mark>	v e i ggqr	iragegiia s
A_terreus	DPLLA	RK <mark>FVEELCRFH</mark> VAS	SSFAT <mark>RRVA</mark> KV <mark>I</mark>	JI T <mark>L</mark> RDKH	ikagegiia
A_flavus	DPSLS	ILFVEELCRFHTAS	SALATRRVATVI	J E L RGQK	iragegiia
U_reesii	DPSLA	KP FVEELCRYHT AS	S <mark>al</mark> at <mark>r</mark> rvakv <mark>i</mark>	JITL NGQL	ikagegiia
C apollinis	HSSLA	TFVEELCRYHTGS	S <mark>GLAMRRVA</mark> KA <mark>I</mark>	I E L GGKQ	ikagegiia
M_gypseum	DLSLV P Q	FVEELCRFHTAS	S <mark>amatrrva</mark> kv <mark>i</mark>	I E L GGKT	ikagegiiaA
M brunnea	DPEK.WA	AP FVEELCRFHT AS	S <mark>alatkrva</mark> ke <mark>i</mark>) V V Y G G K L	ikagegiiaA
M_phaseolina	N P TG. LA B	PAFVEELCRYHTGS	S <mark>amalrrva</mark> kv <mark>i</mark>	V RWGDKT	iragd<mark>gvvl</mark>a
T_cutaneum	DPSLA	AN <mark>MVQELCRYHT</mark> AS	S <mark>amalk</mark> rvale <mark>i</mark>	JIVL GGQT	ikagegiia s
C_reinhardtii	DPAR.LV	PA <mark>ATEEICRFHT</mark> GS	S <mark>SY</mark> AL <mark>R</mark> RLAVA <mark>I</mark>	V Q V DGQL	vkkgegiial
C_variabilis	DPSLL	PCAVREICRYHTAS	S <mark>AY</mark> AL <mark>R</mark> RMALE <mark>I</mark>	V K <mark>V</mark> GDVT	vkage<mark>glia</mark>l
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F_oxysporum	lll				eee	000000000
	320		330	340	350	360
F_oxysporum	NQSANRI	DEE <mark>VF</mark> .EI	N <mark>PD</mark> EFN <mark>M</mark> NRK	.WPPQDPLGF	GFGDHRCIA	EHLAKAELT
F_lichenicola	NQSANRI	AD <mark>IF</mark> .EI	N <mark>PD</mark> EFN <mark>M</mark> NRK	.WPAEDP <mark>LGY</mark>	GFGPHRCIA	EHLAKAELT
C_globosum	NQSANRI	DEE <mark>IF</mark> .EI	N <mark>PD</mark> TFN <mark>M</mark> H <mark>R</mark> K	.WPNEDPLGF	GFGDHRCIA	EHLAKAELR
T_virens	NQSANRI	DAD <mark>IF</mark> .KI	N <mark>PD</mark> EFN <mark>M</mark> NRE	.WPVEDPLGF	G Y G D H R C I A	ETLAKTELS
S_macrospora	NLSANRI	DEE <mark>VF</mark> .KI	D <mark>AD</mark> KFD <mark>M</mark> HRE	.WPERE <mark>LGF</mark>	GWGEHRCIA	EFLAKAEMR
G_graminis	NQSANRI	DAD <mark>VF</mark> GPI	D <mark>PD</mark> VFD <mark>M</mark> HRK	.WPGGGRDP <mark>LGF</mark>	G <mark>F</mark> GPH <mark>RCV</mark> A	EHLAKAELA
A_terreus	NQSANRI	DED <mark>VF</mark> .PI) <mark>PD</mark> TFN <mark>M</mark> HRE	TDSEQN <mark>LAY</mark>	G Y G D H R C I A	EGLARAELE
A_flavus	NQAANRI	PG <mark>VF</mark> .PI	O <mark>PD</mark> TFD <mark>M</mark> FRK	RGPEEA <mark>LGF</mark>	G Y G D H R C I A	EMLARAELE
U_reesii	NQAANRI	DED <mark>IF</mark> .PI) <mark>P D</mark> K F D I H R K	RGSESA <mark>LGY</mark>	G Y G D H R C I A	EGL <mark>SRAEL</mark> E
C_apollinis	NQSANRI	DEA <mark>VF</mark> .PI	N <mark>PD</mark> KFD <mark>M</mark> Y <mark>R</mark> K	RWEEEA <mark>LGF</mark>	G <mark>F</mark> GPH <mark>RCI</mark> A	EWLARAELE
M_gypseum	TQSGNR	AD <mark>VF</mark> .PI	N <mark>P D</mark> T F D <mark>M</mark> Y <mark>R</mark> K	RGAESA <mark>FGF</mark>	GYGEHRCVA	EWLARAELE
M_brunnea	TQSGNR	DEE <mark>VF</mark> GP'	T <mark>ANEF</mark> D <mark>M</mark> K R V	RGSEEA <mark>LGY</mark>	GWGAHRCVA	EWLARAELE
M_phaseolina	NQSGNRI	DAA <mark>IF</mark> .PI	⊃ <mark>РD</mark> MFD <mark>M</mark> H <mark>R</mark> A	RGAEAA <mark>lgy</mark>	G <mark>F</mark> GAH <mark>RCV</mark> A	EWLARCEME
T_cutaneum	NYSGNRI	DAD <mark>AF</mark> .KI	D <mark>PD</mark> VFD <mark>I</mark> R <mark>R</mark> T	.FDKDP <mark>LAF</mark>	GWGPHRCIA	ETLAKVELT
C_reinhardtii	NQSANRI	DES <mark>VF</mark> .PI	D <mark>PD</mark> R F D <mark>I</mark> H <mark>R</mark> Q	SNPQQ <mark>VGF</mark>	G <mark>Y</mark> G T H <mark>∨</mark> C <mark>V</mark> A	EWLARAEIQ
C_variabilis	NQSANR	EE AF .PI	D <mark>PD</mark> AFD <mark>I</mark> R <mark>H</mark> S	PNP.Q <mark>VGY</mark>	G <mark>Y</mark> GTHLCVA	MPLAMAELE

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F_oxysporum	l	22	20	20	20						ک	200	. 1										•	•		•	•					
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F_oxysporum	Т	VF	SΤ	LΥ	QK	F P	D	LΚ	VP	VI	<mark>?</mark> I	GK	I	N	YТ	P 1	LN:	RE	v	GI	V								D	L P V	TI	F
F_lichenicola	Т	VF	ΑT	LF	ΚE	F P	D	LN	IZ	VI	PE	ΓEΚ	I	N	FΤ	Ρ1	ĹG	GC	v	GV	V								D	LP \	TT	F
C_globosum	S	AF	ΤF	LF	ΚK	AP	G	ΓK	LP	VI	2 I	KD	I	E	ΥТ	Ρ1	LQ:	RE	A	FΥ	V	QE	Ε	Q	GΞ	SΝ	ΤI	r R	D	7 P E	N	F
T_virens	Т	VF	SΤ	ΙF	ΚE	LР	Ν	ΓK	LP	VI	P I	SK	I	N	FΤ	Ρ1	LH:	RE	v	GI	Е		•			•	•		D	L P \	V	F
S_macrospora	Α	VF	GΤ	ΙF	ΚK	LP	Ν	ΓK	IP	VI	? N	1 D K	I	E	ΥT	Ρ1	LD:	RE	v	GI	V		•	•		•	•		к <mark>ј</mark>	L P V	T	W
G_graminis	Α	VF	GΤ	LF	KR	LP	G	LE	IF	VI	?]]	ND	L	E	YТ	Ρ]	LV:	RE	v	GΫ	V		•				•		S	LP V	T	W
A_terreus	Α	VF					•						•	•					•			. 0	ΞE	Н			•		Q	JP.	1.	•
A_flavus	Т	VF	SΤ	LF	QΤ	LP	S	ΓK	LP	VI	P	(SΕ	I	Q	WΤ	ΡI	?Т:	RE	v	GI	V		•				•		G	LP V	T	W
U_reesii	Α	VF	SΤ	LF	QR	LP	Ν	LR	LO	FI	P	ISE	I	K	ΥS	ΕF	ΡT1	MC	v	GI	A		•	•		•	•		ΕJ	LP V	V	W
C_apollinis	I	VF	SΤ	LF	QK	LP	Ν	LR	LP	ΙI	PE	ΈE	I	E	ΥT	ΡI	LD:	KE	v	GI	V		•	•		•	•		κ <mark>ι</mark>	LP V	V	W
M_gypseum	I	VF	ΤТ	LF	RR	LP	D	LΚ	LP	VI	PI	DE	v	K	ΥS	DI	? S :	KE	v	GI	Т		•	•		•	•		ΕJ	LP]	T	W
M_brunnea	Ι	VF	ΑT	LW	QK	LP	Ν	ΓK	LP	۷Z	ΥĒ	'AK	V	Ν	ΥS	ΡI	? K :	KD	Ъ	GI	G		•				•		ΕJ	LP V	V	F
M_phaseolina	Ε	VF	GΑ	LV	ΤR	VP	G	LR	LP	LΤΕ	7 <mark>?</mark>	/EE	L	R	WΤ	ΡŻ	ΑT	KD	v	GV	V		•				•		ΕJ	LL	Υ	L
T_cutaneum	Α	VF	ΕТ	LΥ	KR	LP	Ν	ΓK	PZ	VI	? I	ΓED	I	A	ΥS	Ρ1	LD:	KD	v	GI	V		•				•		ΕJ	LP V	T	W
C_reinhardtii	V	AΙ	GΤ	LF	RR	LP	Ν	LR	LP	VI	PE	ΙSQ	I	Q	ΥS	DI	? A :	RE	v	GI	A		•				•		A	L P V	T	W
C_variabilis	С	AF	GG	LL	RR	LP	T	ΙK	LP	V Z	ΙE	, D Ŏ	L	Q	WS	DI	ΡK	AE	v	GL	Т		•				•		G	L P V	T	W
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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 3a 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_oxysorum = 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 = ${\it Macrophomina\ phaseolina, T_cutaneum = Trichosporon\ cutaneum, C_reinhardtii = Chlamydomonas\ rheinhardtii, C_variabilis = Chlorellandtii, C_variabili$ variabilis.



Figure S3. N₂O production in soil microcosms amended with chloramphenicol and streptomycin to inhibit bacterial activity. NO₃- (2 mM) or NO₂- (1 mM) were added as the sole nitrogen source. Acetate (\bullet), formate (\Box), pyruvate (\blacktriangle), or plant residue (\diamond , dashed line) were added as carbon source and electron donor for NO₃- or NO₂- 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. NO3⁻(\blacktriangle), NO2⁻(\blacksquare), and N2O (\circ) dynamics in transfer cultures from Havana and Urbana microcosms amended with ampicillin and kanamycin to prevent bacterial activity. NO2- (1 mM) or NO3- (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).