

Anthropogenic N deposition increases soil C storage by reducing the relative abundance of lignolytic fungi. Elizabeth M. Entwistle, Donald R. Zak, and William A. Argiroff. *Ecological Monographs*.

APPENDIX S3

Table S1. Agaricomycete taxa excluded from our list of “highly lignolytic taxa”. We excluded any classes, families, or genera:

- 1) that are known to be (largely or entirely) comprised of non-saprotrophs or for which the nutritional modes are unknown,
- 2) for which we have no information regarding a role in lignin decay,
- 3) for which there is published evidence that the taxon is either nonlignolytic or weakly lignolytic,
- 4) that are or may be lignolytic, but for which there is insufficient evidence that they are *highly* lignolytic,
- 5) for which existing evidence regarding their capacity to decay lignin is contradictory,
- 6) that are represented in our data set by fewer than 20 sequences regardless of their physiology,
- 7) that were “unclassified” at the order or class level (most not shown below),
- 8) that were “unclassified” at the genus level (not shown below) and did not belong to families selected as “highly lignolytic taxa” (see Table S2).

We list the current (to the best of our knowledge) taxonomic placement of any taxa we excluded. If this differed from the classification we obtained from the Ribosomal Database Project fungal LSU rRNA v7 classifier (Liu et al. 2012) files, we documented this in the notes column.

Taxa within Agaricomycetes	Reasons for exclusion with citations	Notes
Auriculariales	5. Inconsistent role in lignin decay. Observations of lignin removal range from none to very high for different species, strains, substrates, and studies (Worrall et al. 1997, Osono and Takeda 2006, Liers et al. 2011, Suhara et al. 2012). Species of this group are of interest for their role in lignin decomposition because they have high numbers of lignin-decomposing genes (Floudas et al. 2012, Nagy et al. 2015), including dye-decolorizing peroxidases (Liers et al. 2010). Despite their seemingly high	

	potential for lignin decay, we have excluded Auriculariales because actual measurements for their role in this process show a high degree of variation.	
Agaricales/ Agaricaceae	4 & 5. Probably not highly lignolytic. <i>Agaricus</i> removed ~50% of lignin mass from straw over 70 days (Durrant et al. 1991), but the ability of <i>Agaricus</i> to decay fresh needle litter was observed to be weak (Osono et al. 2006). <i>Agaricus</i> species possess genes for lignolytic enzymes (Hildén et al. 2013, Floudas et al. 2015) and may be adapted for late-stage litter decay (Morin et al. 2012). <i>Lepiota</i> , the genus to which most of the Agaricaceae sequences in our data set belonged, is much less well-studied, in this regard. <i>L. cristata</i> was able to oxidize lignin model compound ABTS, but did not bleach humic acids nor oxidize Mn ²⁺ (Steffen et al. 2000). No other information regarding the role of <i>Lepiota</i> in lignin decay could be found.	
Agaricales/ Amanitaceae	1. Largely mycorrhizal, although some species are saprotrophic litter decomposers with some lignin decomposing genes (Kohler et al. 2015)	
Agaricales/ Bolbitiaceae	4. Probably not highly lignolytic. <i>Agrocybe</i> comprised the preponderance of sequences from the Bolbitiaceae in our data set. <i>Agrocybe aegerita</i> produced low-molecular weight compounds, which are evidence of lignin decomposition, but caused a low amount of lignin loss on a mass basis compared to other fungi studied (Liers et al. 2011).	
Agaricales/ Clavariaceae	1. Includes saprotrophs and biotrophs, but the dominant genera in our data set (<i>Clavaria</i> , <i>Clavulinopsis</i> and <i>Ramariopsis</i>) are all biotrophic (Birkebak et al. 2013)	
Agaricales/ Cortinariaceae/ <i>Cortinarius</i>	1. Ectomycorrhizae	
Agaricales/ Hymenogastraceae/ <i>Dermocybe</i>	6. < 20 sequences	
Agaricales /Cortinariaceae/	6. < 20 sequences	

<i>Quadrispora</i>		
Agaricales/ Entolomataceae	2. Little is known about their role in decomposition or their capacity for lignin decay. Saprotrophs, especially of grasslands (Lynch and Thorn 2006, Griffith and Roderick 2008) and some mycorrhizal species (Smith et al. 2013).	
Agaricales/ Hydnangiaceae/ <i>Laccaria</i>	1. Ectomycorrhizae	<i>Laccaria</i> classified to Agaricales/Tricholomataceae , but belongs in Agaricales/Hydngangiaceae (Matheny et al. 2006, Kirk et al. 2008)
Agaricales/ Hygrophoraceae	1. <i>Hygrocybe</i> , the genus that dominates Hygrophoraceae sequences in our data set, is biotrophic (Seitzman et al. 2011). <i>Gliophorus</i> is also biotrophic (Seitzman et al. 2011).	<i>Gliophorus</i> classified to Agaricales/Tricholomataceae, but should be in the Agaricales/Hygrophoraceae (Seitzman et al. 2011)
Agaricales/ Hymenogastraceae/ <i>Flammula</i>	2. No information regarding lignin decay available. <i>Flammula alnicola</i> and <i>Flammula conissans</i> cause root and butt root, with ~2% mass loss on wood over a 6 month incubation. <i>F. alnicola</i> produces a rot described as vivid yellow to yellow brown (Denyer 1959).	classified to Agaricales/Cortinariaceae. In the past, <i>Flammula</i> was included in the Strophariaceae (Kirk et al. 2008), but others have found that it does not belong to the Strophariaceae (Moncalvo et al. 2002, Matheny et al. 2006) and is more appropriately placed in Agaricales/Hymenogastraceae (Matheny et al. 2006)
Agaricales/ Hymenogastraceae/ <i>Galerina</i>	4. Insufficient information regarding their role in lignin decay. <i>Galerina marginata</i> is a white-rot organism with a high number of genes for lignin and crystalline cellulose decay (Floudas et al. 2015, Kohler et al. 2015) and other species in the genus <i>Galerina</i> are also wood decay fungi (Muraoka et al. 1999). A laccase from a <i>Galerina</i> species efficiently	classified to Agaricales/Cortinariaceae. This is correct according to some sources (Kirk et al. 2008), but others have placed it in the

	oxidize lignin model compound ABTS (Ibrahim et al. 2011) and decolorized synthetic dyes (Mendoza et al. 2014). <i>Galerina pseudomycesopsis</i> nor <i>Galerina mycesopsis</i> were able to utilize lignin in BIOLOG plates (Ibrahim et al. 2011); however, this is a poor means of assessing its lignolytic activity because fungi do not utilize lignin on its own. However, we were unable to locate measurements of lignin decay for this genus.	Agaricales/Hymenogastraceae using molecular phylogenetics (Matheny et al. 2006)
Agaricales/ Hymenogastraceae/ <i>Hebeloma</i>	6. < 20 sequences	classified to Agaricales/Cortinariaceae. This is correct according to some sources (Kirk et al. 2008), but molecular phylogenetic studies have placed it in the Agaricales/Hymenogastraceae (Moncalvo et al. 2002, Matheny et al. 2006)
Agaricales/ Inocybaceae/ <i>Inocybe</i>	1. Ectomycorrhizae	<i>Inocybe</i> classified to Agaricales/Cortinariaceae, but should be in Agaricales/Inocybaceae (Matheny 2005, Kirk et al. 2008)
Agaricales/ Lycoperdaceae	6. < 20 sequences	
Agaricales/ Marasmiaceae/ <i>Moniliophthora</i>	1 & 6. < 20 sequences. <i>Moniliophthora</i> is a plant pathogen (Aime and Phillips-Mora 2005)	
Agaricales/ Pleurotaceae	6. < 20 sequences	
Agaricales/ Pluteaceae	3, 4, & 5. The straw mushroom, <i>Volvariella volvacea</i> , has a high number of genes for degradation of lignin and utilization of crystalline cellulose (Floudas et al. 2015). In a decomposition experiment, <i>V. volvacea</i> caused a 20% mass loss of lignin in straw over a 21 day incubation, with the ratio of lignin-to-mass lost of 1.4, indicating some selectivity for lignin decomposition over other constituents of plant litter (Chang-Ho and Yee 1977). However, none of our sequences classified to the genus	

	<p><i>Volvariella</i>. Instead of the Pluteaceae sequences classified to the genus <i>Pluteus</i>, for which there is very little known regarding its lignolytic abilities. One study found that <i>Pluteus petasatus</i> demonstrated no measurable laccase or MnP activity in enzyme assays and had low production of H₂O₂, a necessary cosubstrate for lignolytic peroxidases. Additionally, <i>P. petasatus</i> did not decolorize two aromatic compounds (Eichlerová et al. 2006). <i>Pluteus</i> has been described as a tertiary decomposer that colonizes after primary decomposers have broken down lignocellulose (Stamets 2004).</p>	
Agaricales/ Psathyrellaceae	<p>3 & 4. Psathyrellaceae also do not appear to cause high amounts of lignin decay. <i>Coprinellus radians</i> exhibited low oxidative enzyme activity, removed low amounts of lignin, and did not produce low molecular weight compounds during over 72 days on wood (Liers et al. 2011). When inoculated on wood that had already been rotted by other fungal species first, <i>Coprinopsis</i> species did not cause significant additional mass loss, but <i>Coprinellus</i> species did, suggesting that at least some Psathyrellaceae might be late stage decay fungi (Oliver et al. 2010). <i>Coprinopsis cinerea</i> possesses 17 laccases in its genome (Kilaru et al. 2006), but has only has a nonlignolytic general peroxidase and no lignolytic class II peroxidases (Ruiz-Duenas et al. 2009).</p>	
Agaricales/ Schizophyllaceae	<p>4. The Schizophyllaceae are wood-rot fungi, but are likely less lignolytic than many other white-rot fungi. <i>Schizophyllum commune</i> has fewer lignolytic genes than most white-rot species which may reduce its lignolytic capabilities compared to other white-rot species (Floudas et al. 2015). <i>S. commune</i> does attack the secondary cell wall which is the lignified part of plant cells. However, it leaves the middle lamella intact, much like a soft-rot species (Floudas et al. 2015). Additionally, <i>S. commune</i> appears to cause a relatively low mass loss in wood decay studies (Martínez et al. 2000, Floudas et al. 2015).</p>	
Agaricales/ Strophariaceae/ <i>Hypholoma</i>	<p>6. < 20 sequences</p>	

Agaricales/ Strophariaceae/ <i>Pholiota</i>	6. < 20 sequences	
Agaricales/ Tricholomataceae/ <i>Armillaria</i>	6. < 20 sequences	
“ “ <i>Cotobrusia</i>	6. < 20 sequences	
“ “ <i>Filoboletus</i>	6. < 20 sequences	
“ “ <i>Flagelloscypha</i>	6. < 20 sequences	
“ “ <i>Hemimycena</i>	6. < 20 sequences	
“ “ <i>Hydropus</i>	6. < 20 sequences	
“ “ <i>Hygroaster</i>	6. < 20 sequences	
“ “ <i>Hymenogloea</i>	6. < 20 sequences	
“ “ <i>Infundibulicybe</i>	6. < 20 sequences	
“ “ <i>Micromphale</i>	6. < 20 sequences	
“ “ <i>Mycenella</i>	6. < 20 sequences	
“ “ <i>Mycenoporella</i>	6. < 20 sequences	
“ “ <i>Omphalina</i>	6. < 20 sequences	
“ “ <i>Pseudobaeospora</i>	6. < 20 sequences	
“ “ <i>Ripartites</i>	6. < 20 sequences	
“ “ <i>Setulipes</i>	6. < 20 sequences	
“ “ <i>Tephrocybe</i>	6. < 20 sequences	
“ “ <i>Tricholoma</i>	1. Ectomycorrhizae	
Agaricales/ Tricholomataceae/ <i>T richolomella</i>	6. < 20 sequences	
Agaricales/ Tubariaceae/ <i>Tubaria</i>	2. <i>Tubaria</i> species have been isolated from lignin rich substrates like wood, needles, and dead roots (Martínez et al. 2000, Matheny et al. 2007), but little is presently known regarding its ability to degrade lignin. <i>Tubaria furfuracea</i> was able to decolorize Remazol Brilliant Blue R (RBBR) dye, a substrate used to evaluate ligninolytic activity in studies examining fungi for biotechnological and bioremediation applications, in	Tubaria were classified to Agaricales/Strophariaceae, but now have their own family, the Tubariaceae (Matheny et al. 2006, Vizzini 2008).

	one study (Okino et al. 2000), but not in another (Machado et al. 2005).	
Amylocorticiales/ Amylocorticiaceae/ <i>Anomoporia</i>	3. Brown-rot. Because the classifier includes both brown-rot and white-rot taxa in sequences used to define this genus (see note), we verified that sequences classified to <i>Anomoporia</i> represented brown-rot taxa by obtaining the unique sequences classified to this genus in mothur (Schloss et al. 2009) and conducting searches with these sequences using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Tool (BLAST®) online portal. All searches produced hits with 94-95% identity to <i>Anomoporia kamtschatica</i> (GenBank AY586630), a brown-rot species (Niemelä et al. 2007).	These sequences were classified to Polyporales/Coriolaceae/Anomoporia. It is now placed in the Amylocorticiales (Binder et al. 2010). Furthermore, the classifier includes both brown-rot and white-rot taxa in Anomoporia, but the genus has since been split into Anomoporia for brown-rot species and Anomoloma for white-rot species (Niemelä et al. 2007)
Boletales	1 & 3. Largely mycorrhizal with some brown-rot species (Floudas et al. 2012)	
Cantharellales	1, 3, & 4. Ectomycorrhizae, orchid mycorrhizae, lichen-associated fungi, plant pathogens, and saprotrophs. Saprotrophs from Cantharellales have been observed highly cellulolytic but not lignolytic (Boberg et al. 2011). Rot has not been characterized as white-rot but as “uncertain” or ancestral soft-rot (Floudas et al. 2015, Nagy et al. 2015)	“Agaricomycetes incertae sedis/Tricellortus” and to “Polyporales/Polyporales incertae sedis/Aphyllorphoralean” were also determined to represent Cantharellales. Sequence defining Tricellortus in classifier produced 93-99% NCBI BLAST matches to <i>Minimedusa</i> , <i>Sistotrema</i> , <i>Clavulinaceae</i> . Sequence defining Aphyllorphoralean produced 96-97% identity matches in NCBI BLAST to <i>Sistotrema</i> and <i>Clavulina</i> .
Geastrales/ Geastraceae	6. < 20 sequences	
Geastrales/ Sphaerobolaceae/ <i>Sphaerobolus</i>	4 & 5. Lack of information regarding their role in lignin decay. <i>S. stellatus</i> is often found as a saprotroph on mulch and is also responsible for turfgrass disease (Baetsen 2013). <i>S. stellatus</i> has a high number of oxidoreductases (Nagy et al. 2015), has been observed to decay lignin	

	(Baetsen 2013), and has demonstrated high selectivity for lignin over other constituents of plant cells (Worrall et al. 1997). In media, <i>S. stellatus</i> strongly degraded lignin (Robinson et al. 1993). However, <i>Sphaerobolus</i> species on natural substrata have not caused high mass loss of lignin (Valmaseda et al. 1990, Worrall et al. 1997, Suhara et al. 2012), although this may be because <i>Sphaerobolus</i> are slow growing (Conway et al. 2000). While <i>Sphaerobolus</i> are lignolytic, data available are currently too limited and contradictory to determine if they are highly lignolytic.	
Gomphales/ Gomphaceae/ <i>Kavinia</i>	4. Lack of information regarding their ability to decay lignin. <i>Kavinia</i> are resupinate fungi often found on wood (Nordén and Paltto 2001, Kout and Hajšmanová 2015). <i>Kavinia</i> species possess strong laccase activity (Harkin et al. 1974) and have been described as white-rot (Ginns and Lefebvre 1993) or uncertain rot (Hibbett et al. 2014). However, no measurements of their ability to decay lignin presently exist in literature.	
Gomphales/ Gomphaceae/ <i>Phaeoclavulina</i>	1. Little known regarding their ecology	
Gomphales/ Gomphaceae/ <i>Ramiricium</i>	6. < 20 sequences	
Hymenochaetales	4. Lack of information regarding their ability to decay lignin. Hymenochaetales have unique manganese peroxidases (Morgenstern et al. 2010) and at least one species (<i>Fomitiporia mediterranea</i>) has a high number of genes for lignolytic enzymes (Floudas et al. 2012). However, we were unable to find measurements of their ability to decay lignin in the literature.	
Phallales	6. < 20 sequences	
Polyporales/ Meripilaceae	6. < 20 sequences	
Polyporales/ Meripilaceae/	6. < 20 sequences	classified to Polyporales/Coriolaceae.

<i>Rigidoporus</i>		Coriolaceae is no longer recognized as a family. <i>Rigidoporus</i> but is currently placed in the Meripilaceae (Kirk et al. 2008).
Polyporales/ Phanerochaetaceae/ <i>Ceriporia</i>	3. Many <i>Ceriporia</i> species are highly lignolytic white-rot fungi. However, <i>Leptoporus mollis</i> and <i>Ceriporia reticulata</i> are brown-rot species which were recently placed phylogenetically in the middle of this genus (Floudas and Hibbett 2015). In order to investigate whether the <i>Ceriporia</i> in our data set were more closely related to white-rot or brown-rot <i>Ceriporia</i> species, we obtained all of the unique sequences which were placed in this genus, using mothur 1.31.2 (Schloss et al. 2009). Of the 666 unique sequences classified to <i>Ceriporia</i> in our data set, we selected 10 and conducted a search through the NCBI BLAST® online portal. For all 10 sequences, the top BLAST hit was <i>Ceriporia reticulata</i> (99% identity, GenBank KP135204), a brown-rot species (Floudas and Hibbett 2015). Therefore, the <i>Ceriporia</i> sequences in our data set most likely represent brown-rot species.	classified to Polyporales/Coriolaceae/ <i>Ceriporia</i> . However, Coriolaceae is no longer recognized as a family and <i>Ceriporia</i> belongs to the Phanerochaetaceae (Kirk et al. 2008, Floudas and Hibbett 2015)
“ “ <i>Ceriporiopsis</i>	6. < 20 sequences	classified Polyporales/Coriolaceae, but is currently placed in the Polyporales/Phanerochaetaceae (Kirk et al. 2008).
Polyporales/ Polyporaceae	6. < 20 sequences	
Polyporales/ Polyporaceae/ <i>Cerrena</i>	6. < 20 sequences	Classified Polyporales/Coriolaceae. Coriolaceae no longer recognized as a family. Now placed in the Polyporales/Polyporaceae (Kirk et al. 2008).
“ “ <i>Hapalopilus</i>	6. < 20 sequences	Classified Polyporales/Coriolaceae. Coriolaceae no longer recognized as a family. Now placed in the

		Polyporales/Polyporaceae (Kirk et al. 2008).
“ “ <i>Oligoporus</i>	6. < 20 sequences	Classified Polyporales/Coriolaceae. Coriolaceae no longer recognized as a family. Now placed in Polyporales/Fomitopsidaceae (Kirk et al. 2008).
Russulales/ Russulaceae	1. Ectomycorrhizal	
Sebacinales	1. Largely mycorrhizal	
Thelephorales	1. Ectomycorrhizal	
Trechisporales	7 & 4. Sequences associated with Trechisporales appear to have been classified only as far as class (Agaricomycetes) and were placed as unclassified at the order level with the RDP v. 7 classifier. Therefore, when we excluded unclassified Agaricomycetes, we excluded sequences in our data set from species in the Trechisporales. A recent genomic analysis (Nagy et al. 2015) has placed two members of this order among white-rot fungi. High laccase activity (Nobles 1958, Harkin et al. 1974, Kreetachat et al. 2016) and high peroxidase activity (Harkin et al. 1974) have been reported for some species from this group, but not from others (Harkin et al. 1974). Rot from species in the Trechisporales has previously been described as white-rot (Harkin et al. 1974, Gilbertson et al. 1975, Huckfeldt and Schmidt 2006) or as an unknown rot (Nobles 1958). However, we were unable to locate any studies measuring lignolysis by Trechisporales. Thus, Trechisporales are lignolytic, but we have insufficient data to be determine if they are highly lignolytic.	Some sequences that were classified as Agariomycetes/unclassified represent Agaricomycetes/Trechisporales

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