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Title:
Characterization of main sulfur source of wood-degrading basidiomycetes by S K-edge X-ray Absorption Near Edge Spectroscopy (XANES)

Running Title:
Wood-degrading basidiomycetes shift sulfur oxidation status in wood

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Abstract

The main wood degraders in aerobic terrestrial ecosystems belong to the white- and brown-rot fungi, where their biomass can be created on wood decay only. However, total sulfur (S) concentration in wood is very low and only little is known about the different S compounds in wood today. S-starved brown-rot fungi *Gloeophyllum trabeum* and *Oligoporus placenta* were incubated on sterilized pine wood blocks whereas *Lentinus cyathiformis* and the white-rot fungi *Trametes versicolor* were incubated on sterilized beech wood blocks. After 19 weeks of incubation, the S-oxidation status was analyzed in wood, degraded wood and in biomass of wood-degrading fungi by synchrotron based S K-edge XANES, and total S and sulfate was quantified. Total S and sulfate content in pine wood blocks were approx. 50 and 1 g g^{-1}, respectively, while in beech wood approx. 100 and 20 g g^{-1} was found, respectively. S in beech was dominated by sulfate-esters. In contrast, pine wood also contained larger amounts of reduced S. Three out of four selected fungi caused a reduction of the S oxidation state in wood from oxidized S (sulfate-ester, sulfate) to intermediate S (sulfonate, sulfoxide) or reduced S (thiols e.g. proteins, peptides, enzyme cofactors). Only *O. placenta* shifted thiol to sulfonate. Growth experiments of these fungi on selective minimal media showed that in particular cysteine (thiol), sulfonates and sulfate enhanced total mycelium growth. Consequently, wood-degrading fungi were able to utilize a large variety of different wood S sources for growth but preferentially transformed *in vivo* sulfate-esters and thiol into biomass structures.

(257 words)

Keywords: basidiomycetes; fungi; S K-edge X-ray Absorption Near Edge Spectroscopy (XANES); sulfur oxidation status; sulfate-esters**.**

1. Introduction

Sulfur (S) is a macro element that is an absolute requirement for growth just like nitrogen (N) and phosphorus (P). Plants are almost entirely dependent on inorganic sulfate as their S source, which often makes up only as little as 5% of the total soil S (Autry and Fitzgerald, 1990; Kertesz et al., 2007; Kertesz and Mirleau, 2004). Once incorporated into plant biomass, S exists in plants in a considerable variety of functional groups, and it has been studied in a broad range of plants and plant compartments, including wood. Analyses of functional S groups were carried out for spruce (Fairchild et al., 2009; Struis et al., 2008), oak (Sandstrom et al., 2005) and pine (Fors et al., 2008) wood species. All tree species were composed of disulfides and thiol groups (e.g. nucleic acid cysteine in proteins, tripeptide glutathione and enzyme cofactors), sulfoxides, sulfonates, and sulfate-ester and sulfates (Fairchild et al., 2009; Fors and Sandstrom, 2006; Sandstrom et al., 2005). The amount of S functional groups varied between tree species, storage of wood (Sandstrom et al., 2005) and even within one tree stem (Fairchild et al., 2009). Although Novak and colleagues (2009) found no clear correlation between S content in wood and the associated environmental air pollution (Novak et al., 2009), Fairchild and colleagues (2009) demonstrated that wood trees record the atmospheric S input over decades, which was caused by industrial and volcanic air pollution (Fairchild et al., 2009). Air pollution has decreased significantly across Europe and North America over the last 20 years, leading to many sulfate limiting terrestrial environments (Fairchild et al., 2009; McGrath and Zhao, 1995) and as a consequence, wood is nowadays formed almost free of S (Struis et al., 2008).

In natural ecosystems, there is a dynamic equilibrium between the accumulation of woody biomass and its breakdown. White- and brown-rot fungi have evolved the means to decompose large volumes of wood completely (De Boer et al., 2004; Fengel and Wegener, 1984) and play a major role in the aerobic processes of microbial wood degradation. The majority of these fungi are capable to extract, beside the abundant carbon source, macro elements such as N, P and S exclusively from wood. This is a considerable challenge for the wood-degrading fungi since the wood material naturally contains very low amounts of macro nutrients, and artificial addition of such macro elements accelerates wood decay significantly (Schmitz and Kaufert, 1936; Sterner and Elser, 2002; Weißhaupt et al., 2010). Nevertheless, wood-degrading fungi are able to recycle their hyphae and retranslocate macro elements through extensive fungal hyphae networks to minimize nutrient limitations (Tlalka et al., 2008).

Fungal degradation and acquisition of S from wood is not fully understood but may employ several intra- as well as extracellular enzymes. Sulfate-ester groups can be cleaved by several types of sulfatase enzymes (Tabatabai and Bremner, 1970) that are produced by many bacteria and fungi (Kertesz et al., 2007). Several filamentous and saprophytic fungi have been reported to have sulfatase activity (Marzluf, 1997), and plant growth promoting fungi have been isolated from faba bean nodules that had sulfatase activity (Omar and Abd-Alla, 2000). Although the ability of mycorrhizal fungi to desulfurize sulfate-esters has not been investigated to date, the fully sequenced ectomycorrhizal fungus *Laccaria bicolor* has five hypothetical proteins related to sulfatase. In contrast, a link between desulfurization of sulfonate groups and fungi has not been established yet. The white rot fungus *Phanerochaete chrysosporium* is capable of transforming linear alkylbenzensulfonates without desulfonation (Yadav et al., 2001), and fungal laccases and peroxidases used for decolorization of sulfonated dyes do not remove the sulfonate group of the target molecule (Wesenberg et al., 2003), suggesting that desulfonation is a bacterial process (Kertesz et al., 2007). Bacteria have been often reported to support fungal wood decay (De Boer et al., 2004; Jakobs-Schönwandt et al., 2010; Weißhaupt et al., 2010) and this could also be the case with the mobilization of organically bound S.

Functional S groups at low concentrations can be measured by the synchrotron-based spectroscopic method S K-edge X-ray Absorption Near Edge Spectroscopy (XANES) that identifies S oxidation states (-1 to +6), which are representative for disulfides and thiols (reduced S), sulfoxides and sulfonates (intermediate S), and sulfate-esters and sulfates (oxidised S) (Table 1). XANES has already been successfully used to identify S species in different environments such as wood (Fairchild et al., 2009; Fors et al., 2008) and soil (Zhao et al., 2006). The aim of this study was to identify the S species in pine and beech wood, and investigate the transformation of the wood S into the fungal biomass as sole source of S by characteristic wood-degrading fungi alone and with the addition of bacterial strains identified to be effective in the assimilation of sulfonate S.

2. Materials and Methods

2.1 *Incubation of basidiomycetes*

The basidiomycetes *Lentius cyathiformis* (CTB 67-02), *Trametes versciolor* (CTB 863), *Oligopurs placenta* (FPRL 280) and *Gloeophyllum trabeum* (BAM Ebw. 109) were taken from malt extract agar (5 g malt extract, 15 g Agar L^{-1}) of the BAM strain collection (https://www.webshop.bam.de; Germany). Choice of fungi, incubation settings and wood sterilization were carried out according to EN 113 (EN113, 1996). Beech wood was sourced from a forest from Brandenburg (Germany), while pine wood was sourced from a Bavarian forest (Germany) as purchased by a national timber trade. Sap wood blocks were cut to a size of (30 mm x 30 mm) to receive a large cross section area to enhance basidiomycetal wood decay. Sterile beech wood blocks were inoculated with strain *L. cyathiformis* and *T. versciolor*, respectively, whereas sterile pine wood blocks were inoculated with *O. placenta* and *G. trabeum*, respectively. Inoculations were carried out in triplicates and incubated aerobically at sterile conditions, 22°C and 70% air humidity in Petri dishes with wet sterilized cotton as described previously (EN113, 1996) and were kept free of any additional nutrient source.

To minimize S storage of the fungi derived from malt extract agar, wood blocks overgrown with mycelium were cut into quarters (15 mm x 15 mm) and were used to inoculate sterile wood blocks by transferring a colonised quarter wood block on top of a sterile wood block. Three of the four quarter wood blocks were used to inoculate new wood blocks and therefore tripling the number of inoculations after each transfer. Transfers were conducted three times after seven, five and seven weeks of incubation (to obtain 324 wood blocks with fungal decay in total). In parallel, non-inoculated sterilized wood blocks were transferred as negative controls. At the last transfer to three of the wood block incubations of each fungus the bacterial desulfonating strains *Rhodococcus sp.* P14D10 (isolated from wheat rhizosphere, (Schmalenberger et al., 2009)) and *Variovorax paradoxus*^{T} (DSM30034, (Schmalenberger and Kertesz, 2007)), respectively, and a combination of *R. sp.* P14D10 and *V. paradoxus* were added. Each bacterial strain was incubated for 7 days at 20 °C in liquid minimal media (Beil et al., 1995) and approximately 3 x 10^9 to 6 x 10^9 cells were added per g wood block as estimated with a Thoma counting chamber of the respective inoculum.

Wood block samples were used for growth experiments in liquid medium (20 ml) and on plates solidified with low sulfate agarose (Schmalenberger et al., 2010) using *i.*) minimal medium for bacteria minimal media, (Beil et al., 1995), *ii.*) modified Melin-Norkrans (MMN) (Marx, 1969) without malt extract and, *iii.*) modified Rorison medium (Hewitt, 1966). All three media had their S supplemented with defined S sources (see below) as well as without any S source as negative control. The application of an array of minimal media was conducted in order to find a malt extract free medium (due to high S content in malt extract) where the fungi from this study were capable to grow sufficiently. Fungal growth after 24 days of

incubation at 22°C on solid media was ranked of three independent incubations of each fungus and S source by visual growth.

2.2 *Synchrotron studies*

S K-edge XANES analysis were employed at the XAS beamline (ANKA, Karlsruhe, Germany; http://ankaweb.fzk.de). The X-ray source of the ANKA XAS beamline is a 1.5 Tesla bending magnet. The fixed exit monochromator of the XAS beamline was operated in step by step mode using the Si111 crystal pair. In order to suppress the higher harmonics, a detuning to 70 % of the maximum beam intensity was used. An ionisation chamber to measure the primary flux and an energy dispersive detector (SDD - Silicon Drift Detector) to measure the fluorescence signal were used.
To build a reference library of S compounds and their oxidation state on a wooden matrix for a linear combination fitting of XANES data sets, sterilized pine wood blocks were supplemented with S 1 mg g^{-1} wood (dry weight) of *i.*) 1,4-dithioerythritol, *ii.*) L-cysteine, *iii.*) sodium dodecyl sulfate (SDS), *iv.*) sodium sulfide, *v.*) dimethyl sulfoxide (DMSO), *vi.*) lignosulfonate, *vii.*) toluenesulfonate, *viii.*) pentanesulfonate, *ix.*) sodium sulfate and measured with XANES. 1 mg S g^{-1} wood was chosen to retrieve a clear XANES spectrum for each S source independently from the natural S sources of wood that was not detectable in comparison to the applied standards. Wood matrix was used for standards in order to exclude any potential effects of the wood matrix onto the spectra. However, spectra obtained from this study were in accord with previous studies of pure compounds (Morra et al., 1997; Pickering et al., 2001).

Wood blocks were split in halves (to fit in the sample holder) and measured directly (1.5 x 3 x 0.4 cm). Initial trials revealed that the powdered wood samples on Kapton tape (DuPont, Wilmington, DE) produced lower quality readings in transmission and reflection mode, compared to entire wood block analysis in reflection mode. The fungal and fungal-bacterial surface biomass, respectively, were scratched from the wood blocks. Separated surface biomass and the corresponding wood blocks were measured in reflection mode. Pine and beech wood blocks from the control experiments without microbes were measured as controls. The photon energy of the primary beam was varied around the S absorption edge at 2472 eV and scans were carried out at 2464 eV to 2510 eV in steps of 1 eV (2464-2468) and 0.2 eV (2468-2510) respectively in order to identify the different oxidation states of S (Table 1). Exposure of the sample to the monochromatic beam was 10 s per step and three replicate measurements were collected for each sample (140 min / sample).

2.3 *Quantification of total S content and sulfate*

The wood blocks used for XANES measurements were thereafter milled by a planetary ball mill to receive homogenized samples as described earlier (Noll et al.). The total S content was determined with the total S analyser TS 3000 with UV fluorescence detector (Thermo Fisher Scientific, Waltham, MA). The matrix independent analyses of TS 3000 was complied with the ASTM D5453 methodology for the determination of total S. The samples were pyrolysed at 1000 °C under controlled conditions that ensured complete combustion of S into sulfur dioxide. The procedure was calibrated by using thianthrene doped clay. Water soluble sulfate from the analysed samples was quantified after maceration of sub samples via IC analysis in three replicate measurements as described earlier (Schmalenberger et al., 2010).

2.4 *Data analysis*

XANES spectra were exported as text files and subsequently loaded into the software package IFEFFIT (Newville, 2001) (http://cars9.uchicago.edu/ifeffit/) and were further analysed with the software Athena (Ravel and Newville, 2005) and WinXAS (http://www.winxas.de) (Ressler, 1998). XANES spectra were normalized and after baseline

subtraction a linear combination fit with standards from this study was carried out to identify major shifts in the XANES spectra by reporting only changes in three basic categories of 5-10, 10-20 and over 20 %.

3. Results

3.1 *Fungal growth on minimal media with defined S sources*

All fungal strains grew better on a MMN medium variation from this study with sulfate as sole S source than without S source (Table 2) but only *G. trabeum* showed maximum growth with sulfate. *O. placenta*, *T. versicolor* and *L. cyathiformis* showed maximum growth with cysteine and sulfonates while DMSO and SDS resulted in lower growth rates (Table 2). *L. cyathiformis* showed limited growth on any of the offered S sources. Cultivation in alternative liquid and solidified media without malt extract (minimal medium and Rorison, see materials and methods) did not result into reliable growth of biomass (data not shown).

3.2 *Sulfate and total S concentrations in incubated wood blocks*

Pine wood from this study contained very low concentration of sulfate, which was less than 3% of the total S (Table 3). However, the beech wood in this study contained almost 20 times higher sulfate concentration and double the amount of total S compared to pine wood. Incubation of beech wood with *L. cyathiformis* increased the amount of sulfate, while incubation with *T. versicolor* led to a decreased amount of sulfate. Incubation of fungi on pine wood resulted in increased concentrations of sulfate. In particular, *O. placenta* was characterized with 12 fold higher sulfate concentration whereas *G. trabeum* almost doubled the concentration of sulfate (Table 3). While total S concentrations were maintained at approx. 100 $\mu g\ g^{-1}$ for beech and 50 $\mu g\ g^{-1}$ for pine, incubation with *O. placenta* seemed to increase the level of total S in pine wood, but this increase was not repeated with pine wood incubated with *O. placenta* and the desulfonating bacterium *V. paradoxus*. However, mass loss during incubation of up to 46% was not factored in at this stage.

3.3 *Identification of different S oxidation states employing XANES*

A calibration of the XANES spectra was carried out to correlate the oxidation states of the S species (-2 to +6) to the corresponding absorption maxima (Fig. 1). All S oxidation states of the standards could be distinguished (Table 1). These findings in these three major groups of reduced S (sulfide, disulfide, thiol), intermediate S (sulfoxide and sulfonate) and oxidised S (sulfate and sulfate-ester) were in line with earlier findings recorded by Salomon and colleagues (Solomon et al., 2003).

3.4 *Changes in the S oxidation status in wood after fungal colonization and degradation*

XANES spectra of beech wood showed that about half of the wood S is comprised of oxidised S (sulfate-ester, sulfate) (Fig. 2, Table 4) and about 80% of this oxidized group was related to sulfate-esters (Table 3). The other identified S species were reduced S thiol and intermediate S sulfoxide and sulfonates and made up the other half of the total S with similar amounts of less than 20% each. *T. versicolor* incubated on beech wood showed the preferential use of oxidised S and reduced S thiol while incubation of *L. cyathiformis* on beech wood revealed a preferential utilization of the intermediate S sulfoxide (Table 4, Fig. 2). The fungal biomass of both beech wood-degrading fungi, however, contained a high amount of reduced S thiol.

Pine wood had very low levels of inorganic sulfate (Table 3), and therefore the affiliated oxidised S concentration was almost exclusively comprised by sulfate-esters. This oxidised S sulfate-ester was the largest S fraction in pine wood, followed by the reduced S thiol and the

intermediate S sulfonate and sulfoxides (Table 4, Fig. 3). Pine wood degraded by *G. trabeum* was characterised by a preferential use of oxidised S sulfate-esters resulting into a spectrum dominated by the intermediate S sulfonate signal, and its biomass was also characterized by a sulfonate signal (Fig. 3). In contrast, *O. placenta* preferentially utilized reduced S thiol . The biomass of *G. trabeum* and *O. placenta* contained large amounts of reduced S thiol but . *trabeum* also contained amounts of intermediate S sulfoxides and sulfonates that were higher than the concentrations found in pine wood.

Decayed pine and beech wood showed a relative increase of intermediate S sulfonates compared to the other S groups (Table 4). In contrast, the intermediate S sulfoxide remained before and after fungal decay relatively stable compared to the other S groups.

3.5 *Changes in the S oxidation status in wood after fungal and bacterial colonization and degradation*

Pine wood incubated with both *O. placenta* and the desulfonating bacterial strain *V. paradoxus* and *R.* sp. P14D10, respectively, revealed that the co-inoculated bacteria had only little effect on shifts of the S K-edge XANES signal in degraded pine wood (Fig. 4, Table 4). The co-incubation of *O. placenta* and *V. paradoxus* resulted into a lower reduction of reduced S thiol, a slightly higher level of intermediate S sulfoxides and no increase of intermediate S sulfonates when compared to pine wood decayed by *O. placenta* alone. The co-incubation of *O. placenta* and *R.* sp. P14D10 revealed only a slight increase of the oxidised S when compared to the incubation of pine and fungus alone.

4. Discussion

Up to date, very little is known about the ability of wood-degrading fungi to mineralize sulfur (S) compounds in wood and whether these fungi are in an interaction with sulfonate mineralizing bacteria to access sulfonate S from wood. This study investigated the fungal and fungal-bacterial utilization of different S species from wood *in situ* through the identification of the S oxidation states present using XANES and the fungal abilities to utilize selected S sources *in vitro*.

Early reports have already shown that wood-degrading fungi prefer defined wood species as substrate (Fengel and Wegener, 1984) and the tested wood-degrading fungi differed in their carbon traits significantly to each other (Hibbett and Donoghue, 2001). The tested wood-degrading fungi were described as the main rotters of the respective wood species (Fengel and Wegener, 1984) and were therefore introduced here under the same incubation conditions and wood species as described earlier (EN113, 1996). The mass loss of 88 incubations of *O. placenta*, *T. versicolor, G. trabeum*, and *L. cyathiformis* was in arithmetic average 46%, 39%, 35% and 25%, respectively, after 10 weeks of incubation (personal communication, Dr. Ina Stephan, BAM), indicating that each fungus has its respective efficiency in wood decay. Indeed, comparison of genome, transcriptome and secretome analysis of the brown-rot fungus *O. placenta* and *Phanerochaete chrysosporium* supported that an evolutionary shift took place from white-rot to brown-rot during which the capacity for efficient depolymerisation of lignin was lost (Martinez et al., 2009). In conclusion, S related degradation pathways could also differ between white and brown rotters. Indeed, this study found substantial differences in the way and amount of functional S degradation of wood-degrading fungi at their preferred wood substrate. Changes in the S oxidation states during wood degradation *in situ* indicated that oxidised S sulfate-esters are the preferred S source of the wood-degrading fungi *T. versicolor* and *G. trabeum* (Figs. 2 and 3, Tab. 3). However, *T. versicolor* and in particular *O. placenta* also utilized reduced S thiol e.g. cysteine. Only *L. cyathiformis* preferentially utilized intermediate S sulfoxides (Fig. 2;

Tab. 3). In this study, the bulk of the microbial biomass was separated from the wood surface. As a consequence, the analysis of the corresponding incubated wood blocks includes sub-surface mycelium. Therefore, this study does not attempt to give a quantitative account of the S oxidation states in the degraded wood and reports trends in the changes of the S oxidation states instead (Tab.3). Sulfate-esters have been found to be important for osmoregulation in many plants and fungi (Osteras et al., 1998). For example, choline-O-sulfate has been identified as a potent osmoprotectant in some plants (Csonka and Hanson, 1991; Koshino et al., 1993; Lamark et al., 1996). However, its function in fungi is also related to S storage (Lamark et al., 1991; Landfald and Strom, 1986; Osteras et al., 1998) and would explain the presence of oxidized S in the fungal biomass (Fig. 3) while XANES spectra from the biomass of bacteria used in this study showed only the presence of reduced S (data not shown).

The intracellular subsequent deployment of degraded S compounds differed to each fungus (Tab. 3). All analysed fungi increased the reduced S thiol in the biomass compared to wood, which suggests an incorporation of the wood S into fungal biomass. *G. trabeum* also increased the intermediate S sulfoxide and sulfonate, which can be explained through a storage capability of wood S within the fungus. However, this needs to be explored further. Our results from the fungal *in vitro* cultivation suggest that intermediate S sulfonates can be used as S source by wood-degrading fungi too. The growth of *G. trabeum* on agarose plates without added S source was much lower when compared to agarose plates with the addition of sulfonates. In contrast, XANES spectra taken from wood blocks degraded by fungi only showed no evidence of the use of S from sulfonate. In particular, the XANES spectra of pine wood degraded by *G. trabeum* highlighted the presence of intermediate S sulfonate in comparison to non-degraded pine wood. Interestingly, Chen (1992) found that a chemical reaction of wood with para-toluene sulfonyl chloride to wood bonded sulfonates resulted in decelerated wood degradation by *G. trabeum* (Chen, 1992). These findings suggest that not only the presence but also the way how the sulfonate groups are bonded to the wood material have an impact on its accessibility as S source. Nevertheless, the results from the XANES spectra also suggested a limited utilization of wood sulfonates in co-incubation with the bacterium *V. paradoxus*. This bacterium has been identified in the past to utilize sulfonates in rhizospheres of crops (Schmalenberger et al., 2008; Schmalenberger and Kertesz, 2007) and grassland (Schmalenberger et al., 2010), and could be responsible for utilizing some of the wood sulfonates.

Apart from *L. cyathiformis,* all tested wood-degrading fungi grew better *in vitro* with cysteine or sulfonate as S source than with sulfate, which suggest that their S assimilation pathways could be adapted to organo-S compounds. Indeed, eukaryotes have a protein recycling system which includes the recycling of bound S and is linked to the F-box domain. In *Saccharomyces cerevisiae* the Met30 and Met4 F-box proteins regulate the incorporation of degraded S compounds into biomass structures such as proteins, sulfolipids and FeS cores of enzymes (Jonkers and Rep, 2009). The uptake of proteins with reduced S (e.g. cysteine) could lead to the repression of sulfate uptake and the decrease of the reduction rate of oxidised S compounds. Moreover, incorporation of oxidised S sulfate into fungal biomass is disadvantageous as it needs to be reduced via the assimilatory pathway where two activation stages have to be carried out and eight electrons have to be supplied to reduce sulfate to sulfide (Leustek, 1996). Energetically, it might be much more efficient to transfer the reduced S group from organic S compounds into fungal biomass compounds such as thiol in wood.

In soils, sulfate-esters (oxidised S) and sulfonates (intermediate S) represent the dominant S oxidation status and soils are the habitat of most saprophytic fungi. Therefore, wood-degrading fungi may access, besides S fraction from wood, also organic S fractions from soil. Indeed, many saprophytic fungi are sulfatase active (Marzluf, 1997) and most likely contribute to the soil sulfatase activity that serves as a proxy for soil fertility and health (Tabatabai and Bremner, 1970).

The aim of this study was to identify trends in the utilization of S sources by wood-degrading fungi when growing on wood as sole carbon and nutrient source. The analysis of XANES spectra confirmed the widespread usage of sulfate-esters as S source and revealed as novel S source the fungal use of thiol and to a minor importance also the use of sulfoxide. However, the growth on sulfonates as S source was only found *in vitro* on agarose plates. The co-inoculation with selected desulfonating bacteria on wood showed only minor trends that suggest changes in the S functional groups. The results of this study allowed a first glimpse at the utilization of S sources among wood-degrading fungi, where S utilization is not uniform but highly complex.

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Figure legends:

Fig. 1. Normalised and stacked S K-Edge XANES spectra from reduced and oxidised sulfur (S) sources from standard chemical compounds (bottom up) thiol (cysteine), sulfoxide (dimethylsulfoxide), alkylsulfonate (pentanesulfonate) ,sulfate and sulfate-ester (sodiumdodecylsulfate, sulfate), arylsulfonate (toluenesulfonate) and a combined fit of the standards.

Fig. 2. Normalised and stacked S K-Edge XANES spectra from A) combined fit of the standards, beech wood blocks, beech wood blocks after incubation with *Trametes versicolor* and *T. versicolor* biomass after wood degradation (bottom up) B) combined fit of the standards, beech wood blocks, beech wood blocks after incubation with *Lenthius cyathiformis* and *L. cyathiformis* biomass after wood degradation (bottom up).

Fig. 3. Normalised and stacked S K-Edge XANES spectra from A) combined fit of the standards, pine wood blocks, pine wood blocks after incubation with *Oligoporus placenta* and *O. placenta* biomass after wood degradation (bottom up) B) combined fit of the standards, pine wood blocks, pine wood blocks after incubation with *Gloeophyllum trabeum* and *G. trabeum* biomass after wood degradation (bottom up).

Fig. 4. Normalised and stacked S K-Edge XANES spectra from A) combined fit of the standards, pine wood blocks, pine wood blocks after incubation with *Oligoporus placenta* and *Variovorax paradoxus*, and *O. placenta* and *V. paradoxus* biomass after wood degradation (bottom up) B) combined fit of the standards, pine wood blocks, pine wood blocks after incubation with *O. placenta* and *Rhodococcus* sp. P14D10 and *O. placenta* and *R.* sp. P14D10 biomass after wood degradation (bottom up).

414 **Table 1** Fuctional S groups, configuration, oxidation status and photon energy.
415

Functional S group	Configuration	Oxidation Status	Photon energy (eV)*
Sulfide		-2 (reduced)	2471.8
Disulfide	R-S-S-R	-1 / 0 (reduced)	2472.4
Thiol	R-S-H	-1 / +0.5 (reduced)	2472.4
Sulfoxide	R-(S=O)-R	+2 (intermediate)	2475.4
Sulfonates	R-C-SO_3	+5 (intermediate)	2480.2-2480.4
Sulfate-ester	R-O-SO_3	+6 (oxidised)	2481.6
Sulfate	SO_4	+6 (oxidised)	2481.6

416 * Photon energy were revealed by XANES measurements after addition of representative
417 functional S compounds in wood (see details in Material and Methods).
418
419 **Table 2** Fungal growth in MMN medium with various sulfur sources after 3 weeks of
420 incubation at 25°C. Ranking are means of three replicate measurements.
421

Fungal species	Sulfur free	Sodium sulfate	Cysteine	DMSO	SDS	Pentane sulfonate	Toluene sulfonate
Gloeophyllum trabeum	A	C-D	B-C	C	A	C	C
Oligoporus placenta	B	C	C-D	B-D	A-B	C-D	D
Trametes versicolor	A	C	D	B	-	B	C
Lentius cyathiformis	-	A	A-B	A-B	A-B	A-C	A-B

422

- = no growth
A = Initial growth beyond inoculum
B = Initial growth on medium
C = Substantial growth on medium
D = Medium completely overgrown

423
424
425 **Table 3** Sulfate and total sulfur (S) concentrations in incubated wood blocks. Means ±
426 standard deviation (SD) of two replicate measurements.
427

Analyzed wood blocks	Total S ($\mu g\ g^{-1}$)	± SD	Sulfate-S ($\mu g\ g^{-1}$)	± SD	Sulfate (%)
Pine	50.0	2.1	1.2	0.4	2.4
Beech	106.4	7.5	20.2	0.4	19.0
Beech with *L. cathyiformis*	117.5	33.1	32.9	1.4	28.0
Beech with *T. versicolor*	94.5	25.8	12.7	0.8	13.4
Pine with *O. placenta*	108.0	5.2	15.3	1.2	14.2
Pine with *G. trabeum*	42.6	0.1	2.0	0.1	4.7
Pine with *V. paradoxus*	56.5	7.9	11.4	2.9	20.2
Pine with *R. sp. P14D10*	65.4	3.3	5.6	0.8	8.6
Pine with *O. placenta* and *V. paradoxus*	57.0	3.8	11.6	0.1	20.4
Pine with *O. placenta* and R. sp. P14D10	77.5	17.4	9.5	0.2	12.2

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Table 4 Estimated shifts in XANES spectra (ATHENA linear combination fit). Ranking are means of three replicate measurements.

Analyzed wood blocks	thiol -1/0	sulfoxide +2	sulfonate +5	sulfate (ester) +6
Beech (portion in %)	15-20	10-20	15-20	45-55
Beech with *T. versicolor*	-A	N	+B	-B
T. versicolor (beech)	+C	N	N	-B
Beech with *L. cathyiformis*	+A	-A	N	N
L. cathyiformis (beech)	+C	N	-B	-C
Pine (portion in %)	30-35	10-15	15-20	35-40
Pine with *O. placenta*	-B	N	+A	N
O. placenta (pine)	+B	N	-A	-A
Pine with *G. trabeum*	N	+A	+B	-B
G. trabeum (pine)	+A	+A	+A	-B
Pine with *O. placenta* and *R.* P14D10	-B	N	+A	+A
O. placenta and *R.* P14D10 (pine)	+B	+A	-A	-B
R. P14D10 (pine)	+C	N	-A	-C
Pine with *O. placenta* and *V.paradoxus*	-A	+A	N	N
O. placenta and *V. paradoxus* (pine)	+C	N	-A	-C

N = no changes
+/-A = small changes of 5-10%
+/-B = substantial changes of 10-20%
+/-C = large changes >20%

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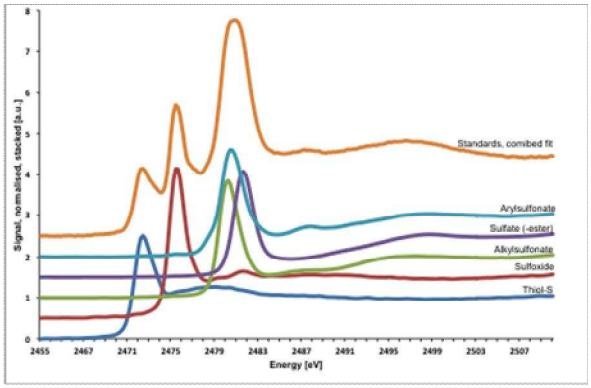
Fig. 1



580

Fig. 2

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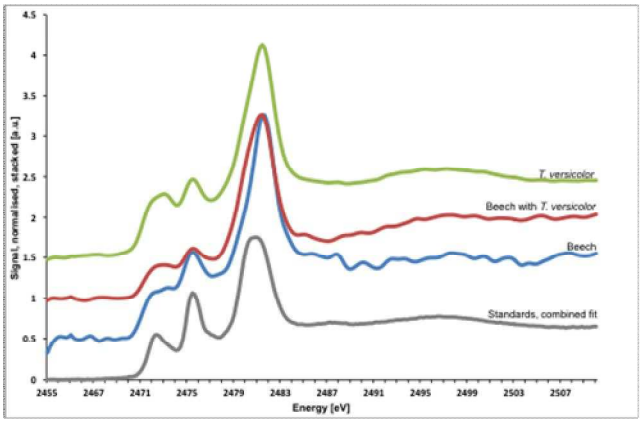

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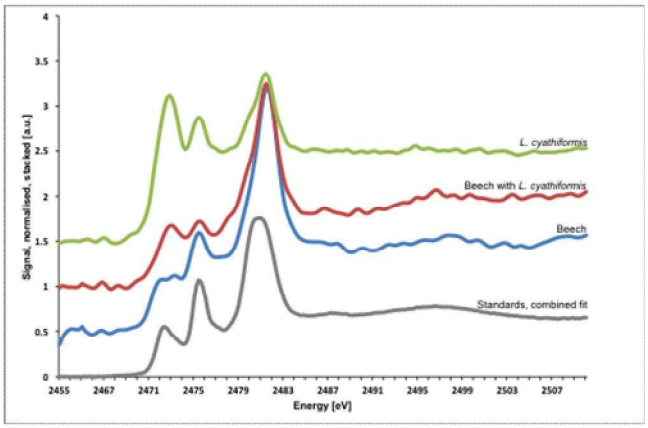

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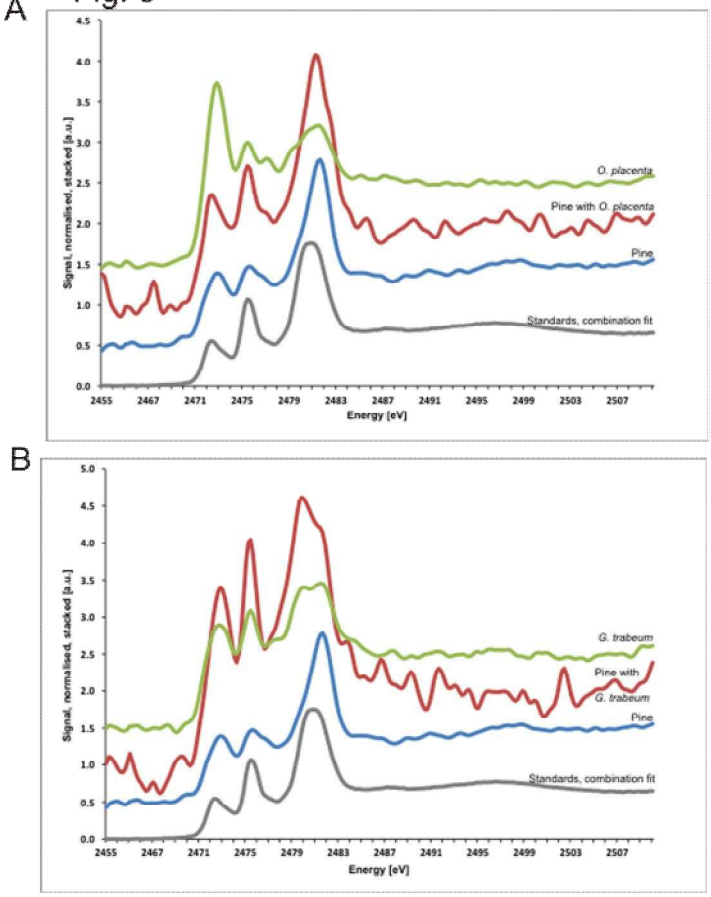
Fig. 3


A
Signal, normalised, stacked [a.u.]
Energy [eV]
O. placenta
Pine with O. placenta
Pine
Standards, combination fit
B
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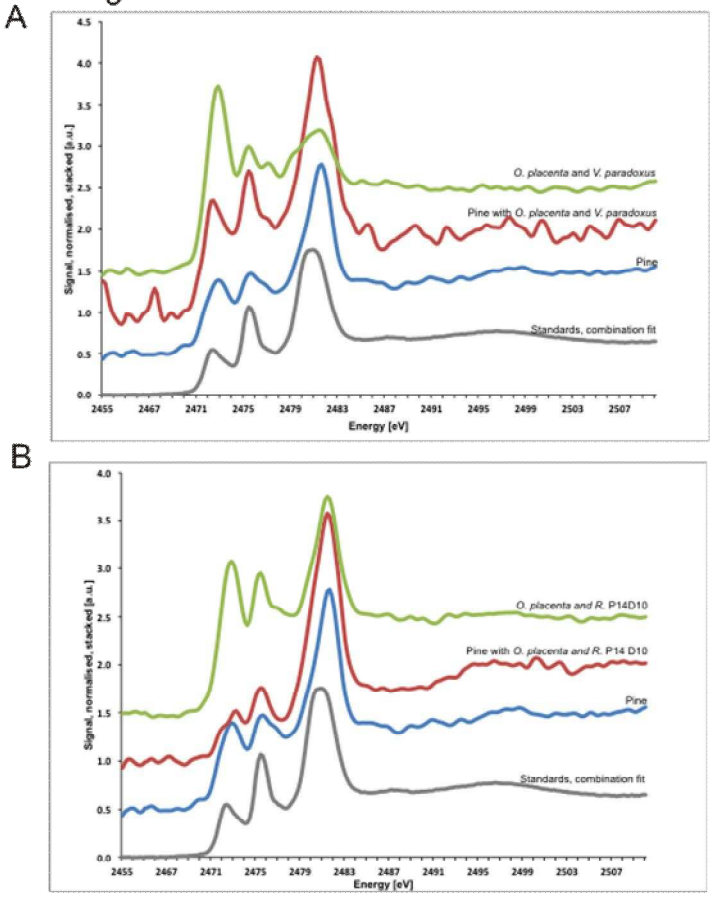
Energy [eV]
G. trabeum
Pine with G. trabeum
Pine
Standards, combination fit

582

Fig. 4


A
Signal, normalised, stacked [a.u.]
Energy [eV]
O. placenta and V. paradoxus
Pine with O. placenta and V. paradoxus
Pine
Standards, combination fit
B
Signal, normalised, stacked [a.u.]
Energy [eV]
O. placenta and R. P14D10
Pine with O. placenta and R. P14 D10
Pine
Standards, combination fit

583