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Characterization of main sulfur source of wood-degrading basidiomycetes by S K-edge X-ray absorption near edge spectroscopy (XANES)

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5 **Title:**

6 **Characterization of main sulfur source of wood-degrading basidiomycetes by S K-edge**
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8
9 **Running Title:**

10 **Wood-degrading basidiomycetes shift sulfur oxidation status in wood**

11
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32
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34 **Abstract**

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

54 **(257 words)**

55

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

58

59

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 alkylbenzenesulfonates 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.

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

121

122

123 2. Materials and Methods

124

125 2.1 Incubation of basidiomycetes

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

140 To minimize S storage of the fungi derived from malt extract agar, wood blocks
141 overgrown with mycelium were cut into quarters (15 mm x 15 mm) and were used to
142 inoculate sterile wood blocks by transferring a colonised quarter wood block on top of a
143 sterile wood block. Three of the four quarter wood blocks were used to inoculate new wood
144 blocks and therefore tripling the number of inoculations after each transfer. Transfers were
145 conducted three times after seven, five and seven weeks of incubation (to obtain 324 wood
146 blocks with fungal decay in total). In parallel, non-inoculated sterilized wood blocks were
147 transferred as negative controls. At the last transfer to three of the wood block incubations of
148 each fungus the bacterial desulfonating strains *Rhodococcus sp.* P14D10 (isolated from wheat
149 rhizosphere, (Schmalenberger et al., 2009)) and *Variovorax paradoxus*^T (DSM30034,
150 (Schmalenberger and Kertesz, 2007)), respectively, and a combination of *R. sp.* P14D10 and
151 *V. paradoxus* were added. Each bacterial strain was incubated for 7 days at 20 °C in liquid
152 minimal media (Beil et al., 1995) and approximately 3 x 10⁹ to 6 x 10⁹ cells were added per g
153 wood block as estimated with a Thoma counting chamber of the respective inoculum.

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

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

164

165 2.2 *Synchrotron studies*

166

167 S K-edge XANES analysis were employed at the XAS beamline (ANKA, Karlsruhe,
168 Germany; <http://ankaweb.fzk.de>). The X-ray source of the ANKA XAS beamline is a
169 1.5 Tesla bending magnet. The fixed exit monochromator of the XAS beamline was operated
170 in step by step mode using the Si111 crystal pair. In order to suppress the higher harmonics, a
171 detuning to 70 % of the maximum beam intensity was used. An ionisation chamber to
172 measure the primary flux and an energy dispersive detector (SDD - Silicon Drift Detector) to
173 measure the fluorescence signal were used.

174 To build a reference library of S compounds and their oxidation state on a wooden matrix for
175 a linear combination fitting of XANES data sets, sterilized pine wood blocks were
176 supplemented with S 1 mg g⁻¹ wood (dry weight) of *i.*) 1,4-dithioerythritol, *ii.*) L-cysteine, *iii.*)
177 sodium dodecyl sulfate (SDS), *iv.*) sodium sulfide, *v.*) dimethyl sulfoxide (DMSO), *vi.*)
178 lignosulfonate, *vii.*) toluenesulfonate, *viii.*) pentanesulfonate, *ix.*) sodium sulfate and measured
179 with XANES. 1 mg S g⁻¹ wood was chosen to retrieve a clear XANES spectrum for each S
180 source independently from the natural S sources of wood that was not detectable in
181 comparison to the applied standards. Wood matrix was used for standards in order to exclude
182 any potential effects of the wood matrix onto the spectra. However, spectra obtained from this
183 study were in accord with previous studies of pure compounds (Morra et al., 1997; Pickering
184 et al., 2001).

185 Wood blocks were split in halves (to fit in the sample holder) and measured directly (1.5
186 x 3 x 0.4 cm). Initial trials revealed that the powdered wood samples on Kapton tape (DuPont,
187 Wilmington, DE) produced lower quality readings in transmission and reflection mode,
188 compared to entire wood block analysis in reflection mode. The fungal and fungal-bacterial
189 surface biomass, respectively, were scratched from the wood blocks. Separated surface
190 biomass and the corresponding wood blocks were measured in reflection mode. Pine and
191 beech wood blocks from the control experiments without microbes were measured as
192 controls. The photon energy of the primary beam was varied around the S absorption edge at
193 2472 eV and scans were carried out at 2464 eV to 2510 eV in steps of 1 eV (2464-2468) and
194 0.2 eV (2468-2510) respectively in order to identify the different oxidation states of S (Table
195 1). Exposure of the sample to the monochromatic beam was 10 s per step and three replicate
196 measurements were collected for each sample (140 min / sample).

197

198 2.3 *Quantification of total S content and sulfate*

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

208

209 2.4 *Data analysis*

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

214 subtraction a linear combination fit with standards from this study was carried out to identify
215 major shifts in the XANES spectra by reporting only changes in three basic categories of 5-
216 10, 10-20 and over 20 %.

217

218

219 **3. Results**

220

221 3.1 *Fungal growth on minimal media with defined S sources*

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

229

230 3.2 *Sulfate and total S concentrations in incubated wood blocks*

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

243

244 3.3 *Identification of different S oxidation states employing XANES*

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

251

252 3.4 *Changes in the S oxidation status in wood after fungal colonization and degradation*

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

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

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

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

276 3.5 Changes in the S oxidation status in wood after fungal and bacterial colonization and 277 degradation

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

286
287

288 4. Discussion

289

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

296 Early reports have already shown that wood-degrading fungi prefer defined wood
297 species as substrate (Fengel and Wegener, 1984) and the tested wood-degrading fungi
298 differed in their carbon traits significantly to each other (Hibbett and Donoghue, 2001). The
299 tested wood-degrading fungi were described as the main rotters of the respective wood
300 species (Fengel and Wegener, 1984) and were therefore introduced here under the same
301 incubation conditions and wood species as described earlier (EN113, 1996). The mass loss of
302 88 incubations of *O. placenta*, *T. versicolor*, *G. trabeum*, and *L. cyathiformis* was in
303 arithmetic average 46%, 39%, 35% and 25%, respectively, after 10 weeks of incubation
304 (personal communication, Dr. Ina Stephan, BAM), indicating that each fungus has its
305 respective efficiency in wood decay. Indeed, comparison of genome, transcriptome and
306 secretome analysis of the brown-rot fungus *O. placenta* and *Phanerochaete chrysosporium*
307 supported that an evolutionary shift took place from white-rot to brown-rot during which the
308 capacity for efficient depolymerisation of lignin was lost (Martinez et al., 2009). In
309 conclusion, S related degradation pathways could also differ between white and brown rotters.
310 Indeed, this study found substantial differences in the way and amount of functional S
311 degradation of wood-degrading fungi at their preferred wood substrate. Changes in the S
312 oxidation states during wood degradation *in situ* indicated that oxidised S sulfate-esters are
313 the preferred S source of the wood-degrading fungi *T. versicolor* and *G. trabeum* (Figs. 2 and
314 3, Tab. 3). However, *T. versicolor* and in particular *O. placenta* also utilized reduced S thiol
315 e.g. cysteine. Only *L. cyathiformis* preferentially utilized intermediate S sulfoxides (Fig. 2;

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

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

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

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

367

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

377

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385

386 **Figure legends:**

387

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

393

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

399

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

405

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

412

413

414 **Table 1** Functional 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 |
|-----------------------------|-------------|----------------|----------|------|-----|-------------------|-------------------|
| <i>Gloeophyllum trabeum</i> | A | C-D | B-C | C | A | C | C |
| <i>Oligoporus placenta</i> | B | C | C-D | B-D | A-B | C-D | D |
| <i>Trametes versicolor</i> | A | C | D | B | - | B | C |
| <i>Lentius cyathiformis</i> | - | 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 (µg g ⁻¹) | ± SD | Sulfate-S (µg 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 <i>L. cathyiformis</i> | 117.5 | 33.1 | 32.9 | 1.4 | 28.0 |
| Beech with <i>T. versicolor</i> | 94.5 | 25.8 | 12.7 | 0.8 | 13.4 |
| Pine with <i>O. placenta</i> | 108.0 | 5.2 | 15.3 | 1.2 | 14.2 |
| Pine with <i>G. trabeum</i> | 42.6 | 0.1 | 2.0 | 0.1 | 4.7 |
| Pine with <i>V. paradoxus</i> | 56.5 | 7.9 | 11.4 | 2.9 | 20.2 |
| Pine with <i>R. sp. P14D10</i> | 65.4 | 3.3 | 5.6 | 0.8 | 8.6 |
| Pine with <i>O. placenta</i> and <i>V. paradoxus</i> | 57.0 | 3.8 | 11.6 | 0.1 | 20.4 |
| Pine with <i>O. placenta</i> and <i>R. sp. P14D10</i> | 77.5 | 17.4 | 9.5 | 0.2 | 12.2 |

428

429

430 **Table 4** Estimated shifts in XANES spectra (ATHENA linear combination fit). Ranking are
 431 means of three replicate measurements.
 432

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

433
 434 N = no changes
 435 +/-A = small changes of 5-10%
 436 +/-B = substantial changes of 10-20%
 437 +/-C = large changes >20%

438
 439
 440

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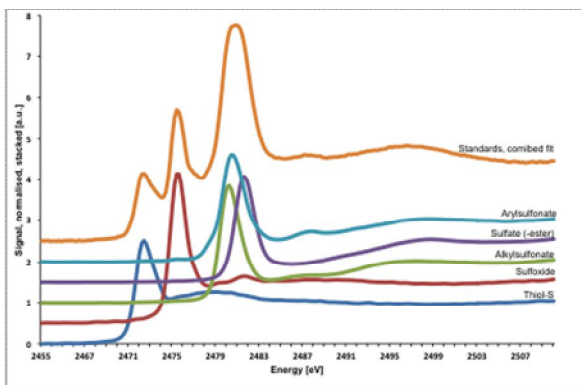
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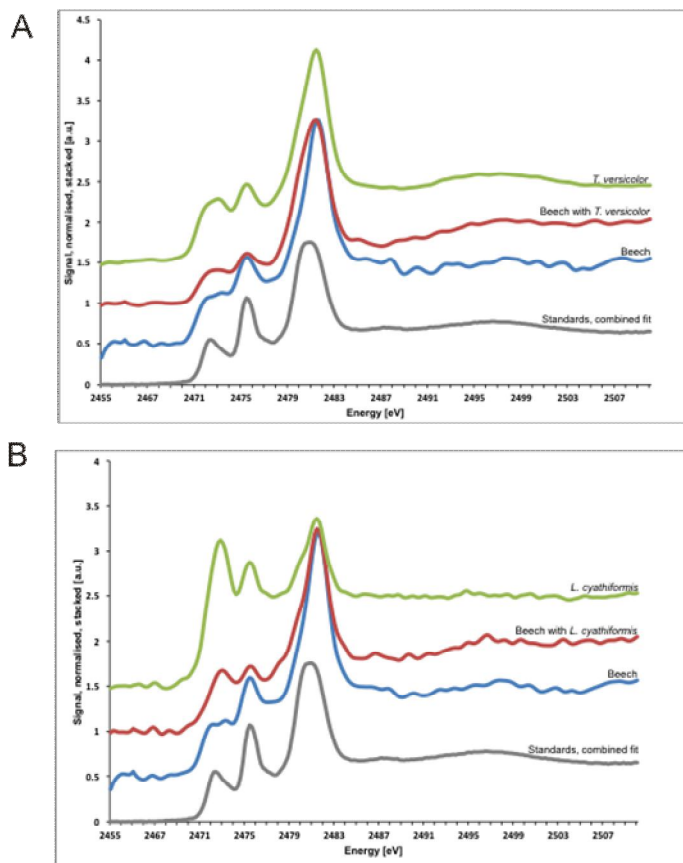
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Fig. 1



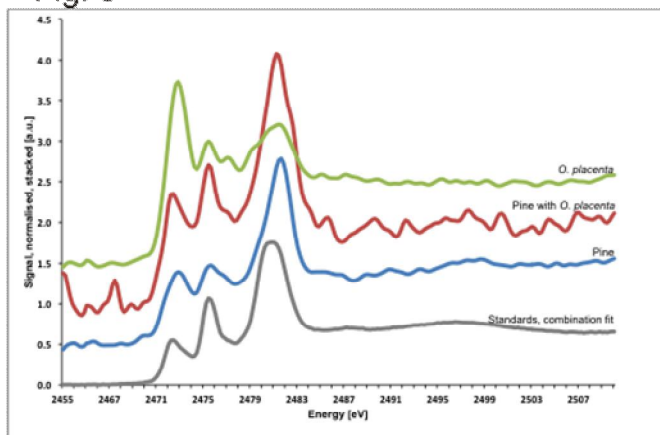
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Fig. 2



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A Fig. 3



B

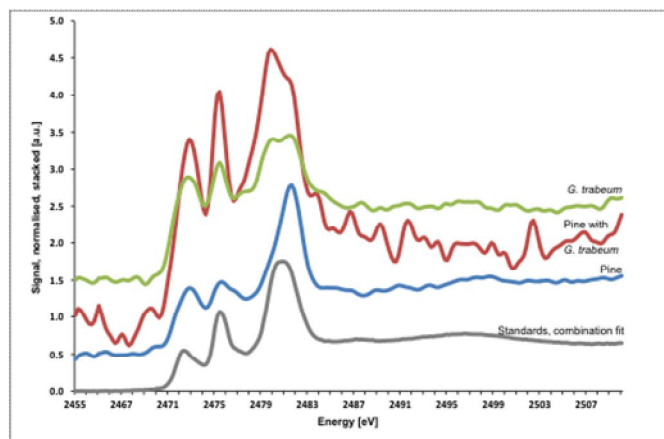


Fig. 4

