

1 ***Trametes versicolor*: potential for atrazine bioremediation in calcareous clay soil,**  
2 **under low water availability conditions**

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15  
16 This manuscript includes 2 figures and 3 tables.

17  
18 **Scientific relevance:** We investigated the feasibility of *T. versicolor* for actively  
19 degrading atrazine (at usual field application rates) in non-sterile calcareous clay soil of  
20 South Portugal, under low water availability (-0.7 and 2.8 MPa) and with scarce organic  
21 matter content. Results strongly suggested that this species could potentially be used for  
22 bioremediation of soil treated with triazine herbicides in semi-arid and Mediterranean-  
23 like ecosystems. As far as we are aware, very little work has looked at the influence of  
24 soil water potential on triazine biodegradation rates by white rot fungi in non-sterile  
25 soil, *T. versicolor* in particular. We therefore consider our work to be an important  
26 contribution in the field of applied environmental microbiology.

27 **Abstract**

28

29 This study has examined the feasibility of *Trametes versicolor* for actively degrading  
30 atrazine ( $0.5 \mu\text{g g}^{-1}$ ) in non-sterile calcareous clay soil (Algarve, Portugal) microcosms  
31 for up to 24 weeks ( $20^{\circ}\text{C}$ ), under low water availability (soil water potentials of  $-0.7$  and  
32  $-2.8$  MPa). Soil respiration, enzymatic (dehydrogenase and laccase) activities and  
33 atrazine quantification by high-performance-liquid-chromatography (HPLC) were  
34 assessed.

35

36 Respiration and dehydrogenase activity (DHA) were significantly ( $p < 0.05$ ) enhanced in  
37 soil containing the inoculant, particularly in the presence of atrazine, indicating that it  
38 remained metabolically active throughout the study. Furthermore, up to 98 and 85% (at  
39  $-0.7$  and  $-2.8$  MPa respectively) of atrazine was degraded in soil containing both  
40 atrazine and the inoculant, compared to 96 and 50% in soil containing atrazine only.  
41 The contribution of *T. versicolor* to atrazine degradation was only significant ( $p < 0.005$ )  
42 under the driest soil treatment conditions. The strategies used for enhancing  
43 colonisation and biodegradation capabilities of the inoculant, as well as the selection of  
44 sawdust as carrier were thus effective. However, there were no differences ( $p > 0.05$ ) in  
45 quantified laccase activity in soil containing the inoculant and the control. Overall, this  
46 study demonstrates that *T. versicolor* is a strong candidate for atrazine bioremediation in  
47 soil with low moisture and organic matter contents, such as that found in semi-arid and  
48 Mediterranean-like ecosystems.

49

50

51 **Keywords:** *Trametes versicolor*; Biodegradation; Atrazine; Soil microcosms; Water  
52 potential; Soil respiration; Enzymatic activity.

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## 60 1. Introduction

61

62 The widespread incorporation of herbicides into soil every year is of major concern,  
63 since they potentially can pose a threat to our health as well as to the quality of soil,  
64 surface water and groundwater resources (Hägglom, 1992; Kearney and Roberts, 1998;  
65 Kuo and Regan, 1999; Ashman and Puri, 2002). Atrazine is a chlorinated aromatic  
66 herbicide heavily used worldwide for control of broad-leaved weeds in agricultural  
67 produce (Ghani *et al.*, 1996; Houot *et al.*, 1998; Ralebitso *et al.*, 2002), as well as in  
68 urban and recreational areas (Gadd, 2001).

69

70 Atrazine and related triazines are moderately persistent in soil (Pointing, 2001) with  
71 reported half-life values ranging from 35 to 50 days, depending largely on soil  
72 environmental conditions (Topp, 2001; Rhine *et al.*, 2003). Microbial metabolism has  
73 long been regarded as the most important mechanism of atrazine degradation in soil  
74 (Armstrong *et al.*, 1967; Gravilescu, 2005). Nevertheless, in conditions of low moisture  
75 and nutrient contents, microbial metabolism is compromised and atrazine persistence  
76 may increase (Weber *et al.*, 1993). Soil water potential has been widely recognised as a  
77 determinant factor controlling soil microbial growth and activity rates. Yet, very little  
78 research has looked at atrazine biodegradation in soil under low moisture regimes  
79 (Moreno *et al.*, 2007).

80

81 The application of white-rot fungi for bioremediation of common environmental  
82 contaminants looks promising. Similar to other white-rot species, *T. versicolor* has  
83 shown to be able to metabolise a wide range of organic compounds (Bumpus *et al.*,  
84 1985; Gadd, 2001). This ability is generally attributed to the production of extracellular  
85 ligninolytic enzymes such as laccase, which is non-specific in regard to its substrate  
86 (Thurston, 1994; Youn *et al.*, 1995; Pointing, 2001; Šašek *et al.*, 2003; Baldrian, 2004).  
87 White rot species can also tolerate a broad range of environmental conditions, including  
88 temperature, nutrient and moisture contents (Maloney, 2001; Magan, 2007). In previous  
89 studies, *T. versicolor* was shown to exhibit good tolerance to water stress conditions  
90 (Mswaka and Magan, 1999; Fragoeiro and Magan, 2005) as well as to triazine  
91 pesticides (Gadd, 2001; Šašek *et al.*, 2003; Fragoeiro and Magan, 2005). Further, the  
92 mycelial growth habit and hyphal extension allow rapid substrate colonisation and

93 utilization of compounds that are not otherwise readily available to the wider microbial  
94 community (Reddy and Mathew, 2001; Magan, 2007).

95

96 So far, most studies involving the use of ligninolytic fungi for bioremediation purposes  
97 have been carried out in liquid media (e.g. Ryan and Bumpus, 1989), often in  
98 bioreactors (Novotný, 2004). *T. versicolor* has been seldom studied in the soil  
99 environment although there are reports of its successful application in sterile soil  
100 (Lamar, 1993) and soil extract broth (Fragoeiro and Magan, 2005). In non-sterile soil,  
101 knowledge is limited on other factors which can influence pesticide degradation, such as  
102 competitive interactions between the introduced fungi and native microbial populations  
103 (Šašek *et al.*, 2003).

104

105 This study aimed to (1) assess the potential of *T. versicolor* for actively degrading  
106 atrazine at 0.5  $\mu\text{g g}^{-1}$  (usual field application rates) in non-sterile calcareous clay soil,  
107 under low water availability conditions (-0.7 and -2.8 MPa). Soil respiration,  
108 dehydrogenase and laccase activities were determined in combination with atrazine  
109 quantification by HPLC, under the study conditions. The selection of soil water  
110 potentials had the water availability range for microorganisms and plants (i.e. -0.03  
111 MPa, field capacity, to -1.5 MPa, wilting point) as reference.

112

113

## 114 **2. Materials and methods**

115

### 116 **2.1. Pre-incubation of *T. versicolor* (R26)**

117 Pre-incubation of the fungal inoculum involved growing the isolate in sterile jars on wet  
118 sterile sawdust (50% water content, used as carrier) at 25°C for up to 3 weeks, until the  
119 substrate was colonised by mycelium. The jar had a vented cap (polypropylene  
120 membrane 0.22  $\mu\text{m}$  pore size) allowing adequate aeration. In order to avoid desiccation,  
121 the jars were placed inside a polyethylene box, where the equilibrium relative humidity  
122 was maintained by a glycerol/water solution (400 ml).

123

### 124 **2.2 Soil preparation, conditions and treatments**

125 The soil used was a calcareous clay soil (top-soil, 0-20 cm) and was collected from an  
126 arable field plot in Lagoa, Algarve, Portugal. The soil had the following main  
127 characteristics, analysed by the National Soil Resources Institute (NSRI, Cranfield  
128 University) and given as mg g<sup>-1</sup> oven dried soil: soil organic carbon (SOC), 12.1; water,  
129 353; sand, 320; clay, 470; silt, 210; pH 6.8; annual average values of precipitation (mm)  
130 and temperature (°C) on site were 400 and 17 respectively; there is no history of  
131 pesticide inputs in the last 4 years. Plant residues and stones were removed manually at  
132 the time of collection and soil was sieved (2 mm) and air-dried at 20°C for 7 days prior  
133 to use. Air-dried soil samples (10 g) were weighed into Universal (25 ml) bottles and  
134 target soil water potentials of -0.7 and -2.8 MPa were set by reference to a soil  
135 adsorption curve and the addition of sterile reverse osmosis (RO) water (Bastos, 2008).

136

137 *i) Atrazine addition to soil*

138 Atrazine was dissolved in RO water and the solution was sonicated for 1 min until  
139 complete dissolution of the herbicide. The amount of water used for dissolution was the  
140 same as that required for setting the target soil water potential treatment, calculated by  
141 reference to the soil water adsorption curve (Bastos, 2008). The solution was then added  
142 to soil (5 g), in order to obtain a final concentration of atrazine of 0.5 µg g<sup>-1</sup>. This  
143 concentration corresponds to usual field application rates of the herbicide (Ghani *et al.*,  
144 1996; Abdelhafid, 2000). The fortified soils were thoroughly homogenised and kept for  
145 1 day at 4°C allowing microbial activity to stabilise at the required water potential  
146 levels, before incubation and analysis.

147

148 *ii) Soil supplemented with sterile sawdust*

149 Wet (50% w w<sup>-1</sup>) finely chopped sterile sawdust was kept overnight at 4°C. It was then  
150 added to air-dried soil (5 g) in order to obtain a concentration of 5% (w w<sup>-1</sup>) and  
151 samples were left equilibrating overnight at 4°C. Conditioning of the treated soil to the  
152 required water potentials was then done by reference to a soil-sawdust calibration curve  
153 (Bastos, 2008). The procedure followed that described in *i*).

154

155 *iii) Soil supplemented with sterile sawdust + atrazine*

156 Soil was amended with sterile sawdust as described in *ii*). Conditioning of the soil to the  
157 treatment water potentials was done by reference to a soil-sawdust adsorption curve and  
158 the addition of sterile RO water supplemented with atrazine, in order to obtain a final

159 concentration of atrazine in soil of  $0.5 \mu\text{g g}^{-1}$ . The procedure followed that described in  
160 *i*).

161

162 *iv) Inoculation of T. versicolor into soil*

163 Sawdust colonised by the test isolate (0.5 g) was added to air-dried soil (5 g) in order to  
164 obtain a concentration of 5% (w w<sup>-1</sup>) and mixed until a homogeneous mixture was  
165 obtained. The procedure followed that described in *ii*).

166

167 *v) Incorporation of T. versicolor + atrazine in soil*

168 The preparation of the homogeneous mixture of *T. versicolor* with sawdust (5% w w<sup>-1</sup>)  
169 and its incorporation into air-dried soil (5 g) was described previously. The procedure  
170 followed that described in *iii*).

171

### 172 **2.3 Incubation of soil microcosms**

173 Treated soil samples and non-treated controls were incubated at 20°C for up to 24  
174 weeks within polyethylene boxes previously thoroughly cleaned. Each box also  
175 contained a glycerol/water solution (400 ml), in order to maintain the equilibrium  
176 relative humidity within each microcosms the same as that of the soil treatments. All  
177 treatments involved in this work are summarised in Table 1.

178

### 179 **2.4 Temporal evaluation of soil respiration**

180 CO<sub>2</sub> evolved from total soil microbial respiration was determined by gas-  
181 chromatography (GC) through static sampling. Following incubation, Universal bottles  
182 containing soil samples were sealed and left for 3 h at 20°C prior to analysis, thus  
183 ensuring detectable volumes of CO<sub>2</sub> in the soil headspace. Headspace (5 ml) was then  
184 injected into a gas chromatographer equipped with a packed column (Porapak Q packed  
185 glass column) and a thermal conductivity detector (Carlo Erba Instruments, GC 8000  
186 Series MFC 800). Five replicates of each treatment were sampled. The GC settings were  
187 the following: column and injector temperatures, 100°C; detector temperature, 180°C;  
188 carrier gas (Helium) at a flow rate of 36 ml min<sup>-1</sup>; the calibration gas consisting of a  
189 standard mixture (10.01% v v<sup>-1</sup> CO<sub>2</sub> in N<sub>2</sub>) was injected three times at the beginning and  
190 after each set of 15 samples. Soil respiration rate was expressed as  $\mu\text{g CO}_2 \text{g}^{-1} \text{soil h}^{-1}$ .

191

### 192 **2.5 Temporal evaluation of dehydrogenase activity**

193 The method for DHA quantification was adapted from von Mersi and Schinner (1991).  
194 It is based on the incubation of soil with the substrate INT (2 (p-iodophenyl)-3-(p-  
195 nitrophenyl)-5-phenyl tetrazolium chloride), followed by the extraction and colorimetric  
196 estimation of the reduction product INF (iodonitrotetrazolium formazan). The  
197 calibration curve ( $Y = 1.0657x + 0.0061$ ) which was firstly produced using a standard  
198 INF solution ( $100 \mu\text{g INF ml}^{-1}$ ), showed a good correlation ( $r^2 = 0.998$ ) between the  
199 concentration of INF and the optical density of the INF solution.

200

201 The INT solution was prepared by dissolving 500 mg of INT into 2 ml of N,N-  
202 dimethylformamide, followed by the addition of 50 ml of RO water. The solution was  
203 sonicated (2 min) and the volume was brought up to 100 ml using RO water. The final  
204 concentration of the substrate solution was 0.5% ( $\text{w v}^{-1}$ ). For every analysis, fresh INT  
205 solution was prepared and stored in the dark until use.

206

207 Soil (0.5 g) at the treatment water potentials was weighed into sterile test tubes and  
208 mixed with 740  $\mu\text{l}$  of Tris-HCl buffer (1 M, pH 7.0) and 1 ml of the substrate solution.  
209 Test tubes were sealed with sterile sponge stoppers and incubated in the dark at 40°C for  
210 2 h. Following incubation, 5 ml of extraction solution (N,N-dimethylformamide:  
211 ethanol in a 1:1 ratio) were added to the mixture and samples were kept in the dark for 1  
212 h. During this time, every sample was vigorously shaken (using the vortex) at 20 min  
213 intervals, ensuring an efficient extraction of the product INF. Aliquots of 2 ml were then  
214 transferred to Eppendorf tubes and centrifuged for 2 min. The supernatant (200  $\mu\text{l}$ ) was  
215 introduced into microplate wells and the INF was determined spectrophotometrically at  
216 450 nm using a Microplate reader (Dynex Technologies Ltd., UK).

217

218 Controls were also prepared for estimating the chemical reduction of INT under the  
219 same conditions. For each treatment, controls were prepared using autoclaved soil  
220 ( $121^\circ\text{C}$ , 20 minutes) and were treated like samples. Five replicates of each treatment  
221 (including respective controls) were sampled. The INT reduction of the control was then  
222 subtracted to that of the samples and DHA was expressed as  $\text{ng INF g}^{-1} \text{ soil } 2 \text{ h}^{-1}$ .

223

## 224 **2.6 Temporal evaluation of laccase activity**

225 Estimating soil laccase activity involved 2 steps: i) extraction of laccase from soil; ii)  
226 quantification of enzymatic activity based on the oxidation of the redox substrate ABTS

227 (2,2-azino-bis-ethylbenthiiazoline-6-sulphonic acid). The procedure described below  
228 was firstly calibrated and optimised using purified commercial laccase from *Rhus*  
229 *vernificera* in crude acetone powder (50 Units mg<sup>-1</sup> solid, minimum) as standard. The  
230 calibration curve obtained ( $Y = 26.33x + 1.643$ ) showed a good correlation ( $r^2 = 0.971$ )  
231 between the concentration of commercial purified laccase (mg ml<sup>-1</sup>) and laccase activity  
232 (U).

233

#### 234 *i) Laccase extraction from soil*

235 The extraction method employed was based on the protocol described by Criquet *et al.*  
236 (1999) with adaptations by Fragoeiro and Magan (2005). Sub-samples (2 g) of treated  
237 soil and non-treated controls were weighed into sterile test tubes and 8 ml of phosphate  
238 buffer in water (10 mM, pH 6.0) were added. The suspension was kept under agitation  
239 (incubator shaker, 250 rpm) at 4°C for approximately 1 h. Aliquots of 1 ml were then  
240 placed into 1.5 ml Eppendorfs and centrifuged (3800 rpm) for 6 min at room  
241 temperature. The supernatant containing the enzyme was stored at -18°C until analyses.

242

#### 243 *ii) Quantification of laccase activity*

244 The method for determining laccase activity using an enzyme extract was based on the  
245 protocol described by Buswell *et al.* (1995) with adaptations by Fragoeiro and Magan  
246 (2005). The reaction mixture performing a total of 300 µl was contained into a 96 well  
247 microtitre plate. It was prepared with 150 µl sodium acetate buffer (0.1 M, pH 5), 50 µl  
248 ABTS (0.55 mM) and 100 µl enzyme extract. The procedure was carried out at ambient  
249 temperature, although the substrate ABTS and the buffer were at 40°C when added to  
250 the reaction mixture. The incubation was performed at 40°C for 1 h. Positive laccase  
251 activity was indicated by a green colourisation of the reaction mixture, characteristic of  
252 the ABTS oxidised form. The product was determined spectrophotometrically at 405  
253 nm using a Microplate reader set in the Endpoint reading mode, with 5 seconds of  
254 agitation at the beginning. Control samples were prepared using boiled enzyme (15  
255 min). Five replicates of each treatment including the respective controls were used. One  
256 enzyme activity unit (U) was defined as the amount of enzyme required for producing a  
257 0.001 increase in the optical density of the reaction mixture per minute, under the  
258 conditions of the assay. Results were expressed as U g<sup>-1</sup> soil.

259

## 260 **2.7 Monitoring atrazine concentration in soil**



261 A two-step procedure was involved in assessing the rate of atrazine degradation in soil  
262 microcosms: i) pesticide extraction from soil and ii) HPLC analysis of soil extracts. The  
263 method employed for atrazine extraction and quantification was adapted from that of  
264 Elyassi (1997) and Fragoeiro and Magan (2005).

265

#### 266 *i) Atrazine extraction from soil*

267 Soil samples corresponding to the SA and SAT treatments (at -0.7 and -2.8 MPa) were  
268 weighed (2 g) into test tubes. Aliquots (3 ml) of methanol (100%) were added to soil,  
269 the tubes were sealed and shaken at 300 rpm in a circular motion shaker for 24 hours in  
270 the dark at room temperature. Following agitation soil was allowed to settle until a clear  
271 supernatant was obtained (30 min aprox.). Aliquots of supernatant (extract) was then  
272 withdrawn with a syringe and filtered using a nylon 0.22  $\mu\text{m}$  syringe filter.

273

#### 274 *ii) HPLC analysis of soil extracts*

275 Extracts were diluted with acetonitrile (75% sample: 25% acetonitrile). A volume of 50  
276  $\mu\text{l}$  was injected into a Gilson HPLC system equipped with a Gilson 117 UV detector  
277 operating at 215 nm, a Gilson 231XL sampling injector, Gilson 306 pump, Gilson 811  
278 C dynamic mixer and an Altima C18 5 mm column (4 mm x 250 mm x 4.6 mm). The  
279 column operated at ambient temperature with a flow rate of 1.5 ml  $\text{min}^{-1}$ . An isocratic  
280 mobile phase system was established using acetonitrile:water at a ratio of 70:30.  
281 Atrazine eluted at approximately 9.8 min. The limit of detection

282

### 283 **2.8 Data handling and statistical treatment**

284 For comparison between means of treatments in respect to respiration, enzymatic  
285 activities and atrazine quantification, analysis of variance (ANOVA) was performed  
286 using STATISTICA (Version 7) at a significance level  $p = 0.05$ . Standard error of  
287 means are shown as vertical bars in figures and indicated in Tables as  $\pm$  SE.

288

## 289 **3. Results**

290

### 291 **3.1. Temporal evaluation of microbial respiration**

292 Figure 1 shows the respiration rate for the clay soil under different soil treatments  
293 incubated at (A) -0.7 MPa and (B) -2.8 MPa (20°C) for up to 24 weeks. Generally, soil

294 treated with atrazine was shown to produce over 40% more CO<sub>2</sub> than non-treated soil  
295 (SS). Throughout the study, soil containing the inoculum alone (ST) showed a  
296 significant ( $p < 0.01$ ) increase in respiratory rates compared to un-inoculated soil (S).  
297 Surprisingly, differences between respiration rates of soil treated with atrazine (SA),  
298 sawdust (SS) and *T. versicolor* (ST) individually, were often not significant  
299 ( $0.05 < p < 0.16$ ) under the treatment soil conditions. Overall, the highest CO<sub>2</sub> evolution  
300 rates ( $0.01 < p < 0.04$ ) were achieved by soil containing both atrazine and the inoculum  
301 (SAT). These were at least 20% higher than in the absence of the inoculant. Maximal  
302 respiration rates occurred generally after week 6, and were followed by a slow but  
303 consistent decrease. Under drier conditions and throughout the study, respiration rates  
304 were generally not statistically different ( $0.05 < p < 0.27$ ) between treatments.

305

### 306 **3.2. Temporal evaluation of dehydrogenase activity**

307 Figure 2 shows the DHA for the clay soil under different soil treatments incubated at  
308 (A) -0.7 MPa and (B) -2.8 MPa (20°C) for up to 24 weeks. Regardless of soil treatment,  
309 the highest DHA levels were achieved under the wettest conditions ( $p < 0.001$ ). Non-  
310 treated soil had the lowest DHA but sawdust and atrazine supplements (individually or  
311 combined) enhanced this enzymatic activity by over 40% at both water potentials  
312 ( $p < 0.001$ ) over the first 6 weeks. However, after 12 weeks and from then onwards, soil  
313 carrying the inoculum alone (ST) showed over 20% higher DHA compared to sawdust-  
314 treated soil whether atrazine was present or not. Activity rates peaked after 6 weeks but  
315 overall, they remained high throughout the study, even under the driest soil conditions.  
316 Nevertheless, irrespective of water potential, there was no significant ( $p > 0.09$ )  
317 difference between soil inoculated with *T. versicolor* in the presence (SAT) and absence  
318 (ST) of atrazine from week 6 onwards.

319

### 320 **3.3. Temporal evaluation of fungal laccase activity**

321 Table 2 shows ABTS oxidation levels in the clay soil under different soil treatments  
322 incubated at (A) -0.7 MPa and (B) -2.8 MPa (20°C) for up to 24 weeks. Interestingly,  
323 substrate oxidation was found to occur in non-treated clay soil in the absence of the  
324 fungus at -0.7 MPa. Further, there was enhanced substrate oxidation ( $p < 0.001$ ) as a  
325 response to atrazine (SA) and sawdust (SS) alone under both water regimes. Differences  
326 between laccase activity in soil containing sawdust only and that carrying the inoculant  
327 were generally only significant ( $p < 0.03$ ) at -2.8 MPa, with the second treatment having

328 over 96% higher laccase production than the first. Under the wettest conditions, the  
329 SAT treatment had only minimal levels of this enzyme, when comparing to the  
330 remaining treatments, including that of soil carrying the inoculum alone (ST). This was  
331 most evident after 6 weeks. Surprisingly, the opposite was observed under drier soil  
332 conditions, with the SAT treatment having nearly 40% higher ( $p < 0.0004$ ) laccase  
333 activity than that of ST, although differences between SAT and SA were not statistically  
334 significant ( $p > 0.06$ ). Very little activity was found after 24 weeks, independent of the  
335 treatment conditions.

336

### 337 **3.4. Monitoring atrazine concentration in soil microcosms**

338 Table 3 shows the remaining atrazine ( $\mu\text{g g}^{-1}$ ) in clay soil incubated for up to 24 weeks  
339 at (A) -0.7 and (B) -2.8 MPa (20°C) in the absence (SA, SSA) and in the presence  
340 (SAT) of *T. versicolor*. Two controls were used in order to reduce bias in respect to the  
341 contribution of the sawdust supplement for atrazine degradation under the study  
342 conditions. The amount of atrazine present in the soil decreased with the incubation  
343 period in all treatments, and this was more rapid during the first 6 weeks.

344

345 In the absence of the fungus,  $0.071 \mu\text{g g}^{-1}$  of atrazine was recovered from sawdust  
346 supplemented soil after 6 weeks, corresponding to around 14% of its initial  
347 concentration. For the same time period, only  $0.023 \mu\text{g g}^{-1}$  of atrazine (i.e. 4% of the  
348 initial concentration) was extracted from soil containing the inoculum. By the end of the  
349 study, residues of the herbicide in soil were decreased to 0.019 and 0.011 in the absence  
350 (SSA) and presence (SAT) of the inoculum, corresponding to 96 and 98% degradation  
351 respectively. At -0.7 MPa, the impact of *T. versicolor* on atrazine breakdown in soil was  
352 only significant ( $p < 0.003$ ) within the first 12 weeks. In contrast, at -2.8 MPa, there was  
353 still a significant ( $p < 0.01$ ) difference between both treatments at the end of the study.

354

## 355 **4. Discussion**

356

357 In this study, *T. versicolor* was inoculated into non-sterile soil containing atrazine at  
358 usual field application rates for up to 24 weeks under low water regimes. Atrazine  
359 quantification by HPLC, combined with the assessment of soil microbial respiration and  
360 dehydrogenase activity allowed estimating the feasibility of this white-rot species to

361 remain metabolically active and degrade atrazine under the study conditions. Laccase  
362 activity was also determined as an indicator of *T. versicolor* relative activity, in order to  
363 evaluate the contribution of this enzyme in the degradation process. An optimal  
364 performance of *T. versicolor* in terms of growth and enzymatic activity is dependent on  
365 its capability to compete with native microflora in contaminated soil (Bumpus, 1993;  
366 Levanon, 1993; Baldrian, 2004). According to Šašek *et al.* (2003), this is an important  
367 aspect since the interaction between both parts can result in either inhibition or  
368 cooperation in the degradation process. In order to enhance *T. versicolor* colonisation  
369 and activity under such conditions, two strategies were employed: pre-incubation of the  
370 fungus on a ligninolytic substrate (wet sawdust) prior to inoculation into soil; use of  
371 sawdust as carrier (5 g inoculant to 95 g soil) but also as nutrient source selective for the  
372 fungus. Other authors have used different carriers and inoculant/soil ratios, ranging  
373 from 5% woodchips-based *T. versicolor* inoculum (Fragoeiro and Magan, 2005) to 50%  
374 straw-based inoculum (Novotný *et al.*, 2003).

375

#### 376 *Temporal soil respiration*

377 Soil respiration was used as an indicator of overall microbial activity and pesticide  
378 breakdown. Increased respiratory activity following incorporation of sawdust and  
379 atrazine (individually or combined) was not surprising as they provide nutrient sources  
380 suitable for native microorganisms (Mandelbaum *et al.*, 1993; Haney *et al.*, 2002;  
381 Moreno *et al.*, 2007). Our results were thus comparable to those in other studies which  
382 used atrazine at similar concentrations (Dzantor and Felsot, 1991; Moreno *et al.*, 2007).  
383 Further, sawdust addition may have also improved aeration throughout soil, favouring  
384 microbial activity in ways equivalent to that reported by Boyle (1995) using alfalfa and  
385 bran.

386

387 Respiratory activity was also enhanced in soil containing the inoculum, indicating that  
388 the test isolate was able to remain metabolically active throughout the study. However,  
389 few significant differences were found between that and soil containing sawdust alone,  
390 which suggests competitive interactions between the inoculant and native microflora.  
391 The highest CO<sub>2</sub> evolution rates were obtained from soil containing both atrazine and  
392 the inoculant, indicating atrazine breakdown by the test isolate, even under limiting  
393 water potentials of -2.8 MPa. Comparable results were obtained by Fragoeiro and  
394 Magan (2008), who employed *T. versicolor* for bioremediation of a pesticide mixture

395 (simazine, dieldrin and trifluraline at 5 ppm) in soil microcosms. Using a sandy loam  
396 soil under similar water potentials, they have also reported maximal CO<sub>2</sub> evolution from  
397 soil containing both the pesticide mixture and *T. versicolor*.

398

399 Overall, respiration rates peaked at around 6 weeks, followed by a consistent decrease  
400 towards the end of the study due to nutrient exhaustion (Balba et al., 1998). There was  
401 evidence that water potential was the limiting factor for soil respiration, as respiratory  
402 activity between treatments was generally not statistically different under the driest soil  
403 conditions. This is consistent with Conant et al. (2004), who found that drier (-1.0 and -  
404 1.5 MPa) soils have substantially lower respiration rates than those moist (-0.03 to -0.05  
405 MPa), partially due to severely restricted bacterial activity.

406

#### 407 *Temporal dehydrogenase activity*

408 Biological dehydrogenation (oxidation) of organic matter under aerobic conditions is  
409 ultimately linked to the respiratory chain and the synthesis of adenosine triphosphate  
410 (ATP) (Trevors, 1982; von Mersi and Schinner, 1991) and is catalysed by  
411 dehydrogenases (Harris and Steer, 2003; Nannipieri *et al.*, 2002, 2003). Besides organic  
412 matter decomposition, intracellular dehydrogenase activity has also been associated  
413 with other key soil functions such as xenobiotic degradation (Min *et al.*, 2001; Acosta-  
414 Martinez *et al.*, 2003). This enzymatic activity has been widely recognised as a good  
415 indicator of microbial activity, since it is linked to viable cells only and has shown to be  
416 positively correlated to respiration under different soil conditions (e.g. von Mersi and  
417 Schinner, 1991; Garcia et al., 1994; Jimenez et al., 2002). In this study, DHA was  
418 determined in order to assess the overall soil oxidative status and this enzymatic activity  
419 in relation to atrazine biodegradation.

420

421 Regardless of soil treatment, the highest DHA levels were achieved under the wettest  
422 conditions, which is supported by previous studies (Quilchano and Maranon, 2002).  
423 Further, supplement addition to soil (sawdust and atrazine, individually or combined)  
424 generally enhanced DHA regardless of soil water potential. In contrast, the low DHA  
425 levels in soil containing the inoculum alone over the first 6 weeks, is likely to reflect  
426 competitive interactions between the inoculant and native microorganisms, agreeing  
427 with respiration data. However, increased DHA from then onwards indicated that the  
428 inoculum remained metabolically active, even under the driest soil conditions.

429 Surprisingly, unlike that observed in the remaining treatments, DHA in the SAT  
430 treatment was the highest over 24 weeks, which is inconsistent with respiratory activity.  
431 This adding to the fact that the SAT treatment did not show improved levels of this  
432 enzyme (when comparing to ST), suggested that atrazine degradation by this white-rot  
433 species may not be coupled to the oxidative metabolism of the fungus. It is possible that  
434 under the treatment environmental conditions, atrazine was not being used by *T.*  
435 *versicolor* for generation of ATP (Haney *et al.*, 2002). This enzymatic activity has been  
436 scarcely studied in relation to atrazine biodegradation by white-rot fungi in soil.

437

438 Previous work has linked single pesticide degradation and DHA activity in soil (e.g.  
439 Min *et al.*, 2001; Moreno *et al.*, 2007). Moreno *et al.* (2007) reported enhanced DHA in  
440 soil containing atrazine in the range of 0.2 to 1000 mg kg<sup>-1</sup> at 28°C. In contrast,  
441 McGrath and Singleton (2000) monitored pentachlorophenol (PCP) biodegradation in a  
442 clay loam. While PCP concentration was found to decrease (from 250 to 2 mg kg<sup>-1</sup>) in  
443 just 6 weeks, levels of DHA remained minimal throughout the study. They suggested  
444 that the generation of toxic PCP biodegradation products may have been inhibitory to  
445 DHA (McGrath and Singleton, 2000). However, it is unlikely for that to explain the low  
446 DHA obtained in this study in soil containing the inoculant. Previous work has shown  
447 that very few toxic simazine breakdown products were originated by this inoculant in  
448 soil extract broth at -0.7 and -2.8 MPa, using the luminescent bacterium *Vibrio fischerie*  
449 (standard toxicity assay) (Fragoeiro, 2005).

450

#### 451 *Temporal laccase activity*

452 Since it is difficult to directly assess fungal growth in soil (Novotný *et al.*, 1999, 2004),  
453 colonisation of white-rot fungi is usually determined indirectly through enzymatic  
454 activity. The ability of such fungi to degrade pesticides has been largely associated with  
455 the production of the glycoprotein laccase (polyphenol oxidase) in the presence of  
456 adequate ligninolytic substrates (Hägglom, 1992; Paszczynski and Crawford, 2000;  
457 Novotný *et al.*, 1999, 2004). Such enzymes have broad substrate specificity towards  
458 aromatic compounds containing hydroxyl and amine groups. ABTS is considered to be  
459 a primary mediator for laccase and therefore its oxidation is generally regarded as an  
460 indication of laccase activity (Youn *et al.*, 1995; Podgornik *et al.*, 2001).

461

462 ABTS oxidation did occur in non-treated clay soil under the study water potentials,  
463 contrary to that found by Fragoeiro and Magan (2008) using a sandy loam. This may be  
464 because other genera of fungi (e.g. *Aspergillus*, *Rhizopus*), actinomycetes (e.g.  
465 *Streptomyces*) and also some bacteria (e.g. *Pseudomonas*, *Bacillus*) are known to  
466 express laccase activity at some extent (Kearney and Roberts, 1998). It suggests that  
467 this enzymatic activity may not be suitable for assessing *T. versicolor* relative activity in  
468 non-sterile soil. The incorporation of sawdust (individually or combined with atrazine)  
469 has shown to stimulate LAC production, which might be a reflection of an active fungal  
470 and actinomycete communities in such soil types (Brown, 1979; Wilson and Griffin,  
471 1975; Harris, 1981; Magan, 1988, 1997; Halverson *et al.*, 2000).

472

473 Very little research has looked at the implications of soil water potential on LAC  
474 activity. In this study, whereas the incorporation of *T. versicolor* into soil did not  
475 resulted in enhanced laccase activity at -0.7 MPa, that enhancement was obtained under  
476 drier soil conditions. This indicates that *T. versicolor* had ligninolytic activity under -2.8  
477 MPa, similarly to that reported by Boyle (1995) and later by Fragoeiro and Magan  
478 (2008). Further, that result suggests that LAC production by the inoculant may be  
479 influenced by competitive interactions with native microflora (White and Boddy, 1992)  
480 at -0.7 MPa, when the wider fraction of the microbial community was metabolically  
481 active. For example, there is evidence of total inhibition of ligninolytic activity in *T.*  
482 *versicolor* when co-inoculated in soil with species of the genus *Trichoderma* (Freitag  
483 and Morrell, 1992). According to Novotný (1999), such interactions may explain why  
484 *T. versicolor* generally produces relatively low levels of laccase in non-sterile soil.

485

486 Under wetter soil conditions, soil containing both atrazine and the inoculant has shown  
487 minimal levels of laccase, compared to soil containing sawdust and atrazine. In contrast,  
488 the opposite was found at -2.8 MPa. Overall, evidence suggests that atrazine  
489 degradation in this soil by *T. versicolor* may have had little or no contribution of laccase  
490 activity under the conditions studied. It is therefore likely that other enzymes may have  
491 been involved at a larger scale. For example, Podgornik and co-workers (2001)  
492 defended that ABTS is also a good substrate for manganese peroxidase (MnP) in *P.*  
493 *chrysosporium* and therefore there is the possibility of this ligninolytic enzyme to have  
494 been equally responsible for ABTS oxidation to a certain degree. Additionally, those  
495 same authors confirmed that Mn(III) complex formation during cultivation of *P.*

496 *chrysosporium* can provide a false-positive for laccase, when ABTS is used as substrate.  
497 Further research is needed on the link between ABTS oxidation and laccase activity  
498 from *T. versicolor*, as well as between such enzymatic activity and pesticide degradation  
499 by this species in non-sterile soil. Similarly, although it is known that *T. versicolor*  
500 produces both MnP and lignin peroxidase (LiP) in culture (reviewed by Tuor *et al.*,  
501 1995), much remains to be done in order to evaluate the contribution of these enzymes  
502 in atrazine biodegradation by *T. versicolor* in the soil environment.

503

504 Contradictory evidence has led to the role of laccase production in the co-metabolism of  
505 pesticides with lignin by white-rot fungi not yet to be well understood (Youn *et al.*,  
506 1995). In this study, laccase production has shown to be highly impacted by soil  
507 treatment, particularly by soil water potential. Bending *et al.* (2002), who used *T.*  
508 *versicolor* for biodegradation of atrazine in liquid culture for up to 42 days, have  
509 reached similar conclusions. Similarly, Mougín *et al.* (1996) have also reported that the  
510 degradation of lindane in soil by *Phanerochaete chrysosporium* was independent of  
511 laccase production by the fungus. In contrast, Fragoeiro and Magan (2008) reported  
512 extremely high laccase activity (797.8 units ml<sup>-1</sup>) by *T. versicolor* in a sandy loam soil  
513 (25°C, -0.7 MPa) treated with a pesticide mixture (simazine, dieldrin and trifluralin, 5-  
514 30 ppm). Besides having been associated also with interspecific interactions of the  
515 fungus (White and Boddy, 1992), Novotný (1999) has further suggested that laccase  
516 production may be influenced by the nature and concentration of the potential  
517 contaminant and soil environmental conditions (Tuor *et al.*, 1995). It is also likely to  
518 vary with the white-rot strain and perhaps with the carrier used (Mougín *et al.*, 1996;  
519 Boyle, 1997) or be dependent on the combination of the aforementioned factors.

520

#### 521 *Monitoring atrazine concentration in soil microcosms*

522 Pesticide degradation in soil was estimated by determining the amount of herbicide  
523 extracted from soil after 6-24 weeks (20°C, -0.7 and -2.8 MPa), compared to its initial  
524 concentration. The decrease in recovered atrazine in the SA and SSA treatments can be  
525 explained by the presence of active native microbial populations, capable of degrading  
526 the herbicide under the study conditions. This was consistent with the enhanced  
527 microbial metabolic activity found for the same time period. Other studies (Haney *et al.*,  
528 2002; Moreno *et al.*, 2007) have reported similar results on the capability of native soil  
529 populations to degrade atrazine added at low concentrations. For example, Moreno *et al.*



530 (2007) have recently demonstrated that 50% of the atrazine added (5 ppm) to clay loam  
531 with freely available water had been degraded by day 16 (28°C) and that no herbicide  
532 was recovered after 45 days.

533

534 However, herbicide breakdown was substantially enhanced in soil containing the  
535 inoculum, particularly within the first 12 weeks. It provides evidence that *T. versicolor*  
536 was able to grow and actively degrade atrazine in non-sterile soil under low water  
537 availability conditions. It also suggests that the pre-incubation of the test isolate and the  
538 use of sawdust as carrier were effective for this species. In this case, it is likely that the  
539 relationship established between the inoculum and native degraders was mainly  
540 cooperative, agreeing with earlier findings by Boyle (1995). In contrast, Tornberg *et al.*  
541 (2003) reported that this species failed to remain viable once inoculated in non-sterile  
542 soil.

543

544 Over 24 weeks, the contribution of *T. versicolor* was found to be no longer significant at  
545 -0.7 MPa. In contrast, surprisingly, its contribution was still significant at -2.8 MPa,  
546 when the remaining fraction of the microbial community had their metabolic activity  
547 limited by water restriction. This clearly shows that this white-rot species is able to  
548 actively biodegrade potential contaminants under environmental conditions, which do  
549 not promote biodegradation by soil native microflora. It is likely to be partially  
550 explained by the mycelial growth habit, allowing rapid and efficient colonisation of soil  
551 while maximising interactions between extracellular enzymatic activity and the  
552 surrounding environment (Maloney, 2001; Reddy and Mathew, 2001). Atrazine  
553 breakdown was less pronounced towards the end of the study, whether *T. versicolor* was  
554 present or not, due to nutrient exhaustion.

555

## 556 **5. Conclusion**

557

558 This study has shown that *T. versicolor* has the potential to be used as a bioremediation  
559 agent for atrazine and related triazine compounds in non-sterile calcareous clay soil,  
560 under low water availability conditions. This may be particularly relevant for  
561 bioremediation strategies in semi-arid and Mediterranean-like ecosystems.

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565 **References**

566

567 Abdelhafid, R., Houot, S., Barriuso, E., 2000. Dependence of atrazine degradation on C  
568 and N availability in adapted and non-adapted soils. *Soil Biology and Biochemistry* 32,  
569 389-401.

570

571 Acosta-Martinez, V., Zobeck, T. M., Gill, T. E., Kennedy, A. C., 2003. Enzyme  
572 activities and microbial community structure in semi-arid agricultural soils. *Biology and*  
573 *Fertilily of Soils* 38, 216-227.

574

575 Armstrong, D. E., Chesters, G. and Harris, R. F., 1967. Atrazine Hydrolysis in Soil. *Soil*  
576 *Science Society of America* 31, 61-66.

577

578 Ashman, M. R. and Puri, G., 2002. *Essential Soil Science: a clear and concise*  
579 *introduction to soil science*. Blackwell Science Ltd., Oxford, UK.

580

581 Balba, M. T., Al-Awadhi, N., Al-Daher, R., 1998. Bioremediation of oil-contaminated  
582 soil: microbiological methods for feasibility assessment and field evaluation. *Journal of*  
583 *Microbiological Methods* 32, 155-164

584

585 Baldrian, P., 2004. Increase of laccase activity during interspecific interactions of white-  
586 rot fungi. *FEMS Microbiology Ecology* 50, 245-253.

587

588 Bastos, A., 2008. Microbial volatile fingerprints: potential use for soil/water diagnostics  
589 and correlation with traditional microbial parameters, PhD Thesis, University of  
590 Cranfield, Silsoe, UK.

591

592 Bending, G.D., Friloux, M. and Walker, A., 2002. Degradation of contrasting pesticides  
593 by white rot fungi and its relationship with ligninolytic potential  
594 *FEMS Microbiology Letters* 212, 59-63.

595

596 Boyle D., 1995. Development of a practical method for inducing white rot fungi to grow  
597 into and degrade organopollutants in soil. *Canadian Journal of Microbiology* 41,

598 345-353.  
599  
600 Boyle, D., 1997. Nutritional factors limiting the growth of *Lentinula edodes* and other  
601 white-rot fungi in wood. *Soil Biology and Biochemistry* 30, 817-823.  
602  
603 Brown, A. D., 1979. Physiological problems of water stress. In: Shilo, M. (Ed.)  
604 Strategies of microbial life in extreme environments. Dahlem Konferezenz, Berlin, 1979.  
605  
606 Bumpus, J. A., Tien, M., Wright, D., and Aust, S. D., 1985. Oxidation of persistent  
607 environmental pollutants by a white- rot fungus. *Science* 228, 1434-1436.  
608  
609 Buswell, J. A., Cai, Y., Chang, S., 1995. Effect of nutrient nitrogen and manganese on  
610 manganese peroxidase and laccase production by *Lentinula (Lentinus) edodes*. *FEMS*  
611 *Microbiology Letters* 128, 81-88.  
612  
613 Conant, R. T., Dalla-Betta, P., Klopatek, C. C., Klopatek, J. F., 2004. Controls on soil  
614 respiration in semiarid soils. *Soil Biology and Biochemistry* 36, 945-951.  
615  
616 Criquet, S., Tagger, S., Vogt, G., Iacazio, G., Le Petit, J., 1999. Laccase activity of  
617 forest litter. *Soil Biology and Biochemistry* 31, 1239-1244.  
618  
619 Dzanter, E.K., Felsot, A.S., 1991. Microbial responses to large concentrations of  
620 herbicides in soil. *Environmental Toxicology and Chemistry* 10, 649-655.  
621  
622 Eliassy, A., 1997. Bioremediation of the pesticides dieldrin, simazine and trifluralin  
623 using tropical and temperate white-rot fungi, PhD Thesis, University of Cranfield,  
624 Silsoe, UK.  
625  
626 Fragoeiro, S., Magan, N., 2005. Enzymatic activity, osmotic stress and degradation of  
627 pesticide mixtures in soil extract liquid broth inoculated with *Phanerochaete*  
628 *crysosporium* and *Trametes versicolor*. *Environmental Microbiology* 7, 348-355.  
629  
630 Fragoeiro, S., Magan, N., 2008. Impact of *Trametes versicolor* and *Phanerochaete*  
631 *crysosporium* on differential breakdown of pesticide mixtures in soil microcosms at two

632 water potentials and associated respiration and enzyme activity. International  
633 Biodeterioration and Biodegradation, article in press.

634

635 Freitag, M., Morrel, J.J., 1992. Changes in selected enzyme-activities during growth of  
636 pure and mixed cultures of the white-rot decay fungus *Trametes versicolor* and the  
637 potential biocontrol agent *Trichoderma harzianum*. Canadian Journal of Microbiology  
638 38, 317-323.

639

640 Gadd, G. (Ed.), 2001 Fungi in Bioremediation. Cambridge University Press.  
641 Cambridge, UK.

642

643 Ghani, A., Wardle, D. A., Rahman, A., Lauren, D. R., 1996. Interactions between <sup>14</sup>C-  
644 labelled atrazine and the soil microbial biomass in relation to pesticide degradation.  
645 Biology and Fertility of Soils 21, 17-22.

646

647 Gravilescu, M., 2005. Fate of pesticides in the environment. Engineering in Life  
648 Sciences 5, 497- 505.

649

650 Häggblom, M. M., 1992. Microbial breakdown of halogenated aromatic pesticides and  
651 related compounds. FEMS Microbiology Reviews 103, 29-72.

652

653 Halverson, L. J., Jones, T. M., Firestone, M., 2000. Release of intracellular solutes by  
654 four bacteria exposed to dilution stress. Soil Society of America Journal 64, 1630-1637.

655

656 Haney, R. L., Senseman, S. A., Krutz, L. J., Hons, F. M., 2002. Soil carbon and nitrogen  
657 as affected by atrazine and glyphosate. Biology and fertility of Soils 35, 35-40.

658

659 Harris, J. A., Steer, J., 2003. Modern methods for estimating soil microbial biomass and  
660 diversity: an integrated approach. In: Collins, H. P., Robertson, G. P., Klug, M. J. (Eds.)  
661 The Utilisation of Bioremediation to reduce soil contamination: Problems and  
662 Solutions. Kluwer Academic Publishers, Netherlands.

663

664 Harris, R., 1981. Effect of water potential on microbial growth and activity in soils in  
665 water potential relations in soil microbiology. *Soil Science Society of America Journal*,  
666 23-96.  
667

668 Houot, S., Barriuso, E., Bergheud, V., 1998. Modifications to atrazine degradation  
669 pathways in a loamy soil after addition of organic amendments. *Soil Biology and*  
670 *Biochemistry* 30, 2147-2157.  
671

672 Kearney, P., Roberts, T. (Eds.), 1998. *Pesticide Remediation in Soils and Water* Wiley  
673 *Series in Agrochemicals and Plant Protection*. John Wiley & Sons Ltd, UK.  
674

675 Kuo, W., Regan, R., 1999. Removal of pesticides from rinsates by adsorption using  
676 agricultural residues as medium. *Journal of Science and Health B34*, 431-447.  
677

678 Lamar, R. T., Evans, J. W., Glaser, J. A., 1993. Solid-phase treatment of a  
679 pentachlorophenol-contaminated soil using lignin-degrading fungi. *Environmental*  
680 *Science and Technology* 27, 2566-2571.  
681

682 Levanon, D., 1993. Roles of fungi and bacteria in the mineralization of the pesticides  
683 atrazine, alachlor, malathion and carbofuran in soil. *Soil Biology and Biochemistry* 25,  
684 1097-1105.  
685

686 Magan, N., 1988. Fungal colonisation and decomposition of cereal straw. *International*  
687 *Biodeterioration* 24, 435-443.  
688

689 Magan, N., 1997. Fungi in extreme environments. In: Wicklow & Soderstrom (Eds.),  
690 *The Mycota IV. Environmental and Microbial Relationships*. Springer-Verlag, Berlin.  
691

692 Maloney, S., 2001. Pesticide degradation. In: Gadd, G. (Ed.), *Fungi in Bioremediation*.  
693 *Cambridge University Press*, Cambridge, UK.  
694

695 Mandelbaum, R., Hadar, Y., Chen, Y., 1993. Simple apparatus to study microbial  
696 activity in organic substrates under constant water potential. *Soil Biology and*  
697 *Biochemistry* 25, 397-399.

698

699 McGrath, R. Singleton, I., 2000. Pentachlorophenol transformation in soil: a  
700 toxicological assessment. *Soil Biology and Biochemistry* 32, 1311-1314.

701

702 Min, H., Ye, Y.F., Chen, Z.Y., Wu, W.X., Du, Y.F., 2001. Effects of butachlor on  
703 microbial populations and enzyme activities in paddy soil.  
704 *Journal of Environmental Science and Health, Part B – Pesticides, food contaminants*  
705 *and agricultural wastes* 36, 581-595.

706

707 Mollea, C., Bosco, F., Ruggeri, B., 2004. Fungal biodegradation of naphthalene:  
708 microcosms studies. *Chemosphere* 60, 636 – 643.

709

710 Moreno, J. L., Aliaga, A., Navarro, S., Hernández, T., Garcia, C., 2007. Effects of  
711 atrazine on microbial activity in semiarid soil. *Applied Soil Ecology* 35, 120-127.

712

713 Mougín, C., Pericaud, C., Malosse, C., Laugero, C., Asther, M., 1996.  
714 Biotransformation of the insecticide lindane by the white-rot basidiomycete  
715 *Phanerochaete chrysosporium*. *Pesticide Science* 47, 51-59.

716

717 Mswaka, A.Y., Magan, N., 1999. Temperature and water potential relations of tropical  
718 *Trametes* and other wood-decay fungi from the indigenous forests of Zimbabwe.  
719 *Mycological Research* 103, 1309-1317.

720

721 Nannipieri, P., Ascher, J., Ceccherini, M. T., Landi, L., Pietramellara, G., Renella, G.  
722 (2003). Microbial diversity and soil functions. *European Journal of Soil Science* 54,  
723 655-670.

724

725 Nannipieri, P., Kandeler, E., Ruggiero, P., 2002. Enzyme activities and microbiological  
726 and biochemical processes in soil. In: Burns, R. G., Dick, R. (Eds.) *Enzymes and the*  
727 *Environment*. Marcell Dekker, New York.

728

729 Novotný, C., Erbanova P, Sasek V, Kubatova A, Cajthaml T, Lang E, Krahl J, Zadrazil  
730 F., 1999. Extracellular oxidative enzyme production and PAH removal in soil by  
731 exploratory mycelium of white rot fungi. *Biodeterioration* 10, 159-168.

732

733 Novotný, C., Svobodová, K., Erbanová, P., Cajthaml, T., Kasinath, A., Lang, E., Šašek,  
734 V., 2004. Ligninolytic fungi in bioremediation: extracellular enzyme production and  
735 degradation rate. *Soil Biology and Biochemistry* 36, 1545-1551.

736

737 Quilchano, C., Mararon T., 2002. Dehydrogenase activity in Mediterranean forest soils.  
738 *Biology and Fertility of Soils* 35, 102-107.

739

740 Paszczynski, A., Crawford, R., 2000. Recent advances in the use of fungi in  
741 Environmental Remediation and Biotechnology. In: Bollag, J.-M. & Stotzky, G. (Eds.)  
742 *Soil Biochemistry* 10, 379-422. Marcel Dekker, New York.

743

744 Podgornik, H., Stegu, M., Zibert, E., Perdith, A., 2001. Laccase production by  
745 *Phanerochaete chrysosporium* – an artefact caused by Mn(III)? *Letters in Applied*  
746 *Microbiology* 32, 407-411.

747

748 Pointing, S., 2001. Feasibility of bioremediation by white-rot fungi. *Applied*  
749 *Microbiology and Biotechnology* 57, 20-33.

750

751 Ralebitso, T.K., Senior, E., van Verseveld, H.W., 2002. Microbial aspects of atrazine  
752 degradation in natural environments. *Biodeterioration* 13, 11-19.

753

754 Reddy, C., Mathew, Z., 2001. Bioremediation potential of white rot fungi. In: Gadd, G.  
755 (Ed.), *Fungi in Bioremediation*. Cambridge University Press, Cambridge, UK.

756

757 Rhine, E. D., Fuhrmann, J. J., Radosevich, M., 2003. Microbial community responses to  
758 atrazine exposure and nutrient availability: linking degradation capacity to community  
759 structure. *Microbial Ecology* 46, 145-160.

760

761 Šašek, V., Glaser, J. A., Bouveye, P. (Eds.), 2003. The utilisation of Bioremediation to  
762 reduce Soil contamination: problems and solutions. *Earth and Environmental Sciences*  
763 19. Nato Science Series. Kluwer Academic Publishers.

764

765 Schmidt, K. R., Chand, S., Gostomski, P. A., Boyd-Wilson, K., Ford, C., Walter, M.,  
766 2005. Fungal inoculum properties and its effect on growth and enzyme activity of  
767 *Trametes versicolor* in soil. *Biotechnology Programs* 21, 377-385.  
768

769 Somerville, L., Greaves, M. P. (Eds.), 1987. *Pesticide effects on Soil Microflora*. Taylor  
770 & Francis, London, UK.  
771

772 Topp, E., 2001. A comparison of three atrazine-degrading bacteria for soil  
773 bioremediation. *Biology and Fertility of Soils* 33, 529-534.  
774

775 Tornberg, K., Baath, E., Olsson, S., 2003. Fungal growth and effects of different wood  
776 decomposing fungi on the indigenous bacterial community of polluted and un-polluted  
777 soils. *Biology and Fertility of Soils* 37, 190-197.  
778

779 Trevors, J. T., Mayfield, C. I., Inniss, W.E., 1982. Measurement of electron transport  
780 system (ETS) activity in soil. *Microbial Ecology* 8, 163-168.  
781

782 Thurston, C.F., 1994. The structure and function of laccases. *Microbiology* 140, 19-26.  
783

784 von Mersi, W., Schinner, F., 1991. An improved and accurate method for determining  
785 the dehydrogenase activity of soil with iodonitrotetrazoliumchloride. *Biology and*  
786 *Fertility of Soils* 11, 216-220.  
787

788 Weber, J.B., 1993. Ionisation and sorption of fomesafen and atrazine by soils and soil  
789 constituents. *Pesticide Science* 39, 31-38.  
790

791 White, N. A., Boddy, L., 1992. Extracellular enzyme location during interspecific  
792 fungal interactions. *FEMS Microbiology Letters* 98, 75-80.  
793

794 Wilson, J M., Griffin, D. M., 1975. Water potential and the respiration of  
795 microorganisms in the soil. *Soil Biology and Biochemistry* 7, 199-204.  
796

797 Youn, H.-D., Hah, Y.C., Kang, S.-O., 1995. Role of laccase in lignin degradation by  
798 white rot fungi. *FEMS Microbiology Letters* 132, 183-188.



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833 Table 1. Summary of the soil treatments involved in this work. Key to treatments: WP,  
 834 water potential; SD, sawdust; Atra, atrazine., Tv, *T.versicolor*.

| 835 |                  | Ref | WP (-MPa)  | Atra (ug) | SD (%) | SD+Tv |
|-----|------------------|-----|------------|-----------|--------|-------|
| 836 | Soil             | S   | -0.7; -2.8 | -         | -      | -     |
| 837 | Soil + Atra      | SA  | -0.7; -2.8 | 0.5       | -      | -     |
| 838 | Soil + SD        | SS  | -0.7; -2.8 | -         | 0.5    | -     |
| 839 | Soil + SD + Atra | SSA | -0.7; -2.8 | 0.5       | 0.5    | -     |
| 840 | Soil + Tv        | ST  | -0.7; -2.8 | -         | -      | 0.5   |
| 841 | Soil + Atra + Tv | SAT | -0.7; -2.8 | 0.5       | 0.5    | 0.5   |

842

843 Table 2. Temporal laccase activity (U g/soil) based on ABTS oxidation in clay soil  
 844 incubated for up to 24 weeks at 20oC at (A) -0.7 and (B) -2.8 MPa as a response to  
 845 different soil amendments.

846 A)

|     | Incubation time (weeks) |             |             |             |
|-----|-------------------------|-------------|-------------|-------------|
|     | 0                       | 6           | 12          | 24          |
| S   | 0.30 ± 0.29             | 2.55 ± 1.06 | 1.88 ± 0.88 | 0.67 ± 0.41 |
| SA  | 9.04 ± 1.41             | 12.0 ± 3.53 | 7.92 ± 1.57 | 0           |
| SS  | 9.50 ± 0.77             | 24.5 ± 9.60 | 16.4 ± 3.04 | 0           |
| SSA | 10.3 ± 1.12             | 28.7 ± 0.79 | 19.9 ± 0.32 | 0           |
| ST  | 8.49 ± 3.53             | 21.2 ± 9.11 | 13.3 ± 2.89 | 0           |
| SAT | 18.5 ± 5.66             | 0.51 ± 0.22 | 0.50 ± 0.51 | 0           |

847

848 B)

|     | Incubation time (weeks) |             |             |    |
|-----|-------------------------|-------------|-------------|----|
|     | 0                       | 6           | 12          | 24 |
| S   | 0.72 ± 0.11             | 1.97 ± 0.80 | 0.86 ± 0.23 | 0  |
| SA  | 0                       | 21.5 ± 4.32 | 15.7 ± 2.03 | 0  |
| SS  | 6.50 ± 0.51             | 11.1 ± 3.88 | 0           | 0  |
| SSA | 8.57 ± 1.01             | 13.4 ± 0.90 | 6.20 ± 0.56 | 0  |
| ST  | 10.0 ± 1.06             | 14.6 ± 3.63 | 9.88 ± 1.54 | 0  |
| SAT | 14.2 ± 1.90             | 23.1 ± 5.69 | 13.4 ± 2.01 | 0  |

849

850 **Table 3. Remaining atrazine (ug/g) in clay soil at (A) -0.7 and (b) -2.8 MPa at 20oC**  
 851 **in the absence (SA, SSA) and presence (SAT) of *T.versicolor*.**

852 (A)

| Incubation (weeks) | Treatment | Remaining atrazine ( $\mu\text{g g}^{-1}$ ) |
|--------------------|-----------|---|
| 0                  | SA        | 0.495 $\pm$ 0.01                            |
|                    | SSA       | 0.500 $\pm$ 0.03                            |
|                    | SAT       | 0.500 $\pm$ 0.02                            |
| 6                  | SA        | 0.080 $\pm$ 0.01                            |
|                    | SSA       | 0.071 $\pm$ 0.02                            |
|                    | SAT       | 0.023* $\pm$ 0.02                           |
| 12                 | SA        | 0.036 $\pm$ 0.03                            |
|                    | SSA       | 0.034 $\pm$ 0.02                            |
|                    | SAT       | 0.016* $\pm$ 0.03                           |
| 24                 | SA        | 0.022 $\pm$ 0.06                            |
|                    | SSA       | 0.019 $\pm$ 0.04                            |
|                    | SAT       | 0.011 $\pm$ 0.05                            |

853 B)

| Incubation (weeks) | Treatment | Remaining atrazine ( $\mu\text{g g}^{-1}$ ) |
|--------------------|-----------|---|
| 0                  | SA        | 0.480 $\pm$ 0.03                            |
|                    | SSA       | 0.495 $\pm$ 0.03                            |
|                    | SAT       | 0.490 $\pm$ 0.01                            |
| 6                  | SA        | 0.262 $\pm$ 0.04                            |
|                    | SSA       | 0.255 $\pm$ 0.01                            |
|                    | SAT       | 0.102* $\pm$ 0.03                           |
| 12                 | SA        | 0.258 $\pm$ 0.02                            |
|                    | SSA       | 0.242 $\pm$ 0.02                            |
|                    | SAT       | 0.084* $\pm$ 0.02                           |
| 24                 | SA        | 0.254 $\pm$ 0.06                            |
|                    | SSA       | 0.237 $\pm$ 0.04                            |
|                    | SAT       | 0.076* $\pm$ 0.03                           |

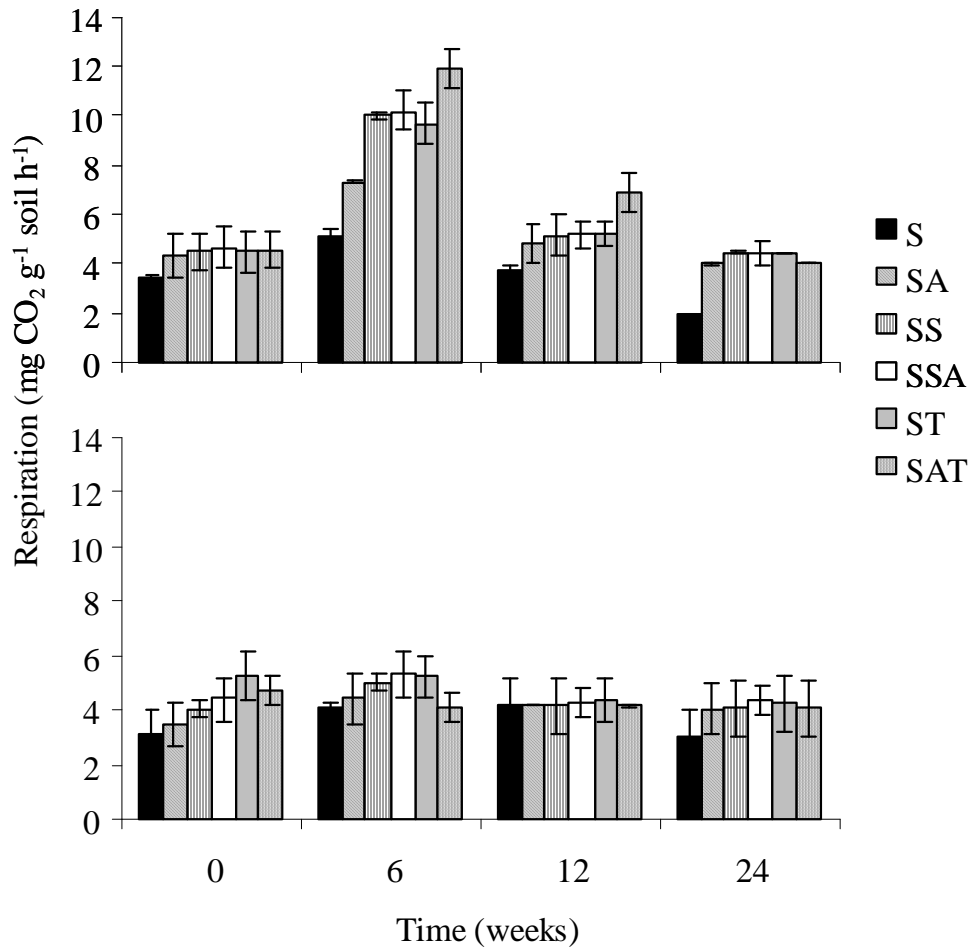
854 \* Statistically different from both controls (SA and SSA) at  $p < 0.05$ .

855

856

857 Figure 1. Respiration rates for the clay soil under different soil treatments for up to 24  
 858 weeks at 20°C under (A) -0.7 and (B) -2.8 MPA. For key to treatments see Table 1.

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# Trametes versicolor: Potential for atrazine bioremediation in calcareous clay soil, under low water availability conditions.

Bastos, Ana Catarina

2009-06

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A.C. Bastos, N. Magan, Trametes versicolor: Potential for atrazine bioremediation in calcareous clay soil, under low water availability conditions, International Biodeterioration & Biodegradation, Volume 63, Issue 4, June 2009, Pages 389-394

<http://hdl.handle.net/1826/3395>

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