1 **Exotic litter of the invasive plant *Ligustrum lucidum* alters enzymatic production and**

2 **lignin degradation by selected saprotrophic fungi.**

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ABSTRACT

Chemical changes in leaf input to forest soils have been reported to affect decay processes. In this work, litter mass loss and decomposition constants (k) during 200 days in solid-state fermentation of the native tree *Celtis tala* and the exotic one *Ligustrum lucidum* with three common litter saprotrophic basidiomycetes were compared. Alterations in litter quality were characterized by solid-state ^{13}C NMR spectroscopy, pH, soluble sugars, ammonium, proteins and phenol content determination, and were associated with extracellular lignocellulolytic enzyme production. Differences in substrate decomposition related to litter type were observed for *Leratiomyces ceres,* achieving a higher k in the exotic *L. lucidum* litter, which might be attributed to the induction of Manganese peroxidase activity. Substrate preference for alkyl C and more degradation of lignified compounds were found in such substrate. Although no statistical differences in mass loss were observed for the rest of the fungi assayed, we detected changes in several of the parameters evaluated. This suggests that exotic invasions may alter ecosystem functioning by accelerating decomposition processes through an increased fungal ligninolytic activity.

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Introduction

Invasion of exotic plants in pristine terrestrial ecosystems is a major environmental problem due to alterations in the vegetal community with the consequent detrimental impact on the activity of resident organisms (Pyšek *et al.* 2012). Plant-litter provides the main contribution of biomass and energy in several environments such as forests, where the substrate quality strongly influences microbial composition and its related activity in soils (Hättenschwiler *et al.* 2005; Wardle *et al.* 2004). Nevertheless, there are scarce data about the effect of the input of senescent leaves from invasive plants on decomposition and humification processes associated with enzyme activity in soils.

Different decay rates among organic matter kept under identical environmental conditions are mostly associated with changes in the content of its major chemical constituents such as nitrogen, lignin and polyphenol, as well as the relative contribution of carbon: nitrogen and lignin: nitrogen (Wardle *et al.* 2004). Leaf litter consists mainly of polysaccharides cellulose and hemicellulose and aromatic polymer lignin, the latter being considered as the most recalcitrant. Fungi are the dominant decomposers in temperate and boreal forest soils (Hättenschwiler *et al.* 2005) and among fungi, saprotrophic basidiomycetes are capable of producing a wide variety of extracellular oxidoreductases and hydrolytic enzymes that allow them to degrade all these three litter components (Purahong *et al.* 2014). This study was carried out with the aim of comparing the abilities of saprotrophic basidiomycetes in decomposition of leaf-litter of both native and invasive plants, based on the hypothesis that litter quality modifies the lignocellulolytic activity of these fungi and may greatly influence litter decomposition rates.

Pereyra Iraola Park is a Biosphere Reserve declared as such by UNESCO in 2007, located in Buenos Aires Province, Argentina. It constitutes the largest green space (10,248

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63   hectares) of the metropolitan area of the City of Buenos Aires and La Plata, acting as a "green

64   lung" for the urban conglomerate. The area has mixed zones belonging to relictual patches of

65   marginal forest, locally known as Selva Marginal, and forested sites with a noteworthy

66   introduction of exotic species. The former is confined to the coastal strip of the De la Plata river

67   and is considered as a reservoir of an extensive spectrum of biodiversity, on the southern limit of

68   distribution of subtropical forests in South America (Kalesnik *et al*. 2013). While *Celtis tala* Gill.

69   ex Planch. (Cannabaceae) is a typical native tree in the Reserve, *Ligustrum lucidum* Ait.

70   (Oleaceae), also known as Glossy privet, it is in fact a monodominant canopy tree native to

71   China, which has increased its coverage in the region since it was imported for ornamental

72   purposes. In heavily invaded stands, *L. lucidum* can exceed 80% of the tree cover, while

73   elsewhere, it is distributed as solitary individuals adjacent to native trees (Aragón and Groom

74   2003; Gavier-Pizarro *et al*. 2012). *L. lucidum* is tolerant to a wide range of soils and light

75   conditions and, in addition to vegetative reproduction it produces a large number of seeds, which

76   are readily dispersed by birds. These features contribute to making *L. lucidum* a successful

77   invader with a broad representation at ecosystem level (Marano *et al*. 2013). Previous studies

78   indicated that it influences the species composition and reduces the diversity in invaded plant

79   communities (Lichstein *et al*. 2004). The aggressive invasion of *L. lucidum* was reported to be an

80   important issue in other countries such as Australia and New Zealand (Cronk and Fuller 1995).

81   However, the influence of *L. lucidum* senescent leaves on the decomposing activity of litter

82   saprotrophic basidiomycetes and their role in litter degradation are still unraveled.

83   The objectives of this work were (i) to evaluate how chemical composition of leaf litter

84   affects mass loss and production of lignocellulolytic enzymes by litter saprotrophic

85   basidiomycete's cultures during solid-state fermentation (SSF) and (ii) to assess if fungi have a

86 substrate preference for decomposing native *Celtis tala* or exotic *Ligustrum lucidum* litter by

87 comparing their decomposition constants under SSF.

88 **Materials and Methods**

89 *Fungal isolates and identification*

90 Fresh basidiomata were collected in the Natural Reserve Pereyra Iraola Park in Buenos

91 Aires Province, Argentina (34°84'S 58°10'W), during the summer of 2014. *Marasmiellus*

92 *candidus, Leratiomyces ceres* and *Marasmius haematocephalus* were classified based on the

93 morphological characteristics of their basidiomata. These fungi were selected because they were

94 frequently found in both litter assayed (Mallerman 2017). To confirm the identities of fungal

95 isolates, total DNA was extracted from agar cultures with the Microbial DNA Kit (MoBio, USA)

96 and then used for PCR amplification (ABI 3130xl Genetic Analyzer). Amplification reactions

97 used the universal fungal-primers ITS1 (5'- TCCGTAGGTGAACCTGCGG -3') and ITS4 (5'-

98 TCCTCCGCTTATTGATATGC -3') for the ribosomal DNA region (White *et al*. 1990). The

99 PCR products separated through electrophoresis in 1.5% agarose gel were sent to a sequencing

100 facility (University of Buenos Aires). Sequences obtained were analyzed using BLAST,

101 compared for homologous NCBI-GenBank database and deposited under the accession numbers:

102 **KX423791** (*Leratiomyces ceres*), **KX423792** (*Marasmius haematocephalus*) and **KX423793**

103 (*Marasmiellus candidus).* For fungal isolation, a sterile piece of mushroom tissue was collected

104 from the interior of each basidiomata, transferred onto Petri dishes containing 20 mL of malt

105 extract agar medium (MEA: malt extract 1.2%, glucose 1% and agar 2%) and grown at 25°C, in

106 darkness. Strains were maintained at 4°C on MEA slants by periodic transfers and specimens

107 were preserved in the culture collection of Natural and Exact Science Faculty, University of

108 Buenos Aires, Argentina (BAFCcult).

Litter decomposition under SSF conditions

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110 *Ligustrum lucidum* and *Celtis tala* senescent leaves (just before abscission) were

111 collected from several individuals in the studied area during the summer of 2014. Litter was air-

112 dried at room temperature and stored at 4°C before use. At the beginning of the experiment,

113 leaves were cut into smaller pieces (size > 20 mm) and approx. 2.5 g were placed in 125 mL

114 Erlenmeyer flasks. To calculate the initial litter dry mass, flasks were oven-dried at 80°C until

115 constant weight. The litter was moistened with 20 mL of distilled water, autoclaved for 20 min at

116 120°C, and inoculated with two 0.6-cm diameter MEA mycelial plugs, taken from the margin of

117 an actively growing colony. Cultures were incubated at 25°C in darkness, sampling them every

118 30 days, performing the last on day 200. Non-inoculated flasks served as controls and to estimate

119 water loss during the incubation period, in which sterile water was replenished every time a

120 sample was taken. Three replicates per treatment were conducted (3 fungi and 1 control x 2 litter

121 x 3 replicates x 6 sampling times). Decomposition constant (k) was estimated by calculating the

122 percentage of dry mass remaining after each culture incubation period (percentage reduction of

123 inoculated litter in relation to the uninoculated one), using a single exponential decay model

124 (Olson 1963): $M_t = M_0 . e^{-kt}$; where M_0 is the initial dry mass, M_t is the dry mass remaining at

125 time t, k is the decomposition constant and t is time.

Chemical composition of the water soluble and solid fractions from litter

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127 On each sampling time, a water soluble fraction (WSF) and a solid fraction (SF) were

128 obtained from both uninoculated and fungal transformed litter, according to Saparrat *et al.*

129 (2008). For this, the content of each flask was mixed on a rotary shaker at 150 ppm for 1 h with

130 20 mL of distilled water, filtered through gauze and centrifuged for 10 min at 5000 × g, 4˚C.

131 Supernatants conformed the WSF, while the SF consisted of the resulting pellet collected

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132   together with the material retained in the gauze. Dry mass content was measured in the SF by

133   oven-drying at 80°C until constant mass (Saparrat *et al*. 2008).

134   In the WSF, the extracellular proteins were assessed using the Bradford (1976) method

135   with the BioRad protein assay reagent (Bio-Rad, Hercules, CA) and bovine serum albumin

136   (BSA) as standard protein. Ammonium-N was determined following the protocols of Beecher

137   and Whitten (1970). Free phenolic water-soluble compounds were extracted according to Box

138   (1983) and Folin-Ciocalteu reagent was used for analysis of total phenolic content, being results

139   expressed as phenol equivalents.

140   The chemical composition of the SF was analyzed by Cross-Polarization Magic-Angle

141   Spinning ^{13}C Nuclear Magnetic Resonance (^{13}C CPMAS NMR) procedure. The ^{13}C CPMAS

142   NMR spectra were obtained using a 7T Bruker Avance II-300 spectrometer (Bruker Instruments

143   Inc., Germany). Litter samples were dried and ground by a 4-mm zirconium rotor and spun at

144   magic angle at 10 kHz. All the solid-state NMR experiments were performed at room

145   temperature. ^{13}C CPMAS NMR was performed with a ramped ^{1}H pulse during a contact time of

146   3 ms to obtain the best signal-to-noise ratio. ^{1}H decoupling was performed during the acquisition

147   with a TPPM15 sequence to improve the resolution. Recording 1 K transients with a recycle time

148   of 3 s represented standard conditions. The operating frequencies for protons and carbons were

149   300.13 and 75.46MHz, respectively. Glycine was used as an external reference for the ^{13}C

150   spectra and to set the Hartmann-Hahn matching condition in the cross-polarization experiments.

151   Because of the substantial amount of instrument time required, only samples of the initial and

152   ending decomposition time (200 days) were analyzed. Relative areas were calculated as the

153   percentage of total intensity from the integral curves of the following carbon chemical-shift

154   regions: alkyl C (0-45 ppm), O-alkyl C (45-110 ppm), methoxyl C (50-60 ppm), aromatic C

155   (110-140 ppm), phenolic C (140-160 ppm), and carboxyl C (160-190 ppm). Deconvolution

156   method of the NMR spectra was performed using the DmFit software (Massiot *et al.* 2002). The

157   degree of humification was calculated as the ratio between the corresponding areas (A) of the

158   spectra alkyl C to O-alkyl C (Baldock *et al.* 1997). The alkyl C to carboxyl C ratio was

159   calculated according to Knicker *et al.* (2000).The aromaticity index (AI) was considered as AI =

160   100 (A (110-160 ppm) /A (0-160 ppm)) (Lorenz *et al.* 2006). Finally, the lignin content was

161   estimated according to Haw *et al.* (1984) as: % lignin = (100%) (183/9.92) l´ lig / ((183/9.92) l´

162   lig + (162/6) l´ carb). The weighting element in this calculation is the ratio of formula weights

163   for lignin and carbohydrate fractions to the number of carbon atoms in the average formulas

164   assumed for their repeating units [for more details see Haw *et al.* (1984)].

165   *Enzyme activities in the WSF*

166   In order to understand the role of enzymes related to the degradation of the main

167   structural litter components: cellulose, hemicellulose and lignin along time, we evaluated the

168   activity of β-1,4-endoglucanase, β-glucosidase, β-1,4-endoxylanase, laccase and MnP. β-1,4-

169   endoglucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91) and β-1,4-endoxylanase (E.C.

170   3.2.1.8) activities were estimated by measuring the reducing sugars released from

171   carboxymethylcellulose, crystalline cellulose or beechwood xylan, respectively, as substrates in

172   50 mM sodium acetate buffer, pH 4.8. Liberated reducing sugars were quantified by the DNS

173   method (Miller 1959) using either glucose or xylose as standards. β-glucosidase (E.C 3.2.1.21)

174   activity was determined in 50 mM sodium acetate buffer, pH 4.8, by measuring p-nitrophenol

175   released from p-nitrophenyl-β-D-glucopyranoside (Wood and Bhat 1988). All enzyme activities

176   were determined at 50ºC. Laccase (EC 1.10.3.2) activity was measured by the oxidation of 5 mM

177   2,6-dimethoxyphenol (DMP) in 0.1 M sodium acetate buffer (pH 3.6), assessing the increase in

178   absorbance at 469 nm (ε = 27.5/mM cm) (Ben Younes *et al*. 2007). The MnP activity (EC

179   1.11.1.13) was assayed by the oxidation of phenol red in 0.2 M sodium succinate buffer pH 4.5,

180   with hydrogen peroxide and manganese sulfate at 610 nm (ε = 22/mM cm) according to

181   Paszczynski *et al*. (1988). Both ligninolytic activities were measured at 30°C. International

182   enzymatic units (U) were used (μmol product/min). Results were expressed in units per gram of

183   dry litter (U/g dry litter). For all spectrophotometric measurements, three technical replicates

184   were performed.

185   ***Statistical analysis***

186   Decomposition constants (*k*) among treatments were analyzed using two-way ANOVA,

187   with litter and fungal species as main factors. Data was logarithmically transformed before

188   analysis to meet the necessary assumptions for parametric testing of normality (Shapiro-Wilks

189   test) and equality of variances (Levene's test). All post hoc comparisons were accomplished with

190   a Tukey´s test and α = 0.05 was used for testing statistical significance. When mass loss was

191   compared in a fungus over time, a one-way ANOVA followed by a Tukey's test was performed.

192   The shifts of individual carbon fractions, ratios and indexes calculated in the ^{13}C CPMAS NMR

193   analysis were compared between initial fresh litter types using a pair t test. A one-way ANOVA

194   followed by the Tukey's test was used to compare the chemical categories among fresh and

195   decomposed litter within each litter type. Statistical analyses were performed using Statistica 7.0

196   software package (Stat-Soft, USA). The correlation structure among chemical variables (based

197   on analytical determinations in the WSF and NMR spectra of the SF from fungal-litter samples

198   at the end of the SSF) and the enzyme activities (detected in the WSF of the same samples) was

199   determined by calculating pairwise Pearson's correlation coefficients. Also, the common

200   structure of these two data tables was examined by Coinertia analysis (CoiA) (Dray *et al*. 2003),

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201 previously used in litter decomposition (Alarcón-Gutiérrez *et al.* 2009). CoiA was performed

202 using the ade4 package in the R environment (R Development Core Team 2008). Principal

203 Component Analysis (PCA) was used as an ordination method in the two data sets and CoiA

204 projected the two PCAs into the same graphical space, in order to visualize variations in

205 individual chemical components among litter types and fungi in relation to enzyme activities. As

206 we had some zero values, a Detrended Correspondence Analysis (DCA) -using *vegan* package in

207 R- was performed first. Because the ordination axes or "gradient lengths" were shorter than two

208 units, the data were linear, and a CoiA with PCA was appropriate for the analysis (Ter Braak

209 1998). The co-linearity was tested using the vif function with *fmsb* package in R. All variables

210 above a threshold of five were excluded from the analyses. In order to identify which factors

211 significantly corresponded to overall enzyme activities, we used the function envfit in *vegan*

212 package in R. The significant variables were analyzed for variance partitioning with varpart

213 function in *vegan*.

214 **Results**

215 *Litter decomposition under SSF*

216 Litter fungal strains *Marasmiellus candidus*, *Leratiomyces ceres* and *Marasmius*

217 *haematocephalus* were able to grow and colonize native *Celtis tala* and exotic *Ligustrum*

218 *lucidum* litter, using them as only substrate source in SSF conditions. The percentages of dry

219 mass remaining after each sampling time are shown in Fig. 1. Litter decay rates varied according

220 to the fungus employed in the SSF. In the native litter, fungal degradation began faster than in

221 the exotic one, excepting litter treated by *L. ceres*, a treatment that, however, showed a

222 significant mass loss after 90 days ($P < 0.01$). In the native litter, *Marasmiellus candidus,*

223 *Leratiomyces ceres* and *Marasmius haematocephalus* caused dry mass loss of $10.97 \pm 1.12\%$,

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224   8.78 ± 3.64% and 24.11 ± 3.19%, respectively, at the end of the fermentation period. In the

225   exotic litter, decay progressed slowly during the first 60 days. Afterwards the rate of substrate

226   decomposition caused by *L. ceres* and *M. haematocephalus* increased till the end of the

227   experiment, reaching 22.90 ± 1.79% and 31.46 ± 2.15% dry mass loss, respectively. On the

228   contrary, in *M. candidus*, a plateau in decomposition rate was detected from day 90 onwards,

229   with 7.52 ± 1.49% final mass loss. Decomposition constants (*k*) followed a decreasing order: *M.*

230   *haematocephalus > L. ceres* growing in the exotic litter *> M. candidus > L. ceres* cultivated in

231   the native one (Table 1). Significant differences in *k* values between litter species were found

232   only for *L. ceres*, causing faster daily decay rate in the exotic litter (1.39×10^{-3} day^{-1}) than in the

233   native one (0.52×10^{-3} day^{-1}).

234   ***Chemical composition of the WSF and SF from litter***

235   The chemical composition of the WSF extracted from native and exotic litter,

236   respectively, was notoriously different. The first one was more alkaline (8.32 ± 0.01 vs. 5.08 ±

237   0.03; *P* < 0.0001) and also had a lower sugar content (4.03 ± 0.59 mg/g vs. 45.07 ± 1.51 mg/g; *P*

238   < 0.0001), proteins (9.48 ± 0.88 mg/g vs. 11.13 ± 0.32 mg/g; *P* < 0.0001), ammonium-N (0.07 ±

239   0.01 mg/g vs. 0.25 ± 0.02 mg/g; *P* < 0.0001) and phenolic compounds (4.77 ± 0.31 mg/g vs. 8.30

240   ± 0.26 mg/g; *P* < 0.0001). Changes in chemical characteristics of leaf litter WSF over time are

241   presented in Fig. 2. The native litter incubated with *Leratiomyces ceres* exhibited a slight

242   increase in pH values. In the exotic litter cultures, strong alkalization of the substrate was

243   observed, especially with *Marasmiellus candidus*, which markedly increased pH within the first

244   30 days of the experiment. Sugar content in the native litter showed few changes along the SSF

245   with *M. candidus* and *L. ceres*, but when decayed by *Marasmius haematocephalus*, a significant

246   increase was observed after day 60. In the exotic litter, water-soluble sugar content was about 10

247   times higher than in the native one, but it was rapidly consumed and almost exhausted within 60

248   days. Ammonium-N values detected in WSF showed a tendency to increase over time. In the

249   case of *Celtis tala* inoculated with *Marasmius haematocephalus* and *Marasmiellus candidus*, the

250   increment occurred in the initial phase of the SSF, and ammonium levels remained constant once

251   maximum values were attained. In *L. ceres*, a peak at day 90 was detected and after 120 days,

252   ammonium concentration started to rise. For *Ligustrum lucidum* treatments, a first decline in

253   ammonium content was observed. Nevertheless, after 120 days, it started to increase when

254   incubated with *Marasmius haematocephalus* and *Leratiomyces ceres*. Water-soluble protein

255   levels registered a maximum at day 30 in the treated native litter and in the exotic one inoculated

256   with *Marasmiellus candidus* as well, although differences were not statistically significant in the

257   first case. A major increase in protein levels was recorded in the native litter incubated with *M.*

258   *haematocephalus* at initial fermentation stages, remaining constant after day 60. Phenolic

259   compounds in WSF showed no significant changes in the native litter decomposed by *L. ceres*,

260   while incubation with *M. candidus* increased total phenolics in WSF at day 200. On the contrary,

261   total phenolics showed a drastic decrease in the exotic litter and to a lesser extent in the native

262   one when decayed by *M. haematocephalus.*

263   ^{13}C CPMAS NMR spectra shown in Fig. 4 correspond to SF from uninoculated *Celtis*

264   *tala* and *Ligustrum lucidum* litter (initial litter samples) and litter obtained after 200 days-SSF

265   with each of the saprotrophic fungi evaluated. All spectra exhibited the same pattern, differing in

266   the relative intensity of the various chemical shift regions. Important differences were found in

267   spectra signals among uninoculated litter types in all the regions that were considered for

268   integration and in all the ratios and indexes estimated as well, except for the O-alkyl C region (*P*

269   = 0.15, Table 2, Table 3). A larger proportion of alkyl C was found in *L. lucidum* (33.67%) rather

10

270 than in *C. tala* (24.22%) uninoculated litter. The intensity in this region can be attributed to

271 aliphatic components, mostly methylene structures of lipids, cutins and waxes as well as short-

272 chains of acids or amino acids. At the end of the SSF, an increase in their relative content was

273 observed in *Ligustrum lucidum* inoculated with *Leratiomyces ceres* and *Marasmius*

274 *haematocephalus*. Methoxyl carbons, principally of lignin, were detected at 45-60 ppm, although

275 around 10% were identified as methoxyl groups in some of the less common monomer units of

276 hemicellulose, specifically the glucuronic acid in xylan (Haw *et al*. 1984). Methoxyl carbons

277 content in fresh *C. tala*, was found to be 4 times higher than in *L. lucidum.* In *L. lucidum* a

278 significant increase in methoxyl carbons content was detected by the end of the SSF, only when

279 litter was inoculated with *Leratiomyces ceres*. Signal of the O-alkyl C region constituted the

280 largest proportion in fresh litter and was attributed to polysaccharides, comprising 55.60% in *C.*

281 *tala* and 48.27% in *L. lucidum.* Mainly, two dominant peaks were found, a wider one at 70-80

282 ppm, an apparent doublet that was reported to correspond to C-2, C-3, and C-5 carbons of

283 cellulose (I) and a peak at 105 ppm that was generally assigned to the C-1 carbon of the

284 anhydroglucose repeating unit of the cellulose (I) (Haw *et al*. 1984). The relative content

285 declined by the end of the SSF mainly in *Ligustrum lucidum* inoculated with *Leratiomyces ceres*

286 (Fig. 1, Table 2). As the decomposition preceded the alkyl C/O-alkyl C ratio, also known as

287 humification degree, it increased in *M. haematocephalus* mainly growing in the exotic but also in

288 the native litter, and in *L. ceres* it grew in the exotic one. No significant differences in the alkyl

289 C/O-alkyl C index were found in the rest of the fungal-litter combinations (Table 3). The

290 intensity in the spectral region from 110 ppm to 160 ppm was due to the aromatic ring carbons of

291 lignin. The phenolic region, between 140 and 160 ppm, characteristic of oxygen-substituted

292 aromatic ring carbons, was negligible in our litter spectra. Almost twice the content of aromatic

293   carbons was detected in fresh exotic *L. lucidum* (4.75%) compared to native *C. tala* (2.45%),

294   which is consistent with a higher aromaticity and lignin index. Aromatic C content decreased by

295   the end of the decomposition period as a consequence of lignin-degrading fungal activity and

296   maximal chemical shifts were registered in *M. haematocephalus*, which completely delignified

297   all *L. lucidum* substrate. Finally, signals at 160-190 ppm were attributed to acetate groups known

298   to be present in hemicellulose or carbonyl species also existing in hemicellulose (e.g., CO_2H

299   groups of uronic acids). In *C. tala* inoculated with *Marasmius haematocephalus*, an increment

300   was observed, while in *L. lucidum* the content of carboxyl carbons declined when it was

301   inoculated with *Marasmiellus candidus*.

302   ***Enzyme activities in the WSF***

303   Endoglucanase activity was significantly different among substrates in every sample time

304   (Fig. 3A, *P* < 0.05) with higher values detected when using the exotic litter and *Leratiomyces*

305   *ceres* being the largest producer (7.55 ± 0.99 U/g). In the native litter, *Marasmius*

306   *haematocephalus* recorded the highest endoglucanase activity. This treatment also showed a β-

307   glucosidase activity that stood out from the rest of fungal-litter combinations, with maximum

308   values of 2.80 U/g approximately recorded upon 120 days (Fig. 3B). Furthermore, *M.*

309   *haematocephalus* and *L. ceres* growing in the exotic litter were the second and third major β-

310   glucosidase producers, respectively. Endoxylanase activity showed a peak at day 30 when

311   cultivating *M. haematocephalus* in the native litter (Fig. 3C, 6.09 ± 0.31 U/g); while a constantly

312   increasing activity was detected in the other treatments with maximal enzyme activity at day 200

313   in *L. ceres* growing in the exotic substrate. Ligninolytic activity was represented mostly by *L.*

314   *ceres* (Fig. 3D, Fig. 3E). When cultivated in the exotic litter, MnP activity was remarkably

315   higher than in the rest of fungal-litter treatments while laccase activity was detected as a first

316   peak produced at day 30. In the native litter, laccase production was greater but the uppermost

317   values were detected at advanced stages of fermentation.

318   *Coinertia Analysis (CoiA)*

319   Some of the chemical variables evaluated at the end of the SSF were highly correlated

320   with the enzyme activities (Supplementary Table S1[1]). The alkyl C/O-alkyl C ratio (humification

321   degree) positively correlated with endoglucanase ($r = 0.82$; $P < 0.01$), endoxylanase ($r = 0.65$; P

322   < 0.05) and MnP activities ($r = 0.62$; $P < 0.05$). The last activity was sensitive to this index

323   despite no significant correlations were observed for its individual components. Endoglucanase

324   ($r = -0.76$; $P < 0.01$) and MnP ($r = -0.84$; $P < 0.001$) activities were also correlated with pH, with

325   greater activities in the acidic values. β-glucosidase activity was strongly correlated with the

326   content of ammonium in the WSF ($r = 0.87$; $P < 0.001$). Nevertheless, laccase activity did not

327   correlate with any of the chemical variables measured. As there was multicollinearity among

328   chemical variables, we decided to select only the non-collinear and "representative" ones to

329   reduce the complexity of the variable matrix for the coinertia analysis. From them, the envfit

330   analysis showed that O-alkyl C proportion, alkyl C/carboxyl C ratio, remaining mass degree, pH,

331   content of phenolics and proteins were the variables that significantly corresponded to overall

332   enzyme activities (Supplementary Table S2). The projected inertia with two axes (Fig. 5A, Ax1

333   and Ax2) represented 90.86% of the total covariance (Fig. 5C). The first axis separated

334   *Leratiomyces ceres* growing on the exotic litter from all the other treatments and responded to a

335   high alkyl C/carboxyl C ratio, more acidic conditions and a major decrease in the phenolic

336   compounds content, principally due to the activities of endoenzymes and MnP (Fig 5B, Fig. 5D).

337   The second axis separated the other fungal species, with *Marasmius haematocephalus* and

338   *Marasmiellus candidus* having opposite scores. *M. haematocephalus* correlated well with β-

339   glucosidase activity and protein content and showed greater mass loss, while *M. candidus* was

340   associated with high chemical scores and low mass loss. Seventy-six percent of the variability

341   was explained jointly by the two sets of variables (Supplementary Fig. S1).

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343   [1] Supplementary material is available with the article through the journal Web site.

344   **Discussion**

345   Below-ground litter decomposition is hierarchically controlled by climate, chemical

346   composition of the substrate and by soil biota (Swift *et al*. 1979; Coûteaux *et al*. 1995; Cadish

347   and Giller 1997; Hättenschwiler *et al.* 2005). In our study, species-specific responses in litter

348   decomposition as a function of litter chemistry could be detected, because species were

349   separately analyzed in environmentally controlled conditions. Litter decomposition rates varied

350   according to the fungus employed in the SSF and only for *Leratiomyces ceres,* significant

351   differences were observed depending on the litter employed, with higher degradation of the

352   exotic *Ligustrum lucidum* litter.

353   Previous studies recording *Ligustrum lucidum* decomposition detected a *k* of 11.20×10^{-3}

354   day^{-1} in a natural lowland stream from Buenos Aires (Marano *et al.* 2013). This value exceeded

355   the *k* value of the native *Pouteria salicifolia* leaf litter, and it was related to the better quality of

356   the substrate (less lignin and phenolic compounds, lower L:N ratio, similar C:N and higher C:L

357   proportion). Aragón *et al*. (2014) proved that in a native secondary forest *L. lucidum* litter

358   decomposed faster than the litter from the native species *Ocotea porphyria* and *Cupania*

359   *vernalis*, reaching a *k* mean value of $3.018 \ year^{-1}$ ($8.26 \times 10^{-3} \ day^{-1}$). Moreover, Fernandez *et al*.

360   (2017) suggested that *L. Lucidum* invasion altered the abundance and composition of fungal

361   decomposers associated with the secondary forest mentioned. Several studies proposed that alien

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362   species possess common litter traits that allow them to decompose faster than the native species

363   co-occurring with them, promoting invasion and nutrient cycling (Allison and Vitousek 2004;

364   Liao *et al*. 2008). However, taking into account that other studies showed opposite results

365   (Godoy *et al.* 2010; Knight *et al.* 2007), generalizations concerning the comparative

366   decomposition of native and exotic litters cannot be made. As far as we know, there are no

367   reports on *Celtis tala* decomposition rates and only its degradation by ascomycetes was

368   investigated (Saparrat *et al.* 2008). In comparison, our *k* for *L. lucidum* was considerably lower

369   than *k* values obtained in other studies in natural environments. Such differences are expected

370   since in our experimental approach, degradation was the result of the activity of a single fungus

371   in axenic conditions. In this work, mass loss obtained at day 200 in SSF ranged from 7.50 up to

372   31.50% (Fig. 1), but reached around 20% after 120 days with some litter/fungus combinations i.e

373   *Marasmius haematocephalus* growing in *C. tala* litter and *Leratiomyces ceres* cultivated in

374   *Ligustrum lucidum* litter. An average of 16.60% mass loss was reported for *Chamaecyparis*

375   *obtuse* needle litter degradation by several litter saprotrophic basidiomycetes after 18 weeks in a

376   pure-culture test (Osono *et al*. 2006). Also a mass loss of 16 to 34% was caused by *Hypholoma*

377   *fasciculare* growing in different litter types for 12 weeks under SSF (Voříšková *et al*. 2011).

378   It is assumed that at early stages of decomposition, mass loss is dominated by the

379   degradation of soluble substances and holocellulose that is not protected by lignin (Berg and

380   McClaugherty 2014). A considerable fraction of soluble substances is also leached to the soils.

381   Concurrently, the sugar content in WSF (resulting from easily degradable C compounds) was 10

382   times higher in the exotic *Ligustrum lucidum* than in the native *Celtis tala* (Fig. 2). This larger

383   content of sugars may have supported the initial growth in *L. lucidum* (higher endoglucanase

384   activities responsible for cellulose degradation were detected as well). At the same time, initial

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385   degradation rates are positively affected by an increase in the levels of key nutrients such as S, P

386   and N (Berg 2014), the latter one considered a critical element for litter-decomposing

387   basidiomycetes. Simultaneously, low values of C:N have been generally associated with fast

388   decomposition (Lambers *et al*. 2008). N content in *C. tala* was reported to be around 1% and

389   C:N ratio equal to 27 (Saparrat *et al*. 2008), while Aragón *et al*. (2014) informed 0.82% N

390   content for *L. lucidum* and a C:N ratio of 53.72. During fungal cultivation on *L. lucidum*,

391   ammonium was not detected in the WSF [excluding the end of SSF by *M. haematocephalus* (Fig.

392   2)] suggesting no net mineralization of the N initially present in the litter (in the form of amino

393   acids and proteins) or a net N immobilization in fungal biomass. It was reported that when C:N is

394   high, a substantial fraction of initial nutrients from organic matter can be immobilized into

395   fungal hyphae and converted to growth (Swift *et al*. 1979). The differences in ammonium-N

396   levels detected may indicate a differential availability of N depending on the type of substrate

397   and therefore, N can be considered limiting in *L. lucidum* compared to *C. tala*. This is in

398   agreement with a greater mass loss in *C. tala* at early stages of SSF. In advanced stages of

399   decomposition, mass loss is more limited and involves the degradation of lignin and lignified

400   compounds (Berg 2014). *L. lucidum* litter showed higher values of relative area of aromatic and

401   aliphatic compounds than *C. tala* as well as a higher content of water-soluble phenolic

402   compounds (Table 2, Fig. 2), compounds that are recalcitrant and are known to delay the decay

403   process (De Marco *et al*. 2012). Lignin, aromaticity indexes and phenolic compounds content of

404   the WSF decreased by the end of culture period in *L. lucidum*, revealing the activity of lignin-

405   degrading fungi (Table 3, Fig. 2). Thus, mass loss was higher in *L. lucidum* during the final

406   stages of SSF. Ammonium detected in *C. tala* at the end of the SSF, derived from organic-N

407 degradation, could affect lignin degradation and decay rate in this substrate. Some litter fungi are

408 known to repress the expression of ligninolytic enzymes when labile N is present (Berg 2014).

409 During the course of decomposition of soil organic matter, a rise in the alkyl C/O-alkyl C

410 ratio has been proposed, as O-alkyl C is converted to alkyl C (Baldock *et al*. 1997). In this work,

411 the index shown to be a reliable predictor as a substantial increment was observed at the end of

412 the SSF in those treatments that recorded the highest percentages of degradation, namely

413 *Marasmius haematocephalus* in both substrates and *Leratiomyces ceres* in the exotic litter (Table

414 3). No significant changes in the alkyl C/O-alkyl C ratio were found in the rest of treatments with

415 respect to the uninoculated litter. Since a loss in the O-alkyl C region is mainly associated to

416 cellulose and/or hemicellulose degradation, our results suggest that at least *M. haematocephalus*

417 and *L. ceres* were active degraders of holocellulose, being the former less selective of substrate

418 type. Therefore, these fungi could have a role in humification due to the increase in the alkyl C

419 domain, which is mostly linked to the accumulation of recalcitrant structural components present

420 in plant litter such as that of waxes, cutin, suberin, lipids and amino acids (Kögel-Knabner *et al*.

421 1992).

422 Changes in the activity of the extracellular enzymes that degrade the main components of

423 soil organic matter have been associated to variations in rates of decomposition and soil carbon

424 storage (Sinsabaugh *et al*. 2008). Extracellular enzyme activities are also sensitive indicators of

425 the effort directed by microorganisms towards obtaining carbon, nitrogen, or phosphorus from

426 specific sources (Veres *et al*. 2015). In our work, we detected a noteworthy difference in β-

427 glucosidase production by *Marasmius haematocephalus* with high titers when growing on *Celtis*

428 *tala* but not on *Ligustrum lucidum* (Fig. 3). The reducing sugars content in the WSF of *L.*

429 *lucidum* at initial SSF stages may suggest a byproduct inhibition mechanism of the β-glucosidase

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430   activity in this substrate (Xiao *et al*. 2004) since when sugar levels declined, at the end of culture

431   period, β-glucosidase activity started to rise. The low levels of sugars present in *C. tala* leaf litter

432   may have allowed *M. haematocephalus* to produce a significant amount of β-glucosidase (high

433   protein levels in the WSF) with a consequent increase in glucose levels after 60 days of

434   cultivation. Accordingly, these variables significantly correlated at the end of the SSF ($r = 0.69$,

435   Table S1). Saparrat *et al.* (2008) found that β-glucosidase activity was correlated with *C. tala*

436   leaf litter decomposition. On the other hand, while in *M. haematocephalus* cultures developing

437   on *C. tala,* delignification was not significant, this fungus caused a complete delignification on *L.*

438   *lucidum* substrate, which could compensate *k* and mass loss values between both litter types.

439   Because laccase and MnP activities were negligible, lignin degradation could involve other

440   peroxidases found, expressed by litter decomposing fungi such as the dye-decolorizing

441   peroxidase (DyP, EC 1.11.1.19) and the unspecific peroxygenase (UPO, EC 1.11.2.1) (Kellner *et*

442   *al*. 2014) or other mechanisms such as Fenton reaction, in which degradation involves the

443   generation of low molecular highly oxidizing species (Regalado *et al*. 1999). MnP production by

444   *Leratiomyces ceres* increased only when the fungus was growing on *Ligustrum lucidum* litter

445   (Fig. 5). Considering that MnP is one of the key oxidative enzymes in litter degradation and

446   lignin content is the component most often identified as a limiting factor in decomposition

447   (Purahong *et al*. 2014), MnP activity found in this treatment could possibly explain the important

448   mass loss values, as it is shown in the coinertia analysis. Laccase activity could be detected as

449   well, being higher in the native litter than in the exotic one. Laccases are only considered as

450   lignin-modifying enzymes because their redox potential is lower than that of peroxidases, and are

451   thought to be involved in polymerization–depolymerization processes of lignin, melanin and

452   humic substances (Liers *et al*. 2011). Since no specific pattern related with degradation was

453   observed in our study, the detected activity could be associated with other functions adjudicated

454   to laccase, such as its role in the morphogenesis and differentiation of sporulating and resting

455   structures in basidiomycetes, pigment formation and detoxification (Mayer and Staples 2002).

456   Environmental pH affects fungal growth and enzyme activity, with the majority of fungi

457   having pH optima in the acidic values (Dix and Webster 1995). Nevertheless, some prefer

458   neutral to slightly alkaline conditions, i.e. some *Coprinus* species. Initial pH of *Celtis tala* was

459   8.32 and values remained alkaline throughout all SSF. Fungi growing on *Ligustrum lucidum*

460   started with an acidic initial pH of 5.08 but a general tendency to alkalization was observed,

461   mainly by *Marasmius haematocephalus* and *Marasmiellus candidus,* the latter raised the pH to

462   8.27 within the first 30 days (Fig. 2). Our results suggest that the enzyme system of some of

463   these fungi was highly active even at alkaline pHs. Thus, the stability of *M. haematocephalus* β-

464   glucosidase growing in *C. tala* was tested in a pH range of 4.8-9, retaining more than 50% of its

465   relative activity after 12 h of incubation (Mallerman 2017). Conversely, *Leratiomyces ceres*

466   might be sensitive to alkaline pHs and favored by the introduction of *Ligustrum lucidum*, which

467   provides a lower pH. In agreement a strong correlation between pH and MnP activity was

468   detected, mainly associated with this fungus ($r = -0.84$, Table S1). Resistant enzymes adapted to

469   alkaline pHs, such of the one found in *M. haematocephalus*, have great potential for basic

470   research and industrial applications. The present work explores the lignocellulolytic abilities of

471   species scarcely investigated until now.

472   Our study involved a long-term, laboratory, microcosm approach to determine the impact

473   of two sorts of leaf-litter, one native and another exotic, on mass loss and activity of selected

474   lignocellulolytic saprotrophic basidiomycetes. Enzymatic assays were conducted using optimal

475   parameters and reproducible assay conditions that do not occur *in situ* (such as incubation

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476   temperature and reaction pH), in order to measure not only the maximum potential enzyme

477   activity (Dick 2011) but also to allow the comparison of enzyme activities among different

478   studies (Nannipieri *et al*. 2012). In addition, the alteration of chemical and physical properties of

479   the litter by autoclaving cannot be ruled out (Berns *et al*. 2008). However, such non-target effects

480   were consistent within a given litter type or fungal culture in our study. Therefore, our findings

481   could not be explained.

482   Litter decay is the result of an active cross-kingdom functional succession and bacteria

483   may also significantly contribute to decomposition (Purahong *et al*., 2016). Enzymes involved in

484   cellulose, hemicellulose, chitin and starch catabolism were detected in litter bacteria proteomes

485   (López-Mondéjar *et al*., 2016). Lignin degradation appears to be limited when compared with the

486   abilities of saprotrophic fungi, nevertheless *Actinobacteria* may add to the solubilisation of

487   phenolics (Větrovský *et al*., 2014). Thus, the lack of bacteria may also contribute to the low *k*

488   rates attained in this work. Bacterial-fungal co-culturing could aid in clarifying their possible

489   interacting roles in lignocellulose complex breakdown during plant litter decomposition.

490   In conclusion, native and exotic litter chemistry differentially affects decomposition rates

491   and enzyme production by common lignocellulolytic saprotrophic basidiomycetes. The

492   introduction of *Ligustrum lucidum* to pristine environments could alter the chemical composition

493   of litter input to forest soils. Preliminary data from our *in-vitro* litter degradation study suggests

494   that an impact on litter decomposition rates should be expected, as some common saprotrophic

495   basidiomycetes such as *Leratiomyces ceres* are present, driving to accelerated litter

496   decomposition through an increased ligninolytic activity. Although *Marasmius haematocephalus*

497   and *Marasmiellus candidus* were less sensitive to alterations in litter quality, the three fungi

498   assayed showed significant changes in their pattern of extracellular lignocellulolytic enzyme

499 production with a concomitant modification in nutrient dynamics. Further research is needed to

500 get a better understanding of the role that basidiomycetous fungi and their extracellular enzyme

501 systems play in degradation of soil organic matter in terrestrial ecosystems, as well as to predict

502 potential effects of plant invasion on ecosystems processes and develop management strategies.

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Table 1. Daily decomposition constants (k) in native *C. tala* and exotic *L. lucidum* litter under SSF conditions.

Regression Parameters	C. tala			L. lucidum		
	M. haematocephalus	*M. candidus*	*L. ceres*	*M. haematocephalus*	*M. candidus*	*L. ceres*
Slope (k)	$1.42 \times 10^{-3} \pm 0.10 \times 10^{-3}$[ab]	$0.6 \times 10^{-3} \pm 0.10 \times 10^{-3}$[bc]	$0.52 \times 10^{-3} \pm 0.08 \times 10^{-3}$[d]	$1.98 \times 10^{-3} \pm 0.16 \times 10^{-3}$[a]	$0.53 \times 10^{-3} \pm 0.06 \times 10^{-3}$[cd]	$1.39 \times 10^{-3} \pm 0.07 \times 10^{-3}$[ab]
y-Intercept	$0.12 \times 10^{-2} \pm 1.08 \times 10^{-2}$	$2.74 \times 10^{-2} \pm 1.04 \times 10^{-2}$	$-0.74 \times 10^{-2} \pm 0.89 \times 10^{-2}$	$5.41 \times 10^{-2} \pm 1.74 \times 10^{-2}$	$1.03 \times 10^{-2} \pm 0.67 \times 10^{-2}$	$-1.56 \times 10^{-2} \pm 0.95 \times 10^{-2}$
Adjusted R^2	0.92	0.69	0.70	0.90	0.78	0.93
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Note: Decomposition constants among fungal-litter treatments were tested using a two-way ANOVA ($n = 3$; $P < 0.05$). Different letters indicate significant differences in decomposition rates among fungal-litter treatments (Tukey test; $\alpha = 0.05$).

Table 2. Relative intensities (%) derived from the ^{13}C CPMAS NMR spectra of litter samples at initial (0 days) and final time (200 days) in SSF.

| Culture time	Fungi	Litter type	Region of spectra (ppm)				
			Alkyl C 0–45	Methoxyl 45–60	O-alkyl C 45–110	Aromatic C 110–140	Carboxyl C 160–190
0 days			24.22 ± 0.07	7.74 ± 0.18	55.60 ± 0.01	2.45 ± 0.06	17.71 ± 0.03
	M. haematocephalus	*C. tala*	26.18 ± 1.00	4.21 ± 2.27	41.24 ± 4.81	2.09 ± 0.71	30.47 ± 3.12
200 days	*M. candidus*		20.73 ± 6.33	5.77 ± 1.94	59.95 ± 10.28	1.78 ± 1.67	17.52 ± 2.26
	L. ceres		23.38 ± 5.08	5.33 ± 2.56	57.55 ± 6.96	1.77 ± 0.85	17.29 ± 1.00
0 days			33.67 ± 1.81	1.65 ± 0.42	48.27 ± 2.51	4.75 ± 0.11	14.30 ± 0.81
	M. haematocephalus	*L. lucidum*	49.95 ± 4.57	2.38 ± 0.13	38.66 ± 4.23	0.00 ± 0.01	11.38 ± 0.34
200 days	*M. candidus*		32.41 ± 3.00	2.60 ± 1.70	56.28 ± 1.50	2.15 ± 0.03	9.16 ± 1.49
	L. ceres		45.87 ± 1.18	6.48 ± 1.19	37.44 ± 1.21	3.61 ± 0.09	13.06 ± 0.12

Note: Data are mean (n = 2) ± standard error. Phenolic C region (140-160 ppm) of spectra was negligible

Table 3. Various ratios, lignin content (%), and aromaticities (%) derived from the ^{13}C CPMAS NMR spectra of litter samples at initial (0 days) and final (200 days) culture time in SSF.

| Culture Time	Fungi	Litter Type	Ratios			%	
			Alkyl C/ O-Alkyl C	Alkyl C/ Carboxyl C	O-alkyl C/ Aromatic C	Lignin	Aromaticity
0 days			0.43 ± 0.01	1.37 ± 0.01	22.65 ± 0.59	4.51 ± 0.13	2.73 ± 0.08
	M. haematocephalus	*C. tala*	0.64 ± 0.10	0.86 ± 0.06	21.32 ± 9.57	4.71 ± 1.65	2.85 ± 1.00
200 days	*M. candidus*		0.36 ± 0.17	1.17 ± 0.21	64.89 ± 66.68	3.35 ± 3.15	2.03 ± 1.91
	L. ceres		0.41 ± 0.14	1.35 ± 0.22	37.76 ± 22.12	3.32 ± 1.55	2.01 ± 0.94
0 days			0.68 ± 0.07	2.28 ± 0.01	10.16 ± 0.29	8.99 ± 0.17	5.44 ± 0.10
	M. haematocephalus	*L. lucidum*	1.31 ± 0.26	4.40 ± 0.53	ND*	0.00 ± 0.01	0.00 ± 0.01
200 days	*M. candidus*		0.58 ± 0.07	3.61 ± 0.92	26.17 ± 0.35	3.81 ± 0.18	2.30 ± 0.11
	L. ceres		1.23 ± 0.07	3.51 ± 0.06	10.36 ± 0.07	6.40 ± 0.17	3.87 ± 0.10

Note: Data are mean ($n = 2$) ± standard error. ND indicate a ratio value tending to infinite as aromatic C was absent in this treatment.

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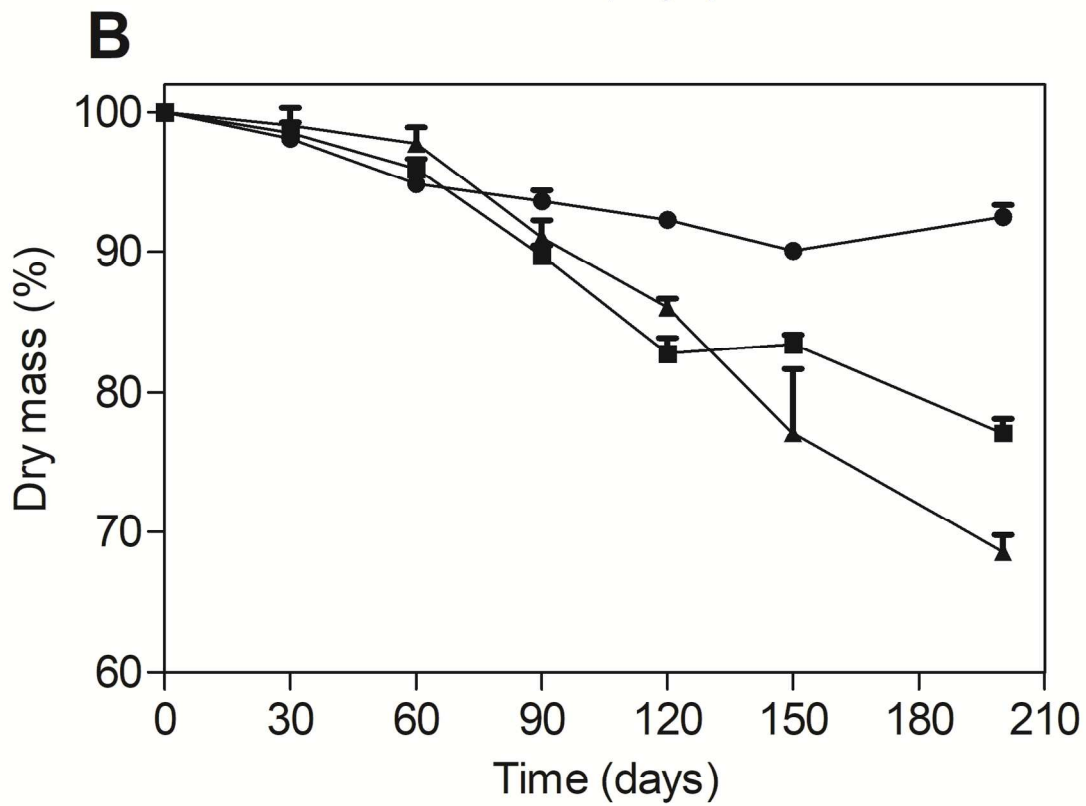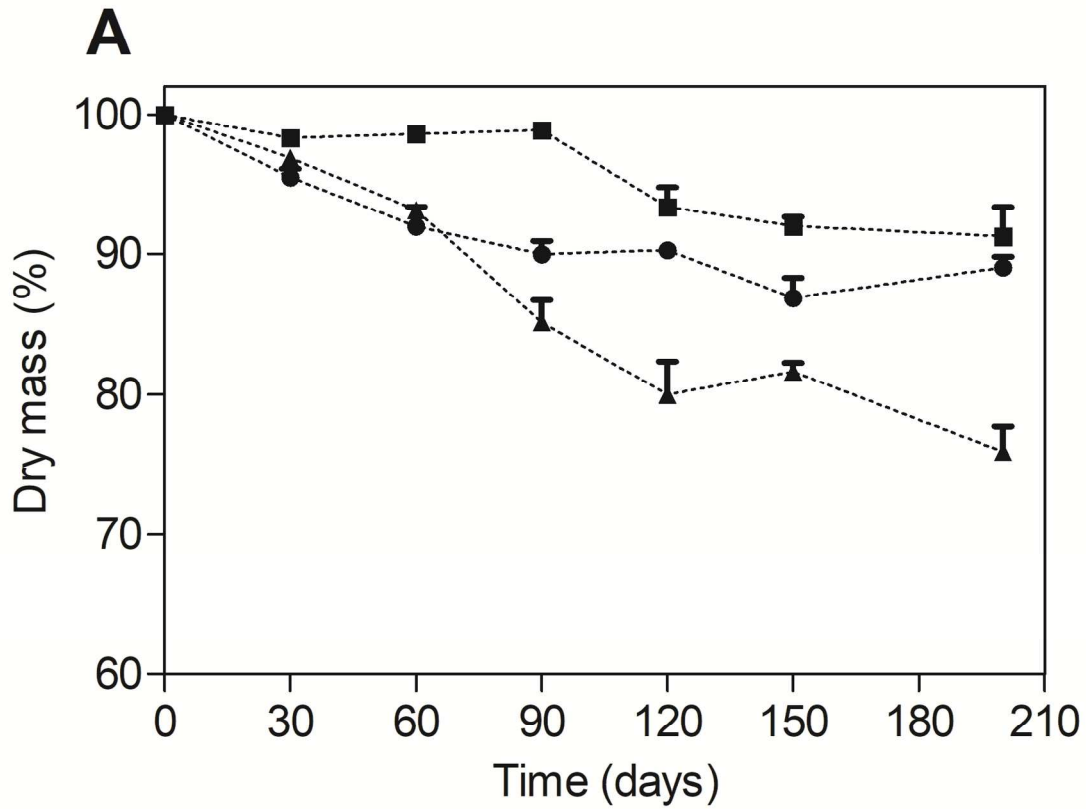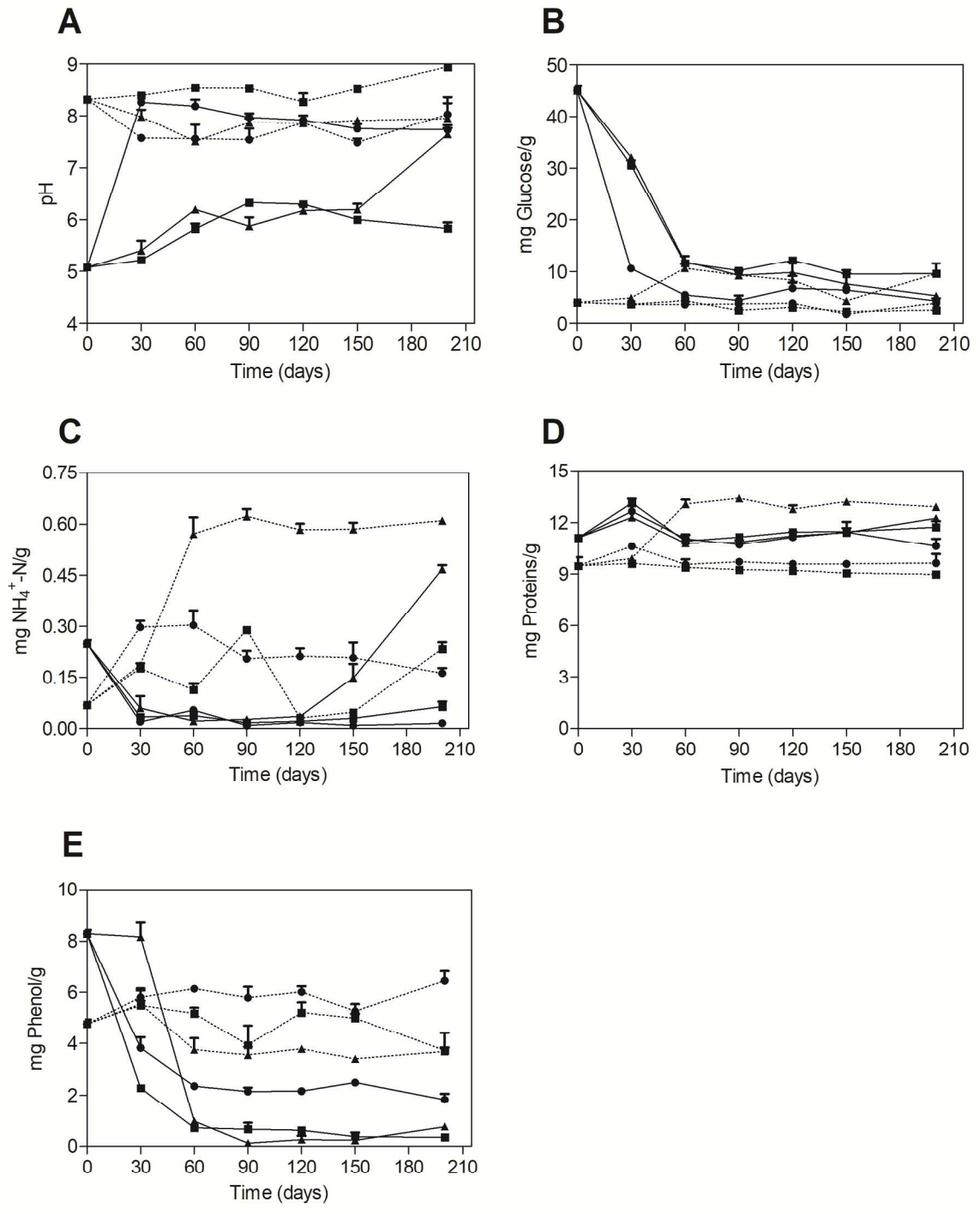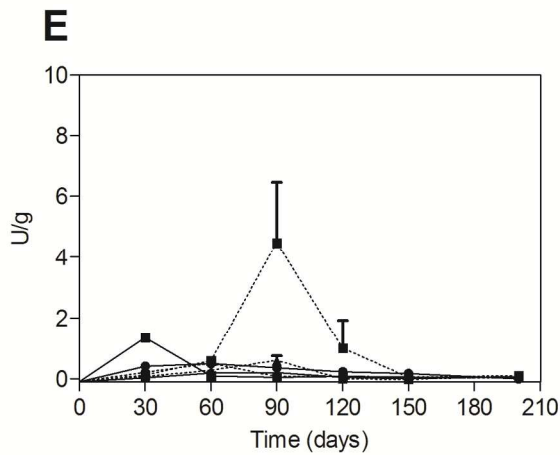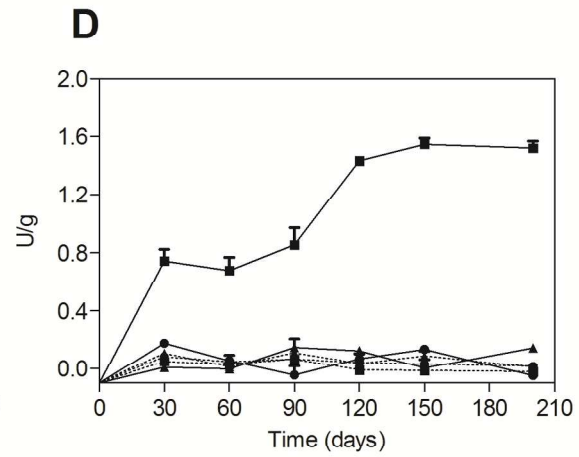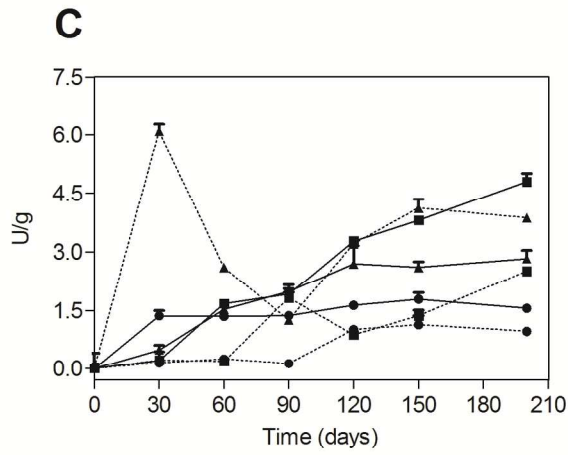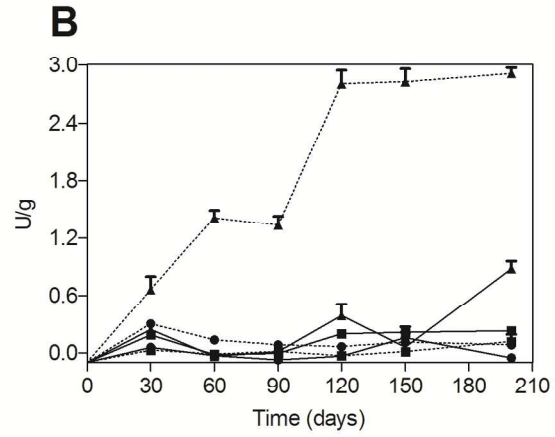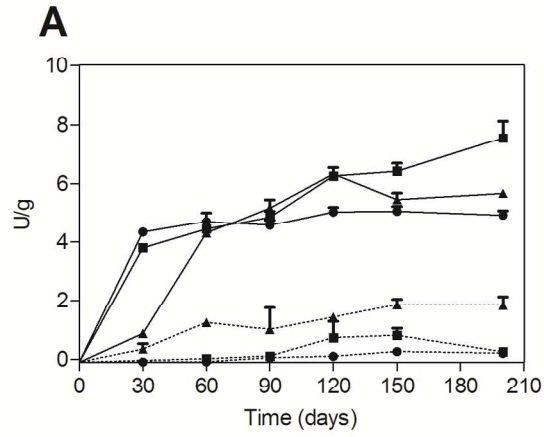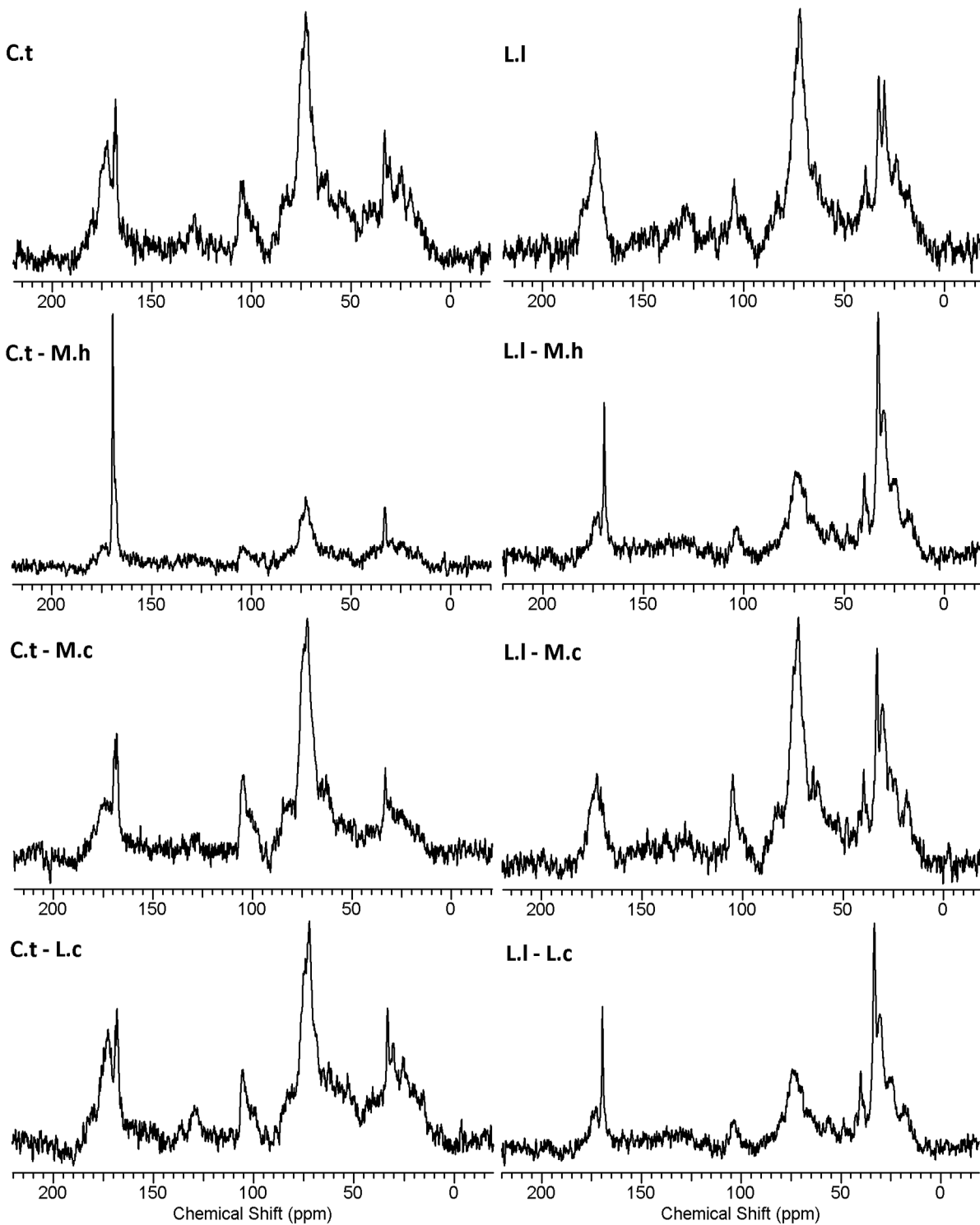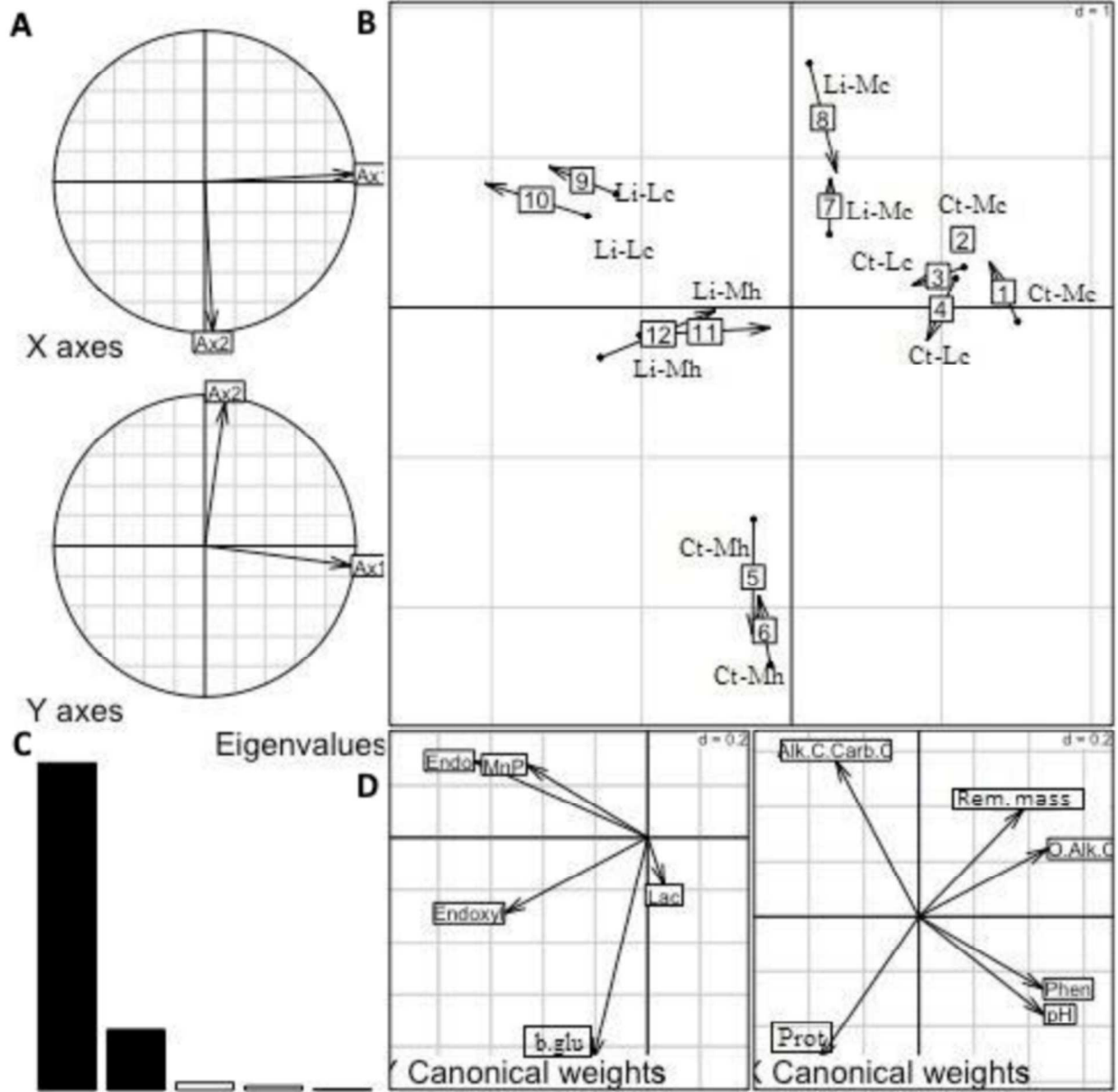
LEGEND TO THE FIGURES

Fig. 1 - Dry mass remaining during 200 days in SSF in (A) *Celtis tala* **and (B)** *Ligustrum lucidum* **litter inoculated with** *Marasmiellus candidus* **(circles),** *Leratiomyces ceres* **(squares), and** *Marasmius haematocephalus* **(triangles). Data are the mean of three replicates, one standard error of the mean is represented.**

Fig. 2 - Characterization of the water soluble fraction during 200 days in SSF with *Celtis tala* **(dotted lines) or** *Ligustrum lucidum* **litter (solid lines) and inoculated with** *Marasmiellus candidus* **(circles),** *Leratiomyces ceres* **(squares) or** *Marasmius haematocephalus* **(triangles): (A) pH (B) Glucose (C) Ammonium-N (D) Proteins (E) Phenolic compounds. Data are the mean of three replicates, one standard error of the mean is represented.**

Fig. 3 - Analysis of enzyme activities related to lignocellulose system during 200 days in SSF with *Celtis tala* **(dotted lines) or** *Ligustrum lucidum* **litter (solid lines) and inoculated with** *Marasmiellus candidus* **(circles),** *Leratiomyces ceres* **(squares) or** *Marasmius haematocephalus* **(triangles): (A) Endoglucanase (B) β-glucosidase (C) Endoxylanase (D) Mn Peroxidase (E) Laccase. Data are the mean of three replicates, one standard error of the mean is represented.**

Fig. 4 - ^{13}**C CPMAS NMR spectra of initial litter samples (C.t and L.l) and after 200 days in SSF with the selected basidiomycetous fungi (C.t-M.c, L.l-M.c, C.t-L.c, L.l-L.c, C.t-M.h and L.l-M.h): C.t,** *Celtis tala***; L.l,** *Ligustrum lucidum***; M.c,** *Marasmiellus candidus***; L.c,** *Leratiomyces ceres***; M.h,** *Marasmius haematocephalus***. Spectra chemical shift range of litter was characterized by the following dominant peaks: alkyl C (0–45 ppm), O-alkyl C**

(45–110 ppm), methoxyl C (50–60 ppm), aromatic C (110–140 ppm), phenolic C (140–160 ppm) and carboxyl C (160–190 ppm).

Fig. 5 - Coinertia analysis using PCA-PCA of the chemical parameters (analytical determinations of the WSF and NMR results of the SF) and the lignocellulolytic enzymes in the WSF produced by the selected basidiomycetous fungi after 200 days in SSF. The analysis represents the individual treatments (from 1 to 12; n = 2) corresponding to the substrate (C.t, *Celtis tala;* **L.l,** *Ligustrum lucidum***) inoculated with the fungal species (M.c,** *Marasmiellus candidus***; L.c,** *Leratiomyces ceres***; M.h,** *Marasmius haematocephalus***). (A) Inertia projection of the chemical and enzymatic parameters (B) Biplot showing the covariance optimization by the coinertia (C) The eigenvalues showing the total variance and the eigenvalues considered in the analysis (D) The Y and X loadings (canonical weights) for PC1 (chemical parameters: Alk.C.Carb.C., Alkyl C/Carboxyl C; Rem. mass, Remaining mass; O. Alk. C, O. Alkyl C; Phen, Phenolic compounds; Prot, Preteins; pH) and PC2 (enzymatic activities: Endo, Endoglucanase; Endoxy, Endoxylanase; b.glu, β glucosidase; MnP, Mn-Peroxidase; Lac, Lacasse).**

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