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1 SOILS, SEC # • RESEARCH ARTICLE

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3 ***In situ* and laboratory non-additive litter mixture effect on C dynamics of *Sphagnum***
4 ***rubellum* and *Molinia caerulea* litters**

5

6

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25

26 **Abstract**

27 *Purpose.* The accumulation of carbon in peatlands originates from the slow rate of *Sphagnum*
28 litter decomposition. Vegetation shifts can alter the *Sphagnum* decomposition rate through a
29 litter mixture effect. This is rarely studied in peatlands. In a site colonized by vascular plants, we
30 examined the effect of mixing litters of *Sphagnum* species with those of *Molinia caerulea* and
31 *Betula* spp on litter C dynamics. We tested that water content and pH may explain the potential
32 non-additive litter mixing effect.

33 *Materials and methods.* Litter bags with *S. cuspidatum* or *S. rubellum* and *M. caerulea* or *Betula*
34 spp were placed in situ, in a *Sphagnum* decomposing environment and retrieved after one year
35 of incubation. In the laboratory, the specific interaction between *S. rubellum* and *M. caerulea*
36 was investigated. Solid, soluble and gaseous forms of C were studied in addition to the
37 fluorescence of the dissolved organic matter (FDOM).

38 *Results and discussion.* Laboratory and field experiments showed that there is a non-additive
39 effect of mixing *S. rubellum* and *M. caerulea* litter on C dynamics. The analyses of FDOM
40 suggested a relatively higher living biomass in the laboratory measured mixture than in the
41 expected one. The in situ *S. rubellum* moist environment could stimulate the decomposition of
42 *M. caerulea* that experiences much drier conditions in its native environment. In the laboratory
43 experiment, *M. caerulea* were kept moist, and no significant difference in water content between
44 the measured and expected mixture was found. Also, pH decreased in the measured mixture,
45 ruling out any direct effect on microbial activity.

46 *Conclusions.* The non-additive mixture effect observed in the laboratory may be triggered by an
47 increase of the microbial biomass. This increase was not explained by direct moisture or a pH
48 effect. The suggested hypothesis that a lower pH could affect the availability of labile organic
49 substrate through increased OM hydrolysis and thus stimulate microbial growth has to be
50 further studied.

51

52 **Keywords** Aromaticity • Fluorescence • Litter-mixture effect • Peatland invading species •
53 Water extractable organic matter

54

55 **1 Introduction**

56 Temperature and water regime perturbations are provoking shifts in the limits of plant
57 distribution and changes in plant abundances (Weltzin et al. 2003; IPCC 2007; Limpens et al.
58 2008). These changes will modify the functioning of ecosystems through the occurrences of
59 new interactions (as some plant distribution limits move northward), or the strengthening of
60 previously weak interactions (increased abundances of previously sparse species; Cornelissen
61 et al. 2001). Among ecosystem processes, litter decomposition will be affected by vegetation
62 changes especially because of litter mixture effects (Gartner and Cardon 2004). Litter
63 decomposition is an important component in the functioning of ecosystems as it influences the
64 carbon (C) and nutrient cycles (Berg and McClaugherty 2003). At the local scale, litter
65 decomposition influences primary productivity through the mineralization of nutrients, and at the
66 global scale, it affects the C cycle through soil organic matter (SOM) stabilization and
67 accumulation (Limpens et al. 2008; Bardgett and Wardle 2010). When a vegetation change
68 occurs, new litter mixture effects are expected to occur. These effects can be detected by
69 comparing the measured values of two or more litters in mixture to theoretical values calculated
70 from the same litters incubated in monoculture (Gartner and Cardon 2004). An additive effect is
71 identified when there is no difference between the measured and expected decomposition rate.
72 When a difference is observed, the litter mixture effect is non-additive. It can be synergistic, i.e.
73 stimulation of the decomposition, or antagonistic, i.e. inhibition of the decomposition. Hoorens et
74 al. (2010) showed that the litter mixture effect may be additive at the plant functional level,
75 because these opposite interactions may take place at the same time. However, such an
76 “averaging” effect may be limited in low biodiversity ecosystems. Identifying such a litter mixture
77 effect in ecosystems and understanding the mechanisms behind it is essential to specify which
78 vegetation change can enhance (increased SOM mineralization) or buffer (increased SOM
79 accumulation) greenhouse gas emissions and/or dissolved organic carbon export.

80 To better understand soil C dynamics, Cotrufo et al. (2010) suggested that simultaneous
81 measurements of (i) CO₂ production, (ii) remaining mass and (iii) water extractable organic
82 carbon (WEOC) should be undertaken. As suggested by Schimel and Weintraub (2003), this
83 latter compartment is thought to be the intermediate pool between remaining mass (solid C) and
84 CO₂ production (gaseous C). Following the suggestions of Cotrufo et al. (2010) and after
85 adapting the model of Schimel and Weintraub (2003) to make it experimentally testable, Gogo

86 et al. (2014) showed in laboratory incubation of peatland plant litters that the WEOC
87 compartment is accurately accounted for when it is assumed that it results from the balance
88 between solid OM decomposition and soluble C respiration, and is quantitatively the most
89 sensitive pool to changes in solid OM decomposition and respiration rates. In the context of the
90 litter mixture effect, quantities as well as the chemical characteristics of WEOC are probably
91 affected. Creamer et al. (2015) showed that litter WEOC is more sensitive to temperature
92 change than WEOC from the mineral soil. This fraction may also be sensitive to other modified
93 conditions such as litter mixture with invading species.

94 Peatlands contain up to a third of the world soil C (Gorham 1991). Their accumulation capacity
95 originates from the particularly low decomposition rates of *Sphagnum* species (Clymo 1983).
96 Any modification in their decomposition rate, through the litter mixing effect caused by
97 vegetation change, would thus undermine the regulatory role of peatlands on the global C cycle
98 (Limpens et al. 2008). Hoorens et al. (2002) showed that an increased atmospheric CO₂
99 concentration can lead to an excess microbial respiration in *Sphagnum recurvum* and *Carex*
100 *rostrata* mixtures. In spite of the importance of such a result and the unfolding vegetation
101 change in peatlands, very few studies have actually addressed the issue of a litter mixing effect
102 in such ecosystems.

103 The two aims of this work are (i) to determine the occurrence of a mixture effect of *Sphagnum*
104 and vascular plant litters and (ii) to identify the mechanisms behind such an effect. For this, two
105 experiments were undertaken: a field experiment to test the effect of mixing *Sphagnum*
106 *rubellum* and *cuspidatum* with *Betula* spp and *Molinia caerulea* on litter remaining mass, and a
107 laboratory incubation experiment testing the effect of mixing *Sphagnum rubellum* and *Molinia*
108 *caerulea* on solid, water extractable and gaseous C from these litters. In the latter experiment,
109 the model developed in Gogo et al. (2014) was implemented to estimate overall exo-enzyme
110 activity and respiration rate. Litter mixture effect can result from nutrient transfer between litters,
111 influences caused by specific compounds released by the litters, changes in microclimatic
112 conditions and modification of trophic levels interactions (Gartner and Cardon 2004;
113 Hättenschwiler et al. 2005). Nutrient content of *Sphagnum rubellum* and *Molinia caerulea* is very
114 low (Gogo et al. 2011). Thus, nutrient transfer may not be a pertinent cause of possible litter
115 mixture effect. Instead, physical changes may occur. *Sphagnum* can affect the pH significantly
116 and these species can retain up to 20 times its weight in water (Rydin and Jeglum 2013). Both

117 parameters are known to have an effect on OM decomposition (Bergman et al. 1999; Prescott
118 2010). To explain potential litter mixture effects in the laboratory experiment, the following two
119 hypotheses were tested: compared to the theoretical expected values, mixing the two litters
120 increases (a) litter water content or (b) pH of the water extract. To test these hypotheses, the
121 water content of all litters was assessed as well as the pH of the water extract. To analyse in
122 greater detail the water extract characteristics, the UV absorption (aromaticity) and the
123 fluorescence properties (differentiation of pools of OM) of the soluble OM were investigated.
124 Fluorescence analyses of DOM gave excitation-emission matrices (EEMs) that were treated
125 using PARAFAC modelling.

126

127 **2 Materials and methods**

128 2.1 Sampling site

129 All litters for *in situ* and lab experiments were collected in La Guette peatland (Sologne, France),
130 a site of the French Peatland Monitoring Service: Service National d'Observation Tourbières. It
131 is located in Neuvy-sur-Barangeon (Cher) in the south-eastern part of the French Centre Region
132 (altitude: 154m, N: 47°19', E: 2°16', Gogo et al. 2011). La Guette peatland is composed of
133 vegetation patches ranging from a transitional fen to wet heathland dominated by *Molinia*
134 *caerulea*, *Erica teralix* and *Calluna vulgaris*. The dominant *Sphagnum* species are *Sphagnum*
135 *cuspidatum* and *Sphagnum rubellum*. In recent decades, the abundance of vascular species
136 already present in the ecosystem, *Molinia caerulea* and *Betula* spp., increased dramatically.
137 Trees covered 15% of the peatland surface in 1980, 50% in 2000 and 69% in 2009. The
138 reasons for such an abrupt increase may be hydraulic works and pine plantation in the
139 surroundings of the site, as well as nutrient inputs.

140

141 2.2 Coupling field and laboratory experiments

142 Studying the effect of mixing litters with *Sphagnum* species is not as straightforward as with
143 vascular plants. In the field, the incubation environment and the *Sphagnum* litter to decompose
144 in the bag are the same material: *Sphagnum* litter. Whether in mixture with *Sphagnum* or in
145 monoculture, the other litter composing the mixture experiences the same incubation conditions.
146 To avoid this issue, Hoorens et al. (2003) and Hoorens et al. (2010) decided to incubate the
147 litter bags in another environment than the natural one, with similar abiotic conditions. This

148 allows a fair contribution of both litters, but as the incubation conditions are not strictly the same
149 as the natural ones, the results are difficult to transpose to the true environment.

150 Gartner and Cardon (2004) suggested that the remaining mass of each litter incubated in
151 mixture should be weighed. Thus, following this recommendation, it was initially planned to
152 separate the litter in mixture samples. However, preliminary tests showed that separating the
153 *Sphagnum* litter which can be composed of very small leaves is a difficult and time consuming
154 task that can lead to biased results as it is difficult to be sure that all the *Sphagnum* leaves have
155 been separated,

156 In this study, it was decided to implement the field experiment in *Sphagnum* hummocks and
157 lawns to test only the effect of the vascular plant litter on *Sphagnum* litter decomposition.
158 Vascular plant litters were also incubated in their native environment. The field experiments
159 were further complemented with a laboratory incubation experiment, where all the litters were
160 incubated in the very same conditions. It has been shown that the initial laboratory respiration
161 rate could give a fair representation of long-term litter decomposability (Aerts and de Caluwe et
162 al. 1997). In addition to respiration, the litter remaining mass and WEOC quantity and
163 characteristics were also measured.

164

165 2.3 Field experiment

166 Litter bags (0.5 mm mesh) were filled with air-dried litter (about 750 mg) of different species:
167 *Sphagnum cuspidatum*, *Sphagnum rubellum*, and leaves of *Betula* spp and *Molinia caerulea*.
168 These samples are referred to as "monoculture". The mixture samples were composed of 50%
169 of *Sphagnum* litter (either *S. cuspidatum* or *S. rubellum*) with 50% of a vascular plant litter
170 (either *M. caerulea* or *Betula* spp). The litter bags (n=4) were placed in situ in March 2009. In *S.*
171 *rubellum* hummocks were placed: *S. rubellum*, *M. caerulea* and *Betula* spp litters in monoculture
172 and in mixture (3 monocultures and 2 mixtures: *S. rub* + *M. cae* and *S. rub* + *Bet. spp*). In *S.*
173 *cuspidatum* lawns were placed: *S. cuspidatum*, *M. caerulea* and *Betula* spp in monoculture and
174 in mixture (3 monocultures and 2 mixtures: *S. cus* + *M. cae* and *S. cus* + *Bet. spp*).

175 In addition, the same protocol was followed to prepare *M. caerulea* and *Betula* spp. litter bags in
176 monoculture that were placed in their native decomposition environment. Litter bags of *M.*
177 *caerulea* were placed in the litter of *M. caerulea* in tussocks. The litter bags of *Betula* spp were

178 placed in the litter of *Betula* spp. Both experiments were located in the same area and
179 undertaken at the same time.

180 The litters were collected in March 2010 after one year of incubation. In the laboratory, the roots
181 were retrieved from the litter bags with tweezers and the litters were gently washed with
182 deionised water. The litter bags were dried at 50°C for 2 days and weighed and the proportion
183 of remaining mass was calculated ($100 \times \text{remaining dry mass} / \text{initial dry mass}$).

184 There was an attempt to separate the litters, but this raised some issues. First, as the samples
185 were extracted with water, some materials were found on the filter and some of these materials
186 were so small that it was impossible to pool them with one or another litter. Furthermore, most
187 of the vascular plant litter remained in one piece, which was absolutely not the case for
188 *Sphagnum* litter, which scattered. This made the recovering of *Sphagnum* litter difficult to
189 undertake without introducing an error in the estimation of the mass remaining, and thus a
190 possible error in the detection of a mixture effect. It was preferred not to separate the litter to
191 have a better guarantee on the whole mixture effect.

192

193 2.4 Laboratory experiment

194 As the field experiment showed significant differences between measured and expected values
195 in the *S. rubellum* and *M. caerulea* mixture, the laboratory experiment focused on these 2 litters.
196 The litters were collected in April 2011 and air dried before incubation, until they reached
197 constant weight. Surface peat water was collected in the same site. There was enough free
198 water to collect it with a beaker and pour it into a flask. Then the water was filtered at 100 µm to
199 get rid of the coarsest particles, without excluding microbial communities.

200

201 2.4.1 Sample preparation and incubation

202 The day after the water was collected from the peatland, air dried *S. rubellum* and *M. caerulea*
203 litter samples were weighed (about 250 mg) and inoculated with 20 ml peat water and left
204 overnight. Then, the excess water of the litters was removed with a tissue and the litters were
205 placed in a 50 ml tube, which contained 5 ml of a potassium sulfate saturated solution (120 mg l⁻¹
206 ¹) to maintain moist conditions (Aerts and de Caluwe 1997). Glass marbles were also placed at
207 the bottom of the tube and covered with a PVC filter (0.5 mm mesh) to avoid contact of the litter
208 with the potassium sulfate solution. A total of 126 samples were prepared: 3 litter types (*S.*

209 *rubellum*, *M. caerulea* and the mixture of the two species, 50% each), 42 samples per litter type,
210 and incubated in controlled conditions. An insulated container (0.3 m x 1 m x 1 m) was built with
211 pieces of PVC covered with aluminum foil to ensure constant environmental conditions during
212 incubation. To obtain a water-saturated air, the atmosphere of the container was filled with air
213 that was pumped through warm water. Sets of samples (*S. rubellum*, *M. caerulea* and the
214 mixture) were randomly put in racks that were placed in the container. The position of the racks
215 within the container was changed every week. Temperature and humidity were monitored and
216 showed that the experimental setup ensured a constant water-saturated atmosphere and an air
217 temperature of 22.5 ± 0.3 °C (n = 32).

218 A total of 126 samples allowed a kinetic study of 7 dates with 6 samples of each litter type at
219 each date. To calculate expected mixture results from additive effect (50% of each litter type),
220 groups were composed of one sample of each type of litter (total of 3 samples per group: 1 *M.*
221 *caerulea* alone, 1 *S. rubellum* alone, 1 mixture of both). At each date, 6 groups were randomly
222 retrieved after 2, 6, 12, 20, 33, 47 and 64 days of incubation. Immediately after the sample had
223 been taken out, the CO₂ production was measured. Then the water extraction of litters was
224 undertaken and finally the sample was dried at 50°C during 2 days before weighing.

225

226 2.4.2 CO₂ production

227 The tubes were placed in a chamber during 20 to 30 minutes. The cover of the chamber was
228 fitted with a mounting flange, which could receive a Vaisala CO₂ infra-red probe (GMP343). The
229 CO₂ concentrations were monitored and recorded directly on a computer. The CO₂ production
230 was calculated from the slope of the increasing concentration with time.

231

232 2.4.3 Water Extractable Organic Carbon (WEOC)

233 After the CO₂ production measurement, the litter was taken out of the tube and placed in an
234 aluminum cup at room temperature. The litter was rinsed twice with 15 ml and once with 10 ml
235 deionised water and the extract was filtered (0.45 µm). The filter was weighed before filtering
236 and was dried 48 h at 50 °C after filtering. The difference in filter mass was added to the mass
237 of litter left in the aluminum cup (litter also dried at 50 °C during 48 h and weighed) to obtain the
238 whole remaining mass. A filtrate subsample was dedicated to dissolved organic carbon (DOC)
239 analysis (Shimadzu TOC 5000), which allowed the calculation of the WEOC (in g C g⁻¹ initial

240 litter dry weight). Another filtrate subsample was dedicated to UV absorption and fluorescence
241 spectroscopy analyses. Before injection into the Shimadzu TOC 5000, the samples were
242 acidified and bubbled to eliminate dissolved inorganic C, leaving only the DOC to be analyzed.
243 Phtalate was used for calibration and each sample was replicated until the coefficient of
244 variation was less than 10% (maximum replication = 5). This procedure was undertaken with
245 each sample (n = 6) from each treatment. Just after extraction, the pH of the extracts was
246 measured on subsamples.

247

248 *2.4.4 Calculation of remaining mass*

249 For both field and laboratory experiments, i.e. laboratory and field incubations, the litters were
250 air-dried until reaching a constant weight. Then, a set of air-dried samples of each litter was
251 dried at 50 °C during 48 h and weighed. This allows the determination of the air to oven dried
252 ratio. This ratio was used to calculate the initial oven dried mass of all incubated air-dried litters.
253 The sum of the litter left after extraction and the litter on the filter gave the remaining mass. This
254 percentage was calculated by dividing the remaining mass at any time by the initial mass of the
255 litter and multiplied by 100. The mass of wet samples was also measured and the water content
256 in g of water per g of remaining mass was calculated.

257

258 *2.4.5 SUVA₂₈₀*

259 The water samples were neutralized with NaOH before measurement as recommended by
260 Weishaar et al 2003). Most of the samples were in the range of 10 to 20 mg L⁻¹, with no
261 samples higher than 31 mg L⁻¹ or lower than 7 mg L⁻¹. UV absorption at 280 nm of all water
262 samples was acquired with a Hitachi U-1900 absorbance spectrophotometer using a 1 cm path-
263 length quartz cell (6030-UV Hellma Analytics). The zero absorbance adjustment was carried out
264 with deionised water before each batch of measurements. Absorbance spectra was measured
265 between 1100 nm and 200 nm. The absorbance at 280 nm was divided by the DOC content of
266 the sample to give the Specific UV Absorption at 280 nm (SUVA₂₈₀ in L cm⁻¹ mg⁻¹). The SUVA₂₈₀
267 informs on the aromaticity of the DOC (Chin et al 1994).

268

269 *2.4.6 Natural dissolved organic matter fluorescence (FDOM) measurement and signal* 270 *processing*

271 Fluorescence properties of DOM have been used to study the mixing of fresh and salt waters in
272 estuaries (Coble 1996; Parlanti et al. 2000, Huguet et al. 2009), as well as humic substances in
273 soil and peat water (Zsolnay et al. 1999; Alberts and Takács 2004; Zaccone et al. 2009). In this
274 study, FDOM was measured to assess whether this technique could differentiate between
275 sources of DOM in decomposition experiments.

276 After UV-vis analysis, the same sample was used to characterize FDOM. Excitation–Emission
277 Matrices (EEMs) were acquired with a Hitachi F-7000 fluorescence spectrophotometer
278 equipped with FL solutions software, using 10 x 10 mm quartz cell (1000-OS Hellma Analytics).
279 For excitation and emission monochromators, slit widths were set at 2.5 nm. EEMs were
280 recorded using a scan speed of 1200 nm/min, at 950 V, over ranges of excitation and emission
281 wavelengths of 250–500 and 300–600 nm, respectively. Excitation and emission wavelengths
282 were stepped by 10 and 1 nm respectively and no inner effect correction was necessary. After
283 subtraction of the blank (deionised water), the EEMs were treated by CP/PARAFAC algorithm to
284 extract independent fluorescence contribution.

285 PARAFAC analysis identified four groups of components in the water extracted organic matter
286 (WEOM) from incubated litter (Table 1) with a CORCONDIA of 68.9%. Three ratios were
287 calculated using the C2 fluorophores corresponding to α or C (Table 1) as the denominator. The
288 ranges of excitation-emission of the four groups were compared to published data (Table 1).
289 The C2 and C3 components were related to humic substances (Table 1). It is assumed that
290 C3/C2 ratio corresponds to α'/α ratio. As both humic substances have α' and α components, it is
291 not possible to use this ratio to distinguish humic substances. The component α is composed of
292 more humified and older compounds than those found in the component α' . An increase in
293 C3/C2 ratio could indicate an increasing proportion of recent material. C1 Em/Ex couples were
294 similar to those found in extracts of IHSS standard humic substances (Alberts and Takács
295 2004) and marine samples corresponding to marine humic-like substances (Coble 1996;
296 Parlanti et al. 2000). The C1/C2 ratio was also calculated and the correlation between the
297 C1/C2 and C3/C2 ratios was significant ($R = 0.87$, $P < 0.0001$). This suggests that, like the
298 C3/C2 ratio, the C1/C2 ratio cannot be used to distinguish humic substances.

299 The C4 component is related to protein-like compounds (Table 1). It is assumed that the C4/C2
300 ratio corresponds to the γ/α ratio used to assess the relative variations of protein-like
301 substances compared to humic substances (Sierra et al. 2005). Zsolnay et al. (1999) observed

302 a relative increase in the γ fluorophores (C4 component in this study) compared to the α
303 fluorophores (C2 component in this study) in soil water extract after fumigation. In the microbial
304 biomass estimation, fumigation is used to achieve lysis of microbial cells, so cellular
305 constituents are released into the soil solution and can be readily extracted with water and
306 measured (Brookes et al. 1985). Low values of the C4/C2 ratio could indicate that protein-like
307 substances are in living microbial cells and not extractable, whereas high values could indicate
308 that microbial cell death has released protein-like compounds in the soil that are extractable. To
309 corroborate this assumption, C4/C2 was related to proxies of microbial activities (catalysis
310 activities and respiration).

311

312 2.5. Model description and parameters estimation

313 As the experimental design was similar to that in Gogo et al. (2014), the data were calculated to
314 allow their use as inputs to the Gogo et al. (2014) model. This model is based on Schimel and
315 Weintraub (2003). It assumes that solid organic matter is catalysed by exo-enzymes into soluble
316 organic matter. Then the soluble matter is absorbed by the microbial and used as an energy
317 source for different microbial function (enzymes production, maintenance, growth) and released
318 into the environment in the form of CO₂ (Schimel and Weintraud 2003). Gogo et al. (2014)
319 simplified this model to make it experimentally testable. It consist of a three compartment: (i) the
320 "L" compartment corresponding to the fraction of C contained in the litter (solid fraction), (ii) the
321 "W" compartment corresponding to fraction of C contained in the WEOC (dissolved fraction) and
322 (iii) the "G" compartment corresponding to fraction of C contained in the cumulative CO₂
323 released by microbial respiration (gaseous fraction). The flux between the L and W
324 compartment corresponds to the exo-enzymes catalysis rate. The flux between the W and G
325 compartment correspond to the respiration. Equations were written to give account of the
326 simultaneous change in time of the state variable (L, W, and G) and the reaction rates. At any
327 time of the course of the experiment the sum of all these three fractions is equal to 1. The three
328 fractions corresponding to the three compartments were experimentally measured. The two
329 reaction rates (catalysis or "c" and respiration or "r") were tuned simultaneously to fit the model
330 to the three experimentally assessed compartments.

331 Gogo et al (2014) showed that allowing the reaction rate to change in the course of the
332 experiment improved the goodness of fit. The reaction rates were allowed to follow a negative

333 exponential decrease with time with the following parameters to describe the shape of the
334 curve: “a + b” is the initial reaction rate, “a” is the final rate, “m” rate of decay with time of the
335 reaction rate (Rovira and Rovira 2010).

336

337 2.6 Statistical analyses

338 Paired *t*-tests and repeated measures ANOVA (with litter type or mixture type as within effect)
339 were conducted with Statistica (2008) to test for differences in response variables between
340 *Sphagnum* spp and vascular plant litters and for differences between measured and calculated
341 values of the mixture. Correlations were calculated on log transformed data when necessary to
342 assess the relationship between two response variables, using Statistica (2008). Model II
343 regression (ranged major axis; Legendre and Legendre 1998) was performed on log
344 transformed data when necessary to calculate equation coefficients.

345

346 **3 Results**

347 3.1 Field experiment

348 *S. rubellum* litter decomposed significantly faster in the presence of *Molinia caerulea* than
349 without ($P < 0.02$; Table 2). This was the only significant non-additive litter mixture effect
350 observed. On the whole, in the field conditions and over a year, *Molinia caerulea* and *Betula* spp
351 decomposed faster than *Sphagnum* species (Table 2). *M. caerulea*, but not *Betula* spp.,
352 decomposed faster in the *Sphagnum* environments than in its native one (Table 3). As a mixture
353 effect was found only with *S. rubellum* and *M. caerulea* and as *Sphagnum* environments
354 affected only *Molinia caerulea*, the laboratory experiment focused on this species.

355

356 3.2 Laboratory experiment

357 3.2.1 C dynamics

358 *S. rubellum* litter mass decreased faster, contained more WEOC and tended to produce more
359 CO₂ than *M. caerulea* litter (Fig. 1a, c, e; Table 4). The measured remaining mass of the *S.*
360 *rubellum* and *M. caerulea* mixture was significantly lower than expected from calculations ($P <$
361 0.002 ; Fig. 1b; Table 4). The non-additive effect of the mixture was also observed on the WEOC
362 and the CO₂ production, with higher measured values than expected (Fig. 1d, f; Table 4).

363 Applying the model of Gogo et al. (2014) gave a good account of the observations with RMSE
364 in the same range as Gogo et al. (2014): between 0.16 and 17.8 in this study (Table 5) and
365 between 0.23 and 14.5 in (Gogo et al. 2014) for *M. caerulea*. Initial exo-enzyme activity and
366 respiration rate in the measured mixture were in the expected range (no more than 10% of
367 difference; Table 5; Fig. 2b, d). However, the rates at which the activities decreased afterward
368 (the "m" parameters in Table 6) were much lower than expected. This implies that both activities
369 in the measured mixture were higher than expected (Fig. 2). The difference was more
370 pronounced for exo-enzyme activities (75% difference) than for respiration (46% difference;
371 Table 6).

372 Contrary to the stated hypothesis, the water content in the measured mixture was not different
373 than the calculated one (Table 4; Fig. 3 a and b). The pH was different and significantly lower in
374 the measured mixture than in the calculated one (Table 4; Fig. 3 c and d).

375

376 3.2.3 Specific Ultraviolet Absorption at 280 nm

377 In all litters, SUVA₂₈₀ decreased with time (Fig. 4). On the whole WEOC from *Molinia caerulea*
378 litter was more aromatic than the *Sphagnum rubellum* WEOC ($P < 0.0001$; Fig. 4ab). Also, the
379 WEOC measured in the mixture was found to be more aromatic than expected (Fig. 4c).

380

381 3.2.4 Fluorescent dissolved organic matter

382 Both C1/C2 and C3/C2 ratios increased with time (Fig. 5a, b, c, d), showing an increased
383 contribution of more recent humic substances. The C1/C2 ratio in *M. caerulea* WEOM was
384 significantly higher than in *S. rubellum* ($t = 6.86$, $P < 0.0001$, $n = 42$; Fig. 5a), but there was no
385 difference between measured and calculated mixtures ($t = 0.035$, $P = 0.97$, $n = 42$; Fig. 5b). In
386 contrast, the C3/C2 ratio was significantly lower in the measured than in the calculated mixture
387 ($t = 3$, $P < 0.005$, $n = 42$; Fig. 5d). However, this ratio in the single litter WEOM was not different
388 ($t = 0.82$, $P = 0.42$, $n = 42$; Fig. 5c).

389 The C4/C2 ratio in all litter types increased with time, showing an increased contribution of
390 protein-like compounds compared to humic substances (Fig. 5e, f; the same pattern is observed
391 when C3 or C1 substitute C2). This increase was particularly high for the *M. caerulea* litter
392 compared to the *S. rubellum* litter ($t = 8.93$, $P < 0.0001$, $n = 42$; Fig. 5e). However, as well as
393 C1/C2, there was no significant difference between measured and calculated mixtures ($t =$

394 0.055, $P = 0.96$, $n = 42$; Fig. 5f). In our study, the C4/C2 ratios were always negatively
395 correlated to the modelled exo-enzyme activity and respiration rates (all $R < -0.89$, all $P < 0.01$;
396 Fig 6). Also, the relationship was in a chronological order: as time passes, the microbial activity
397 decreased, whereas the relative fluorescence of protein-like compounds increased.

398

399 **4 Discussion**

400 4.1 Laboratory synergistic effect of litter mixture on OM dynamics

401 In the laboratory experiment, mixing *M. caerulea* and *S. rubellum* litters increased litter mass
402 loss (decomposition) and CO₂ production (microbial respiration) compared to what can be
403 expected in an additive scenario (Fig. 1; Table 4). Krab et al. (2013) already demonstrated a
404 *Sphagnum*-vascular plant mixture effect on mass loss in the field, but with *S. fuscum* and *Betula*
405 *pubescens*. To our knowledge, such a non-additive effect of mixing *M. caerulea* and *S. rubellum*
406 litters on microbial activity has never been shown before. The mixture effect between *M.*
407 *caerulea* and *S. rubellum* litters could result from a higher exo-enzyme activity and respiration
408 rate in measured than in calculated mixtures during all or most of the incubation time
409 respectively (Fig. 2b, c).

410 WEOC content was also increased by the mixture. The WEOC compartment receives input from
411 litter exo-enzyme catalysis (provoking litter mass loss), and is consumed by the microbial
412 biomass (resulting in output by respiration in the form of CO₂; Schimel and Weintraub 2003;
413 Gogo et al. 2014). As such, WEOC is sensitive to any change in input and output rates (Gogo et
414 al. 2014). The build-up of WEOC built up in the measured mixture, compared to the calculated
415 mixture, can only result from a relatively higher input than output rate. This suggests that in the
416 first stage of decomposition, mixing *S. rubellum* and *M. caerulea* increased exo-enzyme activity
417 more than respiration, compared to what could be expected. The model showed that both rates
418 decreased with time in all types of litter. Thus, the higher WEOC content in the measured
419 mixture could only be caused by an output rate that decreased faster than the input rate (Table
420 6).

421

422 4.2. Increase of aromatic compounds in the WEOC

423 The higher WEOC in the measured mixture was simultaneous to a significant increase of
424 WEOC aromaticity (Fig. 4). Aromaticity variation can be due to (i) input/output of aromatic

425 compounds in the WEOC compartment and/or (ii) input/output of non-aromatic compounds (e.g.
426 carbohydrates), mechanically decreasing/increasing the $SUVA_{280}$.
427 Degradation of lignin, tannin and/or polyphenols of the litters can be a significant source of
428 soluble aromatic compounds into the WEOC. Dignac et al. (2005) showed in a cropland soil that
429 lignin turnover could be faster than the whole soil organic carbon turnover. In the present study,
430 it was shown that decomposition rate of the solid phase in the measured mixture was faster
431 than expected, with thus the potential to draw more aromatic compound into the WEOC than
432 expected. Furthermore, aromatic compounds such as tannin are more difficult to metabolise by
433 the microorganisms, than carbohydrates monomers (Davidson and Janssens 2006). In the
434 present study, it was shown that respiration rate was faster in the measured mixture than in the
435 expected one, leaving into the WEOC probably more aromatic compounds than carbohydrates.
436 The increased of WEOC aromaticity in the measured mixture is thus coherent with the combine
437 effect of increased rate of solid matter decomposition and WEOC respiration.

438

439 4.3 Contribution of fluorescence analysis to the study of litter C dynamics

440 Among the fluorescent components highlighted by the PARAFAC analysis, C4 is the only one
441 that is not attributed to humic substances, but to tyrosine and tryptophane containing materials
442 (i.e. proteins; Coble 1996; Parlanti et al. 2000). These compounds contain nitrogen, which is
443 one of the most limiting nutrients in soils. The occurrence of such fluorophores may be related
444 to a release of exo-cellular enzymes. However, if a high contribution of protein-like fluorophores
445 were to be related to an increased release of exo-enzymes, an increased decomposition rate
446 would be expected when the C4/C2 ratio is high. This was not the case. First, the *S. rubellum*
447 litter decomposed faster than the *M. caerulea* litter, whereas its C4/C2 was much lower (Figs.
448 1a and e and 5e). Second, as time passes, the microbial activity decreased in all samples,
449 whereas the γ fluorophores contribution increased. The occurrence of such fluorophores may
450 also be related to the release into the solution of proteins associated to the decomposing
451 tissues. If this were the case, increased contribution of the γ fluorophores would be associated
452 to an increased mass loss. However, the exact opposite was observed: *M. caerulea*, which
453 decomposition was slow, had a WEOC with the most important γ fluorophore contribution.
454 Furthermore, because of the translocation process (Taylor et al. 2001) *M. caerulea* litter has a
455 very low N content: more than 2 times less than *S. rubellum*, with 1.0 mg g^{-1} and 2.2 mg g^{-1}

456 respectively (Gogo et al 2010). Such a high input of protein-like compounds from such a slow
457 decomposing N poor litter may not be realistic.

458 In peat, the microbial biomass concentrates a significant fraction of the soil total N (peat C/N
459 from 16 to 36, microbial biomass C/N from 6 to 14; Francez et al. 2000). Parlanti et al. (2000)
460 showed in a macro-algae degradation experiment that the γ/α ratio increased in the first 15 days
461 of the incubation corresponding to degradation products of the organisms, probably originating
462 from cell membranes. Zsolnay et al. (1999) also showed that such fluorophores occurred when
463 the microbial cell were lysed, leaving in solution the protein rich cytoplasmic content. As
464 suggested above, protein amount in soil can increase because of exo-enzymes release.
465 However, the expected increase of OM degradation in such case was not observed: microbial
466 activity decreased as the protein-like fluorophores contribution increased. What could happen is
467 that as the microbial biomass dies off, the cell contents are released in the media without being
468 taken up again, provoking a build-up of proteinaceous compounds in the WEOM. Thus, it
469 suggests that the relative increase in the C4/C2 ratio in time could reflect an increasing
470 solubilisation of microbial cell products after their death. The C4/C2 ratio in the context of litter
471 laboratory incubation can be used as an index of the microbial dead/living biomass ratio.

472 As solid OM composition differs between *Sphagnum* species and *M. caerulea* litter (Gogo et al.
473 2011; Gogo et al. 2014), it was expected that the fluorescence of the WEOM produced by solid
474 OM degradation would differ as well. Such a difference would allow identification of the relative
475 contribution of each litter in the mixture, making it possible to identify which litter contributes
476 more to the input into the WEOM. However, the results show that when a difference was found
477 between single litters, there was no non-additive effect in the mixture (C1/C2 and C4/C2, Fig.
478 5a, b, e and f). In contrast, a non-additive effect was detected in the mixture where no difference
479 was found between single litters, (C3/C2, Fig. 5c, d). Thus, in the present case, fluorescence
480 analysis is not a pertinent tool to study chemical changes linked to mixture.

481 Most fluorescent materials (C1, C2, C3) are humic substances (Coble 1996; Parlanti et al. 2000;
482 Alberts and Takács 2004). They are not the direct product of litter exo-enzymatic attack. They
483 were certainly present in the water used to inoculate the litter. Humic substances are known to
484 be recalcitrant to decay (Jenkinson and Rayner 1977). This may explain why the non-additive
485 effect observed on mass loss, WEOC content and respiration was not reflected in these
486 compounds.

488 4.4 Mechanisms of in situ and laboratory litter mixture effect

489 As in the laboratory, there was an in situ synergistic effect of mixing *S. rubellum* and *M.*
490 *caerulea* litters on OM decomposition after one year of incubation (Table 2). This effect of
491 mixing *M. caerulea* with a *Sphagnum* moss on OM decomposition has never been evidenced
492 before. Because the *M. caerulea* decomposing environment is composed of *Sphagnum* litter, it
493 was not possible to test the effect of *Sphagnum* litter on *M. caerulea* litter decomposition. It can
494 however be concluded that the in situ mixture effect observed originated from an effect of *M.*
495 *caerulea* on the rate of decay of *S. rubellum*, which is an additional information compared to the
496 laboratory incubation.

497 The effect of the *Sphagnum* environment on *M. caerulea* can be approached by comparing the
498 decomposition rate obtained in *Sphagnum* and in the native tussock environment. *M. caerulea*
499 litter in its in situ native environment decomposed at a much slower rate than in both *S.*
500 *rubellum* and *S. cuspidatum* environments (Table 3). Van Vuuren and van der Eerden (1993)
501 also found low decomposition rate of *M. caerulea* in its native environment in a heathland (80%
502 of remaining mass after 11 months of incubation). The decomposition of *M. caerulea* litter
503 increased by 17 - 21% in a *Sphagnum* environment compared to their native environment
504 (Table 3). The non-additive litter mixture effect may result from changes in physical properties
505 caused by one litter (Gartner and Cardon 2004). *M. caerulea* is a tufted plant forming tussocks
506 and because of this microtopography most of the litter experiences dry conditions in the first
507 year that limit decomposition. Moisture in the range of 60-75% (wet weight basis) does not limit
508 decay, whereas its variations could influence mass loss in the range of 75-80% and more
509 extensively in the range of 30-60% (Prescott 2010). Microbial activity decreases as the
510 conditions become drier and wetter. A change toward an increase of moisture compared to its
511 native environment would stimulate *M. caerulea* litter decomposition. Through their
512 morphological characteristics, *Sphagnum* mosses are able to retain up to 15 to 20 times their
513 dry weight in water (Rydin and Jeglum 2013, in accordance with Fig. 3), which maintains moist
514 conditions over dry periods. Thus, in field conditions, when *M. caerulea* litter decomposes in a
515 *Sphagnum* environment, it spends more time in favourable conditions for decomposition than in
516 its native environment.

517 Water content seems to explain the differences observed. Although *M. caerulea* did decompose
518 faster than in its native environment, the decomposition rate in *Sphagnum* environment was low
519 (about 70% of mass remaining after one year; Table 3). Hoorens et al. (2003) reported a faster
520 decomposition rate of *M. caerulea* litter in a *Polytrichum commune* decomposing environment
521 (59%). Once a limiting factor was alleviated, other factor influencing OM decomposition may
522 take place. *Sphagnan* a carbohydrate polymer specific to *Sphagnum* environment is thought to
523 preserve OM through different mechanisms (Hájek et al. 2011). A longer experiment and/or
524 comparison of *M. caerulea* litter decomposition in other environments (*M. caerulea* can grow in
525 a wide array of environments) would help to assess the effect of *Sphagnum* environment on *M.*
526 *caerulea* decomposition.

527 As mentioned above, *M. caerulea* is a tufted plant forming tussocks, which implies dry
528 conditions in the first year that limit decomposition. From the in situ experiment, it was
529 suggested that litter moisture is an important factor controlling the effect of the *Sphagnum*
530 environment on the decomposition of *M. caerulea*. In the laboratory conditions, all the litters
531 were subjected to the same conditions. The *M. caerulea* litters in both treatments (in
532 monoculture and in mixture) were moist (unlike native field conditions). A significant correlation
533 was found between microbial activity and water content for all litters (Fig. 6), which supports the
534 role of water content on microbial activity. However, no significant difference in litter water
535 content was found between measured and calculated mixtures (Table 4; Fig. 3a and b). This
536 suggests that additional mechanisms are at work to explain the observed non-additive effect of
537 *S. rubellum* on *M. caerulea* in the laboratory and of *M. caerulea* on *S. rubellum* in the field.

538 Microbial activity can be very sensitive to changes in pH. The optimum pH for CO₂ production in
539 peat is above the pH usually measured in peatlands (Bergman et al. 1999). In our study,
540 although the pH in the mixture extract decreased compared to an additive scenario, microbial
541 activity was enhanced by mixing the two litters. This rules out any direct role of this factor on the
542 mechanism that could explain the synergistic effects observed. However, increase of acidity
543 could have an indirect effect on labile carbon supply to microorganisms. Low pH could
544 accelerate litter OM hydrolysis, releasing both lignin (as suggested above) and carbohydrate
545 monomers. The latter could have stimulated microbial activity, which in turn could have
546 increased litter degradation. Such an hypothesis has to be further explored.

547

548 4.5 Implications and perspectives

549 Hoorens et al. (2010) showed that the litter mixture effect may be additive at the plant functional
550 level, implying that the decomposition rate at the ecosystem level could be calculated from the
551 abundant literature on individual litter decomposition rates. While this may be true for
552 ecosystems supporting enough biodiversity, it is to stress that biodiversity is dramatically
553 decreased in peatlands invaded by *Molinia caerulea*. In the study site, with the exception of
554 some wetter areas dominated by *Rynchospora alba*, *M. caerulea* together with very sparse
555 *Eriophorum angustifolium* dominates the herbaceous strata and covers almost all the site. In
556 some wetter places, where *S. rubellum* can develop, *M. caerulea* is the only herbaceous plant.
557 *M. caerulea* cover increases because of disturbances. In such a situation, the negative and
558 positive non-additive interaction may not cancel out as suggested by Hoorens et al. (2010),
559 leaving only a positive non-additive effect. The ecosystem level decomposition rate estimation
560 using individual litter data in ecosystems where *M. caerulea* dominates should therefore not be
561 used in modelling. Furthermore, the occurrence of a mixture effect between different *Sphagnum*
562 species and different invading vascular plants (Krab et al. 2013; this study) clearly highlights the
563 need to take peatland vegetation change into account when simulating future C stock and
564 fluxes.

565 One must be careful when transposing results obtained in the laboratory to the field. That is why
566 here laboratory experiment was coupled to in situ incubation. Both experiment showed an
567 acceleration of decomposition in litter in mixture. However, the laboratory experiment was much
568 shorter than the field experiment and mixture effect observed in both experiment may not
569 originate from the same process.

570 One aim of this study was to elucidate the mechanisms behind the mixture effect. However,
571 direct effect of the variations of the factors studied (water content and pH) could not explain the
572 observation. As an alternative, the results of the present study suggest that the interaction
573 between labile OM and microbial biomass activity may be the key interaction to focus on in
574 further research. This is crucial because such a synergistic effect can jeopardize the C storing
575 capacity of *Sphagnum* peatlands in the context of vegetation change. Considering the vast
576 areas covered by *Sphagnum* species, especially in Siberia and Canada and the increasing
577 abundance in Northern peatlands of *M. caerulea* and other colonising vascular plant species
578 (e.g. *Betula spp*, *Pinus spp*, *Phragmites australis*), the litter mixture effect of invading species

579 litter with *Sphagnum* spp litters definitely requires more attention. For example, other mixing
580 experiment should be undertaken with *Sphagnum* species and other vascular plant to assess to
581 which extent the mixture effect is occurring in these ecosystems.

582

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591

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692

693 **Tables**

694

695 Table 1. Major components found by a PARAFAC analysis of the fluorescence excitation-
696 emission matrices of the water extractable samples, and the assumed corresponding origin of
697 the substances of each component from different literature sources. M and β were usually
698 attributed to marine humic substances, but were also found in extract of humic substances
699 standard (Alberts and Takács, 2004).

PARAFAC Components	Coble et al. (1996)	Parlanti et al. (2001)	Excitation max. (nm)	Emission max. (nm)	Compound type
C1	M	β	300-340	400-440	Humic substances
C2	C	α	330-370	420-480	humic substances
C3	A	α'	250-270	380-480	humic substances + recent materials
C4	B-T	γ	270-280	300-340	Protein-like material

700

701

702 Table 2. Mean (\pm 1 s.e., n = 4) remaining mass (%) after one year *in situ* incubation of
 703 *Sphagnum* and vascular plant litters, in monoculture and in mixture (Mix). Significant difference
 704 between *Sphagnum* and the vascular plant litters decomposition are in italics and significant
 705 difference between measured and calculated decomposition in mixture are in bold italics.

Incubation site	Litter	Mass remaining \pm s.e.	<i>P</i>
<i>S. rubellum</i> hummocks	<i>S. rubellum</i>	86.9 \pm 0.95	<i>0.007</i>
	<i>M. caerulea</i>	68.5 \pm 2.56	
	Mix measured	71.1 \pm 1.74	<i>0.02</i>
	Mix calculated	77.6 \pm 1.39	
	<i>S. rubellum</i>	86.9 \pm 0.95	<i>0.01</i>
	<i>Betula spp.</i>	59.6 \pm 5.36	
	Mix measured	74.0 \pm 1.45	0.88
	Mix calculated	72.9 \pm 2.19	
<i>S. cuspidatum</i> lawns	<i>S. cuspidatum</i>	83.1 \pm 1.66	0.05
	<i>M. caerulea</i>	71.6 \pm 2.74	
	Mix measured	71.5 \pm 4.09	0.16
	Mix calculated	77.2 \pm 1.41	
	<i>S. cuspidatum</i>	83.1 \pm 1.66	<i>0.004</i>
	<i>Betula spp.</i>	63.7 \pm 2.27	
	Mix measured	71.3 \pm 1.46	0.64
	Mix calculated	72.9 \pm 1.96	

706

707

708 Table 3. Mean (\pm 1 s.e., n = 4) remaining mass (%) after one year *in situ* incubation of *Molinia*
 709 *caerulea* and *Betula spp.* in their native decomposition environment, in *S. rubellum* hummock
 710 and in *S. cuspidatum* lawn environments. *P* values correspond to the comparison of remaining
 711 mass of a litter type in *Sphagnum* environment to their native decomposition environment.
 712 Significant are in italics.

Incubation site	Litter	Mass remaining \pm s.e.	<i>P</i>
<i>M. caerulea</i>		86.4 \pm 2.84	
<i>S. rubellum</i> hummocks	<i>M. caerulea</i>	68.5 \pm 2.56	0.0025
<i>S. cuspidatum</i> lawns		71.6 \pm 2.74	0.008
<i>Betula spp.</i>		67.4 \pm 1.61	
<i>S. rubellum</i> hummocks	<i>Betula spp.</i>	59.6 \pm 5.36	0.12
<i>S. cuspidatum</i> lawns		63.7 \pm 2.27	0.57

713

714

715 Table 4. Mean remaining mass after 64 days of incubation (± 1 s.e., $n = 6$, %), and mean water
 716 extractable organic carbon (WEOC) concentration (± 1 s.e., $n = 42$, mg g^{-1} dry weight), CO_2
 717 production (± 1 s.e., $n = 42$, $\mu\text{g C-CO}_2 \text{g}^{-1}$ dry weight h^{-1}), litter water content (± 1 s.e., $n = 42$, g
 718 $\text{H}_2\text{O g}^{-1}$ dry weight) and WEOC pH (± 1 s.e., $n = 42$) over 64 days of the laboratory experiment
 719 of *Sphagnum* spp. and vascular plant litters, in monoculture and in mixture (Mix). Significant
 720 differences are in italics.

	<i>M. caerulea</i>	<i>S. rubellum</i>	P	Mix measured	Mix calculated	P
Remaining mass	96.1 \pm 0.38	92.6 \pm 0.52	<0.0001	91.8 \pm 0.53	93.8 \pm 0.38	<0.0001
WEOC	1.77 \pm 0.12	3.40 \pm 0.09	<0.0001	3.51 \pm 0.16	2.53 \pm 0.09	<0.0001
CO_2 production	19.7 \pm 2.61	29.1 \pm 4.58	0.06	35.9 \pm 3.89	24.2 \pm 2.78	0.01
Water content	1.28 \pm 0.21	17.2 \pm 0.66	<0.0001	8.08 \pm 0.66	8.96 \pm 0.37	0.09
pH	5.09 \pm 0.04	4.52 \pm 0.08	<0.0001	4.62 \pm 0.06	4.80 \pm 0.04	0.01

721

722

723 Table 5. Relative mean square error (RMSE) achieved when the Gogo et al. (2014) model was
724 fitted to the data of this study.

	Remaining mass	WEOC	Cumulative CO ₂	Sum
<i>S. rubellum</i>	0.76	17.8	6.72	25.27
<i>M. caerulea</i>	0.16	11.5	5.89	17.58
Mixture measured	0.20	13.6	3.91	17.74
Mixture calculated	0.14	12.75	4.37	17.27

725

726

727 Table 6. Model parameters and percentage of change between measured mixture and expected
 728 mixture: (measured – expected) / expected x 100.

Parameters		<i>S. rubellum</i>	<i>M. caerulea</i>	Mix. Measured	Mix. Calculated	% of difference
Exo-enzyme catalysis	m_c	0.063	0.081	0.016	0.065	-75.5
	a_c	0.0011	0.0004	8.27E-07	0.0007	
	b_c	0.0015	0.0019	0.0026	0.0018	
	initial rate ($a_c + b_c$)	0.0026	0.0023	0.0026	0.0024	8.1
Respiration	m_r	0.012	0.027	0.010	0.019	-46.1
	a_r	5.52E-10	0	6.68E-10	1.38E-10	
	b_r	0.36	0.51	0.38	0.42	
	initial rate ($a_r + b_r$)	0.0122	0.0271	0.3793	0.4215	-10.0

729

730

731 **Figure captions**

732 **Fig. 1** Measured (mean, $n = 6$, dots) and modelled (lines, modelling based on Gogo et al., 2014)
733 mass loss (a, b), WEOC (c, d) and cumulative CO_2 (e, f) in *Sphagnum rubellum* (black circles),
734 *Molinia caerulea* (white triangles), and litters in mixture (measured – dark grey diamonds, and
735 expected – light grey squares)

736

737 **Fig. 2** Kinetics of the exo-enzyme catalysis rate (a, b) and respiration rate (c, d) in *Sphagnum*
738 *rubellum* (a) and *Molinia caerulea* (b) litters in monoculture and in measured (c) and expected
739 mixture (d)

740

741 **Fig. 3** Relationship between SUVA_{280} ($\text{L cm}^{-1} \text{mg}^{-1}$, $\pm 1\text{s.e.}$, $n = 6$) and dissolved organic carbon
742 (DOC, mg L^{-1}) of the WEOC extracted from *Sphagnum rubellum* (a) and *Molinia caerulea* (b)
743 litters in monoculture and in measured (c) and expected mixture (d). The black line corresponds
744 to the “conservation” line, where aromatic content experienced no variation (either input =
745 output $\neq 0$ or input = output = 0)

746

747 **Fig. 4** Kinetics of the C1/C2 (a, b), C3/C2 (c, d) and C4/C2 (e, f) ratios obtained after the
748 PARAFAC analysis of the excitation-emission matrices of the WEOM extracted from *Sphagnum*
749 *rubellum* (black circles) and *Molinia caerulea* (white triangles) litters in monoculture and in
750 measured and expected mixture (measured – dark grey diamonds, and expected – light grey
751 squares)

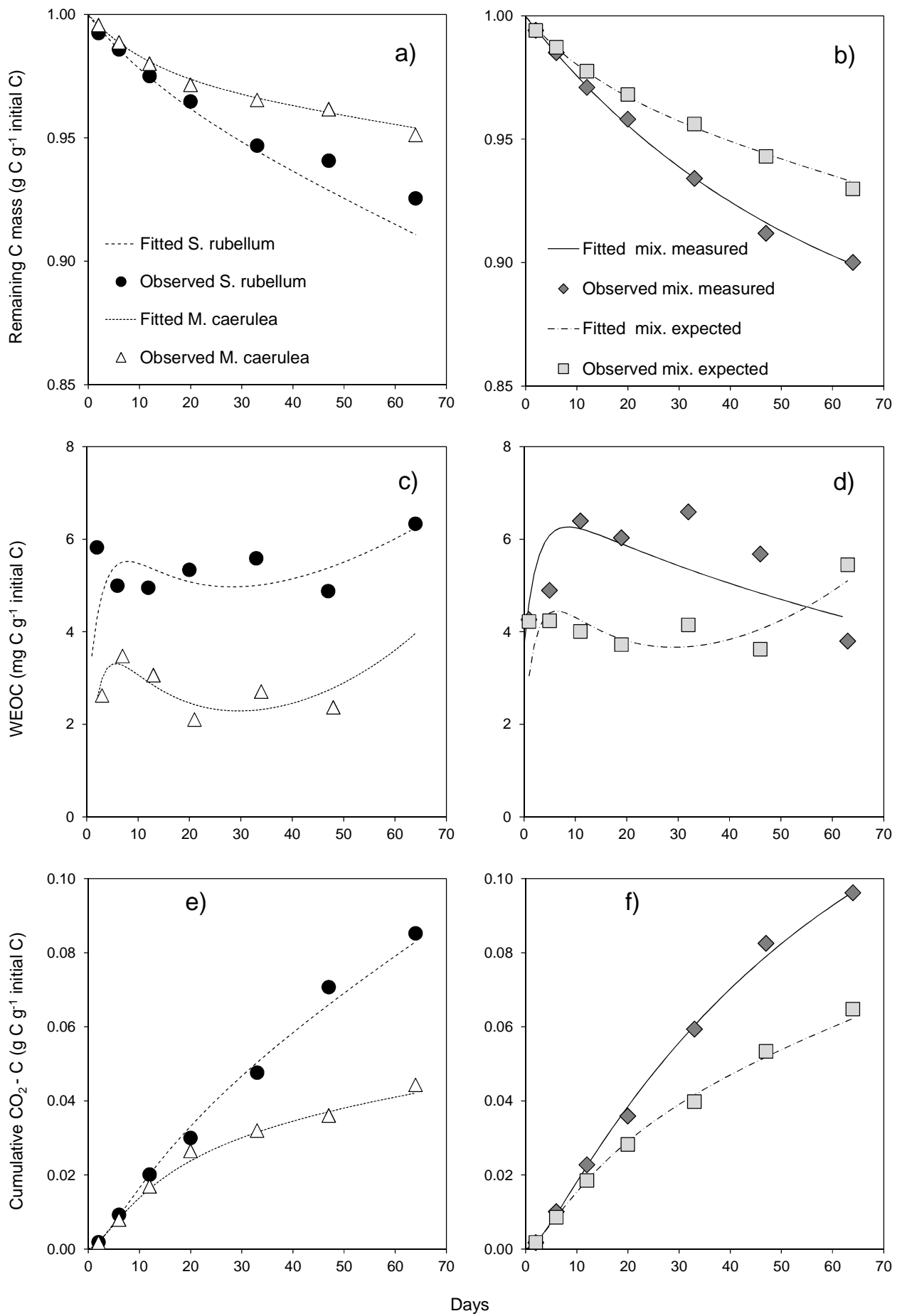
752

753 **Fig. 5** Relationship between modelled enzyme activity (a, b) and respiration (c, d) and the
754 C4/C2 ratio in *Sphagnum rubellum* (black circles) and *Molinia caerulea* (white triangles) litters in
755 monoculture and in measured and expected mixture (measured – dark grey diamonds, and
756 expected – light grey squares). All correlations are significant ($P < 0.05$)

757

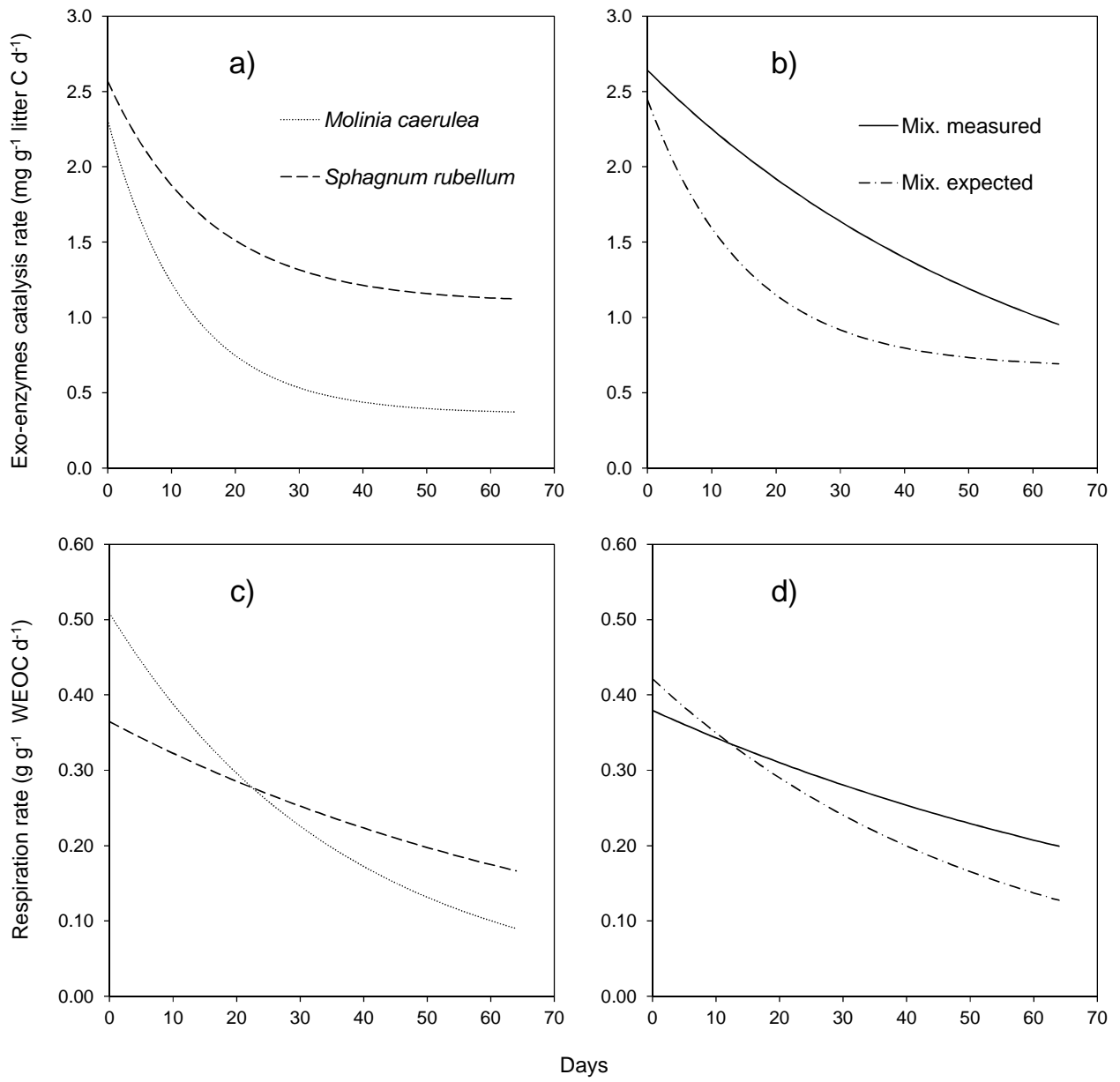
758 **Fig. 6** Relationship between modelled enzyme activity (a, b) and respiration (c, d) and the litter
759 water content in *Sphagnum rubellum* (black circles) and *Molinia caerulea* (white triangles) litters
760 in monoculture and in measured and expected mixture (measured – dark grey diamonds, and
761 expected - light grey squares). All correlations are significant ($P < 0.05$).

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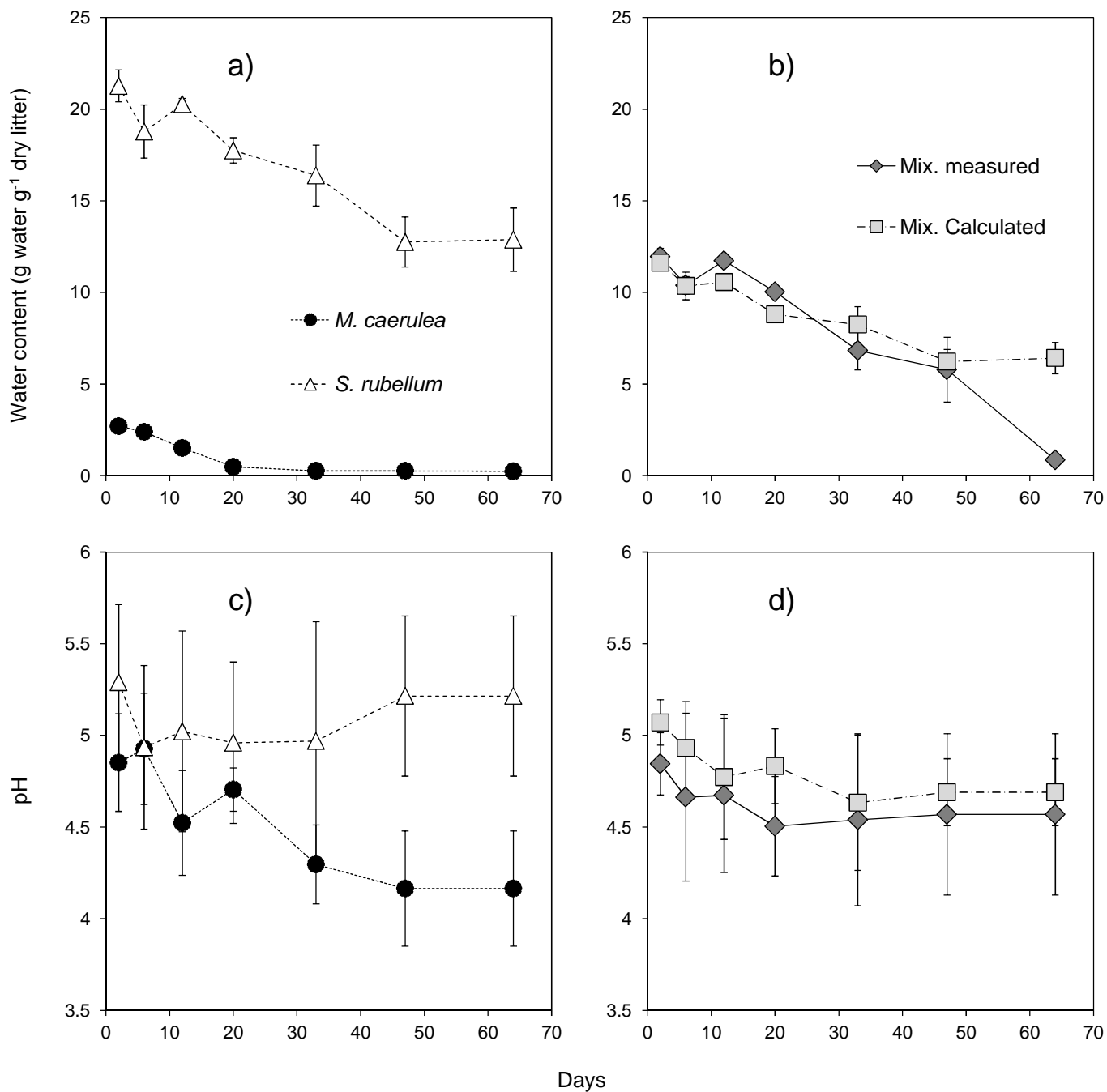
764 Figure 1.



765

766 Figure 2.

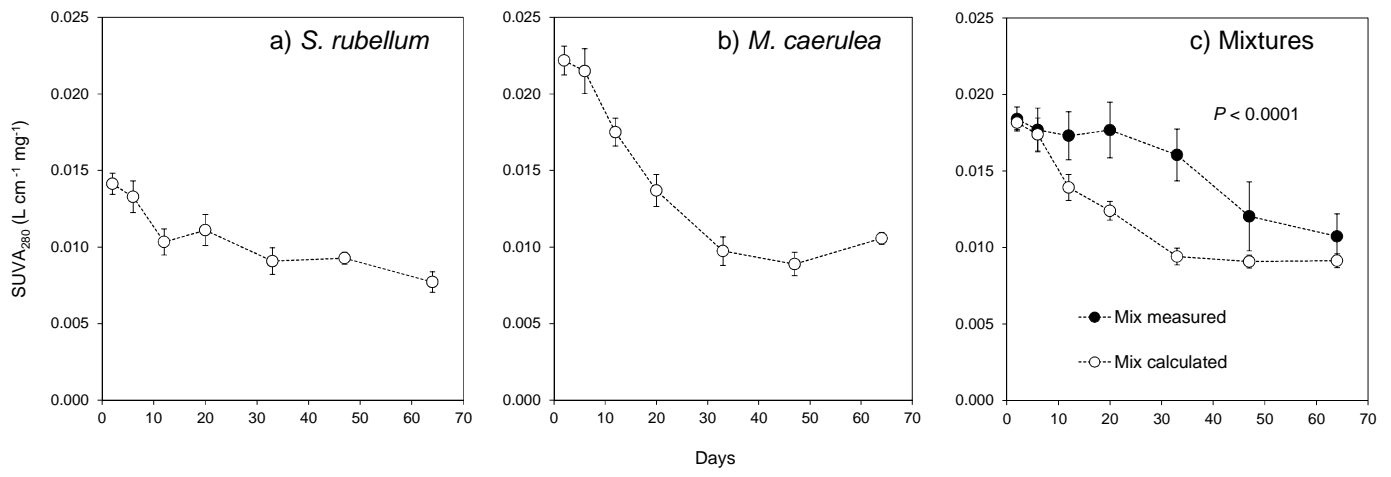
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769 Figure 3.

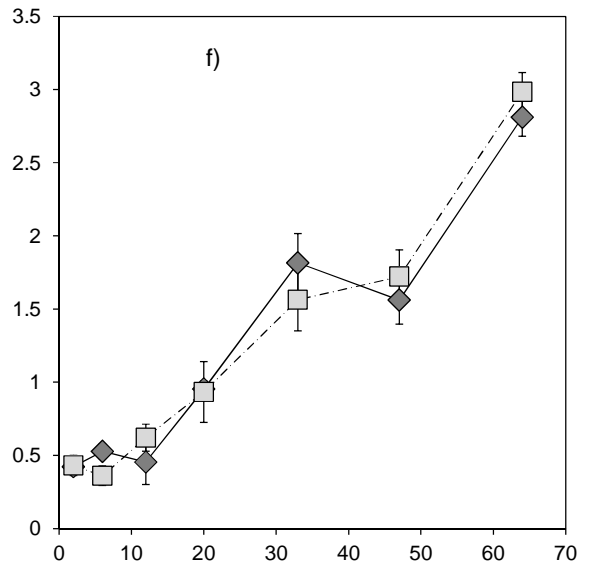
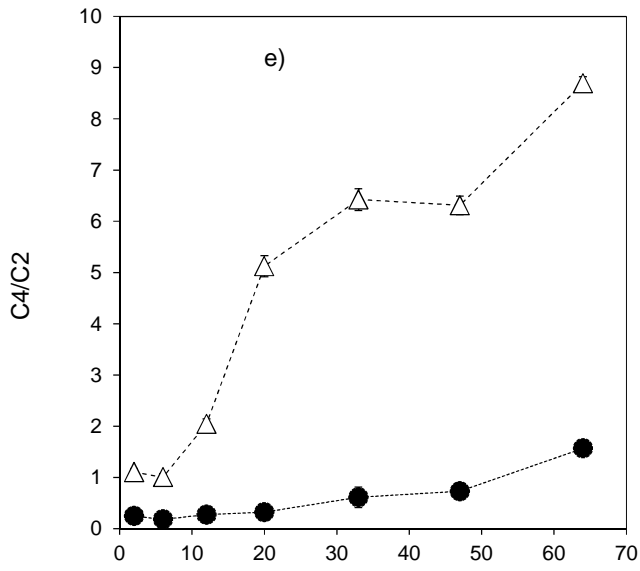
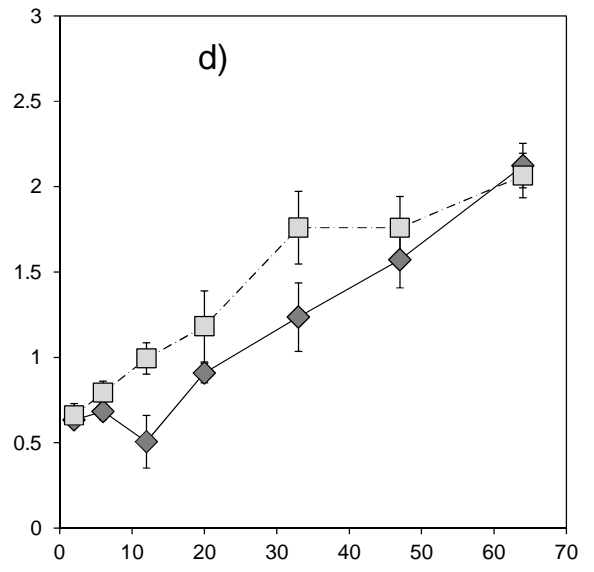
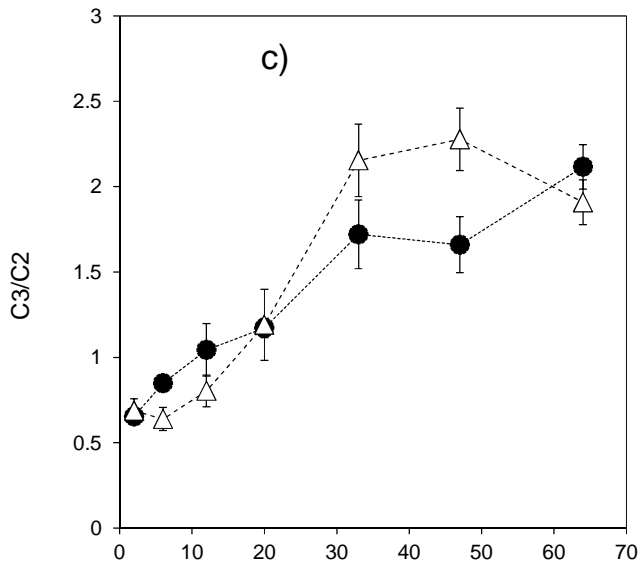
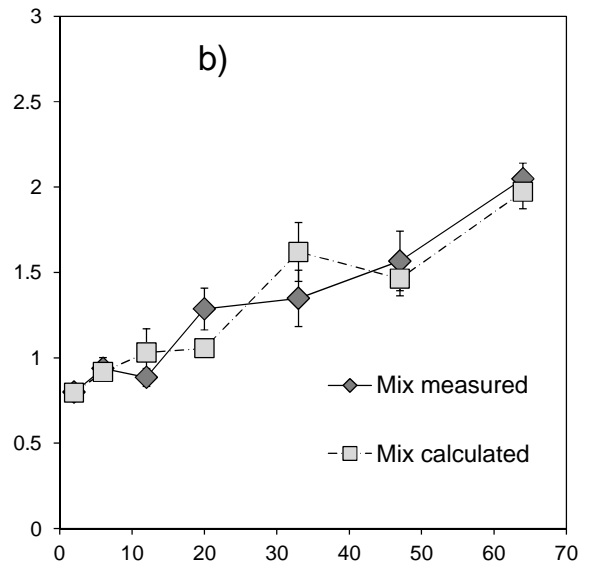
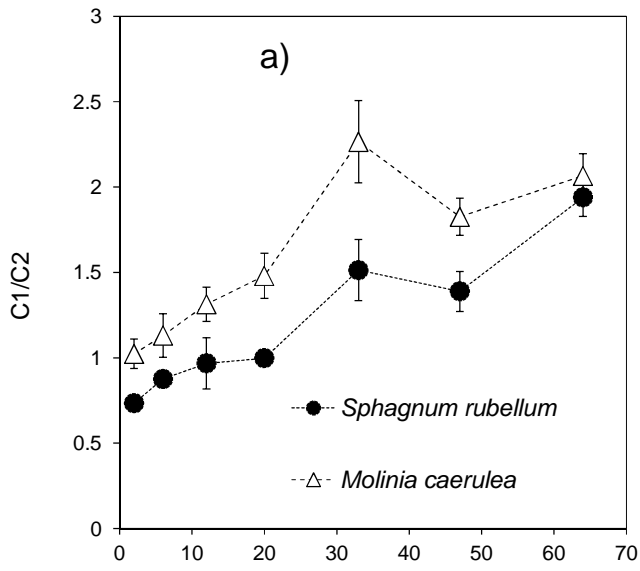
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772 Figure 4.

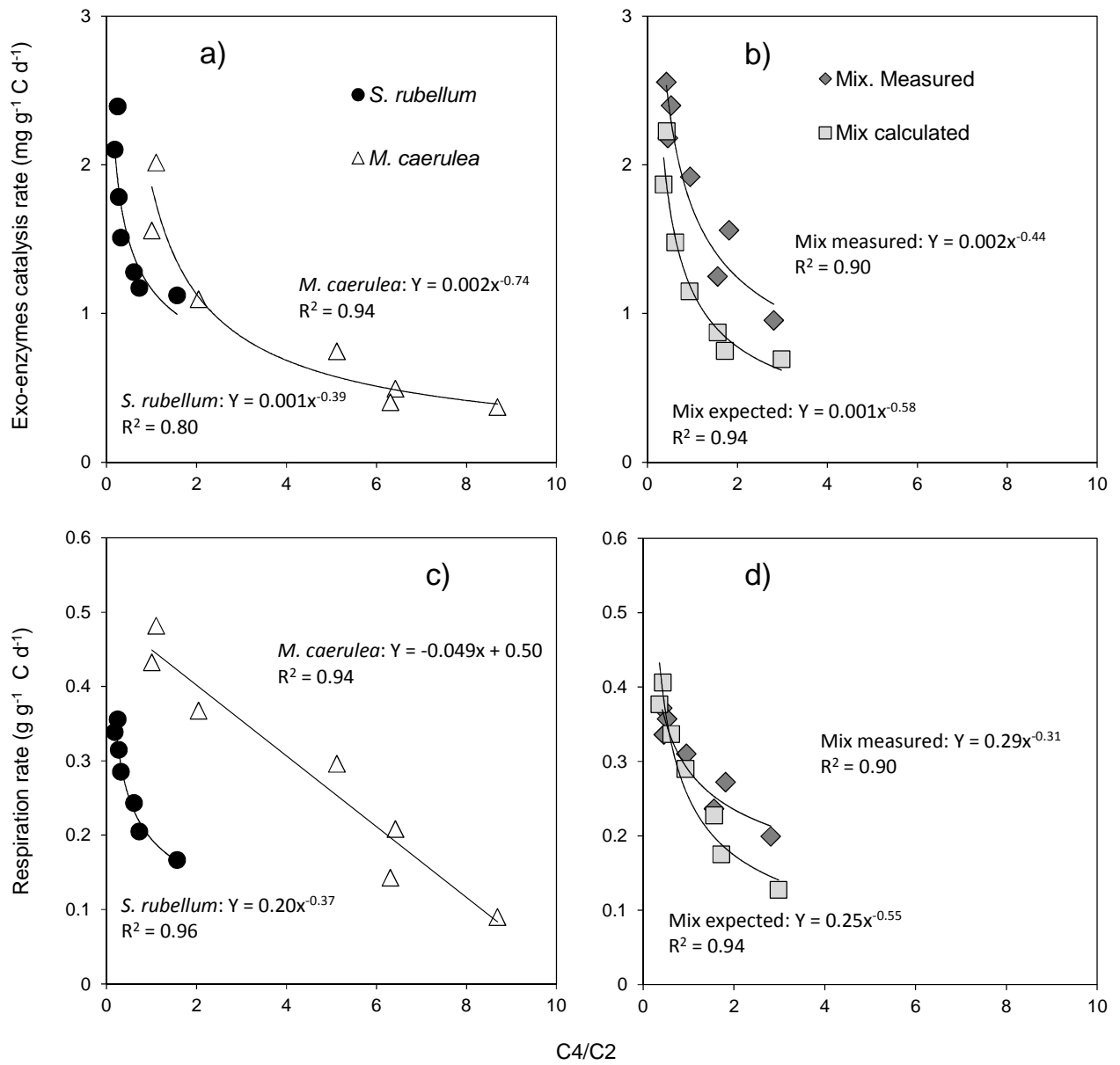
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Days

774

775 Figure 5.



776

777 Figure 6.