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

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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) CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub>  
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<sup>-1</sup>  
206 <sup>1</sup>) 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  $22.5 \pm 0.3$  °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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> infra-red probe (GMP343). The  
229 CO<sub>2</sub> concentrations were monitored and recorded directly on a computer. The CO<sub>2</sub> 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 CO<sub>2</sub> 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<sup>-1</sup> 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 Phthalate 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 SUVA<sub>280</sub>*

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<sup>-1</sup>, with no  
261 samples higher than 31 mg L<sup>-1</sup> or lower than 7 mg L<sup>-1</sup>. 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 (SUVA<sub>280</sub> in L cm<sup>-1</sup> mg<sup>-1</sup>). The SUVA<sub>280</sub>  
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  $\alpha$  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  $\alpha'/\alpha$  ratio. As both humic substances have  $\alpha'$  and  $\alpha$  components, it is  
291 not possible to use this ratio to distinguish humic substances. The component  $\alpha$  is composed of  
292 more humified and older compounds than those found in the component  $\alpha'$ . 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  $\gamma/\alpha$  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  $\gamma$  fluorophores (C4 component in this study) compared to the  $\alpha$   
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 CO<sub>2</sub> (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 CO<sub>2</sub>  
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 CO<sub>2</sub> 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 CO<sub>2</sub> 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, SUVA<sub>280</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub>; 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<sub>280</sub>.  
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  $\gamma$  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  $\gamma$  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  $\gamma$  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<sup>-1</sup> and 2.2 mg g<sup>-1</sup>



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  $\gamma/\alpha$  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 CO<sub>2</sub> 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  $\beta$  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	$\beta$	300-340	400-440	Humic substances
C2	C	$\alpha$	330-370	420-480	humic substances
C3	A	$\alpha'$	250-270	380-480	humic substances + recent materials
C4	B-T	$\gamma$	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	0.007
	<i>M. caerulea</i>	68.5 $\pm$ 2.56	
	Mix measured	71.1 $\pm$ 1.74	<b>0.02</b>
	Mix calculated	77.6 $\pm$ 1.39	
	<i>S. rubellum</i>	86.9 $\pm$ 0.95	0.01
	<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	0.004
	<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 ( $\pm 1$  s.e., n = 6, %), and mean water  
 716 extractable organic carbon (WEOC) concentration ( $\pm 1$  s.e., n = 42, mg g<sup>-1</sup> dry weight), CO<sub>2</sub>  
 717 production ( $\pm 1$  s.e., n = 42,  $\mu\text{g C-CO}_2 \text{ g}^{-1}$  dry weight h<sup>-1</sup>), litter water content ( $\pm 1$  s.e., n = 42, g  
 718 H<sub>2</sub>O g<sup>-1</sup> dry weight) and WEOC pH ( $\pm 1$  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 <sub>2</sub> 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 <sub>2</sub>	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  $\text{CO}_2$  (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  $\text{SUVA}_{280}$  ( $\text{L cm}^{-1} \text{mg}^{-1}$ ,  $\pm 1\text{s.e.}$ ,  $n = 6$ ) and dissolved organic carbon  
742 (DOC,  $\text{mg L}^{-1}$ ) 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)

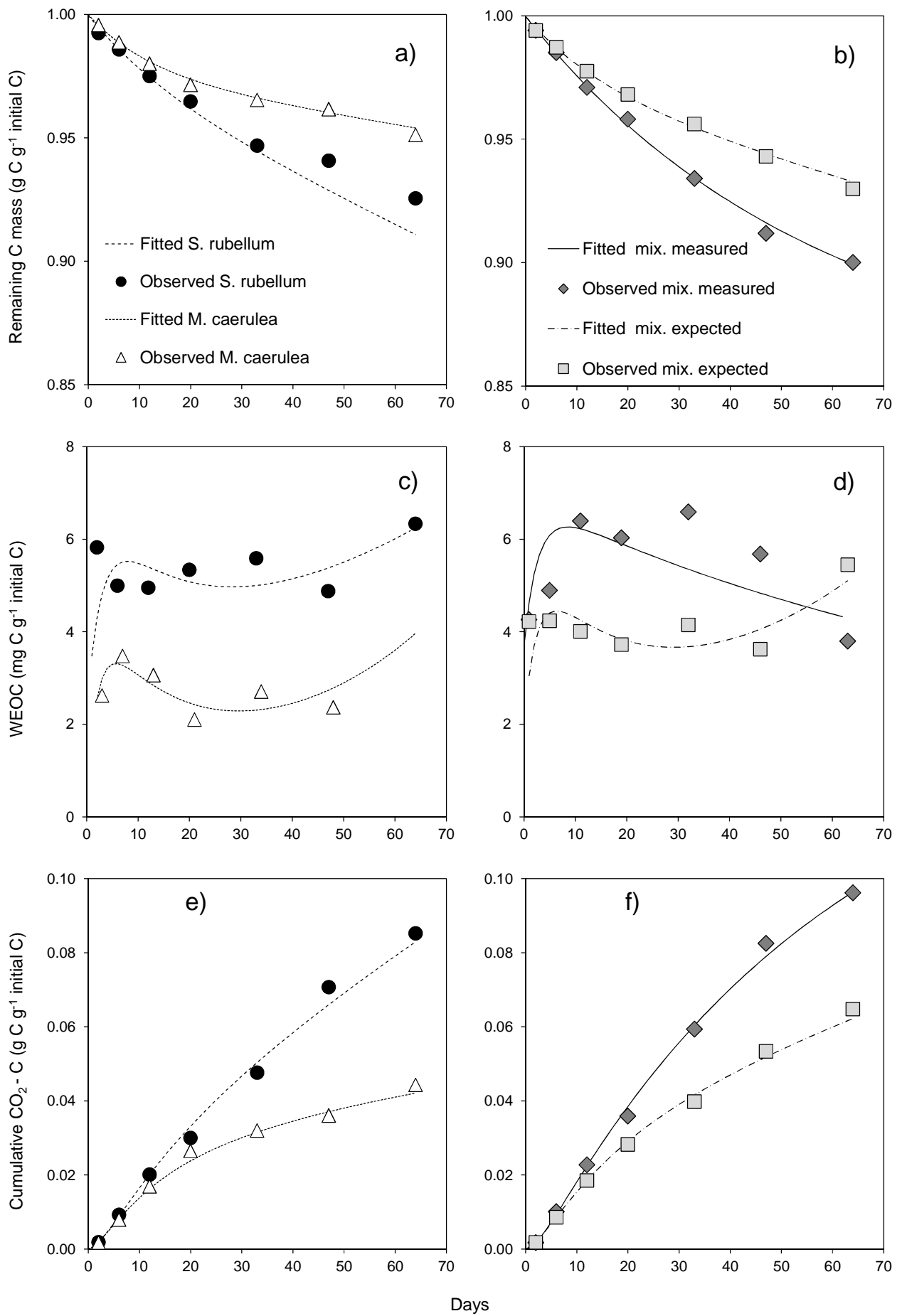
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753 **Fig. 5** Relationship between modelled enzyme activity (a, b) and respiration (c, d) and the  
754 C4/C2 ratio in *Sphagnum rubellum* (black circles) and *Molinia caerulea* (white triangles) litters in  
755 monoculture and in measured and expected 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 ( $P < 0.05$ ).

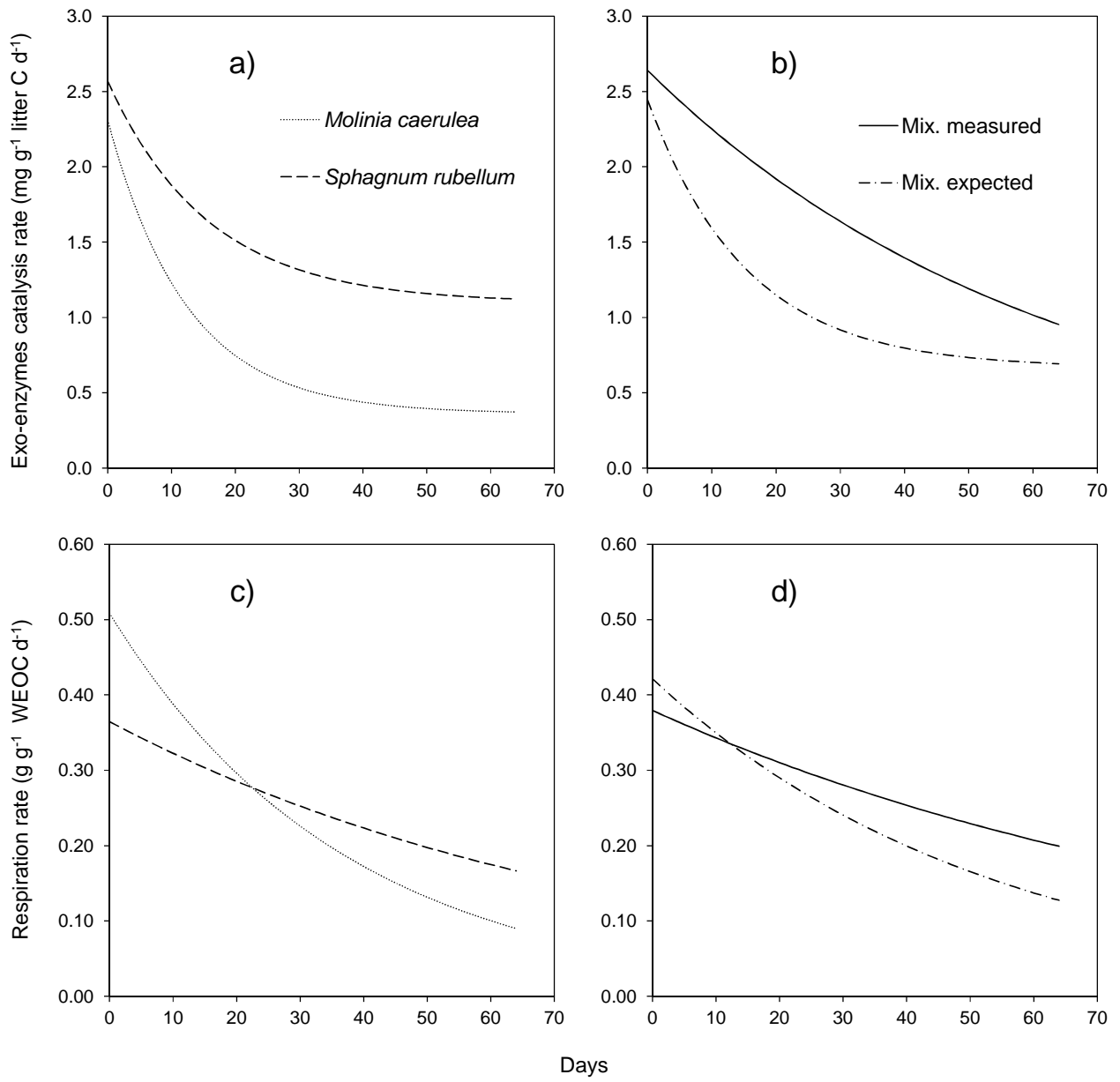
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764 Figure 1.

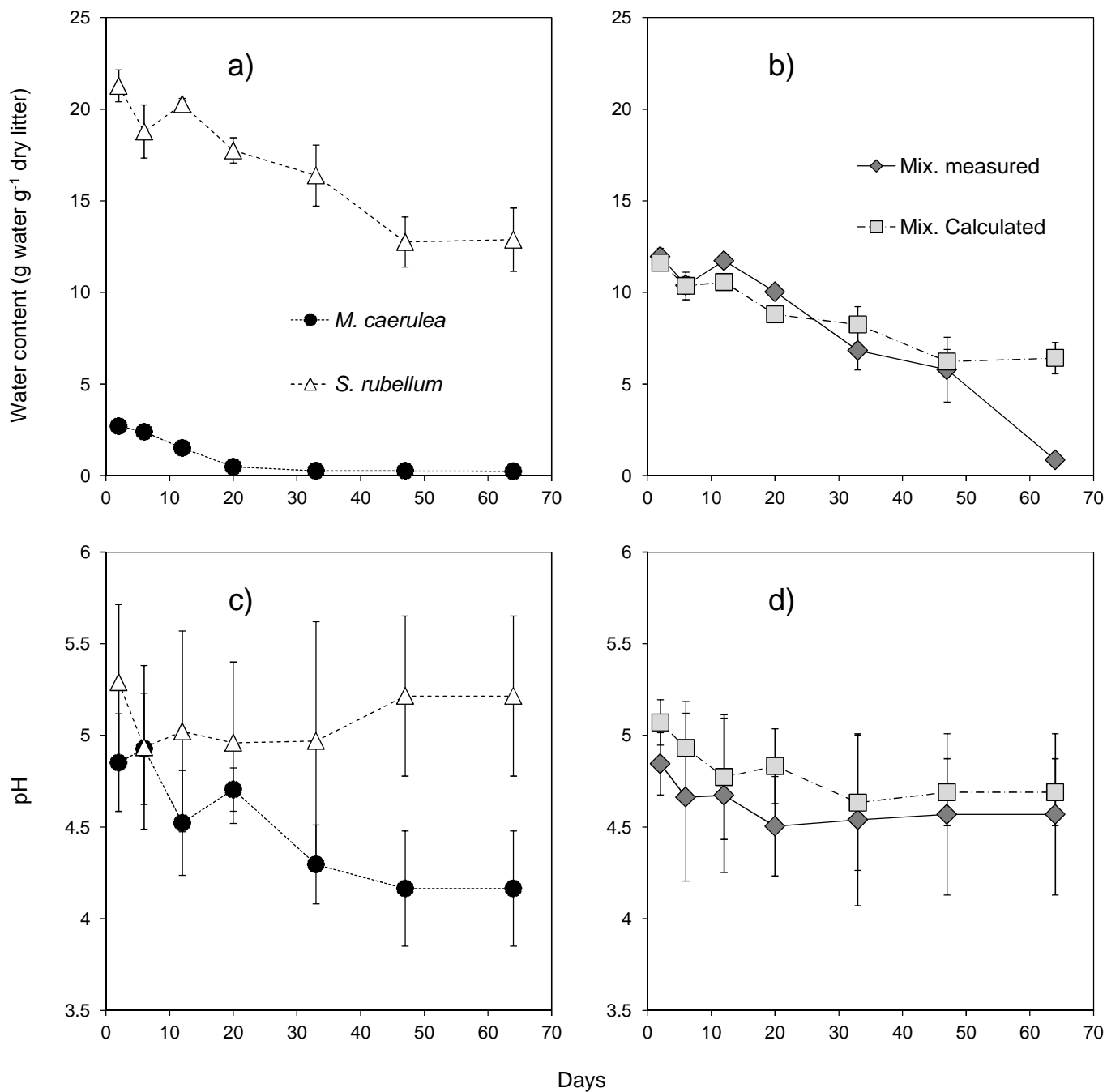




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766 Figure 2.

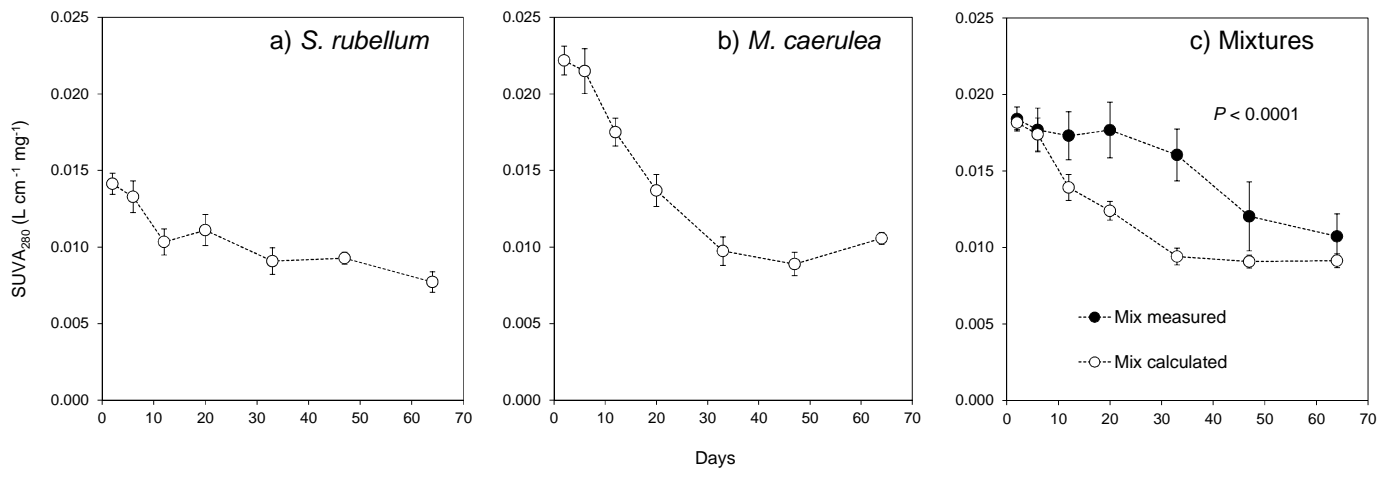
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769 Figure 3.

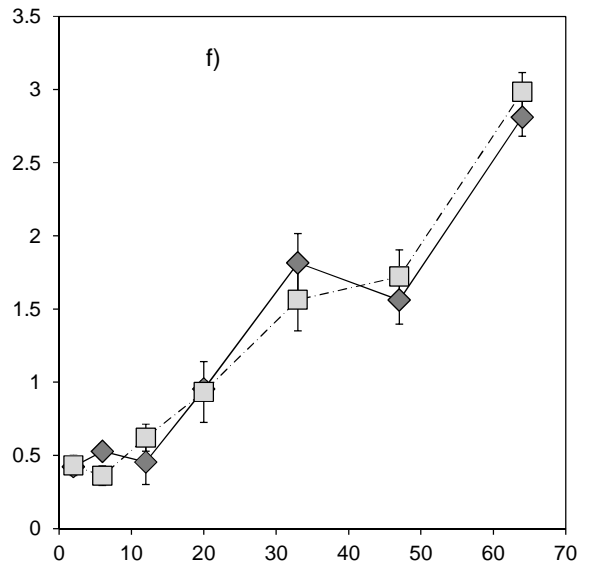
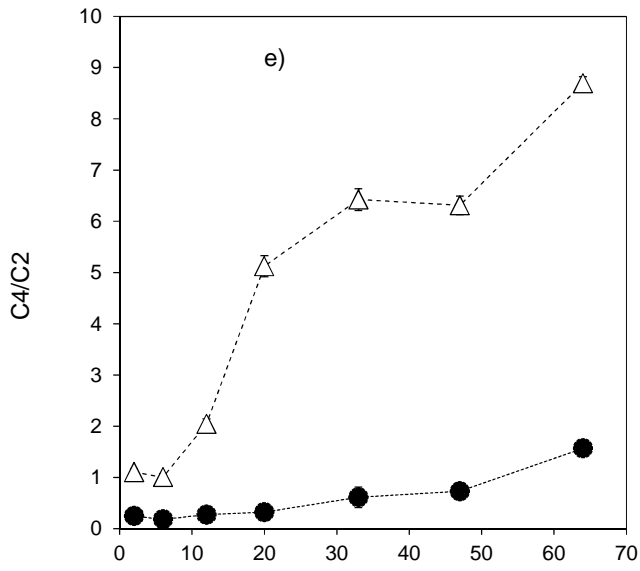
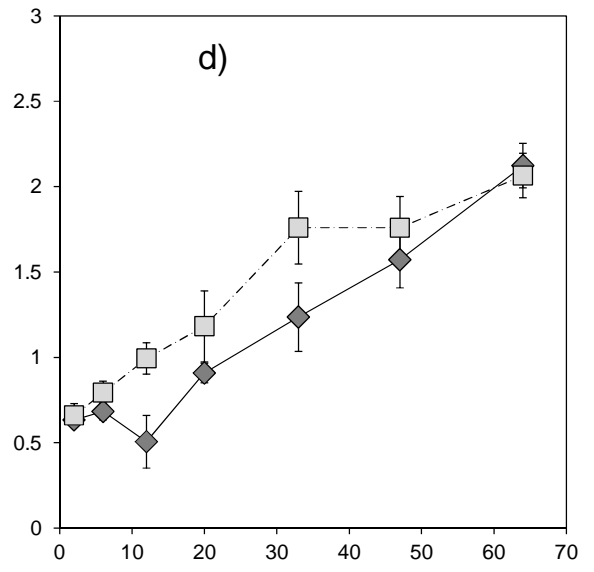
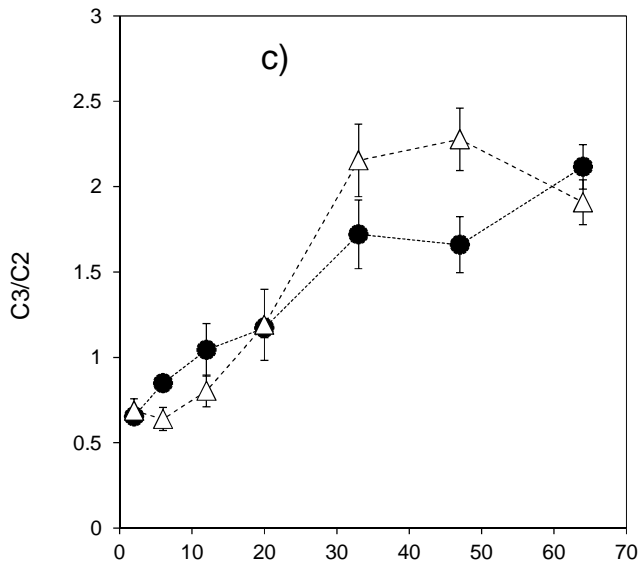
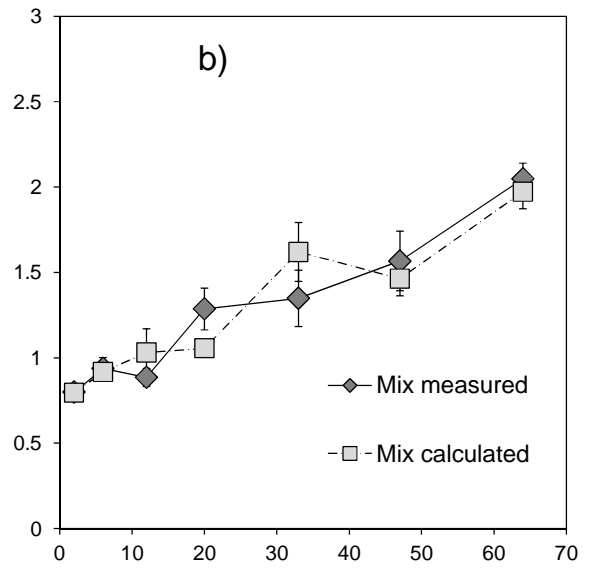
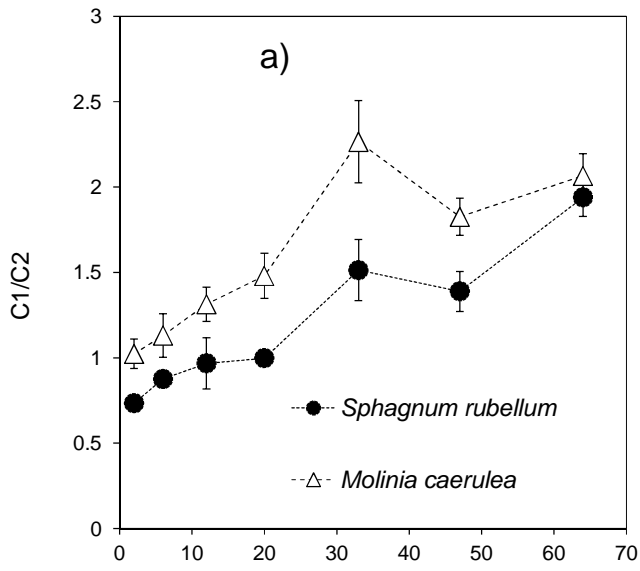
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772 Figure 4.

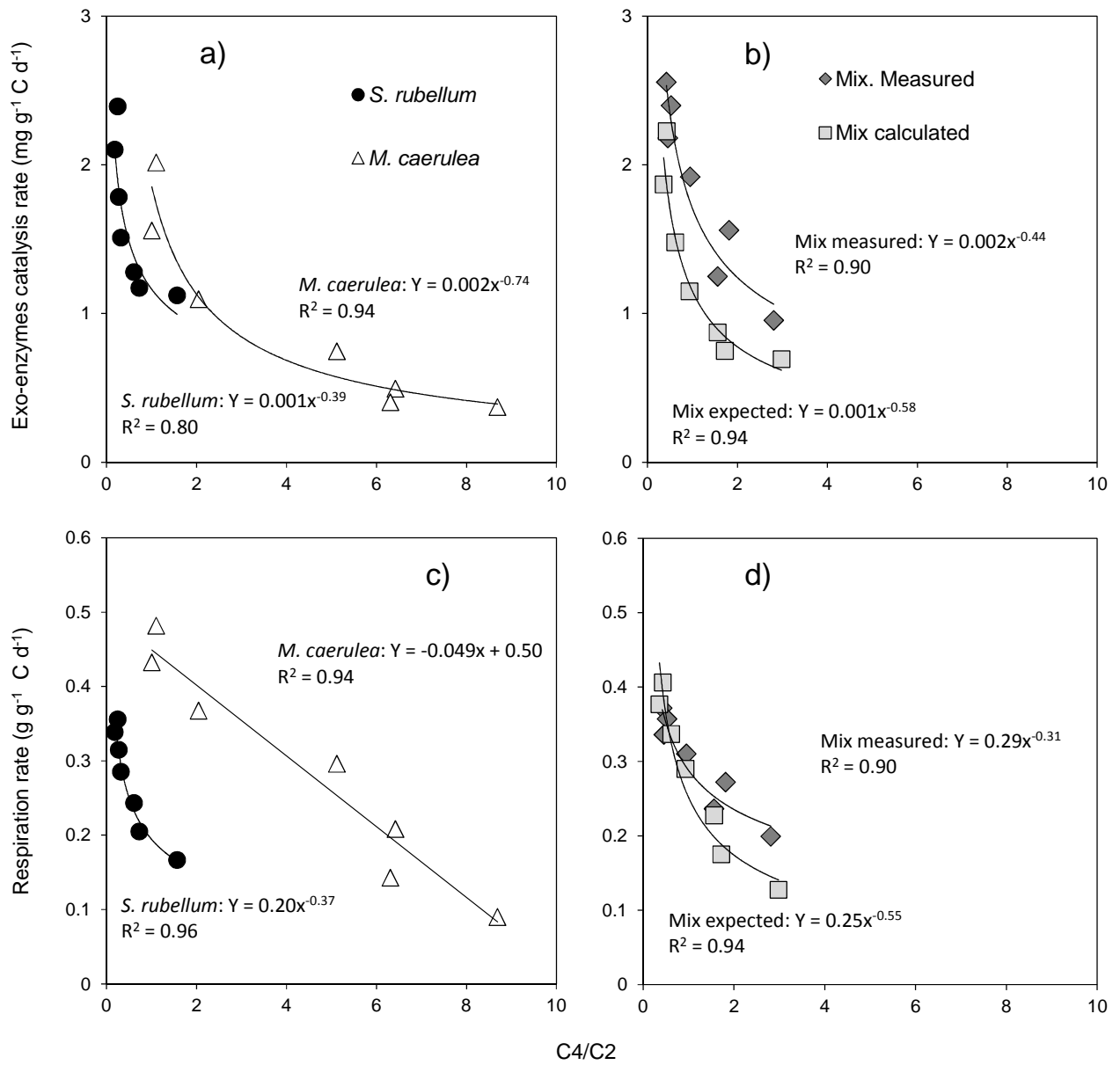
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Days

774

775 Figure 5.



776

777 Figure 6.