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► **To cite this version:**

Mathilde Chomel, Virginie Baldy, Marie Guittonny, Stephane Greff, Annie Desrochers. Litter leachates have stronger impact than leaf litter on *Folsomia candida* fitness. *Soil Biology and Biochemistry*, 2020, 147, pp.107850. 10.1016/j.soilbio.2020.107850 . hal-02571920

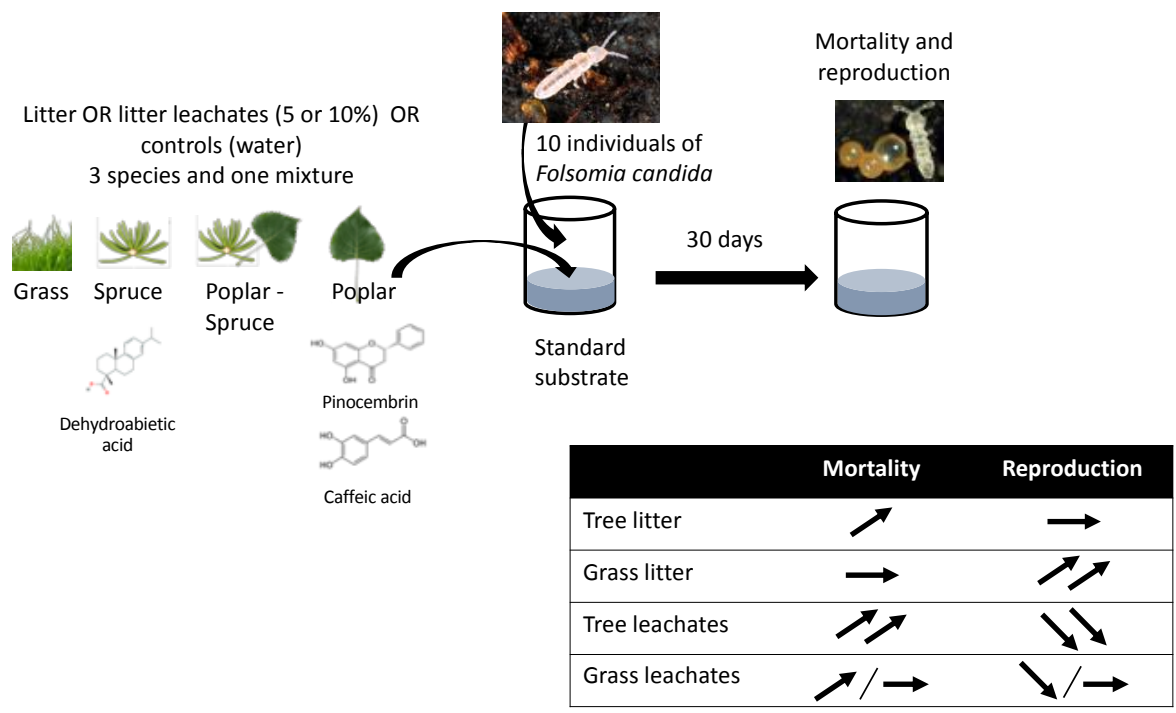
**HAL Id: hal-02571920**

**<https://amu.hal.science/hal-02571920>**

Submitted on 11 Dec 2020

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Change of fitness of *F. candida* in comparison with controls

Tree litter leachates increased mortality of *Folsomia candida* in comparison with the litter treatment or the controls, probably due to the solubility of secondary metabolites contained in the leaves and released in a free form in leachates.

## Highlights

- Tree litter leachates have stronger influence than litter itself on *Folsomia candida* fitness
- Tree litter leachates have stronger effect on *F. candida* fitness than grass litter due to their content in secondary metabolites
- Soluble phenolic compounds released by litter leaching can drive *F. candida* fitness
- Litter compounds leaching appears to be an important process in aboveground-belowground interactions

1 **Litter leachates have stronger impact than leaf litter on *Folsomia candida* fitness**

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15 Key words: secondary metabolites; dissolved organic compound; Collembola; mortality;  
16 reproduction; metabolomic

17

## 18 **Abstract**

19 It is well known that soil physico-chemical conditions and the nature of organic matter have  
20 important effects on soil micro-arthropod communities, including collembolans. However,  
21 mechanisms by which the physical or chemical quality of litter influence collembolan  
22 communities remain unclear. Plant secondary metabolites are partially released in soils  
23 through leaf and litter leaching and decomposition, and can have a strong influence on soil  
24 communities and their activity. In order to disentangle effects of the water-soluble compounds  
25 contained in the litter versus its physical effect, a microcosm experiment was set up exposing  
26 the collembola species *Folsomia candida* to either litter or litter leachates mixed to the  
27 substratum. Litter from three species with different chemical properties and one mixture  
28 (hybrid poplar, white spruce, grass and a mixture of poplar – spruce litter) and two  
29 concentrations of litter leachates (at 5 % and 10 % concentration) were used in microcosm  
30 experiments. After 30 days of incubation, reproduction and mortality rates of *F. candida* were  
31 assessed. Results showed that the tree litter leachates had a stronger impact on collembolan  
32 fitness compared to the litter itself, with a net reduction of survival and reproduction rates.  
33 Between 78 and 100% of mortality was observed in microcosms that received tree leachates,  
34 indicating a strong influence of the soluble compounds contained in tree leaves on  
35 collembolan. In contrast, collembolan reproduction was positively affected by the grass litter  
36 or 10% grass litter leachates compared to control (water). Our findings help to understand  
37 how chemical properties and leaf leaching may have important impacts on micro-arthropods  
38 communities and litter decomposition processes.

## 39 **1. Introduction**

40 Physical and chemical characteristics of plant leaves exert an important control on litter  
41 decomposition processes, mainly through their impact on the activity of litter-feeding

42 invertebrates and microorganisms. For example, previous food choice experiments showed  
43 that earthworms or isopods were sensitive to the quality of the litter (Hättenschwiler and  
44 Bretscher, 2001; Joy and Joy, 1991; Rief et al., 2012). During the first stages of leaf litter  
45 decomposition, an important amount of soluble compounds is lost through leaching,  
46 representing up to 30% of total leaf litter carbon, and released into the soil (Berg and  
47 McClaugherty, 2008). These water-soluble compounds are in a great proportion labile and  
48 readily available, and are an important nutrient source for decomposers and microbial  
49 communities (Joly et al., 2016; Marschner and Kalbitz, 2003). However, secondary  
50 metabolites can also be leached out from green foliage and decomposing litter, and released  
51 into the soil (Gallet and Pellissier, 1997; Rice, 1984). These compounds are defense  
52 compounds to prevent herbivory or parasitism (i.e. terpenes and phenolic compounds), or to  
53 resist against inter- and intraspecific competition in allelopathic mechanisms (Chomel et al.,  
54 2016; Fernandez et al., 2013, 2006). Once released in the environment through leaching or  
55 litter decomposition they are in a free form that may resist degradation and can have strong  
56 impacts on the growth and activity of decomposers, from macro-arthropods to micro-  
57 organisms (Chomel et al., 2016; Hättenschwiler and Vitousek, 2000; Kuiters, 1990). Some  
58 studies showed that microbial activity is highly responsive to litter leachates input with a  
59 change in the soil microbial community (Cleveland et al., 2007; Joly et al., 2016; Wieder et  
60 al., 2008). However, little is known about their effect on soil fauna communities.

61 Collembolans are important members of the soil decomposer food web and have important  
62 direct or indirect roles on the decomposition process (Petersen and Luxton, 1982). Some  
63 species are microbivores and stimulate or regulate litter fungal colonization by grazing on the  
64 fungal hyphae, while others are detritivores and directly participate to organic matter  
65 transformation by fragmenting and ingesting the litter; in both cases they derive nutritional  
66 benefits from the decomposing litter source (Das and Joy, 2009). Collembolans are selective

67 with their food, and for example they can perceive chemical cues of fungi at a distance of 1  
68 cm - 2.5 cm (Auclerc et al., 2010; Salmon and Ponge, 2001), prefer some fungal species over  
69 others (Heděnc et al., 2013; Klironomos et al., 1992; Scheu and Simmerling, 2004), and can  
70 suffer a reduction in fitness from the ingestion of specific fungal species (Klironomos et al.,  
71 1999; Scheu and Simmerling, 2004). Furthermore, collembolans raised on fungal-colonized  
72 litter show grazing preferences and reproduction rates that are more affected by litter type  
73 than by fungal species (Heděnc et al., 2013), demonstrating the strong influence of litter  
74 quality on collembolans activity (Das and Joy, 2009). However, few studies have addressed  
75 the direct impact of the chemical composition of litter on the abundance and diversity of soil  
76 mesofauna, the physical structure of the litter often being a confounding factor. Das and Joy  
77 (2009) reported that a collembolan species (*Cyphoderus javanus* Börner) tended to avoid litter  
78 with greater amounts of polyphenols and tannins. Furthermore, individuals that remained in  
79 contact with litters containing greater quantities of these secondary metabolites exhibited  
80 slower growth, lower fecundity and fewer moults (Das and Joy, 2009). Poinso-Balaguer et al.  
81 (1993) found that condensed and hydrolysable tannins extracted from oak leaves were highly  
82 toxic for the collembolans (Poinso-Balaguer et al., 1993). However, these studies related  
83 collembolans fitness to the total content of secondary metabolites, but did not consider their  
84 solubility in water and their bioavailability in natural environments. Some researchers assert  
85 that allelopathic plant-plant interference is most likely due to water-soluble compounds  
86 (Vyvyan, 2002). Following the same idea, these water-soluble secondary metabolites leached  
87 from the canopy or the litter could be of greater importance for the activity of soil organisms  
88 than the litter content itself.

89 Previous *in-situ* studies showed that mixing spruce and poplar in plantations could offer  
90 valuable ecosystem services, such as tree productivity and soil carbon storage compared to  
91 monospecific plantations (Chomel et al., 2014). Mixture of different litter species can enhance

92 their respective decomposition rate due to the transfer of nutrient between litter species, the  
93 complementarity of diverse resources for the decomposers, or the dilution of secondary  
94 metabolites (Gartner and Cardon, 2004; Gessner et al., 2010; Hättenschwiler et al., 2005).  
95 Similar positive results were observed for mixtures of leachates, leading to a stimulation of  
96 microbial activity (Zheng et al., 2014). In our *in-situ* studies, although we did not find higher  
97 decomposition in a mixture of poplar and spruce litter, some differences with tree species  
98 were observed in the mixed plantations and the collembolan abundance was (i) lower under  
99 poplar trees than spruce trees (Chomel et al., 2015a), (ii) greater in grass litter compared to  
100 poplar or spruce litter (Chomel et al., 2015b), and (iii) increased when grass litter was added  
101 to poplar or spruce litterbags. To understand if these patterns were due to the leaf chemistry of  
102 the different species, since poplar, spruce and grass litters have different qualities, controlled  
103 experiments are necessary. In this study we used the model collembolan species *Folsomia*  
104 *candida* Willem (Collembola: Isotomidae), which is among the most intensively studied of all  
105 species of Collembola (Hopkin, 1997). This parthenogenetic species is widely distributed in  
106 many environments, including forests (Fountain and Hopkin, 2005) and is commonly found in  
107 the litter layer of coniferous and deciduous forests (Christiansen and Bellinger, 1980), even  
108 under the boreal climate of Canada (Skidmore, 1995). Moreover, this species is frequently  
109 used in microcosms experiments as it is easy to maintain in laboratory and have a short  
110 reproductive cycle (Fountain and Hopkin, 2005).

111 The aim of this study was to disentangle the effects of litter water-soluble compounds  
112 (released by senescent leaves leaching) from the litter itself on the abundance and activity of  
113 the Collembola *F. candida*. For this purpose, we compared the effect of three litter species  
114 and one mixture (hybrid poplar, white spruce, grass and a mixture of poplar - spruce litter)  
115 and their respective leachates on *F. candida* fitness by using the modified ecotoxicological  
116 test 'Effects of pollutants on reproduction of *Folsomia candida*' proposed by the International



117 Standards Organization (ISO 11267, 1999). During the experiment, *F. candida* were fed with  
118 yeast to tease apart nutrient effects associated to the treatments and focus on secondary  
119 metabolites effects. We tested the following hypotheses: (i) following results from our *in-situ*  
120 studies (*i.e* low abundance of collembola under poplar trees and high abundance in grass  
121 litter), we expected to find a high concentration of phenolic compounds with allelopathic  
122 activities in poplar litter and a low concentration in grass litter; (ii) A gradient of the effect of  
123 litter and leachates on the fitness of *F. candida* should be observed in the following order  
124 from positive to negative: grass > spruce > poplar; (iii) litter leachates have a more  
125 pronounced impact on *F. candida* compared to litter since nutrients and secondary metabolites  
126 would be directly bioavailable for collembolans; (iv) litter and leachate diversity from the  
127 mixture is expected to stimulate *F. candida* fitness in comparison with monospecific litter and  
128 leachates due to complementarity effects induced by mixed species.

## 129 **2. Material and methods**

### 130 *2.1 Litter sampling and leachates preparation*

131 In late September 2010, three types of senescent leaves (poplar, spruce and grass) were  
132 collected from experimental monospecific plantations of hybrid poplar (*Populus*  
133 *maximowiczii* A. Henry x *P. balsamifera* L.) and white spruce (*Picea glauca* [Moench] Voss)  
134 located in Amos (48°36'N, 78°04'W) in the boreal region of Abitibi-Temiscamingue,  
135 Quebec, Canada. Freshly fallen spruce needles and hybrid poplar leaves were collected by  
136 placing a plastic sheet beneath the trees to prevent soil contamination. Aerial parts of grass  
137 species (mainly grasses, *i.e.* *Poa* sp.) naturally present in the plantation were cut at ground  
138 level during the same period. Collected leaf material was homogenized, air-dried and stored at  
139 room temperature prior to the experiment. Leachates of each litter type (poplar, spruce, grass  
140 and poplar/spruce [50:50]) were prepared by soaking 100 g of litter (air-dried mass) in 1 L of

141 deionized water (10% dry mass) for 24 h in darkness. This concentration is the most  
142 commonly used for *in vitro* allelopathy bioassays (Chen et al., 2013; Fernandez et al., 2013).  
143 Although they can be more concentrated than in natural conditions, it allows studying the  
144 potential allelopathic effect of a species (Kil, 1992). However, to be more representative to  
145 actual field concentrations, a 50% diluted solution was also prepared, producing 5% dry mass  
146 leachate.

## 147 *2.2 Folsomia candida* cultures

148 A population of a single clone of *F. candida* was reared in plastic boxes containing a mixture  
149 of permanently water saturated plaster of Paris and activated charcoal in a ratio 9:1 and  
150 maintained at 20°C with food (dry yeast pellets) available *ad libitum*. To synchronize age of  
151 the organisms, oviposition was stimulated by imposing a cold period and then placing the  
152 adults on a new breeding substrate (Fountain and Hopkin, 2005). After oviposition, adults  
153 were removed and eggs hatched 3-4 days later. Individuals became sexually mature 16 days  
154 after hatching, and to ensure a homogeneous cohort of juveniles, all the young individuals  
155 were placed in a large container and fed for the first time at the same time.

## 156 *2.3 Experimental design*

157 To study the effects of litter or leachates on the fitness of *F. candida*, we set up a total of 65  
158 microcosms. The microcosms were set-up according to 2 interacting factors: the litter form  
159 (litter, 5% leachate and 10% leachate) and the litter species (poplar, spruce, grass and poplar -  
160 spruce mixture) and controls consisted of deionized water without litter. Each combination of  
161 factors was replicated five times. The *F. candida* reproduction test was carried out according  
162 to an adaptation of the ISO standard 11267 (ISO 11267, 1999). The microcosms consisted of  
163 120 mL plastic bottles with pierced screw caps containing 32 g of wet artificial soil, made up  
164 with 70% quartz sand, 20% kaolinite and 10% peat, ground, dried, and sieved to 0.5 mm with

165 the pH adjusted to  $6.0 \pm 0.5$  by the addition of  $\text{CaCO}_3$ . It was moistened to 50% of water  
166 holding capacity with distilled water (7 mL). Chemicals to be tested in the ISO norm are  
167 generally pollutants dissolved in water at range of concentrations that will give appropriate  
168 reduction of reproduction levels (LOEC, CE 50). For this experiment, 7 mL of 5 or 10 %  
169 leachates were directly mixed with the soil. For the litter species treatments, 7 mL of distilled  
170 water were added to the substrate, and 1 g of coarsely chopped litter was re-humidified and  
171 placed on the substrate (corresponding to the amount of litterfall per surface area in natural  
172 plantations, Chomel *et al.* 2014). For each microcosm, ten 10/12-days-old juveniles were  
173 introduced and the microcosms were randomly placed in a climate chamber at a constant  
174 temperature of 20°C and continuous darkness for 30 days. They were opened twice a week for  
175 aeration and fed with baker's yeast at the initial time and after 2 weeks. Following the ISO  
176 guidelines, the pots were flooded with water and gently stirred to collect and count the  
177 animals floating at the surface. The number of surviving adults and the juveniles were  
178 recorded using a dissecting microscope.

#### 179 *2.4 Chemical composition of litter and leachates*

180 Litter initial content of major nutrients (C, N, P) and phenolics were estimated from 5  
181 subsamples of each litter. The litter samples were finely ground with a ball mill (MM301,  
182 Retsch Inc., Newtown, PA) prior to analysis. Carbon (C) and Nitrogen (N) concentrations  
183 were analysed in a CHN elemental analyzer (Flash EA 1112 series, ThermoScientific,  
184 U.S.A.). Phosphorus (P) was extracted with 20 mL of nitric acid from remaining dry ash after  
185 combustion of 0.5 g of subsamples at 500 °C for 5 h in a muffle furnace. The pH was adjusted  
186 to 8.5 with a 40 % NaOH solution. A volume of 1 mL of sample, 0.2 mL of mixed reagent  
187 (emetic tartar and ammonium molybdate solution), 0.04 mL of ascorbic acid and 0.76 mL of  
188 distilled water were placed directly in a microcuvette. After 150 min, the reaction was  
189 completed, and phosphorus concentration was measured at 780 nm with a UV/Vis

190 spectrophotometer (Thermo Scientific®, USA). Total phenolics content was measured by the  
191 method of Folin-Ciocalteu (Folin and Denis, 1915): 1 g of ground litter was shaken with 20  
192 mL of distilled water for 90 min. A volume of 0.25 mL of the filtered aqueous extract (or of  
193 the leachate directly) was mixed with 0.25 mL of Folin-Ciocalteu reagent and 0.5 mL of  
194 saturated aqueous Na<sub>2</sub>CO<sub>3</sub> to stabilize the colour reaction, after which 4 mL of distilled water  
195 was added to dilute the extract. After 1 hour, the reaction was completed and measured at 765  
196 nm on a UV/Vis spectrophotometer (Thermoscientific, U.S.A.). Quantitative results were  
197 expressed with reference to gallic acid.

#### 198 *2.5. Litter metabolic fingerprints (untargeted metabolomics)*

199 The metabolomic profile of the litter was analyzed following the method from Hashoum et al.  
200 (2017). A dry mass of 200 mg of sample was suspended in 4 mL of methanol:water (50:50),  
201 and subjected to ultrasonication for 5 min at room temperature. Extracts were then filtered  
202 using a syringe filter (PTFE 13 mm, 0.22 μm, Restek, USA). Analyses were performed with  
203 an UHPLC instrument (Dionex Ultimate 3000 equipped with a RS Pump, an autosampler, a  
204 thermostated column compartment and a UV diode array, Thermo Scientific®, USA) coupled  
205 to an accurate mass spectrometer (qToF) equipped with an ESI source (qToF Impact II,  
206 Bruker Daltonics®, Germany). UHPLC separation was done on an Acclaim C18 column (150  
207 mm x 2.1 mm, 2.2 μm, Thermo Scientific, USA). Because the negative mode gave a better  
208 sensitivity, mass spectra were recorded in this ionization mode in full scan mode from 50 to  
209 1200 amu at 2 Hz. After the dataset normalization (Hashoum et al., 2017), around 6000  
210 features were kept before the filtering steps were applied to ensure data quality and remove  
211 redundant signals using an in-house script on R (Hashoum et al., 2017). At the end, 3030 ions  
212 were kept for data analyses.

#### 213 *2.6 Statistical analysis*

214 For all the chemical data, ANOVA were performed to test differences between two  
215 interacting factors: Litter species (poplar, spruce, grass and mixture poplar-spruce) and litter  
216 form (control, litter, 5% leachate and 10% leachate). Data were ln-transformed before  
217 performing statistical tests if the conditions of normality and homoscedasticity of the residuals  
218 were not met. After the analyses, multiple comparisons (Tukey contrasts) were done. To have  
219 an estimation of the metabolomic diversity of each litter species, the Shannon diversity index  
220 was calculated from all the metabolomic data including 3030 ions (Quinn et al., 2016; Ristok  
221 et al., 2019). To detect compounds that were specific to each litter species we selected the  
222 fifty most discriminating ions (Variable Importance in Projection, VIP) that differentiated the  
223 three litter species using a PLS-DA analysis (Fig S1) with MetaboAnalystR (Chong and Xia,  
224 2018). The features were annotated with constructor software (Bruker Compass DataAnalysis  
225 5.0, Table S1). The most probable raw formulae and fragmentation patterns ( $MS^2$  spectrum in  
226 negative mode) were compared with online databases (Metlin, Massbank of North America,  
227 Pubchem using Metfrag, Table S1). Further, we constructed a heatmap with those VIPs to  
228 visualise the relative intensities of each of these ions according to each litter type with R. A  
229 binomial generalized linear model (GLM) was used to test the difference of collembolans  
230 mortality rate in function of the different litter species (poplar, spruce, poplar/spruce mixture  
231 and grass litters) and litter forms (control, litter, 5% leachate and 10% leachate). Binomial  
232 GLMs are designed to fit proportions or percentages. A negative binomial GLM (*i.e.*, a  
233 specific version of a Poisson model that uses an additional parameter to correct for data over-  
234 dispersion) with a log link function was used to test the difference in reproduction with the  
235 different litter species (poplar, spruce, poplar/spruce mixture and grass litters) and litter forms  
236 (control, litter, 5% leachate and 10% leachate). Negative binomial GLMs are designed to fit  
237 count data (data that usually lacks normality), as it is generally the case when sampling  
238 invertebrate taxa. All statistical analyses were done with R v.3.1.0 (R Core Team, 2017),

239 using the package ‘stats’ for the ANOVA and binomial GLM and the package ‘MASS’ for the  
240 negative binomial GLM.

### 241 **3. Results**

#### 242 *3.1 Chemical composition of litters and leachates*

243 Spruce litter presented greater C concentrations, while grass litter had greater N  
244 concentrations compared to the two other litter types ( $P < 0.01$  and  $P < 0.001$  respectively,  
245 Table 1). Consequently, grass litter had the lowest and spruce litter the greatest C/N ratios,  
246 while poplar had intermediate values ( $P < 0.001$ ). Phosphorus concentrations in the litters  
247 were similar among the different litter species (Table 1). Concerning the total phenolics, grass  
248 litter had more than four-fold lower phenolic concentrations compared to the three other litter  
249 species ( $P < 0.001$ , Fig. 1). For the soluble compounds, however, we observed that grass and  
250 spruce leachates contained lower amounts of phenolic compounds compared to poplar and  
251 mixed litters leachates ( $P < 0.005$ , Fig. 1). The proportion of phenolics in the leachates  
252 relative to the litters differed depending on the litter species; these proportions corresponded  
253 to 36%, 7%, 31%, and 38% of the total litter concentrations respectively for grass, spruce,  
254 poplar-spruce mixture and poplar leachates (Fig. 1). Metabolomic diversity, calculated by the  
255 Shannon index of the whole dataset, was lower in spruce and higher in poplar litters, with an  
256 intermediate diversity in grass litter (ANOVA,  $F = 52.9$ ,  $P < 0.001$ , Table 1). Qualitative  
257 analysis of the 50 most discriminant features of the three litter species is reported in Fig. 2 and  
258 shows that the majority of the features were specific to the poplar litter, and were present in  
259 greater abundance than in the two other litter species. Among them, we identified caffeic acid  
260 derivatives, pinocembrin and several pinocembrin derivatives, salicin, quercetin and  
261 arthromerin A (Table S1). Six features, among which we identified dehydroabietic acid, were  
262 specific to the spruce litter, while rare or absent in the poplar litter. Three compounds were

263 found either in the poplar and the grass litter, among them we identified chlorogenic acid and  
264 ferulic acid (Table S1).

### 265 *3.2 Effects of litter and leachates on F. candida mortality*

266 Mortality of *F. candida* differed across combinations of litter species ( $X^2 = 63.1$ ,  $P < 0.001$ )  
267 and forms ( $X^2 = 259.7$ ,  $P < 0.001$ ) with a significant interaction term of the two factors ( $X^2 =$   
268  $57.5$ ,  $P < 0.001$ ). While the mortality of *F. candida* with the grass litter or the 10% grass  
269 leachate were similar to the mortality observed in the controls ( $P > 0.05$ , Fig. 3), a four-fold  
270 increase in mortality was observed with the grass 5% leachate compared to the control ( $P <$   
271  $0.001$ , Fig. 3). For the spruce litter, we observed an increase in mortality with all the litter  
272 forms compared to the control (Fig. 3). With the litter itself, mortality reached 62%, while it  
273 reached 84% and 78% with the 5% and 10% leachates, respectively. Although the mortality  
274 with the spruce litter leachates tended to be greater than with the litter, differences were only  
275 marginally significant ( $P = 0.053$  and  $P = 0.058$  respectively). While the mortality of *F.*  
276 *candida* with the mixed litter was similar to the mortality observed in the controls ( $P > 0.05$ ,  
277 Fig. 3), a three-fold increase in mortality was observed with the 5% leachate and the 10%  
278 leachate compared to the litter, reaching mortality rates of 93% and 98%, respectively ( $P <$   
279  $0.001$ , Fig. 3). Lastly, for the poplar litter, we observed an increase in mortality with all the  
280 litter forms compared to the controls (Fig. 3). Mortality reached 68% with the litter, while it  
281 was 94 % and 100 % with the 5 % and 10 % leachates, respectively ( $P < 0.001$ , Fig. 3).

### 282 *3.3 Effects of litter and leachates on F. candida reproduction*

283 Reproduction of *F. candida* differed across combinations of litter species (negative Binomial  
284 GLM,  $X^2 = 39.4$ ,  $P < 0.001$ ) and forms (Binomial GLM,  $X^2 = 70.5$ ,  $P < 0.001$ ) with a  
285 significant interaction term (Binomial GLM,  $X^2 = 75.7$ ,  $P < 0.001$ ). Leachates of spruce,  
286 mixed or poplar significantly reduced reproduction in comparison with the litter or the control

287 treatments ( $P < 0.05$ , Fig. 4). The grass litter and 10% leachate increased the reproduction of  
288 *F. candida* by 385 % and 70 %, compared to the control, respectively. However, the grass 5%  
289 leachate decreased the reproduction by 83% compared to the control (Fig. 4).

## 290 **4. Discussion**

291 The aim of this study was to observe the fitness of *F. candida* in the presence of litter or  
292 leachates of different litter species with diverse chemical characteristics. We observed that  
293 leachates generally increased *F. candida* mortality compared to the litters. Additionally, we  
294 observed greater mortality rates of collembolans with tree leachates, which contained a  
295 greater quantity of phenolic compounds, compared to grass leachates. Our results also showed  
296 reproduction rates four times greater with grass litter than for tree litters or controls, in line  
297 with our previous *in-situ* litter decomposition study for which a greater abundance of  
298 organisms was observed when grass litter was added to spruce or poplar litter (Chomel et al.,  
299 2015a).

### 300 *4.1 Effects of litter species and its quality*

301 While we expected an increase of collembolans fitness with the presence of grass litter, we  
302 observed a considerable increase of their reproduction compared to the control and the other  
303 litter species, partially confirming our first hypothesis. Nutritional quality of decomposing  
304 litter depends on the chemical constituents of leaf tissues (Das and Joy, 2009; Heděnec et al.,  
305 2013). Grass litter had relatively low C/N ratios and phenolic compounds contents, indicating  
306 greater concentrations of carbohydrates and proteins. This labile organic matter rich in N  
307 provides a readily available energy source to decomposers (Aber et al., 1990; Aerts, 1997),  
308 and induced better conditions for feeding and reproduction of collembolans (Das and Joy,  
309 2009). While mortality rate with the grass litter was similar than the control, reproduction rate  
310 was much higher. This result confirms the benefit of “high” litter quality to enhance *F.*



311 *candida* fecundity (Booth and Anderson, 1979; Scheu and Simmerling, 2004) and shorten the  
312 period until oviposition starts (Scheu and Simmerling, 2004). Besides C/N ratios, litter  
313 phenolic concentrations could also explain *F. candida* fitness. In comparison with the grass  
314 litter, both poplar and spruce litters had more than four-fold greater concentrations of  
315 phenolics. Within 'low' quality litters, poplar litter had lower C/N ratios but similar total  
316 phenolic concentrations than spruce litter, and both litter types negatively affected the survival  
317 and reproduction of *F. candida*. We observed an increase in mortality of 240 % and 210 %  
318 and a decrease in reproduction of 29 % and 18 %, respectively for poplar and spruce litters,  
319 compared to the control. We identified several specificities among the tree litter species:  
320 spruce litter contained dehydroabietic acid, while pinocembrin and caffeic acid metabolites  
321 were found in poplar litter. Pinocembrin is a flavonoid that has been recognized to have  
322 antimicrobial activities (Rasul et al., 2013), and caffeic acid is also known to have strong  
323 allelopathic and antimicrobial activities (Batish et al., 2008). The terpenoid dehydroabietic  
324 acid is a defense metabolite abundant in resin, thus common in conifers (Phillips and Croteau,  
325 1999). These three secondary metabolites potentially reduce the colonization of the litter by  
326 microorganisms by their antimicrobial activities and can have repulsive effects on detritivores  
327 (Chomel et al., 2016; Das and Joy, 2009). The presence of these secondary metabolites and  
328 the high concentration of total phenolic compounds in tree litters compared to grass litter  
329 could explain their strong and negative impact on *F. candida* survival and reproduction.  
330 However, when these two species of litter were mixed, mortality rate was similar to the  
331 control, confirming our fourth hypothesis. Mixed diets may significantly increase collembolan  
332 fitness by providing a balanced nutrient intake from the different litters present in the mixture  
333 or by a dilution of toxic compounds contained in one of the species (Hättenschwiler et al.,  
334 2005; Scheu and Simmerling, 2004). Conversely, although mixing the two litter types

335 increased survival compared to single species litter, it had no effect on the reproduction of *F.*  
336 *candida*.

#### 337 4.2 Effects of leachates vs litter

338 In agreement with our third hypothesis, leachates had a greater impact on *F. candida* fitness  
339 compared to the litters. Some of the leached compounds can act as easily-available nutrient  
340 sources for decomposers. However secondary metabolites can be toxic and can also have a  
341 greater impact on organisms once released in the environment by leaching or decomposition  
342 (Chomel et al., 2016). It appears that phenolic compounds from the poplar litter were more  
343 water-soluble compared to those from spruce litter, as their leachates contained 38 % of the  
344 total phenolic compounds of the litter, while spruce leachates only contained 7 % of the total  
345 phenolic compounds of the spruce litter. This can be explained by the fact that needles have a  
346 thicker epidermis and hypodermis that reduce the leaching capacity of the inner leaf tissues  
347 (Don and Kalbitz, 2005; Joly et al., 2016). Microcosms that received 10% leachates of poplar,  
348 mixed poplar/spruce or spruce showed 100%, 94 % and 78% of mortality, respectively,  
349 whereas grass leachates only induced 32% of mortality and was not different from the control,  
350 indicating a strong influence of the compounds contained in the tree leaves on *F. candida*.  
351 The greater diversity of poplar compounds and their higher solubility could explain why  
352 poplar litter leachates induced stronger effects compared to spruce or grass litter leachates.  
353 This result is in line with our findings in a previous field experiment where we observed a  
354 lower abundance of soil fauna in poplar plantations compared to spruce or mixed plantations  
355 (Chomel et al., 2015a). There is some evidence that soil microbial activity is highly dependent  
356 on leachates. Indeed, soil respiration has been shown to be quickly responsive to litter  
357 leachates input with a change in the soil microbial community (Cleveland et al., 2007; Joly et  
358 al., 2016; Wieder et al., 2008) and soil respiration is significantly explained by the proportion  
359 of water-soluble compounds contained in the leaf litter layer covering the soil (Fanin et al.,

2011). The differences in *F. candida* fitness observed with the different litter species could also be an effect of the different microbial communities that are present in the leachates. Several studies have shown that leachates can have strong allelopathic activity from one plant species to another (Fernandez et al., 2013, 2006; Rice, 1984) and can have strong effects on plant community diversity and composition (Ma et al., 2020); however, studies on the effect of leachates on soil fauna communities are rare. Leaching of leaves and litter is an important process in ecosystems since a large quantity of compounds can quickly be released by a rainfall event, or gradually released during the litter decomposition process. Nonetheless, litter leachates are rarely considered in studies on aboveground and belowground interactions, whereas this study showed that they may contain bioavailable compounds that can be significant drivers of collembolan fitness.

## 5. Conclusion

Litter species significantly affected *F. candida* mortality and reproduction. Grass litter increased survival and reproduction of collembolans, while tree litters reduced survival. This study also showed that leachates had greater impacts on collembolans fitness compared to litter, with stronger negative effects of tree leachates compared to grass leachates. These results indicate a strong influence of the compounds contained in the trees leaves. Phenolic compounds in poplar leaves were more leachable than in spruce leaves and presented a greater diversity, with the presence of several allelopathic compounds as caffeic acid and pinocembrin, which could explain the stronger negative effect of poplar leachates. This study showed that the release of compounds through leaching of leaves or litter could be an important factor for the activity of soil organisms and for aboveground-belowground interactions in natural ecosystems. Other studies should be conducted with more species of collembola to see if similar patterns are observed.

384 **Acknowledgements**

385 The authors thank to Thomas Tully (Ecole Normale Supérieure, Paris, France) for providing  
386 the initial culture of *Folsomia candida*. We thank Germain Boungou and Amélie Saunier  
387 (IMBE) for the help with the phosphorus and metabolomic analyses. This work was supported  
388 by the Natural Sciences and Engineering Research Council of Canada (NSERC-CRSNG,  
389 grant number 381553-09). LC-MS analysis were conducted at the IMBE ‘Chemical Ecology  
390 and Metabolomics facility’ funded by the CNRS, “Sud Provence-Alpes-Côte d’Azur”  
391 regional council, TOTAL Foundation and the French National Research Agency (ANR).

392 **Declaration of interests**

393 The authors declare to have no competing interests that could have appeared to influence the  
394 work reported in this paper

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554 **Figure captions**

555 Figure 1. Total phenolic concentration of litters (white bars) and 10% leachate (black bars)  
556 expressed in gallic acid equivalent. Mean  $\pm$  SE.

557 Figure 2. Heatmap of the fifty most discriminant ions (Variable Importance in Projection,  
558 VIP) from the LC-MS metabolomic fingerprints of the three litter species. These 50 ions are  
559 the discriminatory compounds between the three species. The colour in the heatmap indicates  
560 log transformed ratio of a given ion vs the average intensity of the ions in all samples.  
561 Negative and positive values are coded with blue and red colours, respectively, and they  
562 indicate under- or over-expression of the specific feature in one species compared to the  
563 others.

564 Figure 3. Mortality of *Folsomia candida* (mean  $\pm$  SE) growing in mesocosms with the  
565 different litter species (grass, spruce, mixture poplar/spruce, poplar) and forms (control, litter,  
566 5% leachates and 10% leachates). Different letters denote significant differences according to  
567 Tukey contrasts.

568 Figure 4. Reproduction of *Folsomia candida* (mean  $\pm$  SE) growing in mesocosms with the  
569 different litter species (grass, spruce, mixture poplar/spruce, poplar) and forms (control, litter,  
570 5% leachates and 10% leachates). Different letters denote significant differences according to  
571 Tukey contrasts.

572

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574

575 **Tables**

576 Table 1. Initial concentrations of C and N, C/N ratio, P and metabolomic diversity of the  
 577 different litter types (mean  $\pm$  SE).

	Litter species			F value
	Poplar	Spruce	Grass	
C (% DM)	38.1 $\pm$ 1.2 (a)	42.3 $\pm$ 1.5 (b)	36.9 $\pm$ 1.1 (a)	5.1 **
N (% DM)	0.5 $\pm$ 0.02 (a)	0.5 $\pm$ 0.02 (a)	0.65 $\pm$ 0.03 (b)	14.9 ***
C/N	76.7 $\pm$ 4 (b)	86.7 $\pm$ 3.5 (c)	58.9 $\pm$ 2.2 (a)	20.6 ***
P (mg g <sup>-1</sup> DM)	0.49 $\pm$ 0.01	0.53 $\pm$ 0	0.48 $\pm$ 0.01	2.3 ns
Metabolomic diversity	3.03 $\pm$ 0.10 (c)	1.06 $\pm$ 0.12 (a)	1.88 $\pm$ 0.18 (b)	52.9 ***

578 Results of ANOVAs are reported on the right side, with significant differences indicated with \*0.05, \*\*0.01,  
 579 \*\*\*0.001. Different letters within a row denote significant differences according to Tukey tests (a<b<c with 0.05  
 580 significance threshold)

Figure 1  
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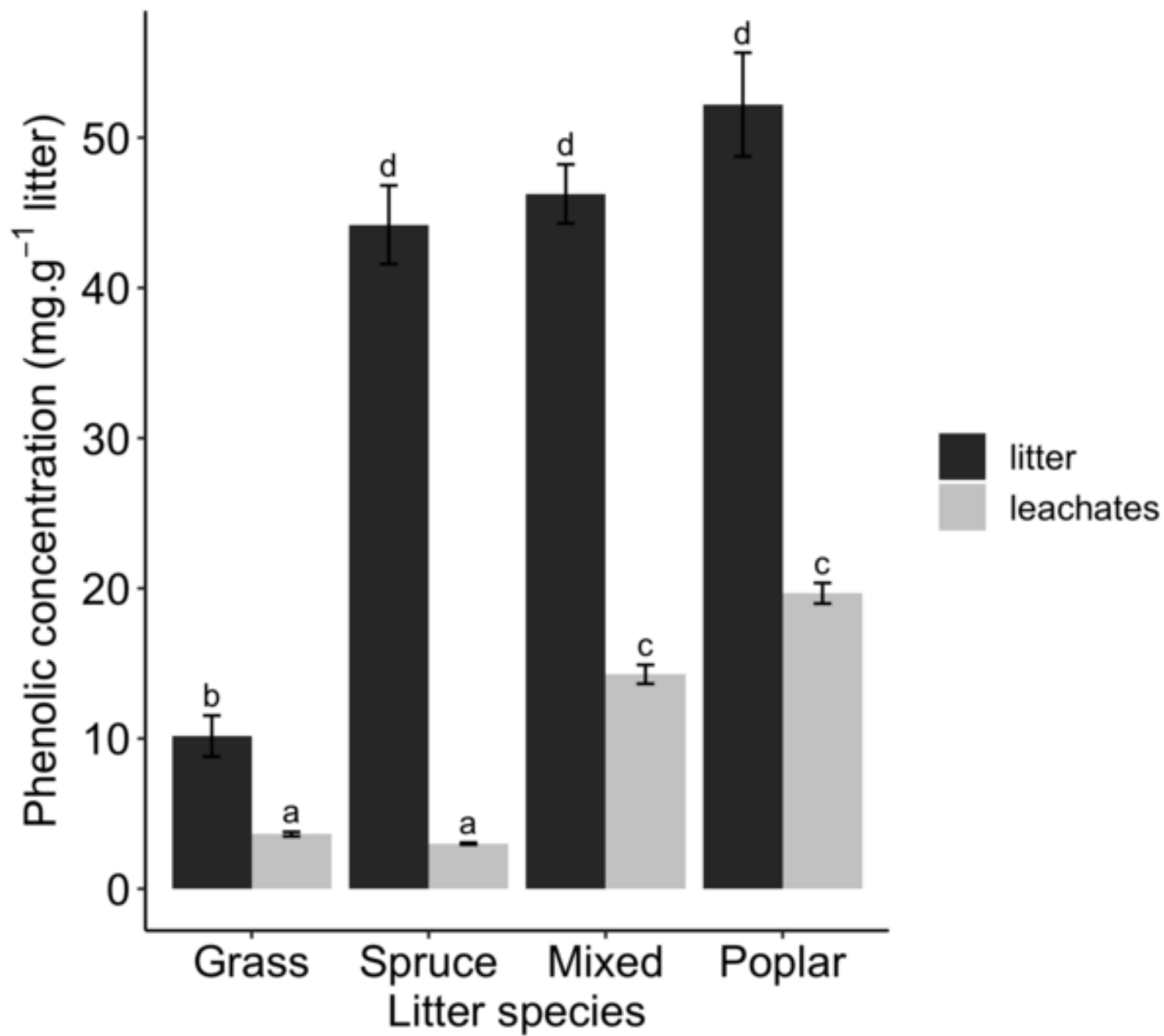


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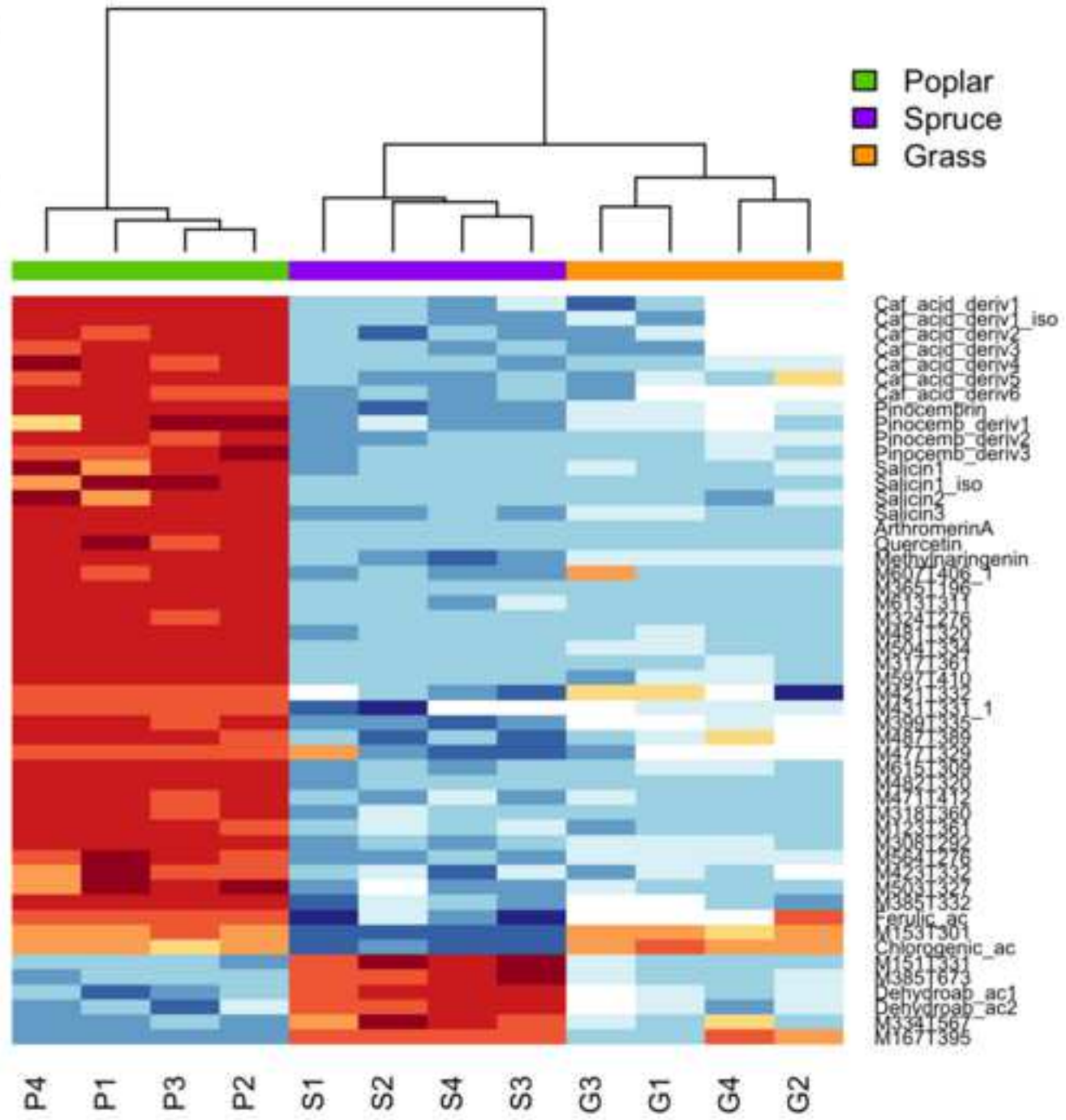
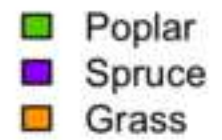
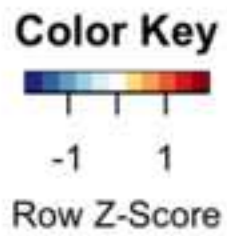


Figure 3  
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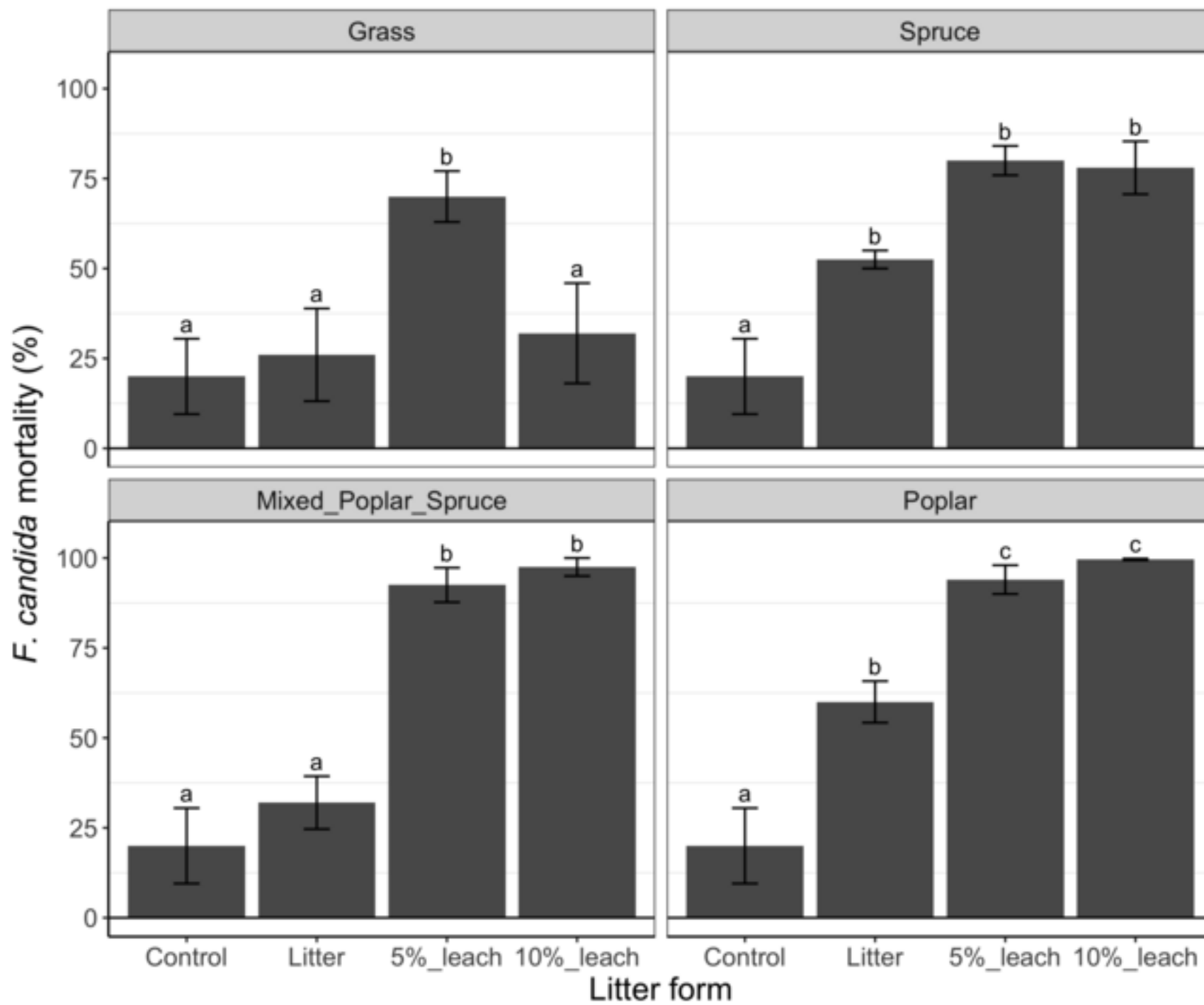
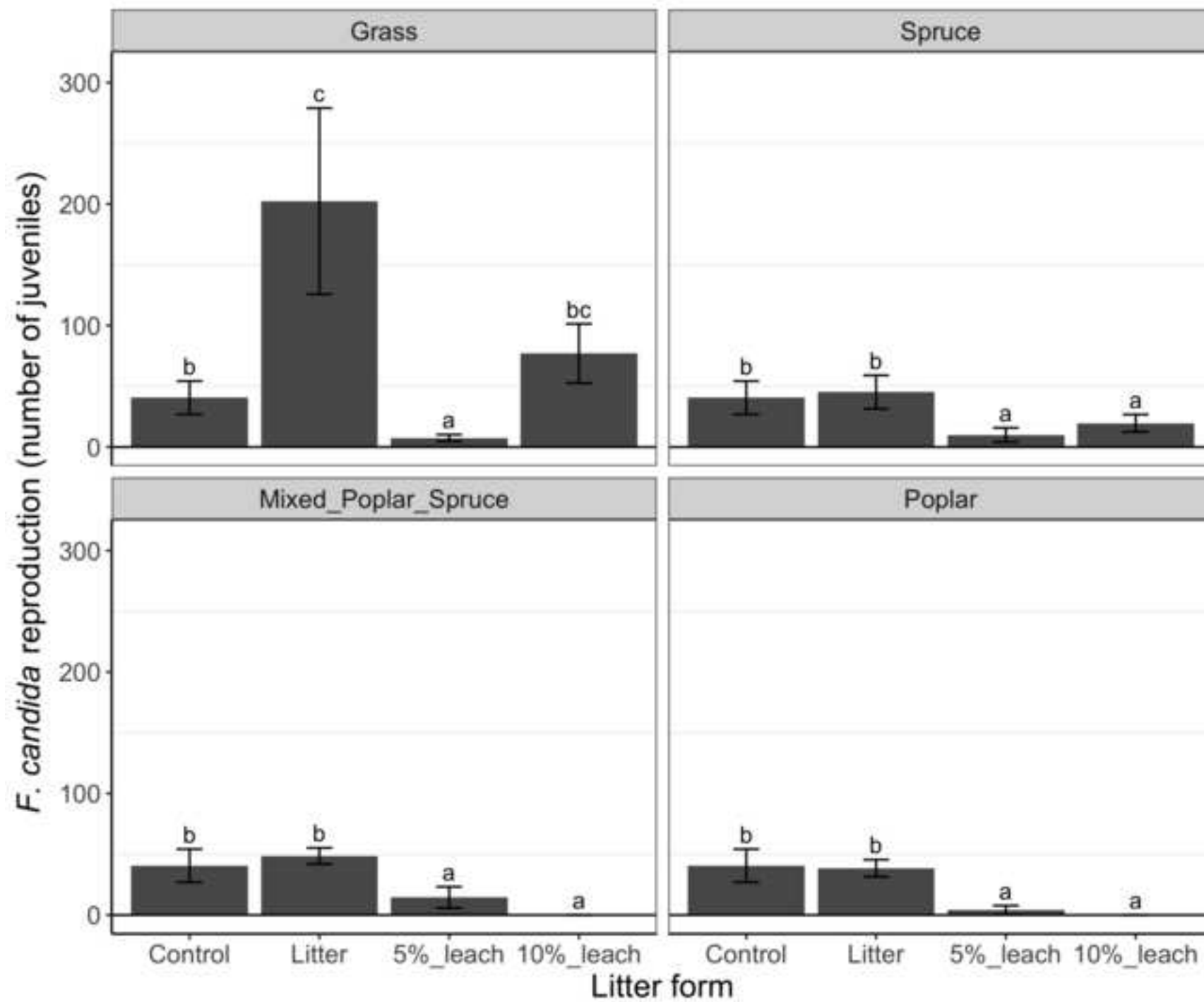


Figure 4  
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