



HAL
open science

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

Mathilde Chomel, Virginie Baldy, Marie Guittonny, Stephane Greff, Annie
Desrochers

► 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

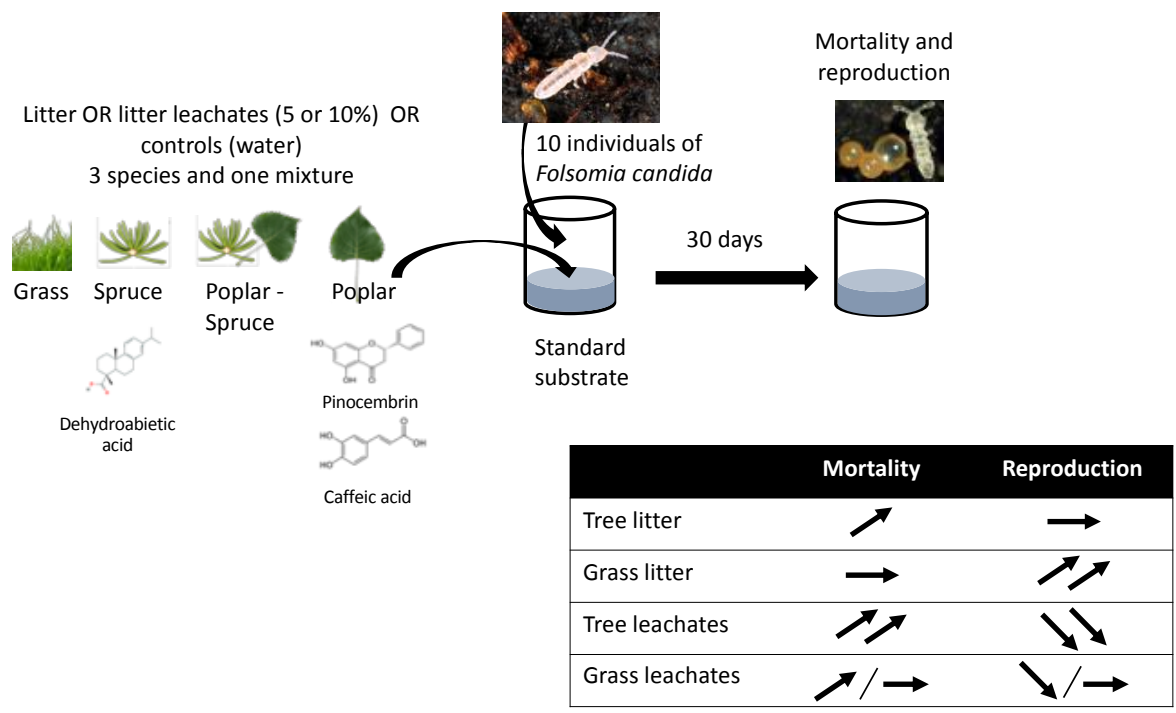
HAL Id: hal-02571920

<https://amu.hal.science/hal-02571920v1>

Submitted on 11 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



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**

2 Mathilde Chomel^{abd*}, Virginie Baldy^b, Marie Guittonny^c, Stephane Greff^b, Annie
3 DesRochers^d

4 ^a Department of Earth and Environmental Sciences, University of Manchester, Oxford Road,
5 Manchester M13 9PT, UK. (present address of the corresponding author)

6 ^b Institut Méditerranéen de Biodiversité et d'Ecologie - Aix Marseille Université, CNRS, IRD,
7 Avignon Université, IMBE UMR 7263, 13331 Marseille Cedex 03, France

8 ^c Université du Québec en Abitibi-Témiscamingue - Institut de recherche en mines et en
9 environnement, 445, boul. de l'Université, Rouyn-Noranda, Québec J9X 5E4, Canada.

10 ^d Université du Québec en Abitibi-Témiscamingue - Institut de Recherche sur les forêts, 341
11 rue principale Nord, Amos, Québec J9T 2L8, Canada.

12

13 * Corresponding author.

14 Email address: mathilde.chomel@manchester.ac.uk

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 ± 0.5 by the addition of 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 Na₂CO₃ 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 Bounou 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

395 **References**

- 396 Aber, J.D., Melillo, J.M., McClaugherty, C.A., 1990. Predicting long-term patterns of mass
397 loss, nitrogen dynamics, and soil organic matter transformation from initial fine litter
398 chemistry in temperate forest ecosystems. *Canadian Journal of Botany* 68, 2201–2208.
- 399 Aerts, R., 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial
400 ecosystems: A triangular relationship. *Oikos* 79, 439–449.
- 401 Auclerc, A., Libourel, P.A., Salmon, S., Bels, V., Ponge, J.F., 2010. Assessment of movement
402 patterns in *Folsomia candida* (Hexapoda: Collembola) in the presence of food. *Soil*
403 *Biology & Biochemistry* 42, 657–659. doi:10.1016/j.soilbio.2009.12.012
- 404 Batish, D.R., Singh, H.P., Kaur, S., Kohli, R.K., Yadav, S.S., 2008. Caffeic acid affects early
405 growth, and morphogenetic response of hypocotyl cuttings of mung bean (*Phaseolus*
406 *aureus*). *Journal of Plant Physiology* 165, 297–305. doi:10.1016/j.jplph.2007.05.003
- 407 Berg, B., McClaugherty, C., 2008. *Plant litter decomposition, humus formation, carbon*
408 *sequestration* (2nd edition). Springer-Verlag, Berlin Heidelberg.
- 409 Booth, R.G., Anderson, J.M., 1979. The influence of fungal food quality on the growth and
410 fecundity of *folsomia candida* (Collembola: Isotomidae). *Oecologia* 38, 317–323.
411 doi:10.1007/bf00345191
- 412 Chen, F., Peng, S.L., Chen, B.M., Ni, G.Y., Liao, H.X., 2013. Allelopathic potential and
413 volatile compounds of *Rosmarinus officinalis* L. against weeds. *Allelopathy Journal*
414 32, 57–66.
- 415 Chomel, M., DesRochers, A., Baldy, V., Larchevêque, M., Gauquelin, T., 2014. Non-additive
416 effects of mixing hybrid poplar and white spruce on aboveground and soil carbon
417 storage in boreal plantations. *Forest Ecology and Management* 328, 292–299.
418 doi:10.1016/j.foreco.2014.05.048
- 419 Chomel, M., Guittonny-Larchevêque, M., DesRochers, A., Baldy, V., 2015a. Home Field
420 Advantage of Litter Decomposition in Pure and Mixed Plantations Under Boreal
421 Climate. *Ecosystems* 18, 1014–1028.
- 422 Chomel, M., Guittonny-Larchevêque, M., DesRochers, A., Baldy, V., 2015b. Effect of mixing
423 herbaceous litter with tree litters on decomposition and N release in boreal plantations.
424 *Plant and Soil* 1–13. doi:10.1007/s11104-015-2648-5
- 425 Chomel, M., Guittonny-Larchevêque, M., Fernandez, C., Gallet, C., DesRochers, A., Paré, D.,
426 Jackson, B.G., Baldy, V., 2016. Plant secondary metabolites: a key driver of litter

427 decomposition and soil nutrient cycling. *Journal of Ecology* 104, 1527–1541.
428 doi:10.1111/1365-2745.12644

429 Chong, J., Xia, J., 2018. MetaboAnalystR: an R package for flexible and reproducible
430 analysis of metabolomics data. *Bioinformatics* 34, 4313–4314.
431 doi:10.1093/bioinformatics/bty528

432 Christiansen, K.A., Bellinger, P., 1980. The Collembola of North America north of the Rio
433 Grande. Part 2. Families Onychiuridae and Isotomidae. Grinnel College, Grinnel, IA.

434 Cleveland, C.C., Nemergut, D.R., Schmidt, S.K., Townsend, A.R., 2007. Increases in soil
435 respiration following labile carbon additions linked to rapid shifts in soil microbial
436 community composition. *Biogeochemistry* 82, 229–240. doi:10.1007/s10533-006-
437 9065-z

438 Das, S., Joy, V.C., 2009. Chemical quality impacts of tropical forest tree leaf litters on the
439 growth and fecundity of soil Collembola. *European Journal of Soil Biology* 45, 448–
440 454. doi:10.1016/j.ejsobi.2009.09.001

441 Don, A., Kalbitz, K., 2005. Amounts and degradability of dissolved organic carbon from
442 foliar litter at different decomposition stages. *Soil Biology and Biochemistry* 37,
443 2171–2179. doi:10.1016/j.soilbio.2005.03.019

444 Fanin, N., Hättenschwiler, S., Barantal, S., Schimann, H., Fromin, N., 2011. Does variability
445 in litter quality determine soil microbial respiration in an Amazonian rainforest? *Soil*
446 *Biology and Biochemistry* 43, 1014–1022. doi:10.1016/j.soilbio.2011.01.018

447 Fernandez, C., Lelong, B., Vila, B., Mévy, J., Robles, C., Greff, S., Dupouyet, S., Bousquet-
448 Mélou, A., 2006. Potential allelopathic effect of *Pinus halepensis* in the secondary
449 succession: an experimental approach. *Chemoecology* 16, 97–105.

450 Fernandez, C., Santonja, M., Gros, R., Monnier, Y., Chomel, M., Baldy, V., Bousquet-Melou,
451 A., 2013. Allelochemicals of *Pinus halepensis* as drivers of biodiversity in
452 Mediterranean open mosaic habitats during the colonization stage of secondary
453 succession. *Journal of Chemical Ecology* 39, 298–311. doi:10.1007/s10886-013-0239-
454 6

455 Folin, O., Denis, W., 1915. A Colorimetric Method for the Determination of Phenols (and
456 Phenol Derivatives) in Urine. *Journal of Biological Chemistry* 22, 305–308.

457 Fountain, M.T., Hopkin, S.P., 2005. *Folsomia candida* (Collembola): A “standard” soil
458 arthropod. *Annual Review of Entomology* 50, 201–222.
459 doi:10.1146/annurev.ento.50.071803.130331

460 Gallet, C., Pellissier, F., 1997. Phenolic compounds in natural solutions of a coniferous forest.
461 Journal of Chemical Ecology 23, 2401–2412.
462 doi:10.1023/b:joec.0000006682.50061.83

463 Gartner, T.B., Cardon, Z.G., 2004. Decomposition dynamics in mixed-species leaf litter.
464 Oikos 104, 230–246.

465 Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H.,
466 Hättenschwiler, S., 2010. Diversity meets decomposition. Trends in Ecology &
467 Evolution 25, 372–380.

468 Hashoum, H., Santonja, M., Gauquelin, T., Saatkamp, A., Gavinet, J., Greff, S., Lecareux, C.,
469 Fernandez, C., Bousquet-Mélou, A., 2017. Biotic interactions in a Mediterranean oak
470 forest: role of allelopathy along phenological development of woody species.
471 European Journal of Forest Research 136, 699–710. doi:10.1007/s10342-017-1066-z

472 Hättenschwiler, S., Bretscher, D., 2001. Isopod effects on decomposition of litter produced
473 under elevated CO₂, N deposition and different soil types. Global Change Biology 7,
474 565–579. doi:10.1046/j.1365-2486.2001.00402.x

475 Hättenschwiler, S., Tiunov, A., Scheu, S., 2005. Biodiversity and litter decomposition in
476 terrestrial ecosystems. Annual Review of Ecology, Evolution, & Systematics 36, 191–
477 218.

478 Hättenschwiler, S., Vitousek, P., 2000. The role of polyphenols in terrestrial ecosystem
479 nutrient cycling. Trends in Ecology & Evolution 15, 238–243.

480 Heděnc, P., Radochová, P., Nováková, A., Kaneda, S., Frouz, J., 2013. Grazing preference
481 and utilization of soil fungi by *Folsomia candida* (Isotomidae:Collembola). European
482 Journal of Soil Biology 55, 66–70. doi:10.1016/j.ejsobi.2012.12.005

483 Hopkin, S.P., 1997. Biology of the Springtails (Insecta: Collembola). Oxford University
484 Press.

485 ISO 11267, 1999. Soil quality - inhibition of reproduction of Collembola (*Folsomia candida*)
486 by soil pollutants. International Organization for Standardization, Geneva,
487 Switzerland.

488 Joly, F.-X., Fromin, N., Kiikkilä, O., Hättenschwiler, S., 2016. Diversity of leaf litter
489 leachates from temperate forest trees and its consequences for soil microbial activity.
490 Biogeochemistry 129, 373–388. doi:10.1007/s10533-016-0239-z

491 Joy, S., Joy, V.C., 1991. Food preference and growth rate of *porcellio laevis* reared on
492 decomposing leaf litter. Entomon 16, 107–113.

493 Kil, B.-S., 1992. Effect of pine allelochemicals on selected species in Korea, in: Rizvi, S.J.H.,
494 Rizvi, V. (Eds.), Allelopathy: Basic and Applied Aspects. Springer Netherlands,
495 Dordrecht, pp. 205–241. doi:10.1007/978-94-011-2376-1_14

496 Klironomos, J.N., Bednarczuk, E.M., Neville, J., 1999. Reproductive significance of feeding
497 on saprobic and arbuscular mycorrhizal fungi by the collembolan, *Folsomia candida*.
498 Functional Ecology 13, 756–761. doi:10.1046/j.1365-2435.1999.00379.x

499 Klironomos, J.N., Widden, P., Deslandes, I., 1992. FEEDING PREFERENCES OF THE
500 COLLEMBOLAN FOLSOMIA-CANDIDA IN RELATION TO MICROFUNGAL
501 SUCCESSIONS ON DECAIVING LITTER. Soil Biology & Biochemistry 24, 685–
502 692. doi:10.1016/0038-0717(92)90047-2

503 Kuiters, A.T., 1990. Role of phenolic substances from decomposing forest litter in plant-soil
504 interactions. Acta Botanica Neerlandica 39, 329–348.

505 Ma, Z., Wang, Y., Gu, Y., Bowatte, S., Zhou, Q., Hou, F., 2020. Effects of Litter Leachate on
506 Plant Community Characteristics of Alpine Grassland in Qinghai Tibetan Plateau.
507 Rangeland Ecology & Management 73, 147–155. doi:10.1016/j.rama.2019.10.003

508 Marschner, B., Kalbitz, K., 2003. Controls of bioavailability and biodegradability of dissolved
509 organic matter in soils. Geoderma, Ecological aspects of dissolved organic matter in
510 soils 113, 211–235. doi:10.1016/S0016-7061(02)00362-2

511 Petersen, H., Luxton, M., 1982. A Comparative Analysis of Soil Fauna Populations and Their
512 Role in Decomposition Processes. Oikos 39, 288–388. doi:10.2307/3544689

513 Phillips, M.A., Croteau, R.B., 1999. Resin-based defenses in conifers. Trends in Plant Science
514 4, 184–190. doi:10.1016/S1360-1385(99)01401-6

515 Poinot-Balaguer, N., Racon, L., Sadaka, N., Lepetit, J., 1993. Effects of tannin compounds
516 on two species of Collembola. European Journal of Soil Biology 29, 13–16.

517 Quinn, R.A., Vermeij, M.J.A., Hartmann, A.C., Galtier d’Auriac, I., Benler, S., Haas, A.,
518 Quistad, S.D., Lim, Y.W., Little, M., Sandin, S., Smith, J.E., Dorrestein, P.C.,
519 Rohwer, F., 2016. Metabolomics of reef benthic interactions reveals a bioactive lipid
520 involved in coral defence. Proceedings of the Royal Society B: Biological Sciences
521 283, 20160469. doi:10.1098/rspb.2016.0469

522 R Core Team, 2017. R: A language and environment for statistical computing. R Foundation
523 for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>.

524 Rasul, A., Millimouno, F.M., Ali Eltayb, W., Ali, M., Li, J., Li, X., 2013. Pinocembrin: A
525 Novel Natural Compound with Versatile Pharmacological and Biological Activities

526 [WWW Document]. BioMed Research International.
527 doi:<https://doi.org/10.1155/2013/379850>

528 Rice, E.L., 1984. Allelopathy. Academic Press, Londres.

529 Rief, A., Knapp, B.A., Seeber, J., 2012. Palatability of selected alpine plant litters for the
530 decomposer *Lumbricus rubellus* (Lumbricidae). Plos One 7, 7.
531 doi:[10.1371/journal.pone.0045345](https://doi.org/10.1371/journal.pone.0045345)

532 Ristok, C., Poeschl, Y., Dudenhöffer, J.-H., Ebeling, A., Eisenhauer, N., Vergara, F., Wagg,
533 C., Dam, N.M. van, Weinhold, A., 2019. Plant species richness elicits changes in the
534 metabolome of grassland species via soil biotic legacy. Journal of Ecology 107, 2240–
535 2254. doi:[10.1111/1365-2745.13185](https://doi.org/10.1111/1365-2745.13185)

536 Salmon, S., Ponge, J.F., 2001. Earthworm excreta attract soil springtails: laboratory
537 experiments on *Heteromurus nitidus* (Collembola : Entomobryidae). Soil Biology &
538 Biochemistry 33, 1959–1969. doi:[10.1016/s0038-0717\(01\)00129-8](https://doi.org/10.1016/s0038-0717(01)00129-8)

539 Scheu, S., Simmerling, F., 2004. Growth and reproduction of fungal feeding Collembola as
540 affected by fungal species, melanin and mixed diets. Oecologia 139, 347–353.
541 doi:[10.1007/s00442-004-1513-7](https://doi.org/10.1007/s00442-004-1513-7)

542 Skidmore, R.E., 1995. Checklist of Collembola (Insecta: Apterygota) of Canada and Alaska.
543 Proc. Ent. Soc. Ontario 126, 45–76.

544 Vyvyan, J.R., 2002. Allelochemicals as leads for new herbicides and agrochemicals.
545 Tetrahedron 58, 1631–1646. doi:[10.1016/S0040-4020\(02\)00052-2](https://doi.org/10.1016/S0040-4020(02)00052-2)

546 Wieder, W.R., Cleveland, C.C., Townsend, A.R., 2008. Tropical tree species composition
547 affects the oxidation of dissolved organic matter from litter. Biogeochemistry 88, 127–
548 138. doi:[10.1007/s10533-008-9200-0](https://doi.org/10.1007/s10533-008-9200-0)

549 Zheng, J., Xu, Z., Wang, Y., Dong, H., Chen, C., Han, S., 2014. Non-additive effects of
550 mixing different sources of dissolved organic matter on its biodegradation. Soil
551 Biology and Biochemistry 78, 160–169. doi:[10.1016/j.soilbio.2014.07.023](https://doi.org/10.1016/j.soilbio.2014.07.023)

552

553

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

573

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 ⁻¹ 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
[Click here to download high resolution image](#)

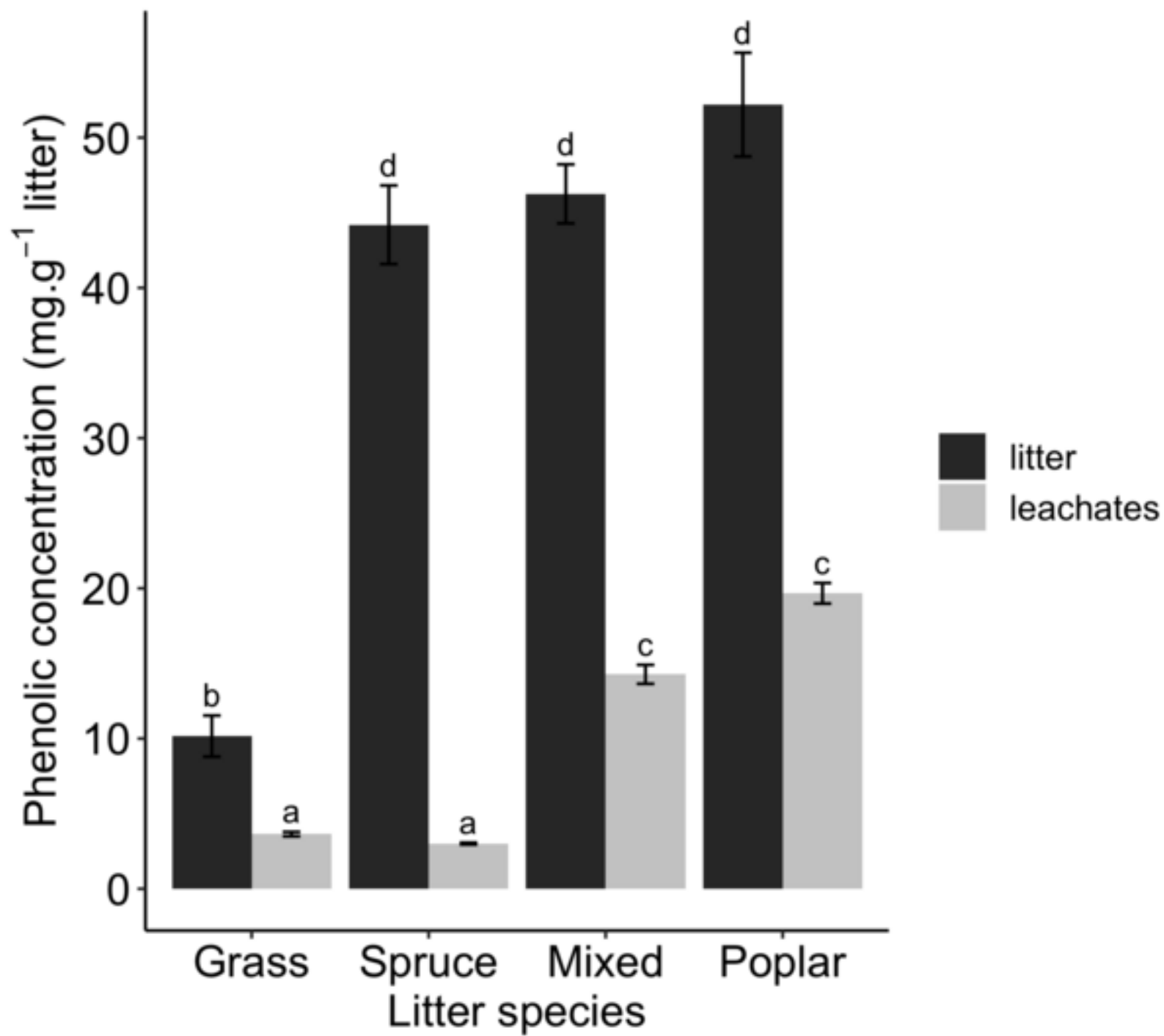


Figure 2
[Click here to download high resolution image](#)

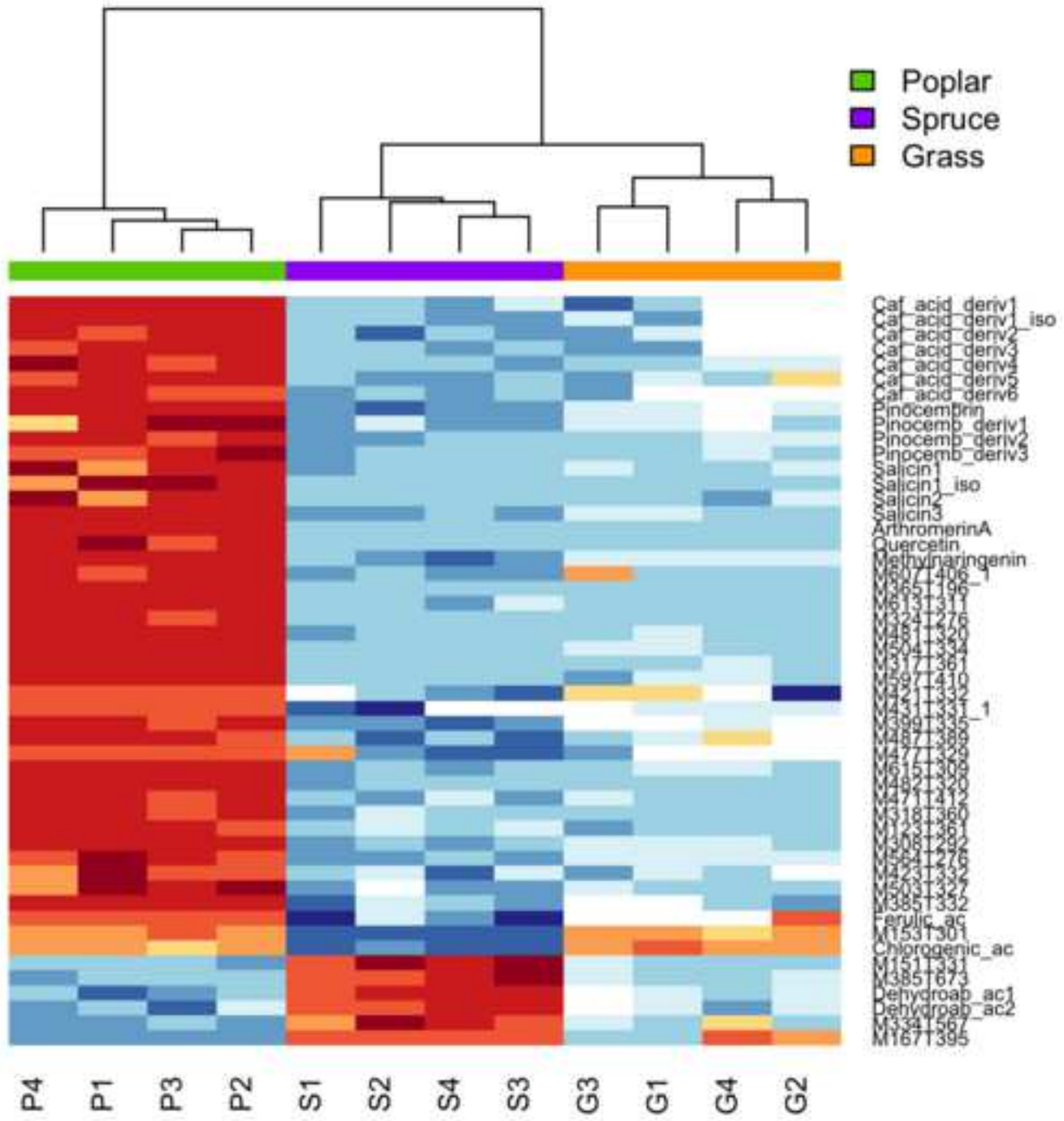
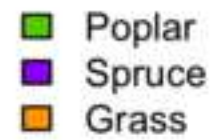


Figure 3
[Click here to download high resolution image](#)

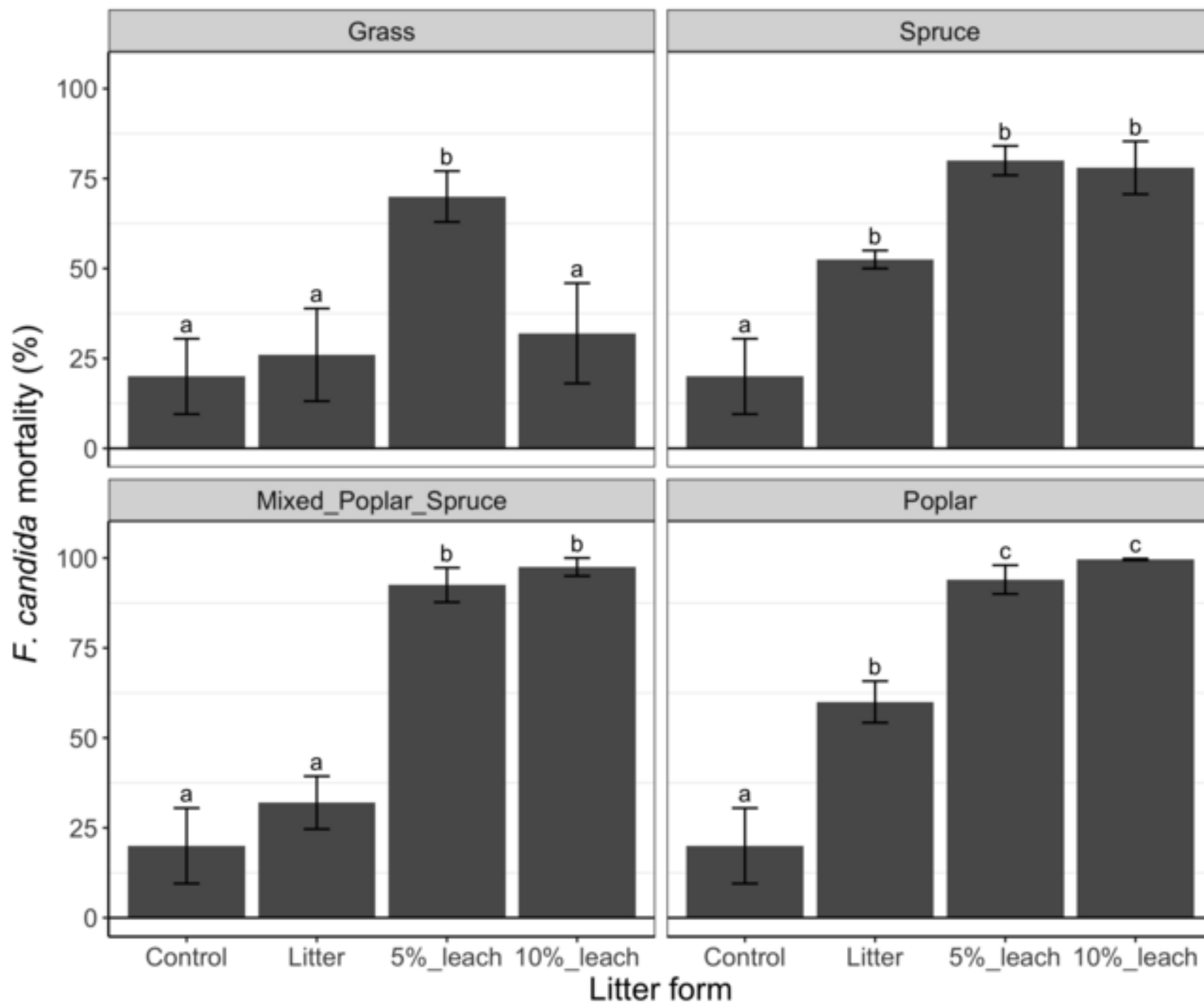
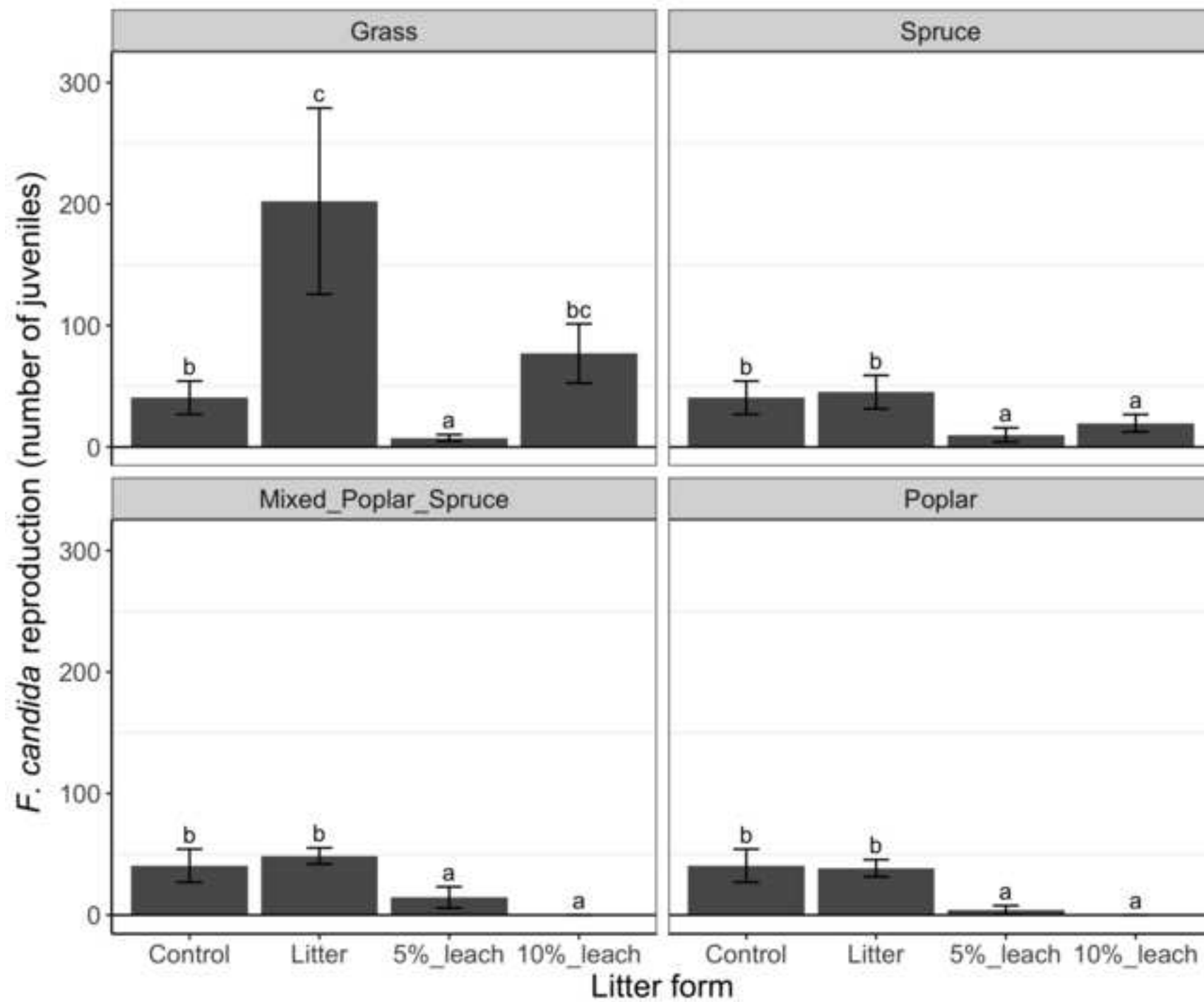


Figure 4
[Click here to download high resolution image](#)



Supplementary Material

[Click here to download Supplementary Material for online publication only: Supplementary Material.docx](#)