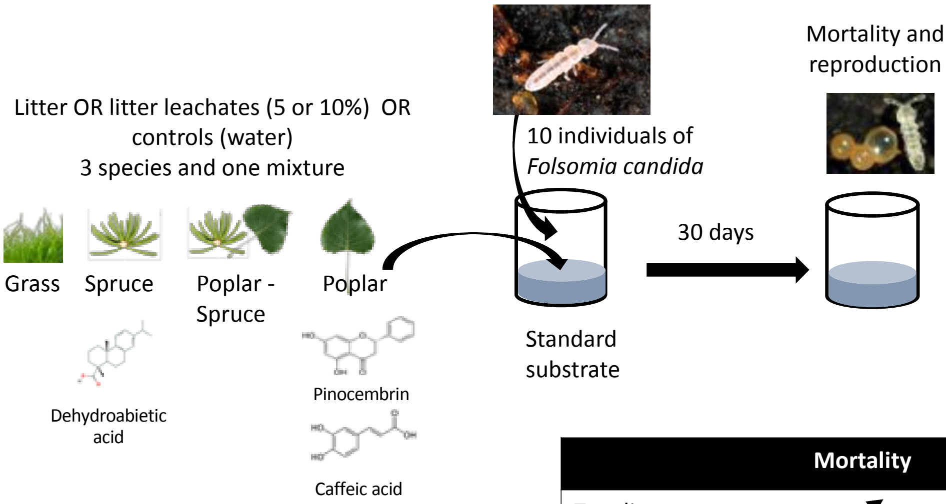
Graphical Abstract (for review)



	Mortality	Reproduction
Tree litter	↗	→
Grass litter	→	↗↗
Tree leachates	↗↗	↘↘
Grass leachates	↗ / →	↘ / →

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

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

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Abstract

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

1. Introduction

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

Collembolans are important members of the soil decomposer food web and have important direct or indirect roles on the decomposition process (Petersen and Luxton, 1982). Some species are microbivores and stimulate or regulate litter fungal colonization by grazing on the fungal hyphae, while others are detritivores and directly participate to organic matter transformation by fragmenting and ingesting the litter; in both cases they derive nutritional benefits from the decomposing litter source (Das and Joy, 2009). Collembolans are selective with their food, and for example they can perceive chemical cues of fungi at a distance of 1 cm - 2.5 cm (Auclerc et al., 2010; Salmon and Ponge, 2001), prefer some fungal species over others (Heděnec et al., 2013; Klironomos et al., 1992; Scheu and Simmerling, 2004), and can suffer a reduction in fitness from the ingestion of specific fungal species (Klironomos et al., 1999; Scheu and Simmerling, 2004). Furthermore, collembolans raised on fungal-colonized litter show grazing preferences and reproduction rates that are more affected by litter type than by fungal species (Heděnec et al., 2013), demonstrating the strong influence of litter quality on collembolans activity (Das and Joy, 2009). However, few studies have addressed the direct impact of the chemical composition of litter on the abundance and diversity of soil mesofauna, the physical structure of the litter often being a confounding factor. Das and Joy (2009) reported that a collembolan species (*Cyphoderus javanus* Börner) tended to avoid litter with greater amounts of polyphenols and tannins. Furthermore, individuals that remained in contact with litters containing greater quantities of these secondary metabolites exhibited slower growth, lower fecundity and fewer moults (Das and Joy, 2009). Poinsot-Balaguer et al. (1993) found that condensed and hydrolysable tannins extracted from oak leaves were highly toxic for the collembolans (Poinsot-Balaguer et al., 1993). However, these studies related collembolans fitness to the total content of secondary metabolites, but did not consider their solubility in water and their bioavailability in natural environments. Some researchers assert that allelopathic plant-plant interference is most likely due to water-soluble compounds (Vyvyan, 2002). Following the same idea, these water-soluble secondary metabolites leached from the canopy or the litter could be of greater importance for the activity of soil organisms than the litter content itself.

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

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

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

2. Material and methods

2.1 Litter sampling and leachates preparation

In late September 2010, three types of senescent leaves (poplar, spruce and grass) were collected from experimental monospecific plantations of hybrid poplar (*Populus maximowiczii* A. Henry x *P. balsamifera* L.) and white spruce (*Picea glauca* [Moench] Voss) located in Amos (48°36’N, 78°04’W) in the boreal region of Abitibi-Temiscamingue, Quebec, Canada. Freshly fallen spruce needles and hybrid poplar leaves were collected by placing a plastic sheet beneath the trees to prevent soil contamination. Aerial parts of grass species (mainly grasses, *i.e. Poa* sp.) naturally present in the plantation were cut at ground level during the same period. Collected leaf material was homogenized, air-dried and stored at room temperature prior to the experiment. Leachates of each litter type (poplar, spruce, grass and poplar/spruce [50:50]) were prepared by soaking 100 g of litter (air-dried mass) in 1 L of deionized water (10% dry mass) for 24 h in darkness. This concentration is the most commonly used for *in vitro* allelopathy bioassays (Chen et al., 2013; Fernandez et al., 2013). Although they can be more concentrated than in natural conditions, it allows studying the potential allelopathic effect of a species (Kil, 1992). However, to be more representative to actual field concentrations, a 50% diluted solution was also prepared, producing 5% dry mass leachate.

2.2 Folsomia candida cultures

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

2.3 Experimental design

To study the effects of litter or leachates on the fitness of *F. candida*, we set up a total of 65 microcosms. The microcosms were set-up according to 2 interacting factors: the litter form (litter, 5% leachate and 10% leachate) and the litter species (poplar, spruce, grass and poplar - spruce mixture) and controls consisted of deionized water without litter. Each combination of factors was replicated five times. The *F. candida* reproduction test was carried out according to an adaptation of the ISO standard 11267 (ISO 11267, 1999). The microcosms consisted of 120 mL plastic bottles with pierced screw caps containing 32 g of wet artificial soil, made up with 70% quartz sand, 20% kaolinite and 10% peat, ground, dried, and sieved to 0.5 mm with the pH adjusted to 6.0 ± 0.5 by the addition of $CaCO_3$. It was moistened to 50% of water holding capacity with distilled water (7 mL). Chemicals to be tested in the ISO norm are generally pollutants dissolved in water at range of concentrations that will give appropriate reduction of reproduction levels (LOEC, CE 50). For this experiment, 7 mL of 5 or 10 % leachates were directly mixed with the soil. For the litter species treatments, 7 mL of distilled water were added to the substrate, and 1 g of coarsely chopped litter was re-humidified and placed on the substrate (corresponding to the amount of litterfall per surface area in natural plantations, Chomel *et al.* 2014). For each microcosm, ten 10/12-days-old juveniles were introduced and the microcosms were randomly placed in a climate chamber at a constant temperature of 20°C and continuous darkness for 30 days. They were opened twice a week for aeration and fed with baker's yeast at the initial time and after 2 weeks. Following the ISO guidelines, the pots were flooded with water and gently stirred to collect and count the animals floating at the surface. The number of surviving adults and the juveniles were recorded using a dissecting microscope.

2.4 Chemical composition of litter and leachates

Litter initial content of major nutrients (C, N, P) and phenolics were estimated from 5 subsamples of each litter. The litter samples were finely ground with a ball mill (MM301, Retsch Inc., Newtown, PA) prior to analysis. Carbon (C) and Nitrogen (N) concentrations were analysed in a CHN elemental analyzer (Flash EA 1112 series, ThermoScientific, U.S.A.). Phosphorus (P) was extracted with 20 mL of nitric acid from remaining dry ash after combustion of 0.5 g of subsamples at 500 °C for 5 h in a muffle furnace. The pH was adjusted to 8.5 with a 40 % NaOH solution. A volume of 1 mL of sample, 0.2 mL of mixed reagent (emetic tartar and ammonium molybdate solution), 0.04 mL of ascorbic acid and 0.76 mL of distilled water were placed directly in a microcuvette. After 150 min, the reaction was completed, and phosphorus concentration was measured at 780 nm with a UV/Vis spectrophotometer (Thermo Scientific®, USA). Total phenolics content was measured by the method of Folin-Ciocalteu (Folin and Denis, 1915): 1 g of ground litter was shaken with 20 mL of distilled water for 90 min. A volume of 0.25 mL of the filtered aqueous extract (or of the leachate directly) was mixed with 0.25 mL of Folin-Ciocalteu reagent and 0.5 mL of saturated aqueous Na_2CO_3 to stabilize the colour reaction, after which 4 mL of distilled water was added to dilute the extract. After 1 hour, the reaction was completed and measured at 765 nm on a UV/Vis spectrophotometer (Thermoscientific, U.S.A.). Quantitative results were expressed with reference to gallic acid.

2.5. Litter metabolic fingerprints (untargeted metabolomics)

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

2.6 Statistical analysis

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

3. Results

3.1 Chemical composition of litters and leachates

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

3.2 Effects of litter and leachates on F. candida mortality

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

3.3 Effects of litter and leachates on F. candida reproduction

Reproduction of *F. candida* differed across combinations of litter species (negative Binomial GLM, $X^2 = 39.4$, $P < 0.001$) and forms (Binomial GLM, $X^2 = 70.5$, $P < 0.001$) with a significant interaction term (Binomial GLM, $X^2 = 75.7$, $P < 0.001$). Leachates of spruce, mixed or poplar significantly reduced reproduction in comparison with the litter or the control treatments (P < 0.05, Fig. 4). The grass litter and 10% leachate increased the reproduction of *F. candida* by 385 % and 70 %, compared to the control, respectively. However, the grass 5% leachate decreased the reproduction by 83% compared to the control (Fig. 4).

4. Discussion

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

4.1 Effects of litter species and its quality

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

candida fecundity (Booth and Anderson, 1979; Scheu and Simmerling, 2004) and shorten the period until oviposition starts (Scheu and Simmerling, 2004). Besides C/N ratios, litter phenolic concentrations could also explain *F. candida* fitness. In comparison with the grass litter, both poplar and spruce litters had more than four-fold greater concentrations of phenolics. Within 'low' quality litters, poplar litter had lower C/N ratios but similar total phenolic concentrations than spruce litter, and both litter types negatively affected the survival and reproduction of *F. candida.* We observed an increase in mortality of 240 % and 210 % and a decrease in reproduction of 29 % and 18 %, respectively for poplar and spruce litters, compared to the control. We identified several specificities among the tree litter species: spruce litter contained dehydroabietic acid, while pinocembrin and caffeic acid metabolites were found in poplar litter. Pinocembrin is a flavonoid that has been recognized to have antimicrobial activities (Rasul et al., 2013), and caffeic acid is also known to have strong allelopathic and antimicrobial activities (Batish et al., 2008). The terpenoid dehydroabietic acid is a defense metabolite abundant in resin, thus common in conifers (Phillips and Croteau, 1999). These three secondary metabolites potentially reduce the colonization of the litter by microorganisms by their antimicrobial activities and can have repulsive effects on detritivores (Chomel et al., 2016; Das and Joy, 2009). The presence of these secondary metabolites and the high concentration of total phenolic compounds in tree litters compared to grass litter could explain their strong and negative impact on *F. candida* survival and reproduction. However, when these two species of litter were mixed, mortality rate was similar to the control, confirming our fourth hypothesis. Mixed diets may significantly increase collembolan fitness by providing a balanced nutrient intake from the different litters present in the mixture or by a dilution of toxic compounds contained in one of the species (Hättenschwiler et al., 2005; Scheu and Simmerling, 2004). Conversely, although mixing the two litter types increased survival compared to single species litter, it had no effect on the reproduction of *F. candida*.

4.2 Effects of leachates vs litter

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

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Declaration of interests

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

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Figure captions

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

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

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

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

Tables

Table 1. Initial concentrations of C and N, C/N ratio, P and metabolomic diversity of the different litter types (mean ± SE).

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

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

Figure 1
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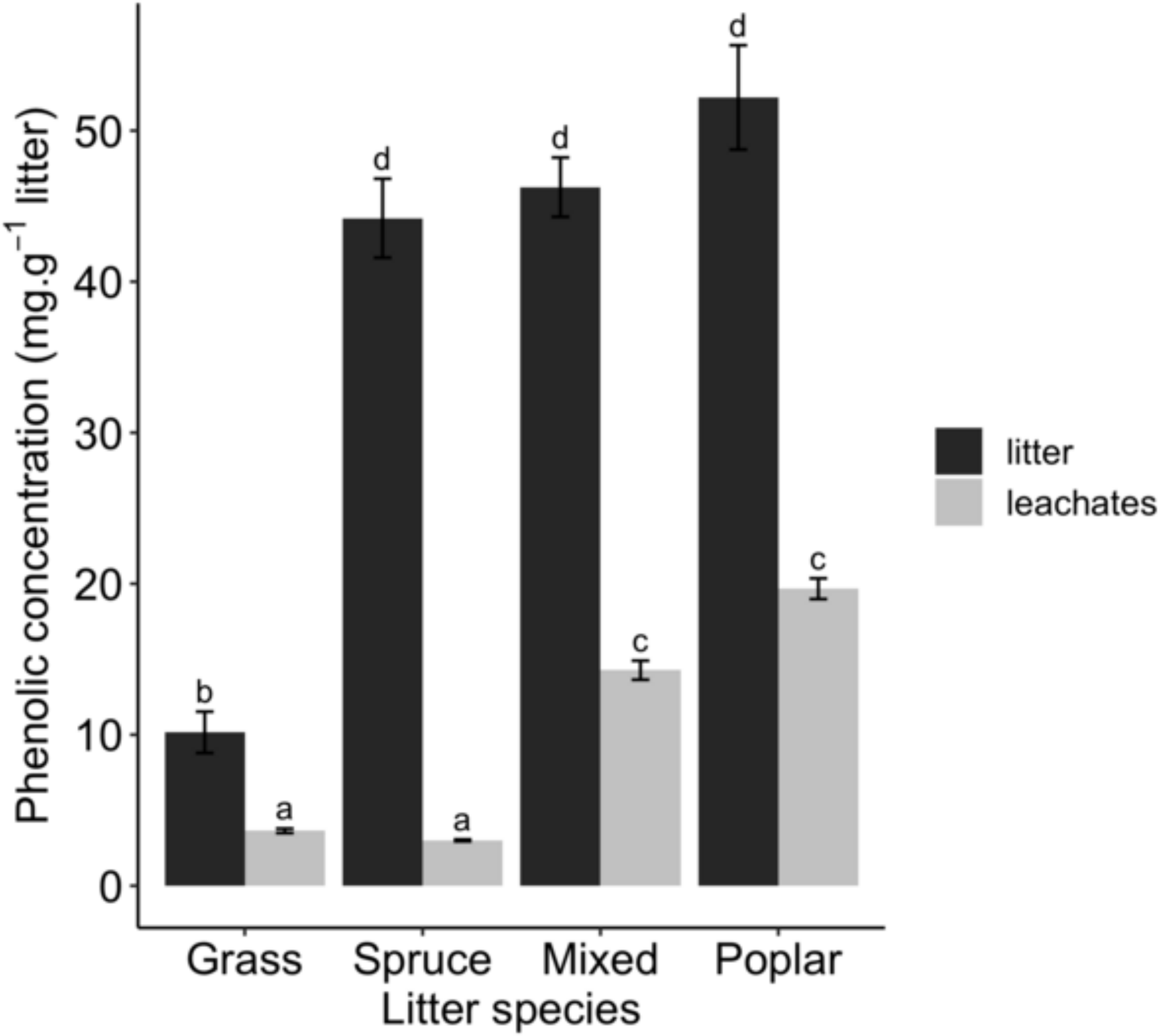

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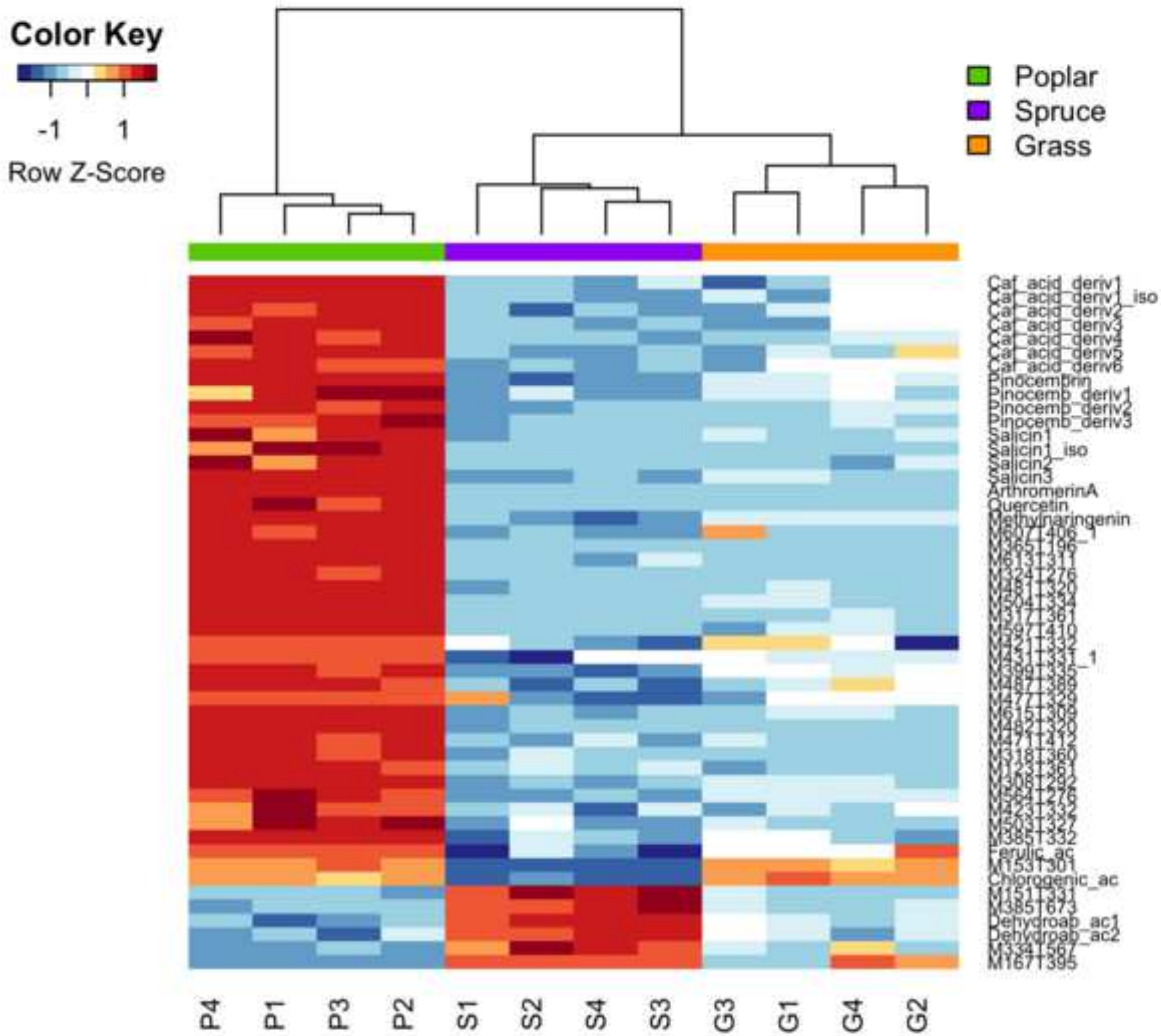

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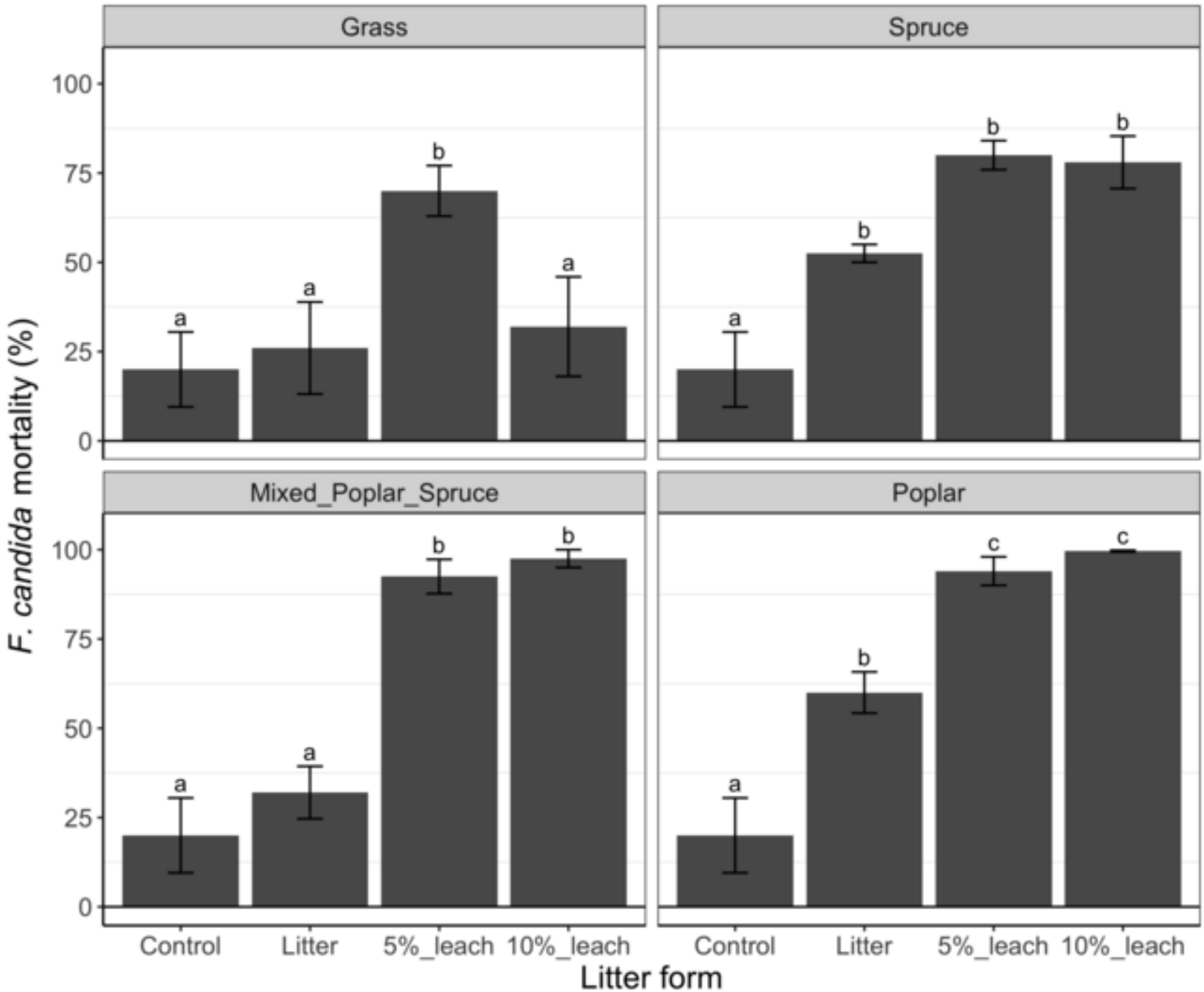

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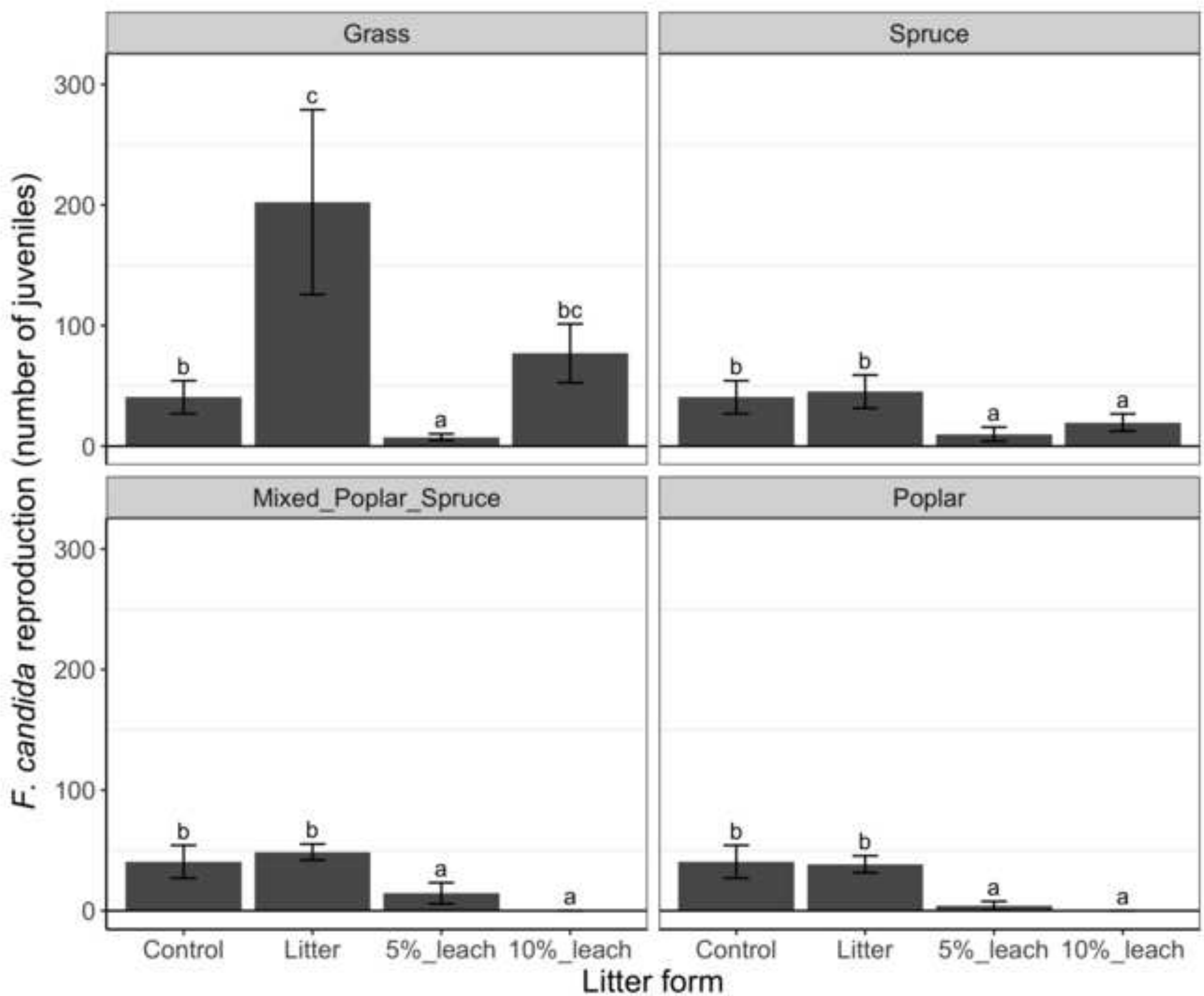

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