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Sylvain Coq, Johanne Nahmani, Elena Kazakou, Nathalie Fromin,  
Jean-François David

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1 **Do litter-feeding macroarthropods disrupt cascading effects of land use on**  
2 **microbial decomposer activity?**

3

4 Sylvain Coq <sup>al\*</sup>, Johanne Nahmani <sup>a1</sup>, Elena Kazakou <sup>ab</sup>, Nathalie Fromin <sup>a</sup>, Jean-François David <sup>a</sup>

5 <sup>1</sup> Equal contribution

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8 <sup>a</sup> CEFÉ, Univ Montpellier, CNRS, EPHE, IRD, Univ Paul Valéry Montpellier 3, Montpellier, France

9 <sup>b</sup> Montpellier Supagro, Montpellier, France

10

11

12 \*Corresponding author. Tel.: +33 4 67 61 32 36

13 E-mail address: [sylvain.coq@cefe.cnrs.fr](mailto:sylvain.coq@cefe.cnrs.fr)

14

15 Declarations of interest: none

## 16 **Abstract**

17 Plant traits are known to control litter decomposition rates through afterlife effects on litter quality.  
18 Land-use practices that modify plant traits, e.g. livestock grazing and soil fertilization, also have  
19 cascading effects on litter decomposition. However, almost all studies of these afterlife effects ignored  
20 the role of soil detritivores in the decomposition processes. We explored how the feeding activities of  
21 a macroarthropod modify microbial activity in leaf litter. Dead leaves from two grassland species,  
22 *Bromopsis erecta* and *Potentilla verna*, were collected in fertilized or unfertilized grazed plots and  
23 fertilized or unfertilized ungrazed plots. We determined how intraspecific variation in litter quality in  
24 response to sheep grazing and soil fertilization (i) influences the consumption and assimilation of leaf  
25 litter by the millipede *Glomeris marginata*, and (ii) affects the activity of microbial decomposers,  
26 assessed by substrate-induced respiration (SIR), in leaf litter before consumption and in faecal pellets  
27 and litter remains processed by *Glomeris* under all treatments. In the absence of millipedes, microbial  
28 activity was significantly higher in leaf litter from fertilized plots. *Glomeris* consumed larger amounts  
29 of leaf litter from fertilized grazed plots, owing to increased consumption of the otherwise poorly  
30 palatable *Bromopsis*, and produced larger amounts of faecal pellets when fed on this food. However,  
31 irrespective of the food consumed, SIR in faecal pellets was found to be similar in all treatments.  
32 Moreover, SIR in litter remains unconsumed at the end of the experiment was reduced to low and  
33 similar levels in all treatments. Overall, homogenization of microbial activity by *Glomeris* suppressed  
34 differences in SIR between leaf litter from fertilized and unfertilized plots, in both *Bromopsis* and  
35 *Potentilla*. Our results suggest that studies that assess afterlife effects of plant traits on decomposition  
36 using methods that exclude soil macrofauna may prove inadequate in ecosystems with abundant  
37 populations of detritivores.

38 **Keywords:** Aboveground-belowground linkages, afterlife effects, litter decomposition, litter-feeding  
39 macroarthropods, microbial activity

40

## 41 **Introduction**

42 Linkages between aboveground and belowground communities play a key role in the functioning of  
43 terrestrial ecosystems (Ohgushi, Wurst, & Johnson, 2018). Among the variety of processes mediating  
44 these interactions, the inputs and decomposition of aboveground plant litter are of prominent  
45 importance (Bardgett & Wardle, 2010). Plant traits such as nutrient contents and traits related to  
46 defence largely control leaf litter decomposition rates, as a result of afterlife effects that cascade down  
47 from living plants to litter quality and microbial decomposer activity (Cornelissen et al., 2004;  
48 Cornwell et al., 2008; Fortunel et al., 2009; Freschet, Aerts, & Cornelissen, 2012). Moreover, any  
49 land-use practice or aboveground biotic interaction that modifies plant traits can potentially affect  
50 decomposition processes via the same cascading mechanisms. Livestock grazing, for example,  
51 considerably alters leaf litter quality not only through shifts in plant community composition, but also  
52 within species, through physiological responses of individual plants to herbivores (Bardgett & Wardle,  
53 2010; Olofsson & Oksanen, 2002; Semmartin, Aguiar, Distel, Moretto, & Ghersa, 2004; Semmartin &  
54 Ghersa, 2006). The resulting effect on litter mass loss is highly variable, but it has been argued that  
55 positive effects of herbivory on decomposition should dominate in fertile ecosystems and negative  
56 effects in less productive situations (Bardgett & Wardle, 2010; Pastor, Cohen, & Hobbs, 2006).  
57 Therefore, both fertilization and grazing are expected to affect the tissues of living plants and generate  
58 afterlife effects on litter quality and decomposition (Bumb et al., 2018).  
59 However, this approach linking decomposition to plant traits generally ignores the role of the soil  
60 fauna, despite its demonstrated impact on litter mass loss at a global scale (García-Palacios, Maestre,  
61 Kattge, & Wall, 2013). Most of the above-mentioned studies were done using fine-mesh litter bags or  
62 microcosms that excluded soil macrodetritivores, which are important regulators of decomposition  
63 processes (David, 2014; Lavelle & Spain, 2001). Saprophagous macroarthropods such as millipedes,  
64 woodlice and insect larvae can consume up to 40% of the annual litterfall in ecosystems in which they  
65 are abundant (Carcamo, Abe, Prescott, Holl, & Chanway, 2000; Coulis, Hättenschwiler, Coq, &  
66 David, 2016; David & Gillon, 2002; Frouz, Jedlička, Šimáčková, & Lhotáková, 2015). Ignoring this  
67 substantial contribution may lead to misleading conclusions. A variable proportion of the ingested  
68 food is assimilated by the animals and the rest is returned to the soil as faecal pellets. When  
69 macrodetritivores are present, a substantial part of the material lost due to litter consumption is not

70 fully decomposed and accumulates as faecal pellets, whose decomposition dynamics differs from that  
71 of leaf litter (Joly, Coulis, Gérard, Fromin, & Hättenschwiler, 2015; Maraun & Scheu, 1996;  
72 Špaldoňová & Frouz, 2014). Joly et al. (2015) showed that when macroarthropods were fed on a wide  
73 range of tree leaf litter, microbial activity assessed using substrate-induced respiration was much less  
74 variable in fresh faecal pellets than in the food offered. Homogenization of microbial activity in faecal  
75 pellets could thus counteract the afterlife effects that operate in the absence of fauna. Moreover,  
76 macroarthropods may also affect the decomposition of leaf litter remains they leave unconsumed,  
77 through (i) selective consumption of leaves strongly colonized by microorganisms (Gunnarsson, 1987;  
78 Harrop-Archibald, Didham, Standish, Tibbet, & Hobbs, 2016; Zimmer, Kautz, & Topp, 2003), (ii)  
79 reduction of the microbial biomass by grazing fungi from the surface of leaves (Bignell, 1989;  
80 Crowther, Jones, & Boddy, 2011; Harrop-Archibald et al., 2016), and (iii) dissemination of microbial  
81 propagules in the litter layer (Lilleskov & Bruns, 2005). All these direct and indirect effects of soil  
82 macroarthropods can potentially affect the decomposition rate of unconsumed leaf litter — and  
83 eventually blur the relationships between land use, plant traits and litter decomposition that would be  
84 expected in the absence of fauna.

85         In the present study, we explored possible interactions between detritivores and changes in  
86 litter quality driven by land use during leaf litter decomposition. Specifically, we tested how the  
87 feeding activities of a millipede modify the effects of intraspecific variation in litter quality on  
88 microbial activity in leaf litter from a Mediterranean grassland. In the same study ecosystem, Coq et  
89 al. (2018) had previously shown that sheep grazing and soil fertilization interactively increased  
90 nutrient concentrations in the leaf litter of two dominant plant species, the grass *Bromopsis erecta* and  
91 the forb *Potentilla verna*. Using the same litter species, we examined (i) whether changes in litter  
92 quality in response to these land-use practices affect both microbial activity in leaf litter and the  
93 amounts of food consumed and assimilated by the millipede *Glomeris marginata*, a common  
94 detritivore in the study area; (ii) whether intraspecific variability in microbial activity is reduced in  
95 millipede faecal pellets in comparison to unprocessed leaf litter, as predicted by Joly et al. (2015); and  
96 (iii) whether microbial activity in unconsumed leaf remains left behind by the millipede is modified in  
97 comparison to unprocessed leaf litter. Using land use as a driver of intraspecific variations in litter

98 quality, our study assesses the extent to which a detritivore species interacts with afterlife effects of  
99 plant traits.

100

## 101 **Materials and methods**

### 102 *Study site and land-use treatments*

103 The study site is located at the Domaine de La Fage, Aveyron, southern France, an experimental  
104 station of the French National Institute for Agricultural Research (INRA) (43°55' N, 3°05' E, 800 m  
105 above sea level). This grassland area, which was described in detail by Bumb et al. (2016) and Chollet  
106 et al. (2014), is traditionally grazed by sheep. From 1978 onwards, three paddocks were fertilized with  
107 inorganic nitrogen (65 kg/ha  $\text{NH}_4\text{NO}_3$  every year) and phosphorus (40 kg/ha  $\text{H}_3\text{PO}_4$  every three years  
108 until 2003), while other paddocks remained in their original condition. In March 2012, three 10 m x 10  
109 m sheep enclosures were set up in two unfertilized and two fertilized paddocks, so that four land-use  
110 treatments were used on the study site: fertilized plots with sheep grazing (F+/G+), fertilized plots  
111 with sheep exclusion (F+/G-), unfertilized plots with sheep grazing (F-/G+) and unfertilized plots  
112 with sheep exclusion (F-/G-).

113

### 114 *Leaf litter and animal collection*

115 In autumn 2012, i.e. a complete growing season after the start of sheep exclusion, fully senesced leaf  
116 litter from two plant species, *Bromopsis erecta* (Huds.) Fourr., 1869 (Poaceae) and *Potentilla verna*  
117 L., 1753 (Rosaceae), was collected directly from plants in the four types of land-use treatments. More  
118 precisely, in the fertilized and in the unfertilized paddocks, litter was collected both inside (ungrazed  
119 treatment) and outside (grazed treatment) the sheep enclosures. These two species were chosen  
120 because they were abundant under the four land-use treatments. It proved, however, impossible to  
121 collect sufficient amounts of leaf litter in 100 m<sup>2</sup> plots to conduct all analyses and experiments  
122 separately for each plot. As it was necessary to pool material from several plots, similar amounts of  
123 leaf litter were collected on all plots subjected to the same land-use treatment and were thoroughly

124 mixed to form samples representative of each treatment at the site scale. Litter quality was therefore  
125 determined on single, composite litter pools from each land-use treatment. Because previous studies in  
126 the same site (Bumb et al. 2016; Bumb et al. 2018) found no significant differences in litter traits  
127 among plots under the same land-use treatment, we argue that the litter pools used in our experiment  
128 were representative of the land-use treatments. Leaf litter was dried at 40 °C for 4 days and stored.  
129 Samples of each litter type were analysed for carbon, nitrogen, phosphorus, tannin content, as well as  
130 for litter thickness and structural resistance (Table 1 and Appendix A). The detailed analysis of this  
131 dataset on litter traits was presented in Coq et al. (2018).

132 As millipedes are by far the most abundant litter-feeding macroarthropods in this area  
133 (unpublished data), adult individuals of *Glomeris marginata* (Villers, 1789) (Glomeridae), one of the  
134 five millipede species present in the study site, were collected during the same period. This species,  
135 which occurs in a large array of ecological conditions, consumes a variety of leaf litter and is easily  
136 kept in the laboratory. Moreover, it is increasingly studied as a model detritivore organism.

137

#### 138 *Litter consumption and assimilation measurements*

139 To evaluate the effects of litter quality changes on food consumption and assimilation by millipedes,  
140 leaf litter from each plant species and each land-use treatment was offered separately to *G. marginata*.  
141 In contrast to Coq et al. (2018), who measured litter consumption by millipedes using binary choice  
142 cafeteria experiments, consumption was measured in the present study with trials using single litter  
143 types. This methodology allows measuring assimilation rates, as well as collecting faecal pellets  
144 produced from a single litter type. Seven days before the start of the experiment, in January 2013, two  
145 groups of randomly selected *G. marginata* individuals were transferred into large plastic boxes  
146 containing either *B. erecta* or *P. verna* leaf litter, for habituation. One day before the start of the  
147 experiment, the millipedes were starved to empty their gut. Three hundred milligrams of each type of  
148 leaf litter were then placed into 115 x 85 x 43 mm lidded, transparent plastic boxes (Caubert, Yèbles,  
149 France, Reference LAB3) and watered with 0.7 mL of distilled water. One pre-weighed *G. marginata*  
150 was added in half of the boxes, the other boxes being kept as controls containing only leaf litter. Each  
151 treatment was replicated five times for a total of 80 boxes [2 litter species x 4 land-use treatments x 2

152 fauna treatments (*G. marginata* and controls) x 5 replicates]. All boxes were kept at a constant 18 °C  
 153 under a 11 h:13 h light:dark cycle for one week and water content was adjusted twice during the  
 154 experiment. Faecal pellets were removed daily from the boxes, dried at 40 °C to prevent mass loss due  
 155 to microbial decay, and weighed. The total production of faecal pellets over the duration of the  
 156 experiment was the sum of daily collections. Leaf litter remaining at the end of the experiment was  
 157 dried at 40 °C and weighed. The amount of litter consumed in each box was calculated after David  
 158 (1998), as

$$159 \quad C = (M_0 - M_0D - M_f) / \sqrt{1-D}$$

160 with  $M_0$  = initial litter dry mass,  $M_f$  = final litter dry mass.  $D$  was the mean proportion of litter  
 161 decomposed in the absence of macrodetritivores, and was calculated as  $D = (M'_0 - M'_f) / M'_0$ , with  
 162  $M'_0$  and  $M'_f$  the initial and final litter dry mass in control boxes. Mean litter mass loss in the presence  
 163 and absence of millipedes are provided in Appendix B. The amount of litter assimilated was calculated  
 164 as the difference between the amount consumed and the weight of faecal pellets produced. Mean  
 165 faecal pellet production is presented in Appendix B. Because *G. marginata* individuals differed in  
 166 body weight, we performed an ANCOVA with individual consumption as the response variable, body  
 167 weight as a continuous predictor and land-use treatments and litter species as fixed effects. The weight  
 168 of *G. marginata* had a strong impact on consumption ( $F = 42.2$ ,  $P \ll 0.0001$ ). Weight-specific  
 169 consumption and assimilation rates (in  $\text{mg g}^{-1} \text{day}^{-1}$ ) were therefore calculated by dividing the daily  
 170 amounts consumed and assimilated by the live weight of the millipede. Assimilation efficiency was  
 171 measured as the ratio between assimilation rate and consumption rate converted to percent.

172

### 173 *Microbial activity measurements*

174 Because assimilation measurements (which require drying faecal pellets) and assessments of microbial  
 175 activity (which are destructive for faecal pellets) cannot be made from the same pool of faecal pellets,  
 176 a similar experiment was repeated the following week to assess how microbial activity in leaf litter  
 177 varied among land-use treatments and how it was affected by the feeding activities of millipedes. The  
 178 protocol and the duration of the experiment were the same as described above, except that daily  
 179 collections of faecal pellets and final litter material were kept in the fridge at 4 °C instead of being



180 dried at 40 °C. The dry weight of this material was determined indirectly, from the percentage water  
181 content of sub-samples. Substrate-induced respiration (SIR) was used to assess the respiratory capacity  
182 of physiologically active microorganisms in control litter, faecal pellets of *G. marginata* and  
183 unconsumed litter remains (Beare, Neely, Coleman, & Hargrove, 1990). The method measures the  
184 glucose mineralization potential of microorganisms in plant residues under optimal conditions and  
185 accounts for changes in both the biomass and composition of microbial communities (Fanin,  
186 Hattenschwiler, Barantal, Schimann, & Fromin, 2011). The MicroResp™ system (Macaulay Scientific  
187 Consulting, Aberdeen, UK) was used. Samples of about 4-5 mg of fresh material were weighed with  
188 an accuracy of 0.01 mg, placed in the wells of a 96-deepwell microplate (Fisher Scientific) with 50 µl  
189 of a 2.5 g L<sup>-1</sup> glucose solution, and incubated for 2 hours at 25 °C. The plates were then covered with a  
190 detection plate containing Cresol red CO<sub>2</sub>-sensitive gel, secured with a clamp, and incubated for four  
191 additional hours. The optical density in the detection plate was read at 590 nm before and after  
192 incubation with a Victor 1420 Multilabel Counter (Perkin Elmer, Massachusetts, USA) and converted  
193 to SIR rates (µg CO<sub>2</sub>. mg<sup>-1</sup> dry litter or faecal pellet. h<sup>-1</sup>). For each type of material, three replicates per  
194 box (or only two when faecal pellet production was insufficient) were analysed.

195

### 196 *Statistical analyses*

197 The main effects and interactions of litter species, fertilization and sheep grazing on each litter trait  
198 (C/N, C/P, N/P, condensed tannins, litter thickness and structural resistance) were tested using three-  
199 way ANOVAs, followed by post-hoc Tukey tests. The main effects and interactions of litter species,  
200 fertilization and sheep grazing on the consumption rate, assimilation rate and assimilation efficiency of  
201 *G. marginata* were also tested using three-way ANOVAs, followed by post-hoc Tukey tests.  
202 SIR data were analysed using mixed models, because for each type of organic matter (*i.e.* control  
203 litter, faecal pellets and litter remains) three samples per microcosm were analysed. Moreover, faecal  
204 pellets and litter remains were collected from the same microcosms, while control litter was  
205 necessarily sampled from a distinct microcosm without millipede. This prevented from analysing SIR  
206 data in a single full model. We therefore combined two lines of analyses. First, we ran mixed models  
207 separately for each type of organic matter, with litter species, sheep grazing, fertilization and their

208 interactions as fixed effects and microcosm as a random effect. We used the *lmer* function from the  
209 lme4 package (Bates et al. 2015), followed by the *Anova* function from the car package. When  
210 interaction terms were not significant, they were sequentially removed from the model and the  
211 analysis was run again until all remaining terms of interaction were significant. Post-hoc tests were  
212 then performed with the *emmeans* function from the emmeans package (Lenth, 2019). Second, to  
213 compare the different types of organic matter, we calculated the differences between SIR in faecal  
214 pellets or litter remains and SIR in control litter. More precisely, for each sample of faecal pellets and  
215 litter remains, we subtracted the mean SIR value measured in control litter from the corresponding  
216 species and land-use treatment. This new variable was referred to as the difference in SIR relative to  
217 controls. Using this difference as a response variable, we ran a mixed model with the type of organic  
218 matter (faecal pellets or litter remains), litter species, sheep grazing, fertilization and their interactions  
219 as fixed effects and microcosm as a random effect, using the same procedures and functions as  
220 described above. We then performed post-hoc tests with the *emmeans* function from the emmeans  
221 package (Lenth, 2019). Additionally, for each type of organic matter (faecal pellets or litter remains)  
222 produced from leaf litter of each plant species, we tested whether the difference in SIR relative to  
223 controls differed significantly from 0 using Student's t-tests. Finally, correlations between  
224 consumption rate, assimilation rate and SIR in control litter were tested using Pearson's correlation  
225 coefficients. All analyses were performed using the R software, version 3.4.4 (R Core Team, 2018).

226

## 227 **Results**

228 Leaf litter consumption by *G. marginata* was much higher on *P. verna* (on average  $42.3 \pm 2.0 \text{ mg.g}^{-1}$   
229  $\cdot \text{d}^{-1}$  across all treatments) than on *B. erecta* ( $18.0 \pm 2.7 \text{ mg.g}^{-1} \cdot \text{d}^{-1}$  across all treatments) (Figs. 1A and  
230 C, Table 2). However, litter species showed a significant interaction with sheep grazing and a  
231 marginally significant interaction with fertilization. Post-hoc tests revealed that a higher consumption  
232 of *P. verna* was observed for almost all land-use treatments, except for the fertilized and grazed  
233 treatment, in which the consumption of *B. erecta* litter was close to that of *P. verna* litter (Fig. 1A and  
234 C).

235 On the whole dataset, consumption was significantly affected by sheep grazing, but again, this effect  
236 strongly depended on litter species and fertilization, as indicated by the significant interactions  
237 between grazing and litter species and between grazing and fertilization (Table 2). When each species  
238 was considered separately, the consumption of *P. verna* litter was not significantly affected by the  
239 land-use treatments (Fig. 1C, Table 2). In contrast, grazing increased the consumption of *B. erecta*  
240 litter from fertilized plots by 270% (Fig. 1A, Table 2), whereas it only led to a slight and non-  
241 significant increase in the consumption of this litter sampled in unfertilized plots. (Fig. 1A, Table 2).

242 The assimilation of leaf litter by *G. marginata* was higher on *P. verna* (on average  $6.5 \pm 0.8 \text{ mg.g}^{-1}.\text{d}^{-1}$   
243 across all treatments) than on *B. erecta* ( $3.8 \pm 0.6 \text{ mg.g}^{-1}.\text{d}^{-1}$  across all treatments) (Table 2, Figs. 1B  
244 and D). Unlike consumption rate, assimilation rate did not vary significantly among land-use  
245 treatments, neither for *B. erecta* nor for *P. verna*. (Figs. 1B and D, Table 2). Therefore, the increased  
246 consumption of *B. erecta* litter from fertilized and grazed plots did not result in larger amounts of food  
247 assimilated but in an increased production of faecal pellets. The assimilation efficiency of *G.*  
248 *marginata*, which did not vary significantly with plant litter species and land-use treatments (Table 2),  
249 was on average 19%.

250 Across all treatments, SIR rates in control leaf litter averaged  $0.27 \pm 0.02 \mu\text{g CO}_2.\text{mg}^{-1}.\text{h}^{-1}$  and  $0.22 \pm$   
251  $0.03 \mu\text{g CO}_2.\text{mg}^{-1}.\text{h}^{-1}$  for *B. erecta* and *P. verna*, respectively, and this difference was not significant  
252 (Figs. 2A and D, Table 3). In both species, there was substantial variation among land-use treatments,  
253 with significantly higher SIR rates in litter from fertilized plots than in that from unfertilized plots  
254 (Figs. 2A and D, Table 3). Fertilization increased SIR by 87% in *B. erecta* litter and by 304% in *P.*  
255 *verna* litter. We did not detect any correlation between SIR in control litter and the consumption and  
256 assimilation rates measured in *G. marginata*, neither when both litter species were considered together  
257 ( $r = 0.08$ ,  $P = 0.70$  and  $r = -0.16$ ,  $P = 0.46$ , respectively), nor when they were analysed separately.

258 The SIR rates of faecal pellets were on average higher when they were produced from *B. erecta* litter  
259 ( $0.33 \pm 0.04 \mu\text{g CO}_2.\text{mg}^{-1}.\text{h}^{-1}$  across all treatments) than from *P. verna* litter ( $0.22 \pm 0.01 \mu\text{g CO}_2.\text{mg}^{-1}.\text{h}^{-1}$   
260 across all treatments, Table 3, Figs. 2B and E). However, the difference in SIR relative to controls

261 did not significantly differ from 0 for faecal pellets, neither when the two litter species were  
262 considered together, nor when they were analysed separately (Fig. 3). Interestingly, while land use had  
263 a strong and significant effect on SIR in control litter, SIR rates in faecal pellets no longer differed  
264 significantly among land-use treatments (Figs. 2B and E, Table 3). This homogenization of SIR in  
265 faecal pellets resulted from increased SIR rates when *G. marginata* was fed litter from unfertilized  
266 plots, while SIR rates remained stable (*B. erecta*) or decreased (*P. verna*) when *G. marginata* was fed  
267 litter from fertilized plots (Fig. 2, Appendix D).

268 Finally, in boxes with *G. marginata*, the SIR rates of litter remains that were not consumed at the end  
269 of the experiment were reduced to similar levels in the two litter species (on average  $0.11 \pm 0.01 \mu\text{g}$   
270  $\text{CO}_2.\text{mg}^{-1}.\text{h}^{-1}$  for *B. erecta* and  $0.14 \pm 0.01 \mu\text{g CO}_2.\text{mg}^{-1}.\text{h}^{-1}$  for *P. verna* across all treatments) (Figs. 2C  
271 and F, Table 3). The difference in SIR relative to controls was significantly stronger for litter remains  
272 than for faecal pellets (Fig. 3, Appendix C), and was significantly negative when the two litter species  
273 were considered together or separately (Fig. 3). This decrease in SIR in litter remains compared to  
274 control litter, by 60% in *B. erecta* and by 37% in *P. verna*, was primarily due to drastic decreases  
275 affecting litter materials from fertilized plots (Appendix C and D). This reduction led to the  
276 homogenization of SIR in the litter remains of *P. verna*, with no significant differences among land-  
277 use treatments (Fig. 2F, Table 3). Differences in SIR rates among treatments were also markedly  
278 reduced in the litter remains of *B. erecta* compared to control litter (Table 3, and compare Figs. 2A  
279 and C).

280

## 281 **Discussion**

### 282 *Litter quality, microbial activity and land use*

283 This study first shows that, in the absence of detritivores, leaf litter sampled from plots with distinct  
284 land-use practices exhibits different levels of microbial activity. Substrate-induced respiration (SIR),  
285 which measures the glucose mineralization potential of microorganisms in plant residues and  
286 correlates quite well with the potentially active microbial biomass (Beare et al., 1990), varied

287 intraspecifically and was clearly higher in litter from fertilized plots. As SIR rates are often positively  
288 correlated to mass loss rates (Cookson, Beare, & Wilson, 1998; Joly et al., 2015; Neely, Beare,  
289 Hargrove, & Coleman, 1991), faster leaf litter decomposition is expected in response to NP  
290 fertilization in the study ecosystem — at least in the early stages of decomposition. This was  
291 confirmed in a recent microcosm study of leaf litter decomposition over eight weeks under controlled  
292 conditions, in which a higher mass loss rate was recorded in material from fertilized plots (F+/G+)  
293 compared to unfertilized plots (F-/G+) (Bumb et al., 2018). In a shorter study, Coq et al. (2018) also  
294 recorded a higher mass loss rate in *B. erecta* leaf litter from F+/G+ plots. Changes in litter quality may  
295 have driven the impact of land-use on microbial activity reported in the present study. As shown in  
296 Table 1, NP fertilization was associated with substantial nitrogen and phosphorus enrichment in *B.*  
297 *erecta* and *P. verna* leaf litter, with lower C/N and C/P ratios in material from fertilized plots.  
298 Moreover, synergistic effects of fertilization and sheep grazing resulted in even lower C/N ratios (*B.*  
299 *erecta*) and C/P ratios (*P. verna*) in fertilized and grazed (F+/G+) plots. It is likely that such effects of  
300 land use on litter quality were cascaded down to the microbial community colonizing leaf litter.

### 301 *Macroarthropod feeding activity and land use*

302 Millipedes are present in the grassland studied and litter consumption by macroarthropods can  
303 also respond to land-use changes (Mancilla-Leytón, Cambrollé, & Vicente, 2017; Reis et al., 2018). In  
304 our study, the comparatively high consumption of *P. verna* litter by *G. marginata* did not vary among  
305 land-use treatments, but the consumption of *B. erecta* litter was much higher when the food came from  
306 fertilized and grazed (F+/G+) plots compared to other plots. This result fully confirms the food  
307 preferences of three millipede species observed by Coq et al. (2018) in a choice experiment. The  
308 particularly low C/N ratio in *B. erecta* litter from F+/G+ plots is likely to have played a role. The litter  
309 C/N ratio has long been recognized as one important food quality parameter for macroarthropods,  
310 notably in grassland ecosystems (David, Malet, Coûteaux, & Roy, 2001; Rushton & Hassall, 1983).  
311 Although litter colonization by microorganisms is another possible driver of litter consumption by  
312 macroarthropods (Gunnarson, 1987; Harrop-Archibald et al., 2016; Zimmer et al., 2003), which was  
313 not explored in Coq et al. (2018), we did not detect any correlation between SIR rates in control litter

314 and the consumption rate of *G. marginata*. This suggests that the low C/N ratio influenced the  
315 palatability of *B. erecta* litter directly, i.e. through the N concentration of plant tissues, rather than  
316 through an increased microbial biomass in N-enriched decomposing leaves. In contrast to the  
317 consumption rate, the assimilation rate of *G. marginata* did not differ significantly among land-use  
318 treatments, whether with *B. erecta* or *P. verna*. The high consumption of *B. erecta* litter from fertilized  
319 and grazed plots was not accompanied by a high assimilation of this material and resulted in an  
320 increased production of faecal pellets.

### 321 *Changes in microbial activity following litter processing by macroarthropods*

322         A major finding of this study was that the feeding activities of *G. marginata* profoundly  
323 affected microbial activity in leaf litter, both in the parts that were eaten, digested and egested as  
324 faecal pellets, and in the parts that were not consumed. However, the faunal effects differed somewhat  
325 between the two types of materials. In faecal pellets derived from each plant species, there was  
326 primarily homogenization of SIR among land-use treatments: the comparatively low SIR rates of leaf  
327 litter from unfertilized plots increased in faeces derived from this food source, while the higher SIR  
328 rates of leaf litter from fertilized plots remained stable (*B. erecta*) or even decreased (*P. verna*) in  
329 faeces derived from this other food. Both higher and lower SIR rates have already been measured in  
330 faecal pellets of *G. marginata* compared to leaf litter (Joly et al., 2015; Maraun & Scheu, 1996), and  
331 Joly et al. (2015) have shown that such variations lead to homogenization of SIR in faecal pellets  
332 derived from different tree litter species. No interspecific homogenization was demonstrated in our  
333 study, since SIR values in control litter, averaged across land-use treatments, were not significantly  
334 different between species. Clearly, however, there was intraspecific homogenization of SIR in the  
335 egested material, so that marked differences in SIR between leaf litter from fertilized and unfertilized  
336 plots completely disappeared in faecal pellets.

337         In leaf litter remains that were not consumed by *G. marginata* at the end of the experiment,  
338 microbial activity was even more affected than in faecal pellets, with decreased SIR in comparison  
339 with control litter. The decrease mainly affected leaf litter from fertilized plots, in which high SIR  
340 rates were recorded in control litter but much lower rates following millipede activity. The effects of

341 macroarthropods on unconsumed litter remains are complex, probably species-specific (David, 2014),  
342 and they have been reported to result in higher (Frouz & Šimek, 2009), unchanged (Coulis,  
343 Hättenschwiler, Fromin, & David, 2013) or lower microbial respiration (Špaldoňová & Frouz, 2014).  
344 In our study, the effects of *G. marginata* on the SIR of litter remains were overall negative, due to the  
345 strong reduction in SIR values in litter from the fertilized plots. Two different, but not mutually  
346 exclusive, mechanisms may underlie this result. First, as microbial colonization increases the  
347 attractiveness of food to macroarthropods (Gunnarsson, 1987; Ihnen & Zimmer, 2008; Zimmer et al.,  
348 2003), millipedes may have selectively ingested dead leaves or parts of dead leaves with high  
349 microbial activity, and left behind litter remains that were poorer in microorganisms. Second,  
350 macroarthropods are capable to graze on microorganisms, especially fungi, without attacking the  
351 underlying substrate (Bignell, 1989; Crowther et al., 2011; Harrop-Archibald et al., 2016), which  
352 thereby reduces microbial biomass in the grazed material. It is impossible to further clarify the  
353 mechanisms involved in our study, but the results show that they were able to decrease microbial  
354 activity in large amounts of litter remains — much larger than the amounts consumed during the  
355 experiment. Moreover, there was some intraspecific homogenization of SIR in litter remains, which  
356 largely suppressed the positive effects of NP fertilization observed in control litter. A similar result  
357 was obtained by Fanin et al. (2012) in a tropical forest ecosystem, in which positive effects of  
358 fertilization on litter SIR were markedly reduced in coarse-mesh litter bags, i.e. in the presence of  
359 macrofauna.

#### 360 *Potential disruption of aboveground-belowground linkages by macroarthropods*

361         These drastic changes in microbial activity following litter consumption by *G. marginata*  
362 raise an important issue: the impact of soil macro-detrivores on the coupling between above- and  
363 belowground components of ecosystems. Only few studies that have hitherto examined the influence  
364 of aboveground processes on plant litter decomposition considered the role of macroarthropods  
365 (Buchkowski, Schmitz & Bradford, 2019). For example, studies in fine-mesh litter bags excluding  
366 macrofauna have shown that intraspecific changes in litter quality in response to herbivory affect litter  
367 decomposition rates (Olofsson & Oksanen, 2002; Semmartin & Ghersa, 2006; Wang et al., 2018).

368 Similarly, our study has shown that intraspecific changes in litter quality in response to land-use  
369 practices affect microbial activity in leaf litter — but only in control boxes without detritivores. In the  
370 presence of millipedes, despite large differences in the quality of litter offered as food, there was  
371 homogenization of microbial activity both in faecal pellets and unconsumed litter remains, i.e.  
372 disruption of the cascading effects of land-use practices in each of the plant species studied. This  
373 finding of fauna-driven homogenization of microbial activity is further strengthened by similar  
374 observations at the interspecific level. Aboveground processes often result in changes in plant  
375 community composition, and studies in fine-mesh litter bags or microcosms without macrofauna have  
376 shown that the leaf traits of different plant species strongly affect litter decomposition (Cornelissen et  
377 al., 2004; Cornwell et al., 2008; Fortunel et al., 2009; Freschet et al., 2012). These afterlife effects of  
378 plant characteristics on decomposition have been the focus of many studies and have been identified  
379 as a pivotal link between aboveground and belowground functioning (Bardgett & Wardle, 2010;  
380 Wardle et al., 2004). Again, however, it was shown that large differences in microbial activity among  
381 26 species of leaf litter were considerably reduced in faecal pellets of millipedes (Joly et al., 2015).  
382 Although SIR measurements do not necessarily reflect the complex process experienced by organic  
383 matter during decomposition, homogenization of SIR following litter consumption suggests that  
384 variability in subsequent microbial decomposition might also be reduced. Further testing of the fate of  
385 faecal pellets and litter remains would be required to clarify the role of detritivores in decomposition.  
386 Nevertheless, the current evidence suggests that studies that assess the effects of plant traits on  
387 decomposition without considering the fate of the litter transformed by soil detritivores may prove  
388 inadequate in ecosystems with abundant populations of these animals.

389 To conclude, we acknowledge that our study, carried out with only one macroarthropod  
390 species and two leaf litter species under artificial conditions, may be insufficient to reach conclusions  
391 at the ecosystem scale. Moreover, the abundance of soil macrodetritivores is a key determinant of their  
392 functional importance, and possible variations in population density in response to land-use practices  
393 should be taken into account. Nevertheless, our results strongly suggest that these organisms can



394 considerably modify the effects of aboveground processes on microbial decomposers and should  
395 therefore be considered in future studies of afterlife effects of plant traits on decomposition.

396

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407

### 408 **Authors' contributions**

409 SC and JN designed and realized the experiment, NF provided her expertise for SIR measurements,  
410 EK coordinated the setting up of the sheep exclusion in the field. SC and JFD analysed the data and  
411 wrote the manuscript. All authors contributed to revisions.

### 412 **Data accessibility**

413 The data associated with this paper will be available from the Dryad Digital Repository.

414

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- 567

568 **Tables**

569 Table 1. Means and standard errors of litter quality traits for *Bromopsis erecta* and *Potentilla verna* collected under the different land-use treatments. Different  
 570 letters indicate significant differences among treatments and species in post-hoc tests after three-way ANOVAs (Appendix A). Detailed information on the  
 571 methods used for the analyses can be found in Coq et al. (2018).

Treatment	C/N	C/P	N/P	Tannins	Litter thickness	Structural resistance
<i>Bromopsis erecta</i>						
F-/G-	52.1 +/- 0.4 a	1223 +/- 65 a	23.5 +/- 1.2 a	0.11 +/- 0.01 a	202 +/- 54 abc	0.33 +/- 0.08 a
F-/G+	53.1 +/- 0.8 a	1245 +/- 26 a	23.5 +/- 0.3 a	0.15 +/- 0.02 a	161 +/- 31 c	0.22 +/- 0.03 bc
F+/G-	44.8 +/- 2.0 b	578 +/- 74 b	12.9 +/- 1.5 bc	0.1 +/- 0 a	182 +/- 31 bc	0.26 +/- 0.04 ab
F+/G+	35.0 +/- 0.4 c	418 +/- 18 c	11.9 +/- 0.6 c	0.16 +/- 0.01 a	173 +/- 34 bc	0.2 +/- 0.07 bc
<i>Potentilla verna</i>						
F-/G-	52.6 +/- 1.6 a	855 +/- 20 d	16.3 +/- 0.6 d	1.63 +/- 0.24 b	225 +/- 27 ab	0.18 +/- 0.05 bc
F-/G+	50.0 +/- 3.4 a	813 +/- 8 d	16.3 +/- 1.3 d	1.07 +/- 0.07 c	237 +/- 20 a	0.22 +/- 0.05 bc
F+/G-	40.1 +/- 0.5 b	602 +/- 16 b	15.0 +/- 0.5 bd	0.73 +/- 0.16 d	228 +/- 17 ab	0.17 +/- 0.04 c
F+/G+	40.3 +/- 0.1 b	447 +/- 26 c	11.1 +/- 0.6 c	0.67 +/- 0.13 d	206 +/- 46 abc	0.2 +/- 0.04 bc

572



573 Table 2. Results of three-way ANOVAs testing for the main effects and interactions of litter species (L), fertilization (F) and sheep grazing (G) on the  
 574 consumption rate, assimilation rate and assimilation efficiency of *Glomeris marginata* feeding on leaf litter. Significant results are in bold.

575	Source of variance	Consumption rate			Assimilation rate			Assimilation efficiency		
		<i>df</i>	<i>F</i> -value	<i>P</i> -value	<i>df</i>	<i>F</i> -value	<i>P</i> -value	<i>df</i>	<i>F</i> -value	<i>P</i> -value
576	L	<b>1</b>	<b>97.8</b>	<b>&lt; 0.0001</b>	<b>1</b>	<b>6.4</b>	<b>0.02</b>	1	3.4	0.07
577	F	1	2.7	0.11	1	0.0	0.99	1	0.3	0.58
578	G	<b>1</b>	<b>16.1</b>	<b>&lt; 0.001</b>	1	0.4	0.51	1	3.1	0.09
579	L x F	1	3.7	0.06	1	1.2	0.28	1	0.1	0.83
580	L x G	<b>1</b>	<b>6.8</b>	<b>0.01</b>	1	0.2	0.66	1	2.7	0.11
581	F x G	<b>1</b>	<b>11.4</b>	<b>0.002</b>	1	2.0	0.16	1	0.0	0.94
	L x F x G	1	0.3	0.60	1	0.0	0.91	1	0.0	0.98

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583 Table 3. Results of mixed models testing for responses of the SIR variable to litter species (L),  
 584 fertilization (F), sheep grazing (G) and their interactions as fixed effects, with microcosm as a random  
 585 effect. Non-significant interactions were sequentially removed from the models. Separate analyses  
 586 were performed for control leaf litter, faecal pellets of *Glomeris marginata* and unconsumed litter  
 587 remains. Significant results are in bold.

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Source of variance	SIR in control litter			SIR in faecal pellets			SIR in litter remains		
	<i>df</i>	<i>ChiSq</i>	<i>P</i> -value	<i>df</i>	<i>ChiSq</i>	<i>P</i> -value	<i>df</i>	<i>ChiSq</i>	<i>P</i> -value
L	1	1.20	0.27	1	<b>3.95</b>	<b>0.046</b>	1	1.63	0.20
F	1	<b>19.49</b>	<b>&lt; 0.0001</b>	1	0.05	0.82	1	1.77	0.18
G	1	0.56	0.45	1	0.13	0.72	1	2.85	0.09
L x G							1	<b>7.63</b>	<b>0.006</b>

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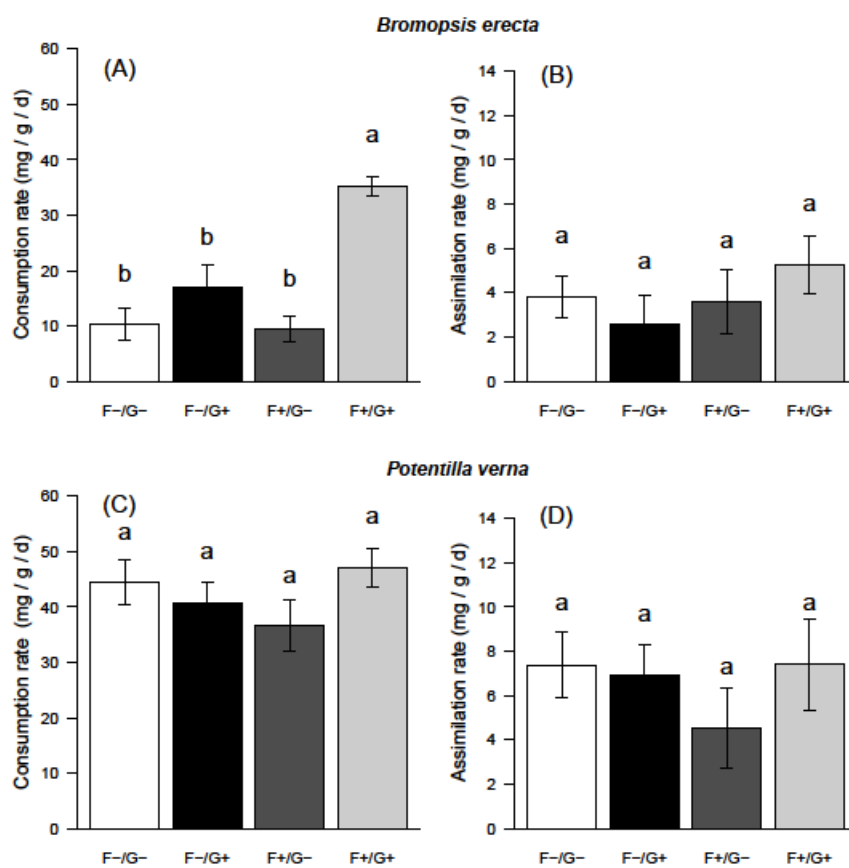
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603 **Figures**

604 Fig. 1. Means and standard errors of the weight-specific consumption rates (A and C) and assimilation  
 605 rates (B and D) of *Glomeris marginata* fed *Bromopsis erecta* and *Potentilla verna* leaf litter. Both litter  
 606 species were collected in plots under four land-use treatments: F<sup>-</sup>/G<sup>-</sup>: unfertilized plots with sheep  
 607 exclusion; F<sup>-</sup>/G<sup>+</sup>: unfertilized plots with sheep grazing; F<sup>+</sup>/G<sup>-</sup>: fertilized plots with sheep exclusion;  
 608 F<sup>+</sup>/G<sup>+</sup>: fertilized plots with sheep grazing. For each variable, different letters above the error bars indicate  
 609 significant differences at P < 0.05.



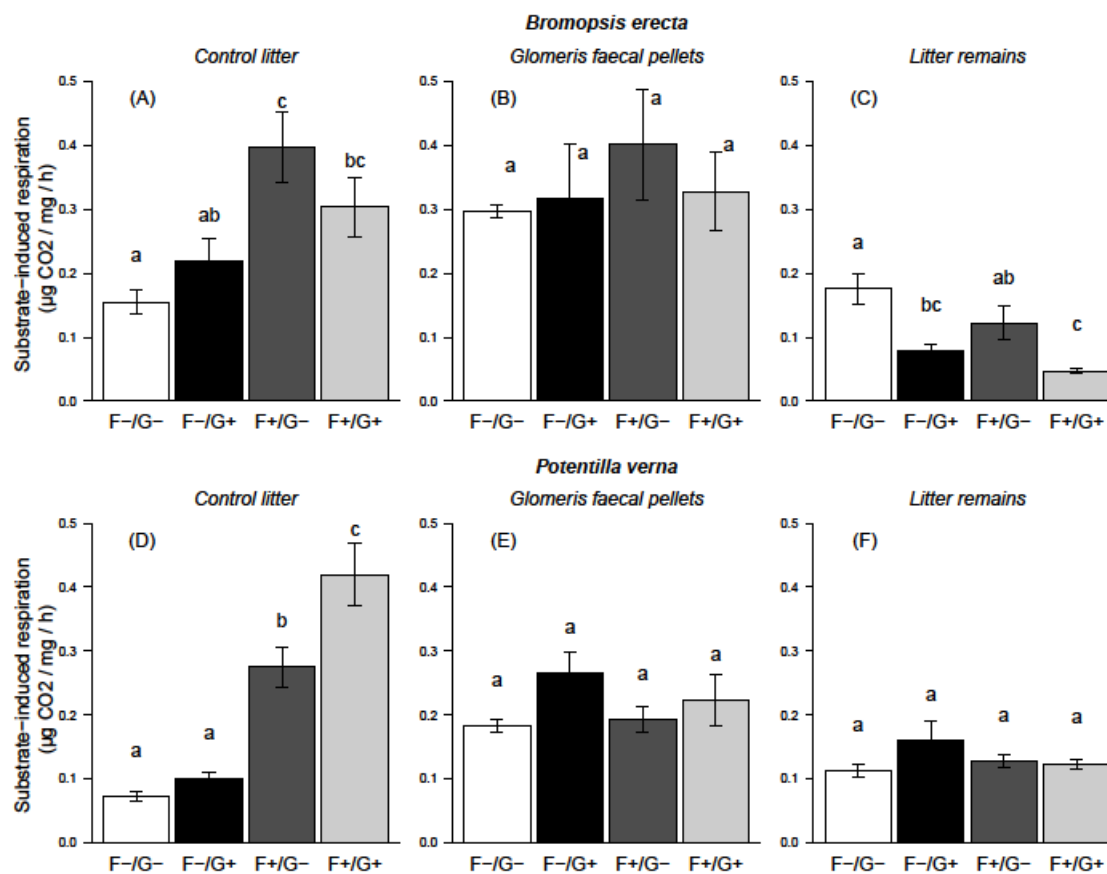
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614 Fig. 2. Means and standard errors of substrate-induced respiration (SIR,  $\mu\text{g CO}_2\cdot\text{mg}^{-1}\cdot\text{h}^{-1}$ ) measured on  
 615 control litter (A and D), faecal pellets of *Glomeris marginata* (B and E) and unconsumed litter remains  
 616 (C and F) when the millipedes were fed *Bromopsis erecta* and *Potentilla verna* leaf litter. Both litter  
 617 species were collected in plots under four land-use treatments: F<sup>-</sup>/G<sup>-</sup>: unfertilized plots with sheep  
 618 exclusion; F<sup>-</sup>/G<sup>+</sup>: unfertilized plots with sheep grazing; F<sup>+</sup>/G<sup>-</sup>: fertilized plots with sheep exclusion;  
 619 F<sup>+</sup>/G<sup>+</sup>: fertilized plots with sheep grazing. Within each type of organic matter, different letters above the  
 620 error bars indicate significant differences at  $P < 0.05$ .



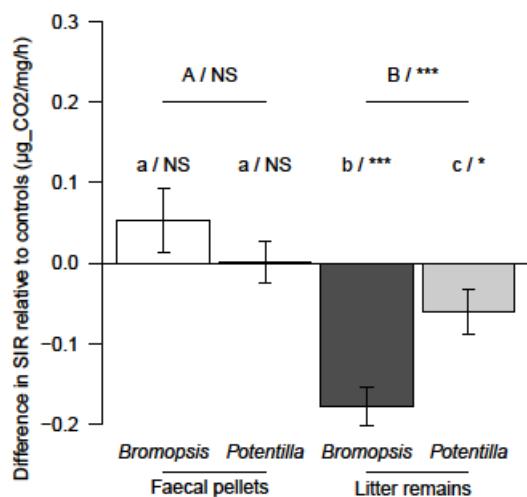
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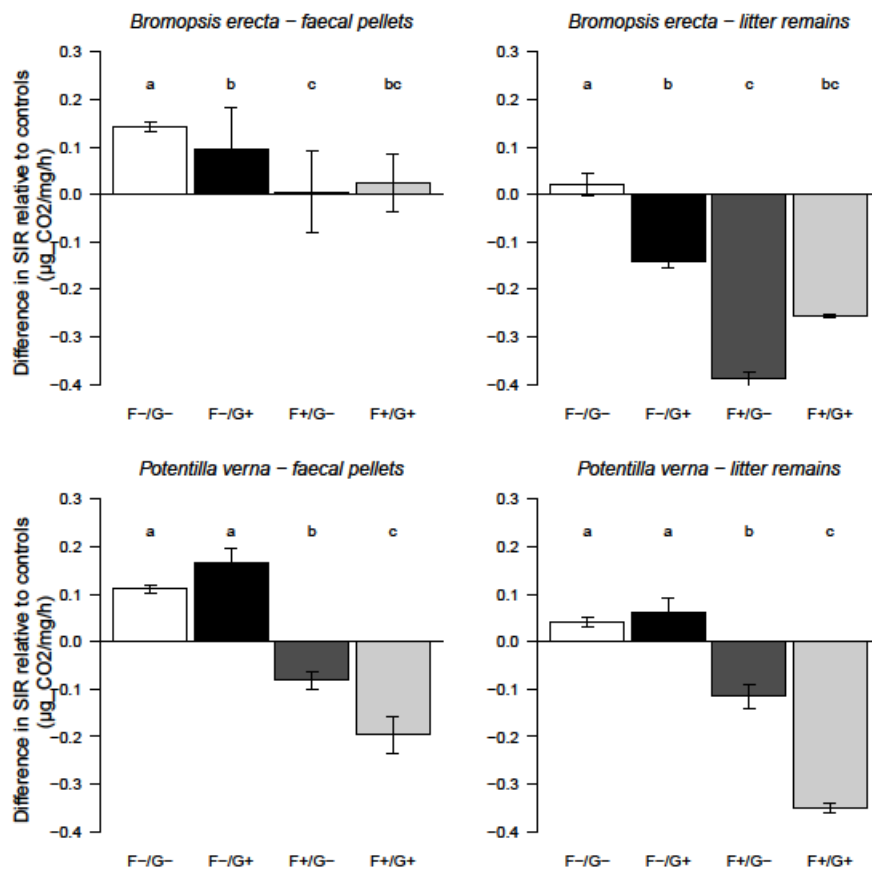
624 Fig. 3. Means and standard errors of the differences between SIR of faecal pellets or litter remains and  
 625 SIR of control litter from the same treatment, averaged across all land-use treatments. Letters indicate  
 626 the results of post-hoc tests comparing the differences in SIR relative to controls in faecal pellets and  
 627 litter remains, when considering both litter species together (top) and separately (below). Asterisks  
 628 indicate which differences differ from 0: NS not significant, \*  $P < 0.05$ , \*\*\*  $P < 0.001$ .

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631 Appendix D. Means and standard errors of the differences between SIR of faecal pellets or litter remains  
 632 and SIR of control litter from the same treatment, for each land-use treatment. Letters indicate the results  
 633 of post-hoc tests, using the emmeans function from the *emmeans* package.



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