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

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Abstract

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

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

Introduction

Linkages between aboveground and belowground communities play a key role in the functioning of terrestrial ecosystems (Ohgushi, Wurst, & Johnson, 2018). Among the variety of processes mediating these interactions, the inputs and decomposition of aboveground plant litter are of prominent importance (Bardgett & Wardle, 2010). Plant traits such as nutrient contents and traits related to defence largely control leaf litter decomposition rates, as a result of afterlife effects that cascade down from living plants to litter quality and microbial decomposer activity (Cornelissen et al., 2004; Cornwell et al., 2008; Fortunel et al., 2009; Freschet, Aerts, & Cornelissen, 2012). Moreover, any land-use practice or aboveground biotic interaction that modifies plant traits can potentially affect decomposition processes via the same cascading mechanisms. Livestock grazing, for example, considerably alters leaf litter quality not only through shifts in plant community composition, but also within species, through physiological responses of individual plants to herbivores (Bardgett & Wardle, 2010; Olofsson & Oksanen, 2002; Semmartin, Aguiar, Distel, Moretto, & Ghera, 2004; Semmartin & Ghera, 2006). The resulting effect on litter mass loss is highly variable, but it has been argued that positive effects of herbivory on decomposition should dominate in fertile ecosystems and negative effects in less productive situations (Bardgett & Wardle, 2010; Pastor, Cohen, & Hobbs, 2006). Therefore, both fertilization and grazing are expected to affect the tissues of living plants and generate afterlife effects on litter quality and decomposition (Bumb et al., 2018).

However, this approach linking decomposition to plant traits generally ignores the role of the soil fauna, despite its demonstrated impact on litter mass loss at a global scale (García-Palacios, Maestre, Kattge, & Wall, 2013). Most of the above-mentioned studies were done using fine-mesh litter bags or microcosms that excluded soil macrodetritivores, which are important regulators of decomposition processes (David, 2014; Lavelle & Spain, 2001). Saprophagous macroarthropods such as millipedes, woodlice and insect larvae can consume up to 40% of the annual litterfall in ecosystems in which they are abundant (Carcamo, Abe, Prescott, Holl, & Chanway, 2000; Coulis, Hättenschwiler, Coq, & David, 2016; David & Gillon, 2002; Frouz, Jedlička, Šimáčková, & Lhotáková, 2015). Ignoring this substantial contribution may lead to misleading conclusions. A variable proportion of the ingested food is assimilated by the animals and the rest is returned to the soil as faecal pellets. When macrodetritivores are present, a substantial part of the material lost due to litter consumption is not

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

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

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

Materials and methods

Study site and land-use treatments

The study site is located at the Domaine de La Fage, Aveyron, southern France, an experimental station of the French National Institute for Agricultural Research (INRA) (43°55' N, 3°05' E, 800 m above sea level). This grassland area, which was described in detail by Bumb et al. (2016) and Chollet et al. (2014), is traditionally grazed by sheep. From 1978 onwards, three paddocks were fertilized with inorganic nitrogen (65 kg/ha NH_4NO_3 every year) and phosphorus (40 kg/ha H_3PO_4 every three years until 2003), while other paddocks remained in their original condition. In March 2012, three 10 m x 10 m sheep enclosures were set up in two unfertilized and two fertilized paddocks, so that four land-use treatments were used on the study site: fertilized plots with sheep grazing (F+/G+), fertilized plots with sheep exclusion (F+/G-), unfertilized plots with sheep grazing (F-/G+) and unfertilized plots with sheep exclusion (F-/G-).

Leaf litter and animal collection

In autumn 2012, i.e. a complete growing season after the start of sheep exclusion, fully senesced leaf litter from two plant species, *Bromopsis erecta* (Huds.) Fourr., 1869 (Poaceae) and *Potentilla verna* L., 1753 (Rosaceae), was collected directly from plants in the four types of land-use treatments. More precisely, in the fertilized and in the unfertilized paddocks, litter was collected both inside (ungrazed treatment) and outside (grazed treatment) the sheep enclosures. These two species were chosen because they were abundant under the four land-use treatments. It proved, however, impossible to collect sufficient amounts of leaf litter in 100 m² plots to conduct all analyses and experiments separately for each plot. As it was necessary to pool material from several plots, similar amounts of leaf litter were collected on all plots subjected to the same land-use treatment and were thoroughly

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

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

Litter consumption and assimilation measurements

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

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

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

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

Microbial activity measurements

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

dried at 40 °C. The dry weight of this material was determined indirectly, from the percentage water content of sub-samples. Substrate-induced respiration (SIR) was used to assess the respiratory capacity of physiologically active microorganisms in control litter, faecal pellets of *G. marginata* and unconsumed litter remains (Beare, Neely, Coleman, & Hargrove, 1990). The method measures the glucose mineralization potential of microorganisms in plant residues under optimal conditions and accounts for changes in both the biomass and composition of microbial communities (Fanin, Hattenschwiler, Barantal, Schimann, & Fromin, 2011). The MicroResp™ system (Macaulay Scientific Consulting, Aberdeen, UK) was used. Samples of about 4-5 mg of fresh material were weighed with an accuracy of 0.01 mg, placed in the wells of a 96-deepwell microplate (Fisher Scientific) with 50 µl of a 2.5 g L⁻¹ glucose solution, and incubated for 2 hours at 25 °C. The plates were then covered with a detection plate containing Cresol red CO₂-sensitive gel, secured with a clamp, and incubated for four additional hours. The optical density in the detection plate was read at 590 nm before and after incubation with a Victor 1420 Multilabel Counter (Perkin Elmer, Massachusetts, USA) and converted to SIR rates (µg CO₂. mg⁻¹ dry litter or faecal pellet. h⁻¹). For each type of material, three replicates per box (or only two when faecal pellet production was insufficient) were analysed.

Statistical analyses

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

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

Results

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

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

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

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

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

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

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

Discussion

Litter quality, microbial activity and land use

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

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

Macroarthropod feeding activity and land use

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

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

Changes in microbial activity following litter processing by macroarthropods

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

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

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

Potential disruption of aboveground-belowground linkages by macroarthropods

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

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

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

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

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Authors' contributions

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

Data accessibility

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

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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

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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 consumption rate, assimilation rate and assimilation efficiency of *Glomeris marginata* feeding on leaf litter. Significant results are in bold.

		Consumption rate			Assimilation rate			Assimilation efficiency		
	Source of variance	<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
	L	1	97.8	< 0.0001	1	6.4	0.02	1	3.4	0.07
	F	1	2.7	0.11	1	0.0	0.99	1	0.3	0.58
	G	1	16.1	< 0.001	1	0.4	0.51	1	3.1	0.09
	L x F	1	3.7	0.06	1	1.2	0.28	1	0.1	0.83
	L x G	1	6.8	0.01	1	0.2	0.66	1	2.7	0.11
	F x G	1	11.4	0.002	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

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

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	3.95	0.046	1	1.63	0.20
F	1	19.49	< 0.0001	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	7.63	0.006

Figures

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

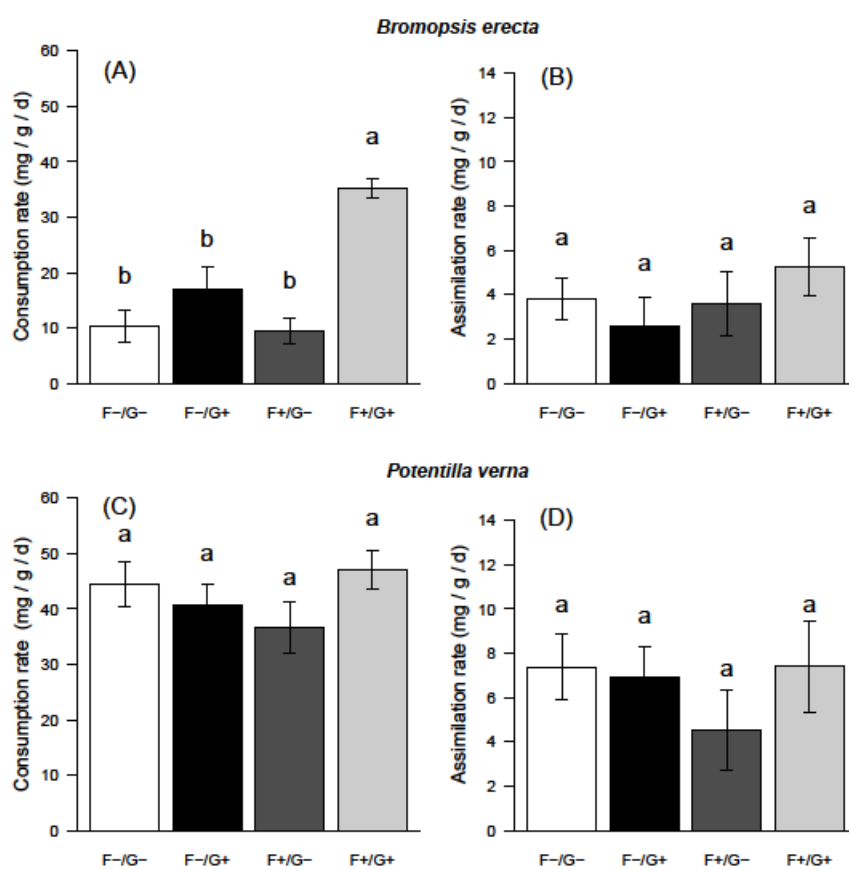


Fig. 2. Means and standard errors of substrate-induced respiration (SIR, $\mu\text{g CO}_2\cdot\text{mg}^{-1}\cdot\text{h}^{-1}$) measured on control litter (A and D), faecal pellets of *Glomeris marginata* (B and E) and unconsumed litter remains (C and F) when the millipedes were fed *Bromopsis erecta*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

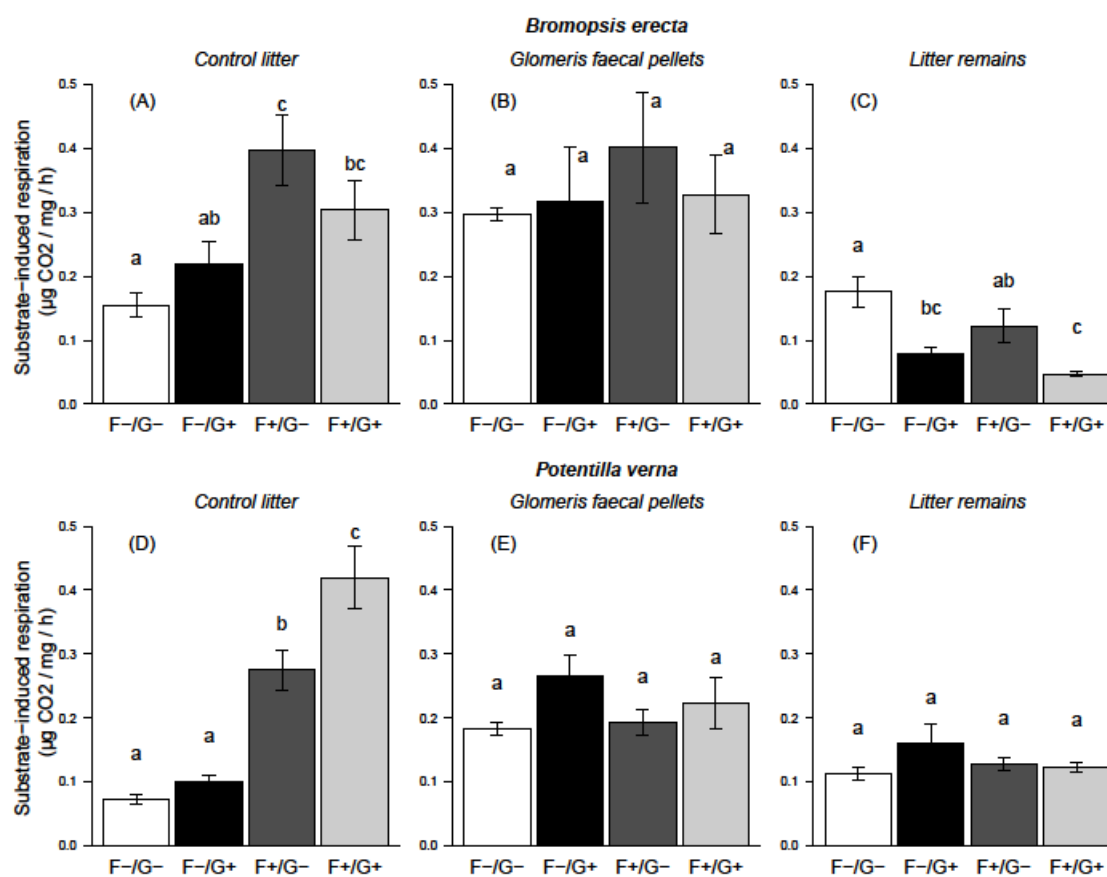
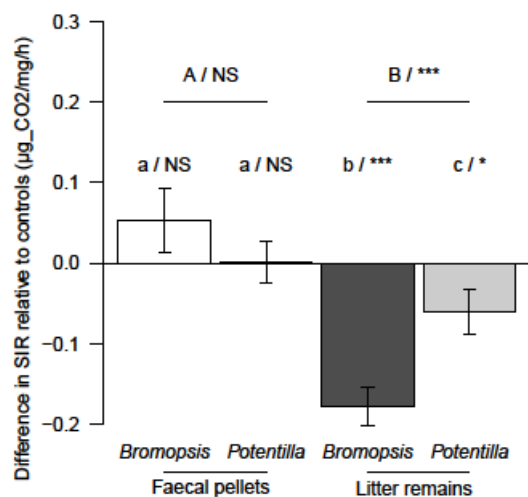


Fig. 3. Means and standard errors of the differences between SIR of faecal pellets or litter remains and SIR of control litter from the same treatment, averaged across all land-use treatments. Letters indicate the results of post-hoc tests comparing the differences in SIR relative to controls in faecal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



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.

