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Nutritive value and physical and chemical deterrents of forage grass litter explain feeding performances of two soil macrodetrivores

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ABSTRACT

Millipedes (Diplopoda) and terrestrial isopods (Isopoda) may play a significant role in soil decomposition. The present study aimed to contribute to the general understanding of feeding performances of macrodetrivores consuming grass litter by answering two questions. Q1: Are grass litter traits indicating nutritive value (i.e. chemical) and traits indicating feeding deterrents (i.e. mainly physical but not necessarily) both necessary to explain individual feeding performances of soil invertebrates consuming grass litter? Q2: Do grass physical traits indicating physical deterrents (e.g. WHC for mechanical aspects) provide more than, less than or the same amount of information about invertebrate individual performances as grass chemical traits indicating mainly chemical but also physical deterrents (e.g. lignin content directly for digestibility and indirectly for mechanical aspects)? We thus designed a laboratory experiment to assess individual feeding performances of two common macrodetrivores (*Armadillidium vulgare* (Latreille, 1804) and *Glomeris marginata* (Villiers, 1789)) in four monospecific treatments of litter from perennial forage grasses (*Brachypodium pinnatum* P. Beauv., *Bromus erectus* Huds., *Festuca rubra* L. and *Holcus lanatus* L.). *A. vulgare* feeding performances were correlated with nutritive values (litter N and P contents) and plant mechanical aspects (LDMC: leaf dry matter content). *G. marginata* performances were correlated with chemical deterrents (cellulose and lignin contents). Thus, (Q1) for grass litters, both traits indicating nutritive value (e.g. N, P) and feeding deterrents (e.g. LDMC, lignin content) are necessary to explain macroinvertebrates feeding performances. We also demonstrated the results depend on the invertebrate species considered. Also, (Q2) chemical deterrents may influence feeding performances of *G. marginata* the most, while physical deterrents related to mechanical aspects may influence those of *A. vulgare* the most. Our study shows that using grass chemical and physical traits that indicate both nutritive value and feeding deterrents can help explain feeding performances of macrodetrivores.

1. Introduction

Soil fauna consists of a huge diversity of life forms, mainly invertebrates (Decaëns et al., 2006). At the ecosystem level, they help deliver ecosystem services such as climate regulation and the production of agricultural goods (Barrios, 2007; Kibblewhite et al., 2008). They strongly influence soil behaviour by contributing to major soil functions such as organic matter decomposition (Barrios, 2007; Kibblewhite et al., 2008). Among soil invertebrates, millipedes (Diplopoda) and woodlice (Isopoda) play a significant role in decomposition processes when they are abundant (David and Handa, 2010;

Wolters, 2000). They can have direct and indirect effects on decomposition by breaking down litter and interacting with soil microorganisms (David, 2014). Understanding relationships between litter quality and macrodetrivore feeding performances is a key step for understanding effects of these animals on decomposition (Coulis et al., 2013; Joly et al., 2015). However, the mechanisms involved are far from being completely understood and predictable, in part because these soil invertebrates have a diverse diet due to their high nutrient requirements (mainly nitrogen (N) and carbohydrates, but also calcium, phosphorus (P) and magnesium) (David and Handa, 2010). David (2014) pointed out that no single litter trait can explain

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macrodetritivore preference; instead, a combination of litter traits indicating nutritive value (e.g. N content, C:N ratio) and feeding deterrents is determinant. Feeding deterrents can be classified as chemical or physical. Chemical deterrents are compounds that decrease litter ingestion or digestion (e.g. secondary compounds such as tannins, alkaloids or glycosides; structural compounds such as lignin). In contrast, physical deterrents decrease the rate at which invertebrates mechanically break down litter (e.g. toughness, thickness, silica spicules) (Levin, 1973; Zimmer et al., 2005). These mechanical aspects are generally indicated indirectly by chemical traits such as cellulose, hemicellulose and lignin contents, which are structural compounds. However they are rarely indicated by physical traits (e.g. toughness, specific leaf area, water holding capacity (WHC)), which are supposed to be much more proximately related to them (Coulis et al., 2015; Joly et al., 2015; Zimmer et al., 2005).

In addition, nearly all of this literature is based on woody plant litter (tree or shrub). To our knowledge, few studies examined herbaceous litter and thus grass litter (David et al., 2001; Paris, 1963; Rushton and Hassall, 1983; Zimmer et al., 2002). Among them, only two studied mechanisms by investigating chemical traits. David et al. (2001) measured consumption and assimilation by *Armadillidium vulgare* (Latreille, 1804) of the litter of five herbaceous species (1 Poaceae) subjected to three decomposition pre-treatments. In this study, consumption was overall positively correlated with litter N content and negatively correlated with the litter C:N ratio. However, the N content and C:N ratio had no significant influence on litter consumption in slightly decomposed litter. David et al. (2001) assumed that feeding deterrents would be removed as decomposition progressed, making the litter more palatable. In another study, in salt marshes, performances of three isopods on three litters (1 grass litter) were predicted by chemical litter traits such as phenolic concentrations (ferulic acid and tannins) and C:N ratios (Zimmer et al., 2002).

It has been theorised for herbivores that “apparent” plants (e.g. trees, grasses) often have low nutritive value and quantitative chemical deterrents (e.g. polyphenols in trees) or physical deterrents (e.g. in grass), whereas “unapparent plants” (e.g. small dicotyledons) often have high nutritive value and rapidly degraded chemical qualitative deterrents (e.g. alkaloids) (Hassall and Rushton, 1984). None of the studies of herbaceous litter mentioned previously investigated physical traits, even though they may help understand effects of physical deterrents (mechanical aspects) on feeding performances of soil invertebrates consuming grass litter, as the theory for herbivores suggests (Hassall and Rushton, 1984).

The present study aimed to contribute to the general understanding of feeding performances of macrodetritivores consuming grass litter. We hypothesised that litter with different functional trait profiles would be consumed differently by soil macrodetritivores. We raised two questions. For each, we developed associated hypotheses. (Q1) Are grass litter traits indicating nutritive value (i.e. chemical) and traits

indicating feeding deterrents (i.e. mainly physical but not necessarily) both necessary to explain individual feeding performances of soil invertebrates consuming grass litter, as previously demonstrated for other litter types (e.g. from woody plants) (David, 2014)? (H1a) Knowledge about both types of grass litter traits is necessary to explain individual feeding performances of invertebrates consuming grass litter. (H1b) Relationships between grass litter traits and individual feeding performances vary among invertebrate species. (H1c) Relationships between grass litter traits and individual feeding performances of an invertebrate species remain the same regardless of the type of biomass forming the litter (e.g. herbaceous, woody). (Q2) Do grass physical traits indicating physical deterrents (e.g. WHC for mechanical aspects) provide more than, less than or the same amount of information about invertebrate individual performances as grass chemical traits indicating mainly chemical but also physical deterrents (e.g. lignin content directly for digestibility and indirectly for mechanical aspects)? (H2) According to the theory for herbivores (Hassall and Rushton, 1984), deterrents in grass litter could rely more on physical defences than on chemical defences. Thus, physical traits provide more information about deterrents than chemical traits.

Consequently, we designed a laboratory experiment to assess feeding performances (consumption, defecation production, assimilation) of two common macrodetritivores, *A. vulgare* and *Glomeris marginata* (Villiers, 1789), on litters of heterogeneous perennial forage grasses. Litters were chosen by a trait-based approach to have a wide spectrum of functional profiles (i.e. nutritive values and physico-chemical deterrents) to provide generality to our conclusions.

2. Materials and methods

2.1. Litter selection, collection and characterisation

We selected litter of perennial forage grasses classified according to the typology of Cruz et al. (2010), developed to distinguish species according to their utility in agriculture. It is based on four traits of leaves (leaf dry matter content (LDMC), specific leaf area, longevity and surface tensile strength) and two traits of whole plants (flowering date and maximum height). It distinguishes functional groups with different growth strategies, biomass accumulation strategies, phenology, frequency of use and feeding values for cattle. We selected four species from different functional groups to have a wide spectrum of functional profiles (i.e. nutritive values and physico-chemical deterrents): *Brachypodium pinnatum* P. Beauv., *Bromus erectus* Huds., *Festuca rubra* L. and *Holcus lanatus* L. (Table 1).

Litter was collected from an experiment consisting of several small plots (60 × 40 cm). A single grass species was sown in each plot in September 2010. Plots were fertilised every year in March or April (75 kg N/ha, 50 kg P/ha and 50 kg K/ha). Plots were mown every year and irrigated. Litter of each species was collected in October 2015 and

Table 1

Traits (means and standard errors (± SE)) used in this study. Carbon content (C, %), nitrogen content (N, %), total phosphorus (P, %), water holding capacity (WHC, %), leaf cellulose content (LCC, %), leaf hemicellulose content (LHC, %), leaf lignin content (LLC, %), leaf dry matter content (LDMC, mg × g⁻¹).

	Litter			Plant leaf				
	Chemical			Physical	Chemical			Physical
	C	N	P	WHC	LCC ^a	LHC ^a	LLC ^a	LDMC ^b
	n = 5	n = 5	n = 5	n = 5	n = 3	n = 3	n = 3	n = 12
<i>Holcus lanatus</i> L.	41.68 ± 0.09	1.05 ± 0.01	0.10 ± 0.03	9.20 ± 0.51	22.01 ± 0.14	26.55 ± 0.67	0.39 ± 0.15	241.83 ± 7.61
<i>Festuca rubra</i> L.	45.01 ± 0.07	1.25 ± 0.00	0.18 ± 0.00	4.56 ± 0.16	28.28 ± 0.79	33.19 ± 0.94	0.36 [*]	228.60 ± 7.12
<i>Bromus erectus</i> Huds.	43.53 ± 0.06	1.11 ± 0.01	0.15 ± 0.04	4.38 ± 0.15	28.72 ± 0.60	31.46 ± 0.51	1.34 ± 0.31	238.80 ± 4.38
<i>Brachypodium pinnatum</i> P. Beauv.	43.75 ± 0.09	0.81 ± 0.01	0.03 ± 0.02	4.15 ± 0.14	24.61 ± 0.61	37.33 [*]	1.01 [*]	329.37 ± 6.07

^a From Cottier et al. (2001)

^b From Cruz et al. (2010).

* Values obtained from 2 replicates only.

February 2016. Only entirely senescent fragments of aerial biomass were kept. Litters were air-dried for a few days and then stored in paper envelopes before the experiment was begun.

A subsample of litter of each species was ground with a plant shredder and then oven-dried at 40 °C for 72 h. Five replicates of 20 mg of ground material were weighed to the nearest µg to quantify carbon (C) and N contents (elemental analyser, Flash 2000 ThermoFisher, NF ISO 10694, NF ISO 13878, NF EN 13137). Five replicates of 7–10 mg were weighed to the nearest µg to quantify the total P content (spectrometric method with ammonium molybdate after persulfate oxidation, adapted NF EN 6878).

Litter WHC was measured according to the following protocol (Coulis et al., 2013), adapted for grass litter. Five replicates of 0.6 g of litter of each species were oven-dried at 40 °C for 72 h before being weighed to the nearest 0.01 g. Litter was then immersed for 24 h in large hermetic plastic boxes (180 × 120 × 75 mm) previously filled with 300 mL of deionised water, then gently collected and put on a 1 mm plastic mesh. The mesh was set up as lids of other empty large plastic boxes using elastic bands. This device allowed the litter to be drained. After draining for 6 h, litter was weighed to the nearest 0.1 mg to measure wet weight (W_w). Litter was then oven-dried at 60 °C for 48 h, and then put in desiccator until being weighed to the nearest 0.1 mg to measure dry weight (W_d). WHC was calculated as $(W_w - W_d)/W_d$.

Table 1 shows mean characteristics of leaves and litters used as traits (Cottier et al., 2001; Cruz et al., 2010). We assumed that plant traits used in the present study were conserved in the litter. C, N and P contents of litter indicate its nutritive value. Physical traits (LDMC and litter WHC) indicate physical deterrents related to mechanical aspects (e.g. toughness). The other chemical traits, leaf cellulose content (LCC), leaf hemicellulose content (LHC) and leaf lignin content (LLC) indicate both chemical and physical deterrents of litter. They indicate litter enzymatic digestibility (Khaled et al., 2006) directly and mechanical aspects indirectly.

2.2. Macrodetritivore selection and collection

Two common macrodetritivores species were studied: the woodlouse *A. vulgare* and the millipede *G. marginata*. Macrodetritivores were collected from a permanent mown meadow in Benque, France (Haute-Garonne, 43°16'24.3"N 0°55'23.3"E) in February and March 2016. The meadow is part of the Long-Term Ecological Research site "Vallées et Coteaux de Gascogne" (LTER_EU_FR_003, a Long-Term Ecosystem Research site). Individuals were kept under laboratory conditions (laboratory room at ambient temperature), in plastic boxes filled with soil, plants and litter from the meadow, before beginning the experiment.

2.3. Experimental design

Hermetic plastic boxes (120 × 90 × 50 mm) were used as microcosms. Each monospecific combination of litter and macrodetritivore species was tested, resulting in 8 treatments (4 litter species × 2 macrodetritivore species) plus 4 controls (litter without a macrodetritivore). Four replicates of each of the 12 treatments were set up, yielding a total of 48 microcosms. Just before the experiment, litters were oven-dried at 40 °C for 72 h and left in a desiccator before being weighed to the nearest 0.1 mg. Each box was filled with 0.5 g of dried litter and one macrodetritivore. At the beginning of the experiment, litter was moistened (to 80% WHC) with deionised water using a pipette. Litter and water were then gently mixed using plastic tweezers. Before the experiment, macrodetritivores were fasted for 5–7 h and then individually weighed to the nearest 0.1 mg. At the beginning of the experiment, individual biomass for a given macrodetritivore species was equilibrated among treatments.

Microcosms were then stored at 17 ± 2 °C under a 10 h light/14 h

dark photoperiod for 7 days. Seven to ten days is a suitable time period to detect consumption preferences and to have an estimation of feeding performances (Gerlach et al., 2012; Joly et al., 2015). On days 2 and 5, cosms were moistened with deionised water as necessary, using a pipette, to reach their initial weight. On days 2, 5 and 7, faecal pellets were gently collected and immediately oven-dried at 40 °C for at least 72 h, placed in a desiccator and weighed to the nearest 0.001 g. At the end of the experiment, the remaining litter and animals were weighed following the previously described protocols.

2.4. Detritivore performance calculation and statistical analysis

Mean individual biomass equalled the mean of initial and final macrodetritivore biomass. Individual consumption rate ($\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$) was estimated using David's (1998) formula as a function of initial and final litter dry mass of a microcosm and its corresponding control. Dejection production rate ($\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$) was the sum of dry weights of the faecal pellets collected (mg) per mean individual biomass (g) per day. Finally, individual assimilation rate ($\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$) equalled the consumption rate minus the dejection production rate.

Analyses of variance (ANOVAs, $\alpha = 0.05$) were performed to test for effects of litter species, macrodetritivore species and their interaction on detritivore performances (consumption, dejection production and assimilation rates, $n = 32$). Normality and homoscedasticity assumptions were tested using Shapiro-Wilk and Bartlett tests respectively ($\alpha = 0.05$), and data were transformed (data of consumption and dejection production rates were square root transformed) to meet these assumptions, whenever appropriate. To search for mechanisms, 48 simple linear regressions ($n = 16$) were calculated, relating each quantitative leaf/litter trait (8 variables, Table 1) to each detritivore performance variable (3 variables), for each of the two macrodetritivores. For these regressions, p-values were deemed significant without ($\alpha = 0.05$) and with Bonferroni correction ($\alpha = 0.05/8 = 0.00625$) since there were 8 different traits and respective tests of a relationship for each of the 6 combinations of detritivore performance variable and macrodetritivore. Using Bonferroni correction decreases the risk of type I error ("false positive"; considering a non-existent relationship significant) but increases the risk of type II error ("false negative"; considering an existing relationship non-significant).

3. Results and discussion

Litter species significantly influenced consumption, dejection production and assimilation rates (Table 2). Macrodetritivore species significantly influenced only the dejection production rate (Table 2). Finally, interaction between litter and macrodetritivore species significantly influenced the consumption rate (Table 2). These results confirmed that both litter and macrodetritivore species are required to fully understand macrodetritivore performances.

We observed no mortality during the experiment. Mean *A. vulgare* performances in our experiment were usually higher than those

Table 2

Results of the ANOVA ($n = 32$) testing respectively the effect of litter species, macrodetritivore species and their interaction on consumption, dejection and assimilation rates. PVE: proportion of variance explained obtained from the ratio between the sum of squares of a variable and the total sum squares. Bold p-values mean $p < 0.05$.

	Litter		Macrodetritivore		Interaction	
	p-value	PVE (%)	p-value	PVE (%)	p-value	PVE (%)
Consumption	0.00076	28.4	0.056	4.8	0.00012	38.2
Dejection	0.0028	22.1	0.0012	15.9	0.00025	33.7
Assimilation	0.049	23.1	0.19	4.5	0.24	11.6

observed on Poaceae in previous studies (David et al., 2001; Rushton and Hassall, 1983) (Fig. 2). For instance, the consumption rate of *Festuca ovina* (L.) and *Koeleria cristata* (L.) ranged from 0 to 14.07 and 1.38–14.06 $\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$, respectively, the defecation production rate from 0 to 13.19 and 1.99–12.73 $\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$, respectively, and the assimilation rate from 0 to 0.88 and -0.16 to 1.33 $\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$, respectively (Rushton and Hassall, 1983). In another study, consumption and assimilation rates of *Lolium rigidum* (Gaudin, 1811) ranged from almost 0 to approximately 20 and from almost 0 to 6 $\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$, respectively (David et al., 2001). To our knowledge, no data on *G. marginata* performances on herbaceous litter exists.

Mean assimilation rates were negative for two treatments: *G. marginata* with *B. erectus* and *G. marginata* with *B. pinnatum* (Fig. 2). Previous studies have observed negative mean assimilation rates for *A. vulgare* on herbaceous litters (David et al., 2001; Rushton and Hassall, 1983). We decided, as authors of these studies did, to keep these negative values to capture the entire range of variability. Negative assimilation rates could have resulted from failing to correctly subtract the consumption by microorganisms when calculating consumption by macrodetritivores in these short-term laboratory experiments. We calculated consumption using David's formula, which estimates macrodetritivore consumption based on the loss of litter mass in the presence and absence of macrodetritivores, assuming that consumption by microorganisms is the same for both (David, 1998). The formula can thus underestimate macrodetritivore consumption and lead to negative assimilation rates, especially when the macrodetritivore consumes too little.

For *A. vulgare*, consumption and defecation production rates were similarly positively correlated with litter N and P contents, and negatively with LDMC (Fig. 1a). At least, 34% of variance in both feeding performances was explained by each of these traits. *A. vulgare* performances could be driven by chemical litter traits which indicate litter

nutritive value (N and P contents). Furthermore, they also could be driven by tissue structure (LDMC). Indeed, LDMC is known to be correlated with leaf tissue density (Cruz et al., 2010). LDMC is also known to be correlated with digestibility (Khaled et al., 2006; Pontes et al., 2007). In our experiment, however, *A. vulgare* performances were not correlated with any plant fibre contents (cellulose, hemicellulose or lignin). Thus, nutritive values and some mechanical aspects related to LDMC may drive feeding performances of *A. vulgare* rather than chemical deterrent aspects of litter. It must be noticed that, in our study, LDMC values for 3 out of the 4 species are very closed to each other, which consequently lowers the power of the correlation (Table 1; Fig. 1a). Our results for the relationships between LDMC and consumption/defecation production rates have to be confirmed, particularly by the use of other grass species with medium LDMC values (see Cruz et al., 2010).

For *G. marginata*, LLC was negatively correlated with consumption, defecation production and assimilation rates (at least 30% of variance explained, Fig. 1b). For the consumption and assimilation rates, relationships remained significant after the Bonferroni correction. LCC was negatively correlated with consumption and defecation production rates, with at least 39% of variance explained. Fibre contents can indicate litter digestibility directly and litter mechanics indirectly; they could be the main drivers of *G. marginata* performances. Neither LDMC nor WHC was correlated with *G. marginata* performances, however, suggesting that litter digestibility could be a driver rather than litter mechanics.

Our results support the H1a hypothesis. For grass litters, both traits indicating nutritive value (e.g. N, P) and feeding deterrents (e.g. LDMC, LCC, LLC) are necessary to explain feeding performances of the soil macroinvertebrates used in this experiment. Furthermore, the hypothesis H1b is supported by our experiment as we also demonstrated that grass litter traits which influence feeding performances were dependent on the invertebrate species considered. In our experiment, *G. marginata*

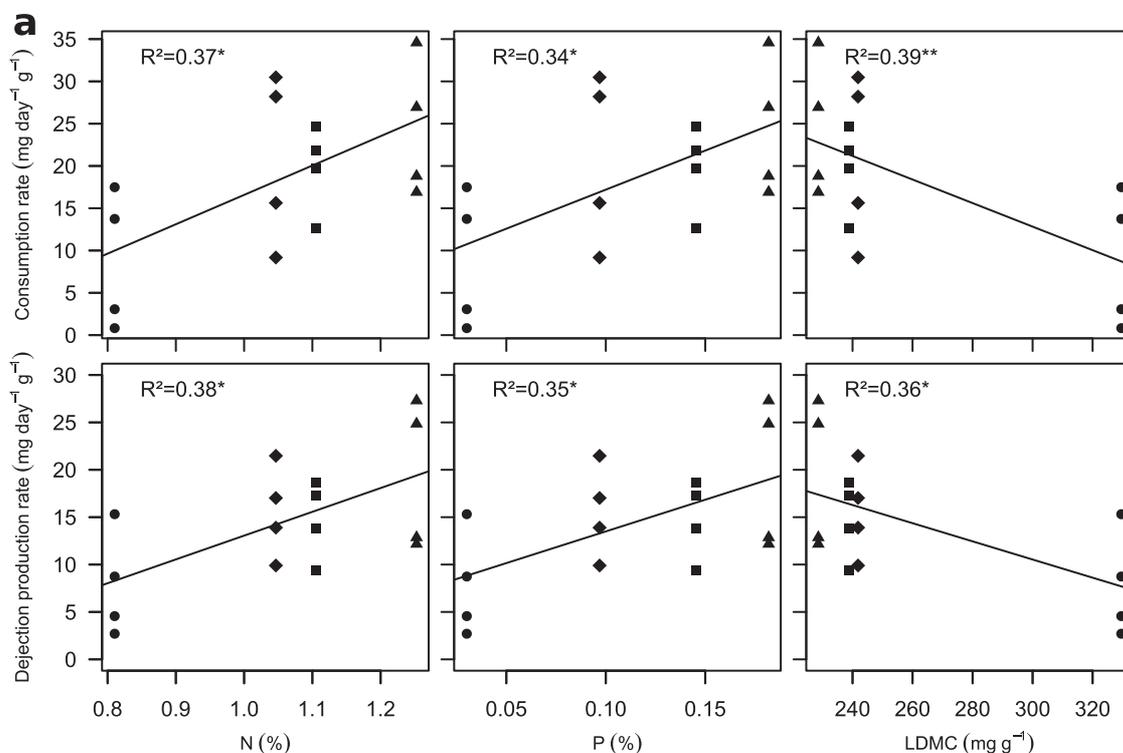


Fig. 1. Significant ($\alpha = 0.05$) linear regressions ($n = 16$) between each trait on respectively consumption rate ($\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$), defecation production rate ($\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$) and assimilation rate ($\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$) of (a) *Armadillidium vulgare* (Latreille, 1804) and (b) *Glomeris marginata* (Villiers, 1789) consuming *Bromus erectus* Huds., *Brachypodium pinnatum* P. Beauv., *Festuca rubra* L. or *Holcus lanatus* L. Nitrogen content (N, %), Phosphorus (P, %), leaf dry matter content (LDMC, $\text{mg} \times \text{g}^{-1}$), leaf cellulose content (LCC, %), leaf lignin content (LLC, %).

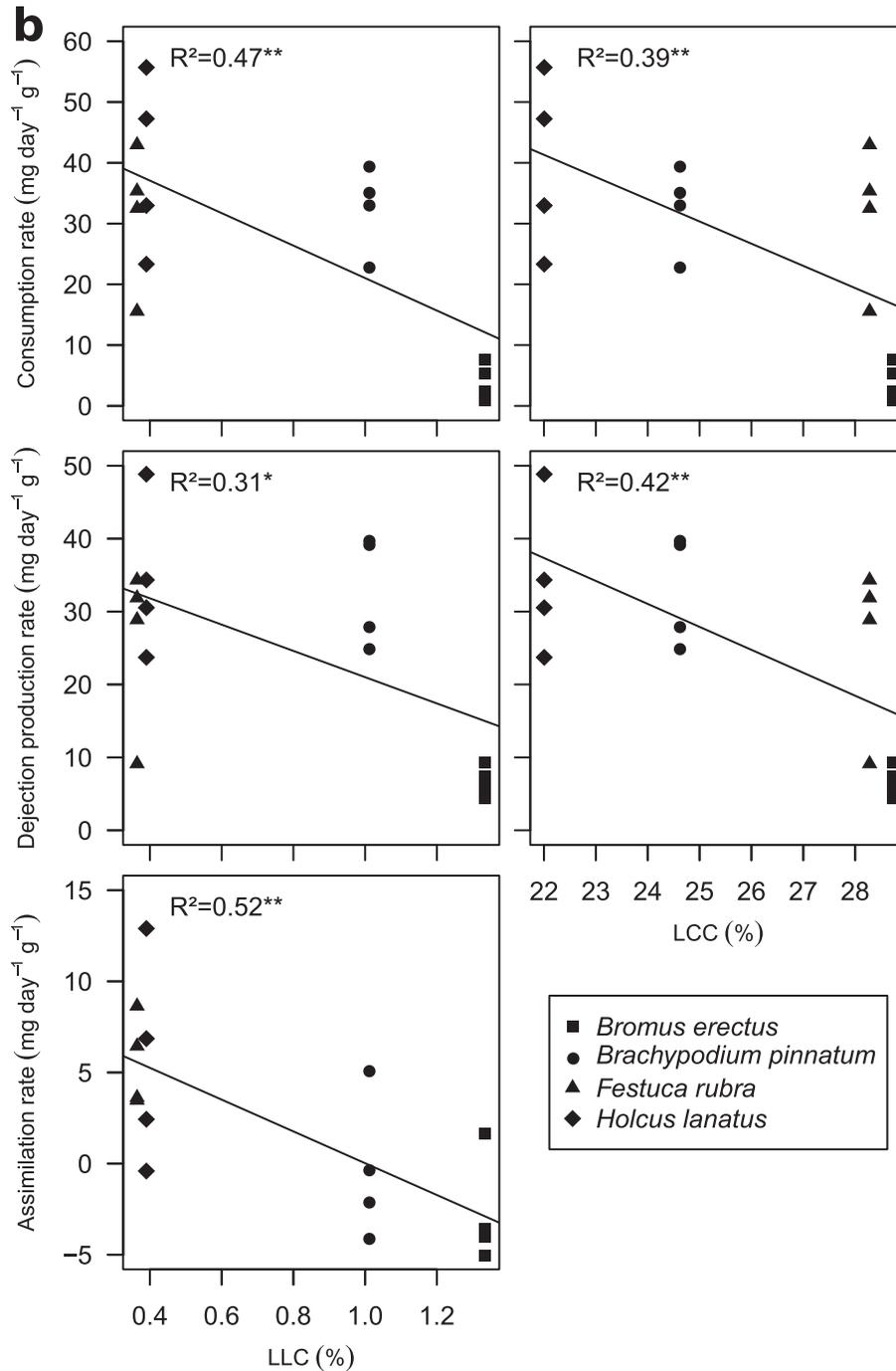


Fig. 1. (continued)

performances were related to traits indicating deterrents such as fibre contents, whereas *A. vulgare* performances were related to traits indicating both nutritive values (N, P) and deterrents (LDMC). In addition, the hypothesis H1c is partially supported by our results. Relationships between grass litter traits and invertebrate feeding performances, considering *A. vulgare* and *G. marginata* separately, are globally similar to those established for woody-plant litters for these two macrodetritivore species. Exceptions were the relationships between *A. vulgare* performances and LDMC (significant in our study) and C content (non-significant in our study but C:N ratio significant in the literature, see below explanations).

In a 7-days experiment, Joly et al. (2015) found a negative correlation between *G. marginata* consumption and hemicellulose contents of 26 tree litters. This result agrees with ours for *G. marginata*. In a 46-days

experiment, Rouifed et al. (2010) found that *G. marginata* consumption was related to the C:N ratio rather than to lignin content, which does not agree with our results. In these two experiments (Joly et al., 2015; Rouifed et al., 2010), litters were first placed on the ground before laboratory experiments to allow initial microbial colonisation and leaching. In the study of Rouifed et al. (2010), this initial exposure removed feeding deterrents, which explained the relationship between invertebrate consumption and litter C:N ratio. In the study of Joly et al. (2015), it did not remove all feeding deterrents, which explained the relationship between invertebrate consumption and litter hemicellulose content. Thus, the lack of initial litter exposure in our experiment could explain why *G. marginata* performances were related mainly to fibre contents (LLC, LCC). This ageing effect was also demonstrated in a 15-days experiment in which *G. marginata* consumed several tree litters

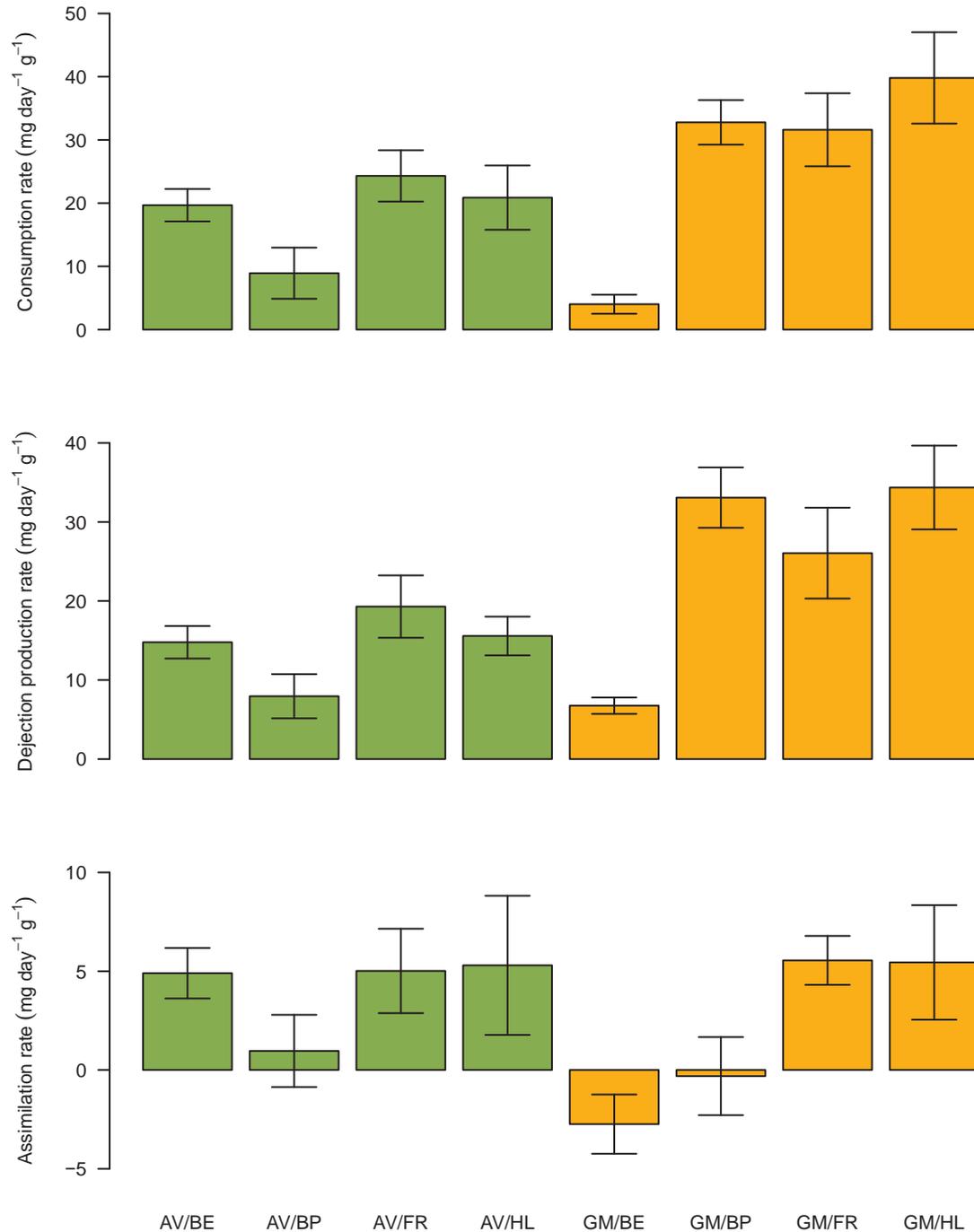


Fig. 2. Means and standard errors (\pm SE) of individual macrodetritivore feeding performances ($n = 4$) *ie.* consumption rate ($\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$), dejection production rate ($\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$) and assimilation rate ($\text{mg} \times \text{day}^{-1} \times \text{g}^{-1}$) of *Armadillidium vulgare* (Latreille, 1804) (AV) and *Glomeris marginata* (Villiers, 1789) (GM) consuming *Bromus erectus* Huds. (BE), *Brachypodium pinnatum* P. Beauv. (BP), *Festuca rubra* L. (FR) or *Holcus lanatus* L. (HL).

(Gerlach et al., 2012).

In an 8-days experiment, David et al. (2001) and Rushton and Hassall (1983) observed *A. vulgare* consumption, dejection production and assimilation rates of grass litter that were lower than those in our experiment. Furthermore, *A. vulgare* consumption was positively correlated with herbaceous litter N content and negatively correlated with litter C:N ratio (David et al., 2001), which partially agrees with our results, in which litter N explained *A. vulgare* consumption and dejection rates, but litter C did not. We have no consistent hypothesis to explain this difference as the N and C contents of litter resulted from multiple factors (*e.g.* plant physiology, fertilisation). One can notice, however, that the range of the C:N ratios is more extended in David

et al. (2001) than in our study. For non-herbaceous litter, C:N ratios and N content are often invoked to explain feeding behaviours of terrestrial isopods (Abelho and Molles, 2009; Gerlach et al., 2014). In addition, chemical feeding deterrents such as secondary compounds (*e.g.* tannins, alkaloids, glycosides) and structural compounds such as lignin also influence feeding performances on non-herbaceous litter (David, 2014; Gerlach et al., 2014; Wood et al., 2012). For grass litters, feeding deterrents could *a priori* be chemical deterrents (*e.g.* polyphenols) but also physical deterrents (Hassall and Rushton, 1984). Our experiment demonstrated that physical traits related to mechanical aspects (LDMC) could be more relevant than chemical traits in indicating deterrents, as fibre contents of grass litter did not explain *A. vulgare* feeding

performances. Finally, although P is a main element of invertebrate diets (David and Handa, 2010), it has rarely been considered to explain feeding performances of invertebrates, even those consuming woody litter (Joly et al., 2015; Zimmer et al., 2005). Our experiment demonstrated that litter P content is important for explaining performances of *A. vulgare* consuming grass litters. One can notice, however, that in our experiment, regression patterns between N and P with *A. vulgare* feeding performances were very similar (Fig. 1a). It remains to be studied whether P content is also important for other terrestrial isopods consuming grass litters and if it always covaries with N.

Our results partially support the H2 hypothesis. Physical traits provided no information about *G. marginata* performances, but fibre contents did. Feeding performances of this relatively large macro-detrivore with large mandibles could be decreased more by litter digestibility than by purely mechanical aspects. In contrast, the physical trait LDMC provided information about *A. vulgare* performances, but fibre contents did not. This isopod could have difficulties consuming certain litters because of mechanical incompatibility between the litter's physical structure and its mouthparts.

Our study shows that using grass chemical and physical traits that indicate both nutritive value and chemical and physical feeding deterrents can help explain feeding performances of macrodetrivores consuming grass litter. In this perspective, silica content (Hassall and Rushton, 1984) or trichomes (Levin, 1973) can constitute physical defences against herbivores. For the species we studied, however, we found silica data for only three of them (*B. erectus*, *F. rubra*, *H. lanatus*), and their contents varied greatly among studies (Aguirre et al., 2014; Cornelissen and Thompson, 1997; Massey et al., 2006). In addition, while silica content is an effective defence against folivorous insects (Massey et al., 2006), no evidence exists that silica content in litter influences macrodetrivore performances. Furthermore, no accurate data about trichomes exists for grass species. Finally, macrodetrivore preferences for litter appear to depend on litter microbes (David, 2014; Ihnen and Zimmer, 2008). Thus, litter phyllosphere traits could also be of great interest.

In conclusion, the present study is an initial step in identifying general trait-based relationships between forage grass litter and macrodetrivores. We focused only on effects of grass litter species with a wide spectrum of functional profiles to explain macrodetrivore performances in a short-term experiment. One perspective for explaining general litter-macrodetrivore interactions would be to relate biomechanical traits of macrodetrivore mouthparts to litter mechanical traits such as toughness. Indeed, one could develop a biomechanical framework for litter-macrodetrivore interactions, as has been done for other interaction networks. For instance, one study explained plant-herbivore (grasshopper) interactions by relating biomechanical traits of grasshoppers to plant traits (Ibanez et al., 2013). Biomechanical traits of soil invertebrates have been rarely studied. Another perspective would be to assess the degree of similarity of chemical element contents (e.g. C, N, P) between macrodetrivores and grass litter. Furthermore, the fates of fragmented litter and faecal pellets were not assessed in our study, although they are of prime importance in decomposition (Coulis et al., 2013; David, 2014; Joly et al., 2015). Microbial dynamics in these processes must also be highlighted, as macrodetrivores can modulate microorganism communities and thus nutrient cycles (David, 2014). Finally, on trait-based results of monospecies treatments, multi-species treatments must be tested. Adding litters from other herbaceous species, such as legumes, must also be considered to fully understand macrodetrivore-litter interactions in meadow ecosystems.

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Appendix A. Supplementary data

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