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Forage grass litter chemical and physical traits explain feeding performances of two soil macrodetritivores

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Abstract

Understanding the relationship between litter quality and macrodetritivore feeding performances is of prime importance. Among soil invertebrates, macrodetritivores such as millipedes (Diplopoda) and terrestrial isopods (Crustacea, Isopoda) could play a significant role in the decomposition process. Furthermore, studies relating herb litter to macrodetritivore performances (consumption, dejection, assimilation) are scarce and rarely used litter traits (especially no physical trait). We thus design a laboratory experiment to answer the following question: what are the pivotal chemical/physical traits informing litter grass quality that shape such macrodetritivore performances? The performances of two common macrodetritivores Armadillidium vulgare (Latreille, 1804) and Glomeris marginata (Villiers, 1789) on heterogeneous litter coming from perennial forage grasses to have a wide spectrum of functional profiles (Brachypodium pinnatum P. Beauv., Bromus erectus Huds., Festuca rubra L. and Holcus lanatus L.) were assessed. We used litter traits to inform litter quality. We also used some conservative plant traits. A. vulgare performances were correlated with nutrient aspects (litter N and P contents) and plant mechanical aspects (leaf dry matter content). G. marginata performances were correlated with plant fiber contents (cellulose and lignin contents).

Keywords: consumption, assimilation, soil macrodetritivore, forage grass litter, functional trait
59 **Highlights**

60 Millipedes and isopods contribution to soil decomposition is partially understood

61 We assessed some of their feeding performances on heterogeneous grass litter species

62 Litter species have been chosen to represent a wide spectrum of functional profiles

63 *A. vulgare* performances were correlated with nutrient and mechanical aspects

64 *G. marginata* performances were correlated with fiber contents
1 Introduction

Soil fauna consists in a huge diversity of life forms mainly represented by invertebrates (Decaëns et al., 2006). At the ecosystem level, they contribute to the delivering of ecosystem services (Barrios, 2007; Kibblewhite et al., 2008) such as climate regulation and the production of agricultural goods. They are highly involved in the soil behaviour by contributing to major soil functions such as organic matter decomposition.

Among soil invertebrates, millipedes (Diplopoda) and woodlice (Crustacea, Isopoda) play a significant role in the decomposition processes when they are abundant (David and Handa, 2010; Wolters, 2000). Their effects on decomposition can be direct and indirect through litter comminution or by interacting with soil microorganisms (David, 2014). Understanding the relationship between litter quality and macrodetritivore feeding performances is a key step for understanding the effect of these animals on decomposition (Coulis et al., 2013; Joly et al., 2015). However the mechanisms implied are far to be completely understood and predictable. For instance, David et al. (2014) pointed out that not a single litter trait can explain macrodetritivore preference. A combination of traits informing the litter nutritive value (e.g. N content, or C-to-N ratio) and the feeding deterrents are determinant. Feeding deterrents could be split into chemical and physical deterrents. Chemical deterrents are compounds that reduce the litter ingestion or digestion (e.g. mainly secondary compounds such as tannins, alkaloids, glycosides or structural compounds such as lignin). Physical deterrents limits litter mechanical breaking (e.g. litter toughness, litter thickness, silica spicules)(Levins, 1973; Zimmer et al., 2005). Theses mechanical aspects were generally indirectly informed by some chemical traits (e.g. cellulose, hemicellulose, lignin contents).

However they were scarcely informed by some physical traits (e.g. toughness, specific...
leaf area, water holding capacity) which were supposed to be more or less directly connected to these mechanical aspects (Coulis et al., 2015; Joly et al., 2015; Zimmer et al., 2005).

In addition, it has been theorize that “apparent” plants (e.g. tree or grasses) could often have low nutrient status and either quantitative chemical deterrents (e.g. polyphenols) or physical deterrents, whereas “unapparent plants” (e.g. small dicotyledonous species) often have a high nutrient status and could rely on rapidly degraded chemical qualitative toxins (e.g. alkaloids) (Hassall and Rushton, 1984). Furthermore, among “apparent plants”, grasses could rely on physical deterrents against herbivory such as trichomes (Levins, 1973), silicon spicules which could likely persist in litter material (Hassall and Rushton, 1984; Massey et al., 2006; Rushton and Hassall, 1983).

The main literature in temperate ecosystems concerns mainly tree or shrub litters but scarcely herb litters. To our knowledge only few studies deal with herb litters (David et al., 2001; Paris, 1963; Rushton and Hassall, 1983; Zimmer et al., 2002). Only few of them used chemical traits. In the work of David et al. (2001) the consumption and assimilation of 5 herb species by *A. vulgare* (2 Asteraceae, 2 Fabaceae, 1 Poaceae) at two atmospheric CO$_2$ levels were measured for several litter submitted to different decomposition pre-treatments (12, 30, 45 days). In this study, the consumption was overall positively correlated with litter nitrogen content and negatively correlated with the litter C-to-N ratio. However, the N content and C-to-N ratio had no decisive influence on litter consumption in slightly decomposed litter. The authors made the assumption that feeding-deterrent factors could be removed as decomposition progressed, making the litter more palatable. The assimilation was only negatively correlated with the C-to-N ratio at the lowest atmospheric CO$_2$ level. Furthermore in
another study, in salt marshes, performances of three isopods (Littorophiloscia)
(Halophiloscia) vittata (Say, 1818), Porcellionides (Metaponorthus) virgatus (Budde-
Lund, 1885), Venezillo (Armidillo) parvus ((Budde-Lund, 1885)) on three litters (2
forest litter and 1 grass litter, Juncus roemerianus Scheele) are predicted by chemical
litter traits such as phenolic concentrations (ferulic acid and tannins) and C-to-N ratios
(Zimmer et al., 2002). Furthermore, no study including herb litters used physical traits.
This present study would like to contribute to the general comprehension of the
interaction between macrodetritivores and herb litters by answering the following
question: what are the pivotal grass litter chemical/physical traits that shape
macrodetritivore feeding performances? Consequently, we designed a laboratory
experiment in which we assess the performances of two common macrodetritivores
Armadillidium vulgare (Latreille, 1804) and Glomeris marginata (Villiers, 1789) on
heterogeneous litter coming from perennial forage grasses, as a first step. We used a
trait-based approach using both litter and plant chemical and physical traits.

2 Materials and Methods
2.1 Litter selection, collection and characterization
We selected litter of perennial forage grasses based on Cruz et al.’ plant classification
(Cruz et al., 2010). This classification was made to dissociate plant species according to
their use values in agriculture. It was made from 6 traits which were obtained from the
leaves: the leaf dry matter content (LDMC), the specific leaf area (SLA), the length of
life duration (LLD) and surface tensile strength (STR); and from the whole plants: the
flowering date (FD) and the maximum height (MH). It discriminated several functional
groups which state different growth strategies, biomass accumulation strategies,
phenology, frequency of use and feeding values for cattle. For our study, we made the
hypothesis that litter from species belonging to these different functional groups will be differently consumed by soil macrodetritivores. We selected four species among different functional groups to have a wide spectrum of functional profiles: 

*Brachypodium pinnatum* P. Beauv., *Bromus erectus* Huds., *Festuca rubra* L. and *Holcus lanatus* L.

Litters were collected from an experimental set-up consisting in several small plots (60*40cm) in which a single plant species has been sown in September 2010. A fertilization was performed every year in March or April (75 kgN/ha, 50 kgP/ha and 50 kgK/ha). A mowing was performed every year and plots were irrigated. Litter of *H. lanatus*, *F. rubra*, *B. erectus* and *B. pinnatum* were collected in October 2015 and February 2016. Only entirely senescent fragments were conserved. Litters were then air-dried during a few days and then conserved in paper envelopes before the beginning of the experiments.

Some litter of each species was crushed using a plant shredder. The crushed material was then oven-dried at 40°C during 72h. Five replicates of twenty milligrams was weighed at the nearest µg to quantify the C and N contents (elemental analyzer, Flash 2000 ThermoFisher). Five replicated of 7-10 mg were weighed at the nearest µg to analyze the P content (spectrometric method with ammonium molybdate after a persulfate oxidation). The litter water-holding capacity (WHC) was measured in accordance with following the protocol. Five replicates of 0.6 gram of litter of each species was oven-dried at 40°C during 72h before being weighed at the nearest 0.01 g.

Litter was then immersed during 24h in large hermetic plastic boxes (180*120*75 mm) previously filled with 300 mL of deionized 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 allows the litter to be drained. After 6 hours of drainage, litter was weighed at the nearest 0.1 mg to assess the moist weight ($W_m$). Litter was then oven-dried at 60°C during 48h, then put in desiccator until being weighed at the nearest 0.1 mg to assess the dried weight ($W_d$). The water holding capacity was calculated as follows: \[ \text{WHC} = \frac{W_m - W_d}{W_d}. \]

Means of litter and leaf characteristics used as traits are presented in the Table 1 (Cottier et al., 2001; Cruz et al., 2010). We made the hypothesis that plant traits used in the present study are conservatives from the plant to the litter. Litter C, N, P contents aimed to mainly inform on the main essential macrodetritivore requirements. Leaf dry matter content (LDMC) and litter water holding capacity (WHC) aimed to mainly inform indirectly on mechanical aspects (e.g. toughness). Leaf cellulose content (LCC), leaf hemicellulose content (LHC) and leaf lignin content (LLC) informed mainly on digestibility and indirectly on mechanical aspects.

### 2.2 Macrodetritivores selection and collection

Two common macrodetritivores species were used: the woodlice *Armadillidium vulgare* (Latreille, 1804) and the millipede *Glomeris marginata* (Villiers, 1789). Macrodetritivores were collected from a permanent mown meadow in Benque, France (Haute-Garonne, 43°16’24.3”N 0°55’23.3”E). The meadow is included into the Long-Term Ecological Research site ‘‘Vallées et Coteaux de Gascogne’’ (LTER EU FR 003). They were collected in February and March 2016. Individuals were conserved in plastic boxes filled with soil, plants and litter from the meadow before the beginning of the experiments.

### 2.3 Experimental design
185 Hermetic plastic boxes were used as microcosms (120*90*50 mm). Each monospecific
186 combination of litter and macrodetritivore species was made, resulting in 12 treatments
187 plus 4 control treatments (litter without macrodetritivore). A total of 64 microcosms
188 were initiated corresponding to 16 treatments and four replicates. Each box was filled
189 with 0.5 gram of dried litter and one individual. Before the experiment, litters were
190 oven-dried at 40°C during 72h, let in a desiccator before being weighed at the nearest
191 0.1 mg. At the beginning of the experiment, litter was moistened (80% WHC) with
192 deionized water using a propette. Litter and water were then gently homogenized using
193 plastic pliers. Before the experiment, macrodetritivores were subject to a diet of 5h-7h
194 before being weighed. They were then individually weighed at the nearest 0.1 mg. At
195 the beginning of the experiment, individual biomass for a given macrodetritivore
196 species was equilibrated among treatments.
197 Microcosms were then stored at 17 ± 2 ºC with a photoperiod (10 light/14h dark) for 7
198 days. At day 2 and 5, cosms were moistened with deionized water if necessary, using a
199 propette, to reach their initial weight. At day 2, 5 and 7, faecal pellets were gently
200 collected and were immediately oven-dried at 40°C for at least 72h, put in a dessicator
201 and weighed at the nearest 0.001 g. At the end of the experiment, remaining litter and
202 animals were weighed following previous described protocols.
203
2.4 Detritivores performances calculation and statistical analysis
204 The average individual biomass was expressed as the mean between the initial and final
205 macrodetritivore biomass. Individual consumption rate (mg.day⁻¹.g⁻¹) was assessed
206 using the David’s formula based on initial and final litter dry mass of a microcosm and
207 its corresponding controls (David, 1998). Dejection production rate (mg.day⁻¹.g⁻¹) was
208 the summed dry weights of the collected faecal pellets (mg) per average individual
biomass (g) and per day. Finally, individual assimilation rate (mg.day⁻¹.g⁻¹) was expressed as the difference between the consumption and the dejection production rates. Analyses of variance (ANOVAs) were performed to test for the effect of litter species, macrodetritivore species and their interaction on detritivore performance (consumption, dejection production and assimilation rates). Normality and homoscedasticity assumptions were tested using Shapiro-Wilk and Bartlett tests respectively, and data transformations were done if necessary. To bury into mechanisms, 48 simple linear regressions were performed, relating each quantitative leaf/litter trait (8 variables) to each detritivore performance variable (3 variables), one for each of the 2 macrodetritivores. For these regressions, p-values were deemed significant without (alpha=0.05) and with a Bonferroni correction (alpha=0.05/8=0.00625) given that there are initially 8 different traits and respective tests for a relationship for each of the 6 combinations of detritivore performance variable and macrodetritivore. Using the Bonferroni correction decreases the risk to make a type I error (‘false positive’; finding a non-existing relationship) but increases the risk to make a type II error (‘false negative’; missing an existing relationship).

3 Results & Discussion

We observed no mortality during the experiment. *A. vulgare* performance mean values in our experiment were the most of the time higher than observed values on *Poaceae* in existing literature (Table 2). For instance, the consumption rate of *Festuca ovina* (L.) and *Koelaria cristata* (L.) ranged from 0 to 14.07 and from 1.38 to 14.06 mg.day⁻¹.g⁻¹ respectively, the egestion rate from 0 to 13.19 and from 1.99 to 12.73 mg.day⁻¹.g⁻¹ respectively and finally the assimilation rate from 0 to 0.88 and from -0.16 to 1.33
mg.day\(^{-1}\).g\(^{-1}\) respectively (Rushton and Hassall, 1983). In another study, the consumption and assimilation rates for *Lolium rigidum* Gaudin (1811) ranged from almost 0 to approximately 20 and from almost 0 to 6 mg.day\(^{-1}\).g\(^{-1}\) respectively (David et al., 2001). The low N content and high C-to-N ratio of *L. rigidum* litter could explain this last result. In our study, litter is coming from a well fertilized experimental set-up which contributed to have high litter N contents. This could be invoked to explain theses higher performances values. To our knowledge no data on *G. marginata* performances on herb litter exist. Mean assimilation rates were negative for two treatments *G. marginata* with *B. erectus* and *G. marginata* with *B. pinnatum* (Table 2). This situation have been already met in the literature in the case of studying *A. vulgare* performances on herb litters (David et al., 2001; Rushton and Hassall, 1983). We decided, as these authors did, to conserve these negative values rather than discarding them as it would avoid to evict arbitrarily a part of variability. To our point of view, the main reason of obtaining negative assimilations could be the failure to correctly remove the microorganism part in the calculation of macrodetritivore consumption in these short-term laboratory experiments. In our experiment, consumption was calculated through the David’s formula which estimated macrodetritivore consumption from a litter mass loss value in presence and in the absence of macrodetritivore, considering that microorganism effect is conservative. It could thus sometimes underestimate the macrodetritivore consumptions and thus led to negative assimilation values especially in the case of low effective consumption. The litter species significantly impacted the consumption, the dejection production and the assimilation rates and contributes to 28.4%, 22.1% and 23.1% to the explained
variance respectively (Table 3). The macrodetritivore species only significantly 
influenced the dejection production rate by contributing to 15.9% to the explained 
variance. Finally, interaction between litter and macrodetritivore species significantly 
impacted both the consumption rate and the dejection production rate by contributing to 
38.2% and 33.7% to the explained variance respectively. These results confirmed that 
both litter and macrodetritivore species are required to fully understand the 
macrodetritivore performances.

For *A. vulgare*, consumption and dejection production rates were similarly positively 
correlated to litter N and P contents, and negatively to LDMC (Table 4). More than 34% 
of variance for both feeding performances was explained by each of these traits. *A. 
vulgare* performances seemed to be ruled by chemical litter traits which inform us on 
litter nutritive value (N and P contents). Furthermore, they also seemed to be ruled by 
the tissue structure (LDMC). Indeed the LDMC is well-known correlated to the foliar 
tissue density (Cruz et al., 2010). Finally, LDMC is also well-known correlated with 
digestibility (Khaled et al., 2006; Pontes et al., 2007). However in our experiment, *A. 
vulgare* performances were not correlated to any plant fiber contents (cellulose, 
hemicellulose or lignin). As a conclusion for *A. vulgare*, it could have been 
demonstrated that it was rather the nutritive aspects and some mechanical aspects linked 
to the LDMC which ruled the feeding performances rather than the chemical deterrent 
aspects of litter compounds.

For *G. marginata*, LLC was negatively correlated to consumption, dejection production 
and assimilation rates (more than 30% of explained variance). For the consumption and 
assimilation rates, p-values were significant after the Bonferroni correction. LCC was
negatively correlated to consumption and dejection production rates with more than 39% of explained variance. Joly et al. (2015) found that *G. marginata* consumption was negatively correlated to hemicellulose contents of 26 tree litters. Fiber contents could informed on litter digestibility and indirectly on litter mechanics which could be the main drivers of *G. marginata* performances. However, neither LDMC nor WHC were correlated with *G. marginata* performances suggesting that the litter digestibility could be rather invoked than litter mechanics. In a similar way, Joly et al. (2015) found no correlation between *G. marginata* performances and respectively tree litter surface specific area (SLA) and WHC.

These results pointed that the use of grass chemical and physical traits could be relevant to explain macrodetritivore performances. Silica content data or information on trichomes may have contributed to a better general comprehension of the effects of herb litter mechanical aspects on macrodetritivores performances. However, for the studied species, we found only silica data for three of our four species (*B. erectus, F. rubra, H. lanatus*) and values are highly variable among studies (Aguirre et al., 2014; Cornelissen and Thompson, 1997; Massey et al., 2006). In addition, if silica content was an effective defence against folivorous insects (Massey et al., 2006), no evidence exist that silica content in litters influence macrodetritivore performances. In addition, no precise trichome data exists for herb species, either on their potential physical deterrent effect or on the potential chemical deterrent effect of the secondary compounds they contain. Much more studies and much more data on these traits could shed light on litter mechanical aspects. Finally, it has been yet demonstrated that litter preferences depend on litter microbes (David, 2014; Ihnen and Zimmer, 2008). Thus, litter phyllosphere traits could be also of great interest.
To conclude, the present experiment is a first step to assess trait-based general rules for the interactions between forage grass litter and macrodetritivores. In our study, we only focused on the effect of litter species with a wide spectrum of functional profiles to explain macrodetritivore performances. An interesting perspective to rise general rules about litter-macrodetritivores interactions could be the use of macrodetritivore biomechanical mouthpart traits to be linked with litter mechanical traits such as toughness. Indeed, a biomechanical framework for litter-macrodetritivore interaction can be drawn as it was done in other interaction networks. For instance, a study succeed to explain plant-herbivores interactions (grasshoppers) from relating biomechanical traits of herbivores with plant traits (Ibanez et al., 2013). Such biomechanical traits are poorly studies for soil invertebrates. Another perspective could be the assessment of the matching between chemical element contents (e.g. C, N, P) between macrodetritivore and litter. Furthermore, in our study the becoming of fragmented litter or the becoming of faecal pellets were not assessed although there are of prime importance in the decomposition process (Coulis et al., 2013; David, 2014; Joly et al., 2015). Microbial evolution in these processes have to be especially highlighted. Furthermore and finally, from the trait-based results of monospecific treatments, plurispecific combination have to be tested.

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