



Positive relationship between plant palatability and litter decomposition in meadow plants

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Abstract: Physical supporting or defense structures of plants, which decrease palatability, remain in plant tissue after a plant's death and so decrease detritus decomposition rates. Consequently, palatability and detritus decomposition rate are expected to be positively correlated. Carbon is the main component of these restricting structures, whereas nitrogen is expected to increase plant attractiveness for herbivores. In this study, we tried to confirm the expected positive relationship between palatability and detritus decomposition rate and to find the species functional traits that are responsible for this concordant response. Some traits are shared by species as a consequence of their common phylogenetic history; consequently, we also studied the effect of phylogenetic correction on the expected relationships.

We assessed the palatability of meadow plant species to a generalist slug *Arion lusitanicus* in an aquarium grazing experiment and detritus decomposition rate in a field litter-bag test. The two characteristics are positively correlated and the relationship is strengthened by phylogenetic correction. The relationship was strongest for the decomposition rates during the first three months of exposition, but weakened when the exposition period was from six months to a year. Palatability was negatively affected by plant carbon content, but no relationship was found between plant palatability and nitrogen content. Similarly, only the relationship of litter decomposition with litter carbon content was significant. The regression tree method was used to detect the influence of species traits on species palatability and detritus decomposition rate. In general, leaf dry matter content, litter carbon content and seed weight were chosen as the best predictors of plant palatability response. Results for the detritus decomposition rate response mainly reflect supporting or defensive structure contents. Litter carbon content, seed weight and plant height are the most apparent common predictors of these variable responses.

In general, our study confirmed the positive relationship between plant palatability and detritus decomposition. Both plant tissue grazing and detritus decomposition are slowed down by plant tissue supportive structures, manifested as high dry matter content or high tissue carbon content.

Nomenclature: Nomenclature for plants follows Kubát et al. (2002).

Abbreviations: LDMC—Leaf Dry Matter Content; PIC—Phylogenetically Independent Contrasts.

Introduction

Herbivory is a common threat to all plant species, and its effect varies among species. At the community level, it affects species coexistence and diversity maintenance (Palmer 1994, for examples see Hulme 1996, Edwards and Crawley 1999, Burt-Smith et al. 2003, Buschmann et al. 2005, Szentesi 2006). According to Strauss and Agrawal (1999), the average damage to plants caused by herbivores is 18% in natural terrestrial ecosystems. The extent of plant consumption is limited by plant physical supporting or defense structures and also by chemical defense (Rhoades 1983, Herms and Mattson 1992, Grime et al. 1996). For example, in typical semi-natural meadow communities, we can expect that broad-leaf dicots might be more attractive for herbivores than monocots, especially graminoids. Although dicots are usually rich in secondary metabolites, graminoid monocots

have narrow, hard leaves with high fiber content and this structural strength makes them less digestible (Grime et al. 1996, Díaz et al. 2001, Cingolani et al. 2005). The final outcome will also depend on the herbivore's ability to cope with one or other type of the plant defense.

Similarly, as these structures protect a plant from herbivore attack, they persist for a long time after the plant's death and decrease plant tissue decomposition (Grime et al. 1996, Cornelissen et al. 2004). Thus, herbivores can influence ecosystem function through selection of basic characteristics of organic matter that enters the soil (Chapman et al. 2003). A high litter decomposition rate was assessed for plants with leaves rich in nitrogen and basic cations (K, Ca, Mg), while plants rich in lignin, tannins or with physical barriers slowly decomposed (Cornelissen 1996, Schädler et al. 2003).

Herbivores affect ecosystem carbon and nitrogen cycling (Cornelissen et al. 2004) through the amount of grazed biomass and waste products, which they return back to the ecosystem or through inducing the production of secondary compounds (Schädler et al. 2003). Carbon, which is the main building element of physical supporting or defense structures, reflects how much structural carbohydrates the plant incorporates in its body (Chapman et al. 2003). High nitrogen content usually indicates high growth rate and high specific leaf area (Cornelissen et al. 1997). However, a plant is not able to grow fast and invest massively in defense at the same time, which results in a trade-off (e.g., Pérez-Harguindeguy et al. 2003, Cornelissen et al. 1999). Total nitrogen content in a plant is several times lower than nitrogen content in an animal body; consequently, nitrogen should be a limiting element for herbivores and they should prefer nitrogen-rich plant tissues (Bryant et al. 1991, Karban and Agrawal 2002). Therefore, on the basis of these statements, we should expect a correlation between palatability, decomposition and carbon or nitrogen content.

Whereas vertebrate herbivory effects are well studied (e.g., Dupré and Diekmann 2001), the influence of invertebrate grazers has received less attention and most of the studies were cafeteria experiments (multiple-choice feeding trials where an herbivore is allowed to feed selectively on plant samples of a whole range of species distributed in random positions on a feeding arena) conducted only on leaf fragments (e.g., Grime et al. 1996, Cornelissen et al. 1999, Schädler et al. 2003) or agar discs (see Fenner et al. 1999), but not on whole plants. This can cause discrepancies between cafeteria and field results, because of different plant architecture and leaf accessibility. According to Pérez-Harguindeguy et al. (2003), who compared field and cafeteria consumption and preference, the main differences were the effects of environmental context (plant accessibility, specialized relationships with specific herbivores) as well as physical barriers (spiny or hairy leaf ridges or stems, plant height and architecture, induced resistance): “when a plant’s foliage is almost inaccessible, herbivory will be extremely low, independent on the plant’s nutritional quality”.

Differences among species that co-occur in an ecological community are the result of modifications to a common ancestor that the species all ultimately share (Webb et al. 2002). Species can have similar trait combinations both because they are phylogenetically related and because they are subject to similar continuing forces of natural selection (Westoby 1999). Thus, we need to know the phylogenetic relationships of compared species to disentangle the effects of phylogeny and environment on species traits, and to deduce their adaptive significance.

Various traits have been used as palatability predictors; published results (e.g., Fenner et al. 1999, Schädler et al. 2003, Cingolani et al. 2005) suggest that plant palatability should increase with plant growth rate, specific leaf area, and decrease with leaf dry matter content, carbon content and leaf toughness. Nevertheless, there is only partial agreement in

the selected predictors among individual studies and often significant predictors in one study are not significant in other studies. Moreover, because of various trade-offs and constraints, traits are correlated among themselves. As a consequence, a trait that is a good predictor need not always be functionally responsible for high/low palatability, but be only well correlated with a trait that has a direct functional effect (but which might not be measured in a study). Palatability is also a consequence of interactions between chemical and structural plant characteristics (Cornelissen et al. 2004), which might obscure any straightforward prediction based on easily measurable plant traits.

The ability to predict species palatability on the basis of combinations of easily measurable plant traits provides many benefits. It undoubtedly improves our understanding of ecosystem functioning mechanisms, but also enables us to predict species vulnerability and plant or vegetation responses to environmental changes, such as land-use shifts (Díaz et al. 2001, Cingolani et al. 2005) or pest expansions. However, as demonstrated by de Bello et al. (2005), the predictive power of an individual trait differs among environments, and consequently, these predictions cannot be easily extrapolated from one region to another. Hendriks et al. (1999) discuss the hypothesis of “context dependent defense”. This means that we need more empirical studies in various environmental situations.

In this study, we were interested in the differentiation of species within a community. We selected an oligotrophic wet meadow community (studied in several previous experiments, so that some species traits were already available) and conducted an experimental assessment of litter decomposition rates and species palatability of its species. Specifically, we attempted to:

- prove that species palatability assessed in a slug grazing aquarium experiment was positively related to detritus decomposition rate;
- test whether palatability and decomposition rate are affected by plant carbon or nitrogen content, and predict these characteristics on the basis of a range of species traits;
- evaluate to which extent these relationships are affected by evolutionary history.

Materials and methods

Two experiments were conducted, one assessing the palatability of plant species to a generalist herbivore, and the other assessing the decomposition rate of the species. Seeds of all of the plant species, plant detritus, and also the slugs, were collected in our experimental locality Ohrazení (where other experiments have also been conducted – e.g., Lepš 1999). Thus, differences in species traits reflect within-site variation. Also, the detritus decomposition experiment was carried out in this locality.

Study site

The study site is a species-rich wet meadow located in the Czech Republic, 10 km southeast from České Budějovice (48°57'11.35" N, 14°35'33.99" E), at 510 m a.s.l. Mean annual temperature of the area is 7.8 °C, and mean annual precipitation is 620 mm (České Budějovice Meteorological Station). The soil has low nutrient levels (total N content: 6–8 g/kg soil, total P content: 400–500 mg/kg soil, C/N = 16–20; Kotorová and Lepš 1999).

Phytosociologically, the vegetation belongs to *Molinion* with some species of *Violion caninae* in drier parts of the meadow (Chytrý 2007). *Molinia caerulea* dominates the vegetation; other common species are *Nardus stricta*, *Holcus lanatus*, *Anthoxanthum odoratum*, *Carex panicea*, *Cirsium palustre*, *Betonica officinalis*, *Potentilla erecta* etc.

The meadow is regularly mown once or twice a year. The meadow was abandoned in the late 1980s and the management was stopped until 1994.

Experimental design

Germination tests. We first performed germination tests to determine how to break seed dormancy and calculate the time period between sowing and the start of germination (to get the germinated seedlings of all species at roughly the same time, see below). Seeds of 37 plant species were collected in our study site in 2005. A number of them were proportionally divided between three Petri dishes filled with cotton wool. Dishes were placed into a freezer (–14 °C), fridge (4 °C) or left at room temperature (20 °C) for 20 days. Then, all of them were put into the room and watered for 3 months. On the basis of the results of this germinability test, twenty species with sufficient germination in at least one of the treatments were chosen for the palatability experiment.

Leaf palatability. The selected twenty species were sown into rectangular aquaria (38 cm × 58 cm area × 38 cm height) placed under outdoor conditions in an experimental garden (Czech Academy of Sciences, České Budějovice). To prevent flooding, aquaria were placed in a wire netting construction with a polythene cover on the top of the construction, so that we only had to water them when necessary. Soil substrate was a 2:1 mix of sand and sterile peat mix. The sowing date of individual plant species corresponded with the germination time set in the previous experiment. Seeds were sown at a density resulting in a continuous ground cover (based on our previous experience). All species started germination within a one week span. Immediately after seedling appearance, we covered the aquaria with a dense but easily translucent net curtain to protect the plants from herbivores living in the surroundings.

There was one aquarium per one plant species. In each aquarium, one control and one target species were sown (each of them in one longitudinal half; see Fig. 1). *Plantago lanceolata*, a common species occurring at the study site, was chosen as the control species. According to Grime et al.

(1996), the species has a medium level of palatability, which we see as ideal for similar studies. Highly palatable species will be easily differentiated (preferred) against *Plantago*, while its presence avoids the possibility that slugs would consume unpalatable species just because of starvation.

After two months of growing, each aquarium was divided transversely by a glass partition. Differences in plant densities in neighboring parts of an aquarium were corrected by thinning to get approximately similar densities in all parts within an aquarium. Five individuals of the generalist slug *Arion lusitanicus* Mabille were added into one half of each aquarium, always in the same arrangement as in Fig. 1. The species originates from western Europe (including the northern part of the Pyrenean peninsula) and is also unspecialized in its native area of distribution. It invaded the Czech Republic in the early 1990s (Dvořák and Horsák 2003), spread quickly, and is now one of the most common slugs in a wide range of habitats. It is considered a pest exhibiting occasional population outbreaks. All slugs were collected from the study site and had been starved for 36 hours before being released into the aquaria. They were allowed to choose any part of a naturally growing fresh plant. They grazed on young plants (older seedlings), which probably corresponds well to their grazing effect in meadow communities (Scheidel and Bruehlheide 2005), but we are aware that relative palatabilities of seedlings and adults to mollusks may differ (Fenner et al. 1999). The slugs lived in the aquaria for six days. Then, they were removed and the plant biomass was clipped, oven dried (80 °C), and weighed. Four biomass samples were obtained from each aquarium.

Species palatability was expressed in percent as a species biomass weight ratio according to the formula (see Fig. 1):

$$\text{Palatability} = (\text{biomass of "target species without slugs" aquarium section} - \text{biomass of "target species with slugs" aquarium section}) / \text{biomass of "target species without slugs" aquarium section}.$$

We did not correct this value using the amount of the control species grazed (the control species was used just as an alternative source of food to prevent grazing of the target species just because of starvation). Whereas the inclusion might help to correct the variability due to varying grazing activity of slugs, it would distort the result in the case where slugs

control species without slugs	control species with slugs
target species without slugs	target species with slugs

Figure 1. Aquarium experiment arrangement. Control species (*Plantago lanceolata*) and one target species were sown each into one longitudinal half of an aquarium. In its transverse half, the aquarium was divided by glass partition (heavy line) into “without slugs” and “with slugs” sections.

grazed first the target species completely and subsequently started to graze intensively the control species.

Plantago lanceolata palatability was expressed as the average of all aquaria palatability values (counted according to the above formula) for this species.

As plants were grown in monocultures and the grazing period lasted for 6 days only, we do not expect any growth response or competitive effects, and consider the resulting amount grazed as just a characteristic of plant palatability.

Litter decomposition. Litter was collected at the study site throughout the summer and autumn 2005. Litter refers to freshly senescent, not decomposed aboveground plant parts without seeds. The litter was air-dried at room temperature (20 °C) for several days, cut into 5 cm long pieces, weighed and put into detritus pockets in a proportional mixture of plant parts. The weight of each pocket was 2 g and the size of each pocket was 8 × 9 cm (inner compartment size). Pockets were sewn from net with a 1.1 mm mesh size. This mesh size is a compromise enabling the access and free movement of soil fauna and preventing litter loss from the bags. 1 g of air-dried litter from each species was left, oven-dried at 60 °C for 48 h and weighed again in order to calculate initial oven-dried weights of the used litter material. For most species, the decrease in weight was between 6 and 8 percent.

Litterbags were placed back carefully into three blocks in the study site. Each block consisted of three squares, and each square represented one of the three litterbag harvests: the first after 3 months, the second after 6 months and the last after 1 year from the day of placement at the locality (29 November 2005). Consequently, each species at each harvest was characterized by three replications, one from each block. For some species, we were not able to collect a sufficient amount of litter to fill the nine litterbags. In those cases when we were not able to fill the required number of litterbags, we omitted one (exceptionally two) of litterbag harvests for this species. Three blocks were used to cover the meadow spatial heterogeneity and also to increase the probability of litterbag survival in the case of disturbance by meadow visitors.

After retrieving, the soil and litter on the outside of the bags were brushed off carefully. Litterbags were emptied immediately and their contents were spread out to remove visible soil animals, attached plant parts (like roots, leaves or moss) or soil particles using tweezers as precisely as possible. The sorted litter material was oven-dried at 60 °C for 48 h and weighed. Percentage dry weight loss after decomposition (percentage of initial dry weight) was assessed for every species in each harvest as an average of the individual litterbag values.

Species detritus decomposition rate (day^{-1}) was defined as r in the formula: $P_t = e^{rt}$, where P_t is the percent of not decomposed litter at time t . The parameter r was estimated as the slope of a line with a fixed zero intercept in the regression of $\ln(\text{harvested species litter weight} / \text{original species litter weight})$ on time, i.e., on the number of days between exposure and harvest of litterbags.

Carbon and nitrogen content analyses. Carbon and nitrogen contents of leaves were measured on both dry field-collected litter and oven-dried biomass from the aquarium leaf palatability experiment (plants from “without slug section” were used), and both were used as predictors for palatability and decomposition rate. Dry material was cut into small pieces and then milled with a ball mill (MM 200, Retsch, Germany) in order to keep all plant parts in the appropriate mixture. Subsamples of 5–6 mg of milled material were weighed and analyzed with an NC 2100 Soil Analyzer (ThermoQuest Italia S.p.A., Italy). During the analysis, carbon and nitrogen contained in organic matter were mineralized to carbon dioxide and molecular nitrogen. These gases were separated with a chromatographic column and their concentrations determined with a thermal conductivity detector. Element contents were expressed as total percentage element content in a weighted sub-sample of organic matter.

Data analyses

Relationships between the two variables were examined first using linear regression, even in the cases where it was not expected that one of the variables is the cause and the other the consequence. In this way, we tried to predict the value of one variable using the other (e.g., species palatability by litter decomposition). This approach enables us to better visualize the relationship using the regression line (i.e., the prediction). Moreover, the significance of the regression is exactly the same as would be the significance of the correlation, and R^2 is just the square of the correlation coefficient. As the expected direction of relationships was derived *a priori* from ecological mechanisms (i.e., we expect a positive relationship between palatability and decomposition, and positive relationships between both palatability and decomposition to nitrogen; however, negative relationships were expected to carbon content), we used corresponding one-tailed tests. Two sample t-test (with correction for unequal variances) was used to compare palatability of monocots and dicots. All the investigated dicot species belong to the eudicots, and consequently form a monophyletic clade. In the paper, we keep the traditionally used name dicots. Similarly, all the monocots belong to the order Poales (families Poaceae, Juncaceae and Cyperaceae). The comparison could be interpreted as comparison of two monophyletic clades, but also corresponds to comparisons usually called “forbs” vs. “grasses and graminoids” in similar studies.

The number of analyzed species/observations can differ between analyses because (1) for some species, for which we have litter decomposition data, there were not enough seedlings to perform the palatability test and (2) for some species tested for palatability, we were not able to collect enough litter in the field. The “pair-wise deletion” approach was used in all of the analyses, i.e., we used all of the species for which the two compared variables were available.

To correct for possible phylogenetic dependence of individual species, we also applied the method of phylogenetically independent contrasts (PIC, see Webb et al. 2002 for a

Table 1. Definitions of plant traits (characteristics) used in the prediction of species palatability and detritus decomposition rate responses via the regression tree method.

Characteristic	Definition
biomass [g/m ²]	dry mass of the species at the locality
height [cm]	the shortest distance between the upper boundary of the main photosynthetic tissues on a plant and ground level
LDMC [mg/g]	oven-dry mass of a leaf divided by its water saturated fresh mass
leaf C content [mg/g]	total amount of carbon per unit of dry leaf mass
leaf N content [mg/g]	total amount of nitrogen per unit of dry leaf mass
leaf P content [mg/g]	total amount of phosphorus per unit of dry leaf mass
litter C content [%]	total percentage carbon content in weighed subsample of species litter
litter N content [%]	total percentage nitrogen content in weighed subsample of species litter
monocot / dicot	characteristic of taxonomic classification
phenology [day]	the Julian day of the start of flowering
plant C content [%]	total percentage carbon content in weighed subsample of species oven-dry biomass
plant N content [%]	total percentage nitrogen content in weighed subsample of species oven-dry biomass
SDMC [mg/g]	oven-dry mass of a stem divided by its water saturated fresh mass
seed weight [mg]	oven-dry mass of an average seed of a species
SLA [mm ² /mg]	one-sided area of a fresh leaf divided by its oven-dry mass

broader discussion of the phylogenetic correction approaches) using the R-software (R Development Core Team 2007). Independent contrasts are widely used to incorporate phylogenetic information into studies of trait relationships (Ackerly 2000). Each PIC provides one replicate for testing whether a divergence in attribute X has consistently been associated with a divergence in Y across separate evolutionary divergences (Westoby 1999). After removing phylogeny effects, we should get the species response to natural selection. The branching structure of the phylogenetic tree was created on the basis of the BIOFLOR database (Klotz et al. 2002, <http://www.ufz.de/biolflor/index.jsp>). Time of divergence of common ancestors – branch lengths were estimated according to the Angiosperm Phylogeny Group website (Stevens 2001) and the following publications: Kellogg (2001), Bremer et al. (2002) and Janssen and Bremer (2004). The computed phylogenetic contrasts of all variables were analyzed using linear regression (as described above).

Species palatability and detritus decomposition rate were predicted on the basis of a larger set of species traits using regression trees (R software; R Development Core Team 2007). This is a non-parametric regression that displays a binary tree built through binary recursive partitioning. The tree is created so that the trait that best distinguishes the dependent variable response splits the species into two groups. After that, the subsequent subsets can be split with any trait again. In this way, the effects of individual traits are not additive, which is probably an important advantage (see de Bello et al. 2005 for discussion of the use of regression trees to predict species responses on the basis of species traits). In interpretations, we have also taken the advantage of the summaries of primary splits and of surrogate splits, which are not displayed in the resulting regression tree, but are provided as a supplement of each analysis. Primary splits summary provides all variables that were good candidates for individual splits (but only one of them was finally selected). Surrogate splits suggest variables that best reproduce the chosen primary split variable (for further details see Breiman et al. 1984). This information enables us to better compare the im-

portance of individual predictors in various trees. We selected 15 traits (Table 1) from the Vista project (Garnier et al. 2007) database compiled from field observations at the study site. The minimum size of the terminal branch was set at 3 observations and the minimum size of a split data group was set at 6 observations.

Results

The most palatable species in the experiment was *Angelica sylvestris*, followed by the highly palatable species *Scorzonera humilis*, *Prunella vulgaris* and *Succisa pratensis*. The lowest palatability was obtained for *Carex leporina*, *Cerastium holosteoides*, *Holcus lanatus* and *Nardus stricta* (Table 2). The most striking feature is the difference between monocots and dicots ($t = 2.661$, $df = 18$, $p < 0.05$); dicotyledonous species were grazed about 38% more than monocotyledonous species.

Palatability is well predicted by the percentage dry weight lost in the first harvest, i.e., after 3 months exposure in the locality ($p < 0.05$, Table 3, Fig. 2). The strength of the relationship weakens with increasing length of exposure, and is only marginally significant for the estimated rate of decay over the whole period (Table 3). Plant species with lower decomposition rates always have a low level of palatability, but plants with higher rates of decomposition (less than 65% of initial dry weight remaining in the first harvest) vary widely in their palatability, with only some being more palatable (Fig. 2). The relationships are much stronger, all being significant, after accounting for phylogenetic relatedness ($p < 0.05$, Table 3).

As expected, plant palatability decreased with biomass carbon content ($R^2_{adj} = 0.140$, $n = 20$, $p_{one-tailed} < 0.05$); surprisingly, the relationship with litter carbon content is slightly stronger ($R^2_{adj} = 0.191$, $n = 20$, $p_{one-tailed} < 0.05$). Conversely, we failed to find the expected positive relationship between palatability and biomass/litter nitrogen content. Also, the test of palatability response to C/N ratio was insignificant. Similarly, when we tried to predict litter decompo-

Table 2. Species palatability as assessed in the aquarium slug grazing experiment, litter decomposition characterized by the remaining part of initial dry weight after the first harvest and species detritus decomposition rate. Palatability is expressed in percent as a species weight ratio according to the formula: (biomass of “target species without slugs” aquarium section - biomass of “target species with slugs” aquarium section) / biomass of “target species without slugs” aquarium section. Control species (*Plantago lanceolata*) palatability result is highlighted by the grey area. “-” indicates that the data are not available for the species.

Species	Palatability [%]	remaining part of initial dry weight after the 1. harvest [%]	Detritus decomposition rate [$\times 10^3 \text{ day}^{-1}$]
<i>Angelica sylvestris</i>	100.0	57.1	-3.2
<i>Scorzonera humilis</i>	93.2	-	-
<i>Prunella vulgaris</i>	90.7	60.9	-2.8
<i>Succisa pratensis</i>	90.4	46.0	-5.8
<i>Lychnis flos-cuculi</i>	59.2	-	-3.4
<i>Betonica officinalis</i>	44.4	46.4	-4.4
<i>Cirsium palustre</i>	26.4	59.4	-4.2
<i>Briza media</i>	25.1	86.1	-1.3
<i>Plantago lanceolata</i>	23.5	-	-
<i>Myosotis nemorosa</i>	23.4	-	-2.4
<i>Luzula multiflora</i>	15.7	-	-1.6
<i>Sanguisorba officinalis</i>	13.6	55.7	-4.6
<i>Anthoxanthum odoratum</i>	11.0	-	-
<i>Danthonia decumbens</i>	4.1	83.2	-1.6
<i>Epilobium palustre</i>	2.7	72.9	-2.2
<i>Deschampsia cespitosa</i>	2.4	-	-
<i>Galium boreale</i>	1.6	71.9	-4.7
<i>Carex leporina</i>	0.0	-	-
<i>Cerastium holosteoides</i>	0.0	-	-
<i>Holcus lanatus</i>	0.0	92.5	-1.6
<i>Nardus stricta</i>	0.0	83.3	-1.5
<i>Achillea ptarmica</i>	-	66.0	-2.8
<i>Agrostis capillaris</i>	-	84.4	-2.4
<i>Carex hartmanii</i>	-	91.1	-1.1
<i>Galium uliginosum</i>	-	-	-4.3
<i>Juncus effusus</i>	-	80.6	-1.8
<i>Lathyrus pratensis</i>	-	70.7	-1.9
<i>Lysimachia vulgaris</i>	-	77.7	-2.2
<i>Molinia caerulea</i>	-	82.9	-1.3
<i>Potentilla erecta</i>	-	51.6	-3.6
<i>Ranunculus acris</i>	-	-	-4.6
<i>Ranunculus nemorosus</i>	-	-	-10.2
<i>Scirpus sylvaticus</i>	-	89.1	-1.4
<i>Selinum carvifolia</i>	-	56.9	-3.7
<i>Tephrosia crista</i>	-	61.6	-2.9
<i>Viola palustris</i>	-	-	-1.4

Table 3. Summary results of linear regression of palatability on litter decomposition, including results of phylogenetically controlled analyses. The effect of dry weight lost after each harvest and total species detritus decomposition rate was examined. “n” refers to number of analysed species, “ R^2_{adj} ” to the adjusted coefficient of determination, “p” to level of significance.

variable	n	R^2_{adj}	p
1. harvest weight loss [% initial dry weight]	12	0.386	<0.01
2. harvest weight loss [% initial dry weight]	15	0.133	<0.05
3. harvest weight loss [% initial dry weight]	13	0.075	<0.1
detritus decomposition rate [$\times 10^3 \text{ day}^{-1}$]	15	0.112	<0.1
PIC 1. harvest weight loss	12	0.435	<0.01
PIC 2. harvest weight loss	15	0.257	<0.05
PIC 3. harvest weight loss	13	0.268	<0.05
PIC detritus decomposition rate	15	0.276	<0.05

sition rate with carbon or nitrogen contents, litter carbon content was the only significant predictor ($R^2_{adj} = 0.431$, $n = 26$, $p_{\text{one-tailed}} < 0.001$). When phylogenetically independent contrasts (PIC) were used, the relationship between palatability and biomass/litter carbon content became non-significant

(with R^2_{adj} close to 0), while the relationship with nitrogen content remained non-significant.

Regression tree analysis selected leaf dry matter content (LDMC) as the fairly best predictor of plant palatability, (high LDMC means low palatability). Other selected predictors were litter carbon content in the left branch of the regression tree and seed weight in the right branch of the tree (see Fig. 3). However, their explanatory power is rather low, and the decrease of relative error for these two predictors was not confirmed by cross-validation. Consequently, the least palatable species should be a plant with high LDMC together with low carbon content (note that low plant carbon content matters only within the group of high LDMC plants) and the most palatable should be a plant with both low LDMC and heavy seeds (again, heavy seeds matter only within the group with low LDMC).

The hierarchical prediction of detritus decomposition rate (Fig. 4) selected litter carbon content as the first splitting rule, with a fairly high explanatory power. The highest de-

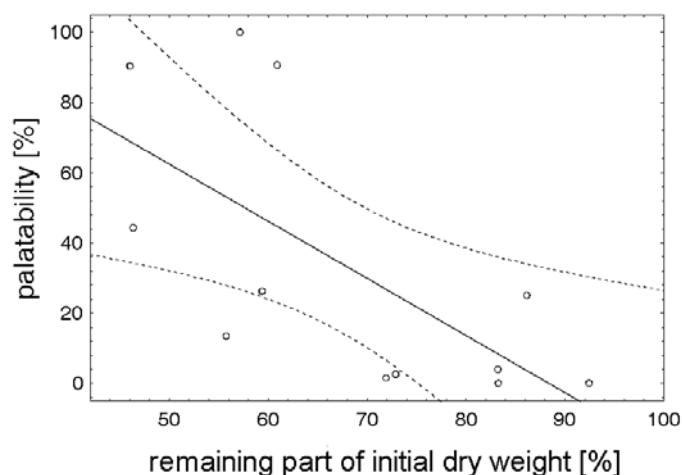


Figure 2. Relationship between plant palatability and litter decomposition characterized by the remaining part of initial dry weight after the first harvest. Dashed lines represent 95% confidence interval.

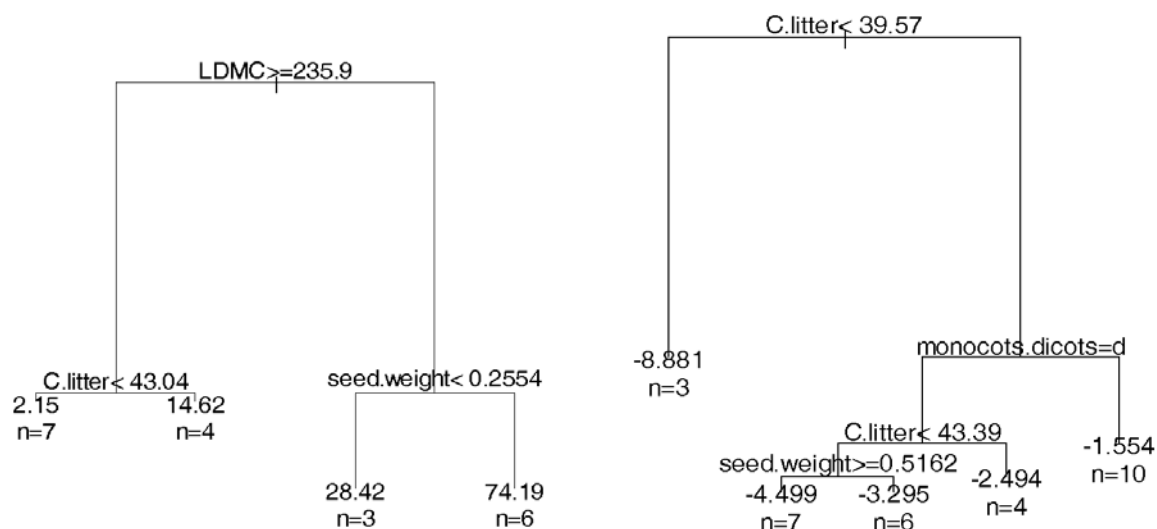


Figure 3. Hierarchical prediction of plant palatability from plant traits using regression trees. Values at the end of branches refer to the predicted level of plant palatability, “n” means the number of species sharing the set of traits defined by the upper nodes. The left branch should be followed if the condition in the node is fulfilled. Traits with threshold values selected as splitting predictors in nodes are: leaf dry matter content (LDMC [mg/g]), litter carbon content (C.litter [%]) and seed weight (seed.weight [mg]). Length of the branches corresponds to the explained variability.

composition rate was exhibited by plants with low litter carbon content. Of the plants with high litter carbon content, the monocots had slower litter decomposition. Seed weight was another obtained splitting variable (see Fig. 4).

A comparison of best improving primary splits of the regression trees pointed to the most apparent common predictors of variables responses: litter carbon content, seed weight and plant height. Plant height was chosen as the second best improving primary split in both regression trees (so it is not presented in the regression trees pictures) and also it was the

Figure 4. Hierarchical prediction of detritus decomposition rate [$\times 10^3 \text{ day}^{-1}$] from plant traits using regression trees. Values at the end of branches refer to the predicted rate of decomposition (more negative values means higher decomposition rate), “n” means the number of species sharing the set of traits defined by the upper nodes. The left branch should be followed if the condition in the node is fulfilled. Traits with threshold values selected as splitting predictors in nodes are: litter carbon content (C.litter [%]), taxonomic classification characteristic (monocots.dicots; m=monocot, d=dicot) and seed weight (seed.weight [mg]). Length of the branches corresponds to the explained variability.

surrogate split best reproducing the best primary splits (not shown in the Figures).

Discussion

Our results confirmed the expected positive relationship between plant palatability and litter decomposition rate. The strength of the relationship decreased with increasing time of litter exposure. Slugs preferentially graze on the soft parts of plant tissue. During the early phase of decomposition, soft plant tissues are broken down, while the remaining tough re-

inforced plant parts (parts containing protective structures both against adverse physical conditions and herbivores) persist and are broken down during the later phases of decomposition. From this point of view, grazing is most similar to early phases of decomposition. Other studies that demonstrated a positive relationship between litter decomposition and palatability (Grime et al. 1996 and Schädler et al. 2003) also used a relatively short litter incubation period (not exceeding 8 or 20 weeks).

Although plants with hard decomposing litter were matched with low palatability levels, some fast-decomposing plant species (mostly dicots) were not preferred by herbivores. In contrast to monocots, dicots are more variable in their physical body structures, architecture or secondary metabolites, all of which can affect plant palatability. According to Cornelissen and Thompson (1997), greater litter weight loss in dicots is significantly correlated with higher total base content, which makes that litter less acidic and so creates a favorable environment for decomposers. When phylogenetic relatedness was taken into account, all relationships were strengthened, which corresponds with the significant results found in other studies (e.g., Grime et al. 1996, Cornelissen et al. 1999, Wardle et al. 2002).

As expected, species with low carbon content were more palatable; surprisingly, litter carbon content was a slightly better predictor than biomass carbon content. Plants which we collected as litter were much older than the plants constituting the aquarium biomass, thus they had better developed their supporting or defensive structures. Consequently, the litter was more variable in its carbon content, which would strengthen the test. However, neither of the relationships was significant after we applied the phylogenetically independent contrasts. This does not rule out the importance of carbon in structural carbohydrates as an anti-herbivore defense, however, we are not able to separate the effects of common ancestors from the adaptive value of the trait.

The significant results of phylogenetically-corrected palatability-litter decomposition relationship tests may be considered as a proof of adaptive significance (in this case probably of traits affecting both processes, i.e., grazing and litter decomposition). On the contrary, higher litter/biomass carbon content in less palatable plants is evidence for the present-day ecological function; however, we can not say anything about its adaptive value. Nevertheless, as noted by Westoby (1999), we should be aware that the power of the PIC test can be very low. For example, a perfectly functional trait that has arisen in only one or a few separate radiations would never show statistical significance in a correlated-divergence analysis. Conversely, a trait repeatedly correlated with an ecological outcome across many ecological radiations or PICs need not necessarily be the true cause (Westoby 1999). In our view, the phylogenetically corrected and uncorrected analyses should always be combined. Whereas assessment of adaptive significance using, e.g., PIC are clearly important for understanding the mechanisms of evolutionary ecology, predictions of species response (e.g., to management

change), based on the uncorrected relationships, are probably more useful.

We found no relationship between both palatability and decomposition and nitrogen content. Likewise, Cornelissen et al. (2004) found leaf nitrogen concentration alone to have poor explanatory value for leaf digestibility, but its predictive power increased when included in a lignin or phenol ratio (which are not available for our plants). Wardle et al. (2002), who investigated the herbivory effect of browsing mammals, concluded that animals preferentially browse vegetation on the basis of carbon chemistry and not nutrient status. Besides, it is possible that the weak prediction of litter nitrogen content resulted from interspecific variation in nitrogen resorption from senescing plants (Cornelissen et al. 2004). Cornelissen and Thompson (1997) found nitrogen to be important only for the decomposition of monocots.

Our study differs from most of the previous palatability assessments in terms of the experimental setup, when whole living plants were subject to slug grazing (in most previous experiments, plant parts, discs, or agar were used). We believe that this approach is important to obtain realistic results and consequently consider our more natural conditions to be an important advantage of our experimental set up. In particular, this approach reflects the whole plant defense, including the accessibility of individual plant parts. However, we are aware that we have to pay for this advantage. Unlike various plant parts, the growth of living plants cannot be weighed in advance and consequently, the amount of grazed biomass is estimated from comparison between the grazed and ungrazed parts of an aquarium. This is less precise than determining the decrease in weight or area of, e.g., plant discs, as done in classical cafeteria experiments. Also, the choice for each of the slugs in our experiment was restricted to two species, less than in usual cafeteria experiments. Both arrangements diverge from a natural system in the availability of particular plant species and the impact of other environmental factors, which can cause some variance between herbivore preferences in cafeterias and consumption in the field (Pérez-Harguindeguy et al. 2003). There were also logistic restrictions to our experimental setup; we needed separate aquaria which were sufficiently large for the slugs to move in for each tested species. Also, it was difficult to get a sufficient number of seeds to germinate at the same time for all species. In the case that the grazing trials for various species would be spread over a longer time period, the grazing activity of slugs might change systematically during this time. Despite all of these problems, we assume that grazing on living plants improves the realism of the experiments considerably.

Mollusks are considered to be the most important grazing invertebrates in temperate grasslands (Fenner et al. 1999). Nevertheless, we are aware that our results only reflect the palatability to our single experimental slug species. Insects are another important group of herbivores at the locality. In comparison to slugs, insects feed on more plant parts in many ways (biting, chewing, sucking, galling, mining), which can cause differences in plant damage and in the level of herbi-

vore impact (Wilf et al. 2001, Karban and Agrawal 2002, our survey at the experimental locality). The experiment of Grime et al. (1996) showed that insects are more selective than mollusks, particularly within monocots. If we were concerned with vertebrate herbivore palatability assessment, the result would also differ, mainly due to the different effect of some traits, physical barriers or preferred plant parts (see e.g., Díaz et al. 2001, Cornelissen et al. 2004, Cingolani et al. 2005).

Some traits predict species responses as a partial effect in multiple regressions or in a hierarchical way within subgroups defined by other traits, but their effects as sole predictors do not have to be significant. Regression trees present a useful tool for analyzing species traits data, because their effects are usually non-additive; the method allows for visualization of effects of trait combinations (de Bello et al. 2005). LDMC, litter carbon content and seed weight were chosen as the best predictors of plant palatability response. The low palatability of high LDMC plants was expected; slugs like the juicy tissue (Hendriks et al. 1999), while the fact that a relatively low amount of carbon predicts also low palatability seems to be surprising. However, we should be aware that this predictor has rather low explanatory power and is relevant only in the high LDMC plant group. This may be because the low carbon content is partially a result of the presence of other less palatable structures or elements, e.g., silica conferring protection from mollusk herbivory (Edwards and Crawley 1999). This idea is confirmed by the presence of *Carex leporina* and *Deschampsia cespitosa* in the mentioned group. Also, the fact that litter and not biomass C content was selected supports this idea; lower C content in litter is probably a good indication of the presence of other defense structures in the species, whereas C in young plants is not so much affected by them. Conversely, the most palatable plants should have low LDMC and heavier seeds. Seed weight itself could hardly affect directly the palatability of leaves, so we suppose that the relationship is due to trade-offs connected with investments into heavy seeds. (We can speculate that energy invested into heavy seeds might be missing for building anti-herbivore defense structures or that high efficiency of photosynthesis needed for producing large seeds is “paid off” by limited defense of photosynthetic structures.) In our case, e.g., *Angelica sylvestris*, *Cirsium palustre* or *Betonica officinalis* form the group of species with firm stems, but large leaves with low dry matter content and relatively large seeds.

Results of the regression tree analysis of detritus decomposition rate response reflect mainly the contents of supporting or defensive structures, which make plants more or less decomposable. Litter carbon content was the main variable responsible for the low decomposition of plants; again, high seed weight predicts faster decomposition (probably for the same reasons as for high palatability). The cause of the slow decomposition of monocots is probably their build (Cornelissen and Thompson 1997, Cornelissen et al. 1999). In this case, *Poaceae*, *Juncaceae* and *Cyperaceae* represent monocotyledonous species whose main features are narrow leaves

with parallel veins, which make them firm and tough. Moreover, silica crystals incrustation is common in *Cyperaceae* and is also often found in *Poaceae*, especially in *Deschampsia*.

Three common predictors of palatability and detritus decomposition rate responses emerged from the comparison of the regression tree analyses (including their primary and surrogate splits): litter carbon content, seed weight and plant height. According to our analyses, tall plants with high litter carbon content and lightweight seeds should decompose slowly and be unpalatable. Díaz et al. (2001) consider plant height and LDMC as the best predictors of plant grazing response. Nevertheless, in their study, tall plants should be preferred by vertebrate herbivores, because small plant height does not allow an herbivore to choose the plant. Here, we regard plant height as a reflection of the amount of supporting structures, which deterred the invertebrate herbivores (slugs). Wardle et al. (2002) found a negative correlation between plant carbon content and deer and goat browsing in the same manner as we did. On the contrary, Schädler et al. (2003) has shown that palatability and detritus decomposition rate were related to litter nitrogen content, specific leaf area and leaf water content, i.e., traits without significant effects in our study.

Our study confirmed the expectation that lower levels of species palatability are accompanied by slower decomposition and the strength of this relationship increases with decreasing time of litter exposure. This relationship was strengthened by phylogenetic correction, thus we can expect that the relationship should be consistent across different lineages. Similar species traits determine the resistance to grazing and low rate of litter decomposition. This supports the species strategy concept of Grime (2001), and is in agreement with the hypothesis that plant species traits are important determinants of ecosystem functioning. For example, we can expect that a change in species trait composition under herbivore pressure would result in decreased litter decomposition and consequently a reduction of nutrient cycling in the ecosystem.

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