Annals of Botany **00**: 1–11, 00
doi: 10.1093/aob/mcx175, available online at www.academic.oup.com/aob

ANNALS OF BOTANY
Founded 1887

Traits determining the digestibility– decomposability relationships in species from Mediterranean rangelands

Iris Bumb[1], Eric Garnier[2], Sylvain Coq[2], Johanne Nahmani[2], Maria Del Rey Granado[2], Olivier Gimenez[2] and Elena Kazakou[1,*]

[1]Montpellier Supagro and Centre d'Ecologie Fonctionnelle et Evolutive (UMR CEFE), CNRS, Université de Montpellier - Université Paul-Valéry Montpellier - EPHE, Montpellier, France and [2]Centre d'Ecologie Fonctionnelle et Evolutive (UMR 5175), CNRS - Université de Montpellier - Université Paul-Valéry Montpellier - EPHE, Montpellier, France
**For correspondence. E-mail elena.kazakou@cefe.cnrs.fr*

Received: 25 August 2017 Returned for revision: 8 September 2017 Editorial decision: 6 October 2017 Accepted: 8 November 2017

• **Background and Aims** Forage quality for herbivores and litter quality for decomposers are two key plant properties affecting ecosystem carbon and nutrient cycling. Although there is a positive relationship between palatability and decomposition, very few studies have focused on larger vertebrate herbivores while considering links between the digestibility of living leaves and stems and the decomposability of litter and associated traits. The hypothesis tested is that some defences of living organs would reduce their digestibility and, as a consequence, their litter decomposability, through 'afterlife' effects. Additionally in high-fertility conditions the presence of intense herbivory would select for communities dominated by fast-growing plants, which are able to compensate for tissue loss by herbivory, producing both highly digestible organs and easily decomposable litter.
• **Methods** Relationships between dry matter digestibility and decomposability were quantified in 16 dominant species from Mediterranean rangelands, which are subject to management regimes that differ in grazing intensity and fertilization. The digestibility and decomposability of leaves and stems were estimated at peak standing biomass, in plots that were either fertilized and intensively grazed or unfertilized and moderately grazed. Several traits were measured on living and senesced organs: fibre content, dry matter content and nitrogen, phosphorus and tannin concentrations.
• **Key results** Digestibility was positively related to decomposability, both properties being influenced in the same direction by management regime, organ and growth forms. Digestibility of leaves and stems was negatively related to their fibre concentrations, and positively related to their nitrogen concentration. Decomposability was more strongly related to traits measured on living organs than on litter. Digestibility and decomposition were governed by similar structural traits, in particular fibre concentration, affecting both herbivores and micro-organisms through the afterlife effects.
• **Conclusions** This study contributes to a better understanding of the interspecific relationships between forage quality and litter decomposition in leaves and stems and demonstrates the key role these traits play in the link between plant and soil via herbivory and decomposition. Fibre concentration and dry matter content can be considered as good predictors of both digestibility and decomposability.

Key words: Afterlife effect, dry matter digestibility, decomposability, fertilization, grazing, plant traits, litter, leaves, stems, rangeland

INTRODUCTION

Plant species control carbon and nutrient cycling as they provide the resources for both herbivores and the functioning of the decomposer subsystem (Hobbie, 1992; Berendse, 1994; Aerts and Chapin, 2000). Several comparative studies have demonstrated that functional traits of living leaves persist through senescence and influence both litter quality and decomposition (Cornelissen, 1996; Cornelissen and Thompson, 1997; Pérez-Harguindeguy *et al.*, 2000). Plant species that are preferentially browsed generally grow more rapidly, accumulate higher concentrations of nitrogen in their tissues and contain lower amounts of secondary metabolites than less preferentially eaten species (Coley, 1988; Grime, 1979; Wardle, 2002). According to the 'afterlife effect hypothesis' (Grime and Anderson, 1986), more palatable species will produce high-quality litter, which promotes the activity of the decomposer subsystem and thus enhances decomposition rates (Cornelissen *et al.*, 1999). Indeed, at the interspecific level mainly structural traits of living leaves and those of leaf litter tend to be strongly positively correlated (e.g. Kazakou *et al.*, 2009; Freschet *et al.*, 2010). Experimental studies supporting the afterlife effects hypothesis found that high leaf consumption by invertebrate herbivores (snails, insects) was related to both low investment in foliar defence and high litter decomposability (Grime *et al.*, 1996; Wardle *et al.*, 1998; Cornelissen *et al.*, 1999; Pérez-Harguindeguy *et al.*, 2000; Cornelissen *et al.*, 2004). In spite of the recognition of the positive covariation between palatability and decomposition, there is only limited evidence to support the view that the defences against invertebrates also operate against larger

vertebrate herbivores and vice versa, reducing leaf digestibility (Bryant *et al.*, 1991). For instance, only one study (Cornelissen *et al.*, 2004) both focused on larger vertebrate herbivores and considered the links between the digestibility of living leaves and leaf litter decomposability and associated traits.

Studies conducted separately on these two processes provide evidence that leaf digestibility and leaf litter decomposability are generally governed by the same leaf traits (see Garnier *et al.*, 2016 for a synthesis): fibre content, which can be considered as an anti-herbivore defence (Coley, 1988) is negatively correlated to digestibility (Al Haj Khaled *et al.*, 2006; Bumb *et al.*, 2016) and decomposability at the interspecific level (Cobo *et al.*, 2002; Wardle *et al.*, 2002; White *et al.*, 2004; Kazakou *et al.*, 2009). Leaf nitrogen concentration is positively related to both processes (e.g. Bidlack and Buxton, 1992; Karn *et al.*, 2006 for digestibility; Pérez-Harguindeguy *et al.*, 2000; Cornwell *et al.*, 2008 for decomposability), whereas leaf toughness (Onoda *et al.*, 2011 for digestibility; Kazakou *et al.*, 2009 for decomposability) and leaf dry matter content (e.g. Pontes *et al.*, 2007; Bumb *et al.*, 2016 for digestibility; Kazakou *et al.*, 2006, 2009; Cortez *et al.*, 2007; Bakker *et al.*, 2011 for decomposability) are negatively correlated to both digestibility and decomposability. The relative importance of each of these traits in explaining the relationship between digestibility and decomposability remains to be determined. We test several biochemical and structural traits for their possible predictive power with respect to leaf digestibility and litter decomposability.

Another point under debate in the literature is whether the relationship between digestibility and decomposition is consistent across different plant organs other than leaves. To date, only a few studies have focused on the digestibility and decomposability of organs other than leaves, such as stems and whole shoots (but see Louault *et al.*, 2005; Al Haj Khaled *et al.*, 2006; Pontes *et al.*, 2007 for shoot digestibility; Bumb *et al.*, 2016 for stem digestibility; Birouste *et al.*, 2012; Freschet *et al.*, 2012, 2013 for stem and root decomposability). However, leaves often represent a small fraction of the plant, and stems in some species may provide important biomass for herbivores and decomposers. With only a few studies available on interspecific variation in stem digestibility (Bumb *et al.*, 2016) and decomposability (Freschet *et al.*, 2012), we do not know whether the covariation between digestibility and decomposability in stems is comparable with that observed in leaves. We postulate that (1) leaves will present higher digestibility and decomposability than stems, that (2) similar traits will affect the two processes, but (3) the magnitude will be not the same. We assume that leaves contain more easily degradable forms of organic nitrogen than organs with predominant support and transport functions, in which most nitrogen might be in structural or defence-related compounds (e.g. microtubules, phloem proteins). Freschet *et al.* (2010) proposed that differences between organ structures also affect their degradation and decomposability. For instance, the typically flat structure of leaves should provide a relatively larger surface for microbial attacks and selective feeding by soil fauna compared with cylindrical organs such as stems. Here, we propose to test these hypotheses by measuring several structural and chemical and traits of green and senescent stems and to evaluate their relative importance in predicting stem digestibility and decomposability.

Previous studies demonstrated that differences in management regimes pertaining to defoliation or fertilizer supply affect vegetation structure and function, leading to differences in digestibility (Bumb *et al.*, 2016) and related traits (Pontes *et al.*, 2007; Carrère *et al.*, 2010). So far, digestibility–decomposability relationships have mostly been established for species belonging to different functional types and very few studies have explicitly addressed the effects of the intensity of herbivory and resources availability on these relationships (Wardle *et al.*, 2004). The originality of the present study is that we propose to explore dry matter digestibility and decomposability relationships for 16 dominant species from Mediterranean rangelands subject to management regimes differing in intensity of grazing and fertilization. We expect that the high-fertility conditions and the high rates of herbivory jointly (1) cause the dominance of fast-growing plants, which are able to compensate for tissue loss by herbivory, and (2) produce highly digestible organs and litter with high decomposition rates.

Figure 1 summarizes the different hypotheses tested: (1) as postulated by the afterlife effect hypothesis, digestibility and decomposability covary positively (bidirectional arrow 1) due to the fact that both are caused by the same traits both across and within management regimes and for all studied organs; (2) digestibility is caused by traits measured only on living organs (arrow 2); (3) traits of living organs cause some traits of litter for all studied organs (arrow 3) and (4) decomposability is caused by traits measured on both living and litter organs (arrows 4 and 5).

MATERIALS AND METHODS

Study site, experimental design and plant species

We chose species from dry calcareous rangelands in southern France located on a limestone plateau (Larzac Causse) at the INRA La Fage experimental station (French National Institute for Agricultural Research) (43°55′ N, 3°05′ E, 790 m above

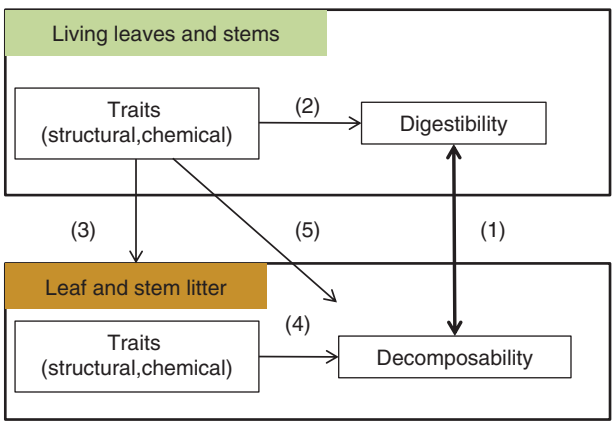

FIG. 1. Scheme showing the putative links between traits of leaves and stems and the two degradation processes studied here: digestibility and decomposition. We tested the hypotheses that (1) digestibility and decomposability covary positively (bidirectional arrow 1); (2) digestibility is affected by traits measured only on living organs (arrow 2); (3) traits of living organs affect some traits of litter for all studied organs (arrow 3) and (4) decomposability is caused by traits measured on both living and litter organs (arrows 4 and 5).

sea level). The climate of the plateau is sub-humid with a Mediterranean influence, with cool wet winters and warm dry summers. Mean annual precipitation of 1070 mm occurs mainly during spring and autumn, and mean monthly temperatures vary from 1 °C in January to 19 °C in August (data from 1973–2006). The vegetation is dominated by perennial herbaceous species (*Bromopsis erecta*), along with loosely scattered shrubs and trees (*Buxus sempervirens* and *Juniperus oxycedrus*) (Bernard, 1996). For the past 35 years, these rangelands have been grazed by a sheep flock (Romane breed) raised outdoors year-round for meat production.

The experimental station is divided into paddocks that vary between 4.7 and 24.5 ha. We chose six of them, with two different management regimes since 1978. The first regime consists of moderate grazing (G+F– hereafter) of 0.20 kg kg^{-1} (proportion of total annual biomass produced that is removed by grazing) and no fertilization. The second regime consists of intensive grazing (G++F+ hereafter) of 0.61 kg kg^{-1} (increased grazing in spring) and fertilization, with additions of mineral nitrogen (65 kg ha^{-1} year) and phosphorus (40 kg ha^{-1} every 3 years, stopped in 2005) since 1978. We chose three plots per paddock, which covered 200–500 m^2, for the present study (replicates).

Based on previous botanical surveys conducted in the paddocks (Bernard-Verdier *et al.*, 2012; Barkaoui *et al.*, 2013; Chollet *et al.*, 2014), we selected 16 species among the most abundant ones: six species were present only in G+F–, six species were present only in the G++F+ and four were present in both treatments (*Bromopsis erecta*, *Pilosella officinarum*, *Potentilla tabernaemontani* and *Poterium sanguisorba*; Table 1, species names from International Plant Names Index).

Sampling for digestibility of living organs and litter decomposability

In spring 2013, we harvested living biomass of these plant species at the period of peak biomass (end of May for G++F+ and mid-June for G+F–). We placed 30–150 healthy individuals per species from each plot into plastic bags with moist tissues (wetted with deionized water) and kept them in a cooler for 24–48 h until further processing in the laboratory. We then sorted each sample into leaves (lamina and sheath) and stems, and dried them at 60 °C for 72 h. We ground them using a ZM100 centrifugal mill through a 1-mm screen.

We collected leaf and stem litter at the season of maximum senescence for each species (summer and autumn 2013). We collected dead or senescing leaves and stems directly from the standing plant, carefully cleaned them and then dried them at 60 °C for 72 h and stored them in the laboratory.

Dry matter digestibility

We measured dry matter digestibility in dried and ground samples of living organs. This was done in two steps. First, we obtained *in vitro* measurements of a reference subsample using a pepsin-cellulase method of Aufrère *et al.* (2007); for more details see Bumb *et al.* (2016). These *in vitro* measurements come both from previously published values (see Bumb *et al.*, 2016 for details) and from our own samples. Second, we developed calibration curves based on near-infrared reflectance spectroscopy (NIRS) using a FOSS NIRSystems 6500 spectrometer (FOSS NIRSystems, Silver Spring, MD, USA). These calibration curves allowed us to predict the *in vitro* digestibility of the remaining samples using only the NIRS values. Near infrared reflectance spectroscopy is a non-destructive and highly precise physical method based on selective absorption of near-infrared electromagnetic waves by organic molecules (Birth and Hecht, 1987). NIRS has proved useful to relate the spectra of samples to their laboratory biochemical values in a number of digestibility studies (Aufrère *et al.*, 1996; Stuth *et al.*, 2003; Andrés *et al.*, 2005). Calibration curves were obtained using modified partial least

TABLE 1. *List of species studied, with occurrence in each treatment, taxonomic group, life cycle and growth form (according to Pérez-Harguindeguy et al. 2013)*

Species	Treatment		Taxonomic group	Life cycle	Growth form
Anthyllis vulneraria	G+F–		Fabaceae	Perennial/annual	Rosette
Brachypodium pinnatum	G+F–		Poaceae	Perennial	Tussock
Carex humilis	G+F–		Cyperaceae	Perennial	Tussock
Helianthemum apenninum	G+F–		Cistaceae	Perennial	Extensive vegetative spread and stemmed herb
Helianthemum canum	G+F–		Cistaceae	Perennial	Extensive vegetative spread and stemmed herb
Stipa pennata	G+F–		Poaceae	Perennial	Tussock
Capsella bursa-pastoris		G++F+	Brassicaceae	Annual	Rosette
Erodium cicutarium		G++F+	Geraniaceae	Annual	Rosette
Geranium molle		G++F+	Geraniaceae	Annual	Extensive vegetative spread and stemmed herb
Plantago lanceolata		G++F+	Plantaginaceae	Perennial	Rosette
Poa bulbosa		G++F+	Poaceae	Perennial	Tussock
Veronica arvensis		G++F+	Plantaginaceae	Annual	Extensive vegetative spread and stemmed herb
Bromopsis erecta	G+F–	G++F+	Poaceae	Perennial	Tussock
Pilosella officinarum	G+F–	G++F+	Asteraceae	Perennial	Rosette
Potentilla tabernaemontani	G+F–	G++F+	Rosaceae	Perennial	Extensive vegetative spread and stemmed herb
Poterium sanguisorba	G+F–	G++F+	Rosaceae	Perennial	Rosette

squares regression with WINISI software (version 4, Infrasoft International, Port Matilda, PA, USA). As measured and predicted digestibility were strongly correlated (Bumb *et al.*, 2016), the NIRS method was used to predict digestibility from spectral data. Chemical parameters known to be related to the nutritional value of the samples were also measured in the 24 samples used for calibrations: *in vitro* dry matter digestibility (g kg^{-1}) was measured by the pepsin-cellulase method of Aufrère *et al.* (2007); total nitrogen concentration (NC; g kg^{-1}) was measured by the Kjeldahl method and fibre content (neutral detergent fibre, NDF %) was measured by the Van Soest sequential detergent method with an amylase and protease pre-treatment (Van Soest *et al.*, 1991). In total, we analysed 119 samples of leaves and 97 of stems (not enough material was available for *Brachypodium pinnatum, Carex humilis* and *Pilosella officinarum*).

Litter decomposability

Within each of the two management regimes, we collected and pooled litters by species independently of the paddock where there were collected. In total we collected leaf litter of 16 species (in total 18 populations, as two species were found in the two management regimes) and stem litter of 14 species (not enough material was available for *Carex humilis* and *Pilosella officinarum*). We incubated leaf and stem litters in microcosms in the laboratory, under controlled temperature and humidity conditions, to measure the potential decomposability of the litter induced by differences in litter quality only. The microcosm type used for this experiment was described by Taylor and Parkinson (1988). Each microcosm chamber, 15 cm high, was made of a 15-cm diameter polyvinylchloride pipe, fitted with a lid and a sealed bottom. We placed 1 kg of standard soil on the grid, 2 cm above the bottom. The soil was a 3:1 mixture of mineral soil and surface organic horizon taken from the CEFE experimental garden in Montpellier. For each of the 16 species, we sealed in nylon litter bags of 1 mm mesh four repetitions of litter samples (between 0.40 and 2.10 ± 0.01 g each) per management regime (Northern Mesh, Oldham, UK). In the microcosm experiment we excluded the mesofauna, and the mass loss observed was the result of microbial decomposition. We weighed each litter sample to get its initial mass. We subsequently moistened the soil to 80 % of field capacity. We kept the microcosms in the dark at 24 °C throughout the experiment and watered them once a week to maintain constant soil moisture during the incubation period. After 8 weeks, we took litter bags out of the microcosms, carefully removed soil particles from the litter bags, dried the litter samples for 48 h at 60 °C and weighed them.

Traits of living and litter organs: sampling and measurements

We assessed living leaf and stem dry matter content (DMC) [calculated as the ratio between the dry mass of the organ and its water-saturated fresh mass (Garnier *et al.*, 2001)] on at least 12 individuals per species in each management regime during spring 2013, collected at the same time as those used for digestibility measurements (more details are given in Bumb *et al.*, 2016).

We conducted chemical analyses on living organs and their litter at the peak period of growth and senescence, respectively, for both leaves and stems. For living leaves and stems, we estimated NC and fibre content (NDF %) by the NIRS method on the same material as that used for digestibility measurement. We based calibrations on NC measured by the Kjeldahl method (Bradstreet, 1954) and fibre content measured by the Van Soest sequential detergent method (Van Soest *et al.*, 1991). We determined the NC of litter leaves and stems with an elemental analyser (model EA 1108; Carlo Erba Instruments, Milan, Italy) while we predicted their NDF by NIRS. We measured phosphorus concentration (PC) colorimetrically with an autoanalyser (Evolution II; Alliance instruments, Frépillon, France), using the molybdenum blue method following digestion with sulphuric acid (Grimshaw *et al.*, 1989) on living leaves and stems, as well as on leaf litter. We measured condensed tannins according to the acid butanol method (Waterman and Mole, 1994). Phosphorus and tannin concentrations were not available for living stems due to insufficient material.

Data analysis

We performed statistical analyses with the R software (R Development Core Team, 2010). To test the influence of management regime, growth form, species (nested in growth form) and their interactions with digestibility and decomposability we used linear mixed models (as implement in the lme4 R package; Bates *et al.*, 2015), in which we treated these effects as fixed and paddock as a random effect on the intercept of the relationship. We performed these analyses for each organ (leaf and stem) of the studied species. We used manual backward elimination for covariate selection using conditional *F*-test *P*-values to assess the significance of effects (as implemented in the R package pbkrtest; Halekoh and Højsgaard 2014). We verified the normality of the distribution of residuals for each model. When a growth form effect was detected, Tukey tests were used to determine significant differences between them. The same procedure was applied to the four plant species that occurred in both treatments to test the influence of management regime for each plant part.

We calculated Pearson and Spearman correlations on data averaged for each species, organ and management regime to test for relationships between digestibility, decomposability and traits. We tested digestibility in relation to traits of living organs only, while for decomposability we tested relationships with traits measured on both living and litter plant material to assess the afterlife effects (Fig. 1). We also tested correlations for each management regime and organ. We estimated intercepts and slopes with ANCOVA to compare the influences of management regime and organ on dependent variables.

We performed principal components analysis (PCA) separately for each organ using the adee4 R package (Dray and Dufour, 2007) to determine the relationships among traits across species, considering separately leaves and stems on the one hand, and traits of living plants and litter on the

other hand. We addressed differences between growth forms using one-way analysis of variance (ANOVA), followed by a Tukey test.

We used multiple regressions to test the relative importance of traits for the prediction of digestibility and decomposability. We used only traits from living plant material to predict digestibility, while we considered traits from living and litter plant material in separate models to predict decomposability. We used a stepwise approach to select the best model and we based this on the corrected Akaike information criterion (package glmulti, function glmulti). We tested the variance inflation factor (VIF) (package car, function vif) in order to avoid collinearity between variables. We considered variables presenting VIF <5 in the model (Kutner *et al.*, 2004). We calculated the partial η^2 (package heplots, function etasq) to provide a measure of the relative importance of each variable representing the proportion of the total variability attributable to a given factor. After selection of variables (the AIC criterion was used), we checked and confirmed the homoscedasticity and normality of the residuals of each best model.

Finally, we calculated Spearman ranking coefficients between living and litter traits (for nitrogen and phosphorus concentrations, NDF and tannin concentrations) on leaves and stems.

RESULTS

Variations in digestibility and decomposability

Species identity and growth forms influenced digestibility (for leaves $F_{4,93.1} = 1.45$**, for stems $F_{2,67.3} = 10.9$***, and Fig. 2A) and decomposability (for leaves $F_{3,197} = 5.05$**, for stems $F_{3,173} = 3.17$*, and Fig. 2B). Rosette plants like *Capsella bursa-pastoris* and *Erodium cicutarium* showed higher digestibility (Fig. 2A) and decomposability (Fig. 2B) than tussock plants like *Stipa pennata*, *Bromopsis erecta* and *Carex humilis*. Extensive vegetative spread and stemmed herbs like *Potentilla tabernaemontani* had digestibility and decomposability similar to rosette plants or intermediate between rosette and tussock plants (Fig. 2).

For most species, digestibility was higher for leaves than for stems (Fig. 2A, 3A), while decomposability was not significantly different between the two organs (Figs 2B and 3B). Differences between organs (Figs 2 and 3), management regimes and growth forms (Fig. 3) were higher for digestibility than for decomposability.

Management regimes influenced digestibility and decomposability of each organ (Fig. 3): species in the G++F+ treatment had higher digestibility and decomposability than those in the G+F− treatment for both leaves and stems (+20.3 % and +26.0 %, respectively, for digestibility; +38.8 % and +20.2 %, respectively, for decomposability).

Relationships between digestibility and decomposability

Digestibility was positively related to decomposability when all data from the 16 plant species were considered in the analysis, irrespective of the organ or management regime considered (Fig. 4). This relationship was also consistent within each management regime or organ (Fig. 4). Common slopes and intercepts were found when leaves and stems were analysed separately (Fig. 4 and data not shown). Digestibility–decomposability relationships typically showed similar slopes for both management regimes, with slight shifts in intercepts: that of the relationship in the G++F+ treatment was marginally higher than that in the G+F− treatment (legend to Fig. 4).

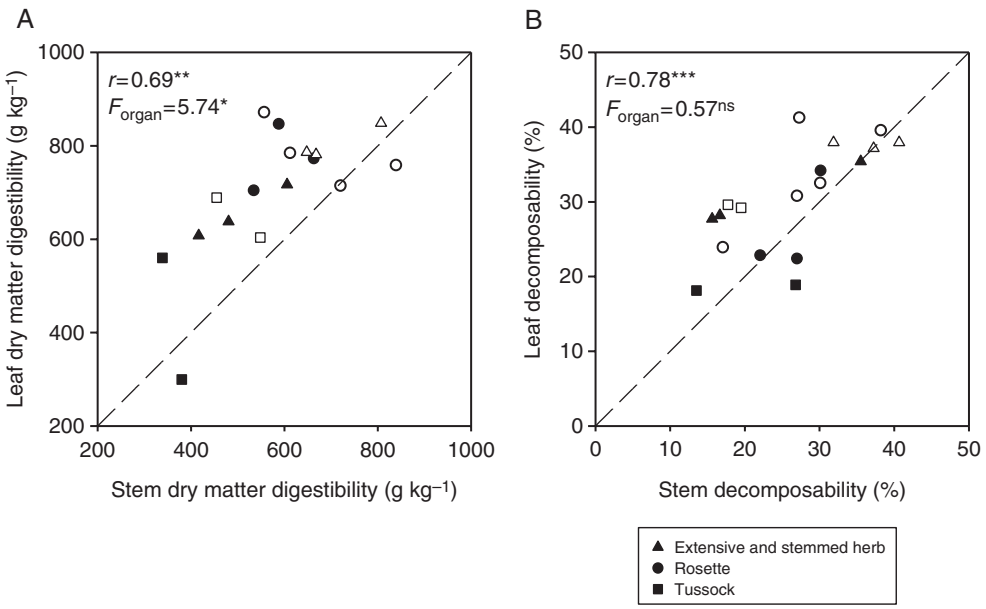

FIG. 2. Relationships between (A) dry matter digestibility and (B) litter decomposability of leaves and stems. The *r* values are Spearman correlation coefficients and *F* values correspond to ANOVA results. Open symbols represent the G++F+ treatment and solid symbols represent the G+F− treatment. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant. Extensive and stemmed herb: extensive vegetative spread and stemmed herb (see Table 1).

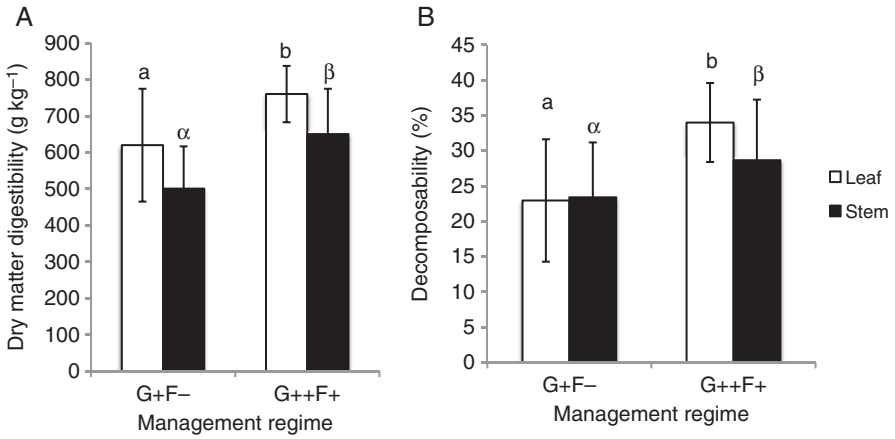

FIG. 3. Average values of (A) dry matter digestibility and (B) decomposability for species of the two management regimes and for leaf and stem. Different letters indicate significant differences among management regimes and organs (*post hoc* Student–Newman–Keuls test).

Relationships between digestibility, decomposability and traits

Axis 1 of the PCA on living leaves accounted for 52.4 % of the variation and was primarily positively associated with DMC and NDF was negatively associated with nitrogen concentration (Supplementary Data Appendix S4). Axis 1 of the PCA for living stems accounted for 75.6 % and was determined by similar traits as for the PCA on living leaves (Supplementary Data Appendix S4). Dry matter content was the trait with the highest loading on axis 1 of the PCA (insets in Supplementary Data Appendix S4), explaining 28 % of the variation for leaves and 35 % for stems. The projection of the points on axis 1 was recovered to test correlations with digestibility for traits from living plant material and decomposability for traits from living and litter plant material. Digestibility was negatively related to this axis 1 for both leaves (Fig. 5A) and stems (Fig. 5B) (see Supplementary Data Appendix S3A, D, F, I for bivariate relationships between traits of living organs and digestibility). Decomposability was also strongly and negatively correlated with this first PCA axis for both leaves (Fig. 5C) and stems (Fig. 5D) (see Supplementary Data Appendix S3B, E, G, J for bivariate relationships between traits of living organs and decomposability). For leaf litter and stem litter we found similar results (Supplementary Data Appendix S4) and decomposability was negatively related to this axis for both leaves (Fig. 5E) and stems (Fig. 5F) (see Supplementary Data Appendix S3C, H, K for bivariate relationships between traits of litter and decomposability).

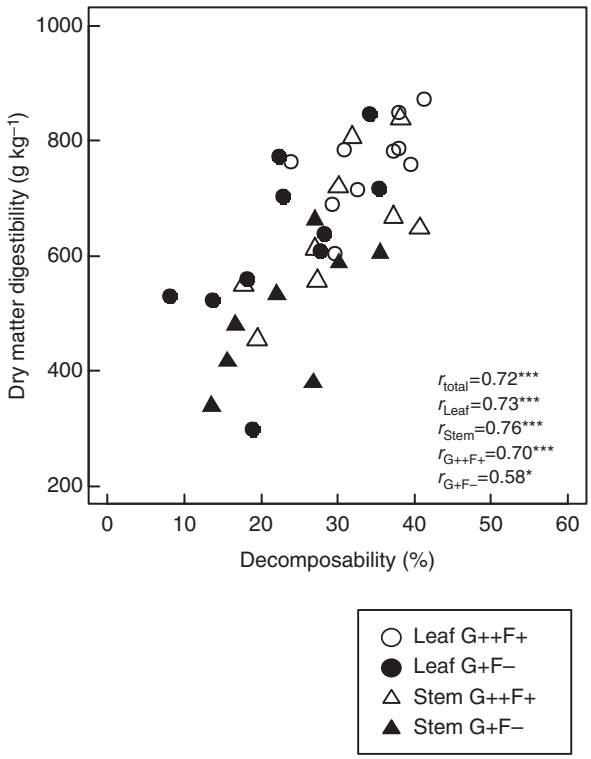

FIG. 4. Relationship between dry matter digestibility and litter decomposability. Solid symbols are for the G+F– treatment, while open symbols are for the G++F+ treatment. The *r* values are Pearson or Spearman correlation coefficients for all data (r_{total}), each organ (r_{leaf} and r_{stem}) and management regime (r_{G+F+} and $r_{G++F–}$). ANCOVA analyses show that the influences of management regimes and organs on slopes and intercepts of the relationships between the two variables are significant only for the effect of management on the intercept (F = 2.99, $P < 0.01$). *$P < 0.05$; ***$P < 0.001$.

Loading of species on axis 1 varied significantly among growth forms. Tussock plants had higher living leaf NDF and DMC and lower NC than rosette plants and extensive and stemmed herbs (F = 11.4***; Fig. 5A, C and Supplementary Data Appendix S4); the PC of their leaf litter was also lower than that of rosette plants and extensive and stemmed herbs (F = 15.9***; Fig. 5E and Supplementary Data Appendix S4). Extensive and stemmed herbs were intermediate for living stems (F = 5.66*; Fig. 5B, D and Supplementary Data Appendix S4). Finally, the litter stems of tussock and rosette plants generally had higher NDF and lower NC than extensive and stemmed herb litter stems (F = 10.1**; Fig. 5F and Supplementary Data Appendix S4).

There was a strong relationship between NDF of leaves and NDF of stems, indicating that fibres were more conserved across the senescing process than nutrients (Supplementary Data Appendix S2).

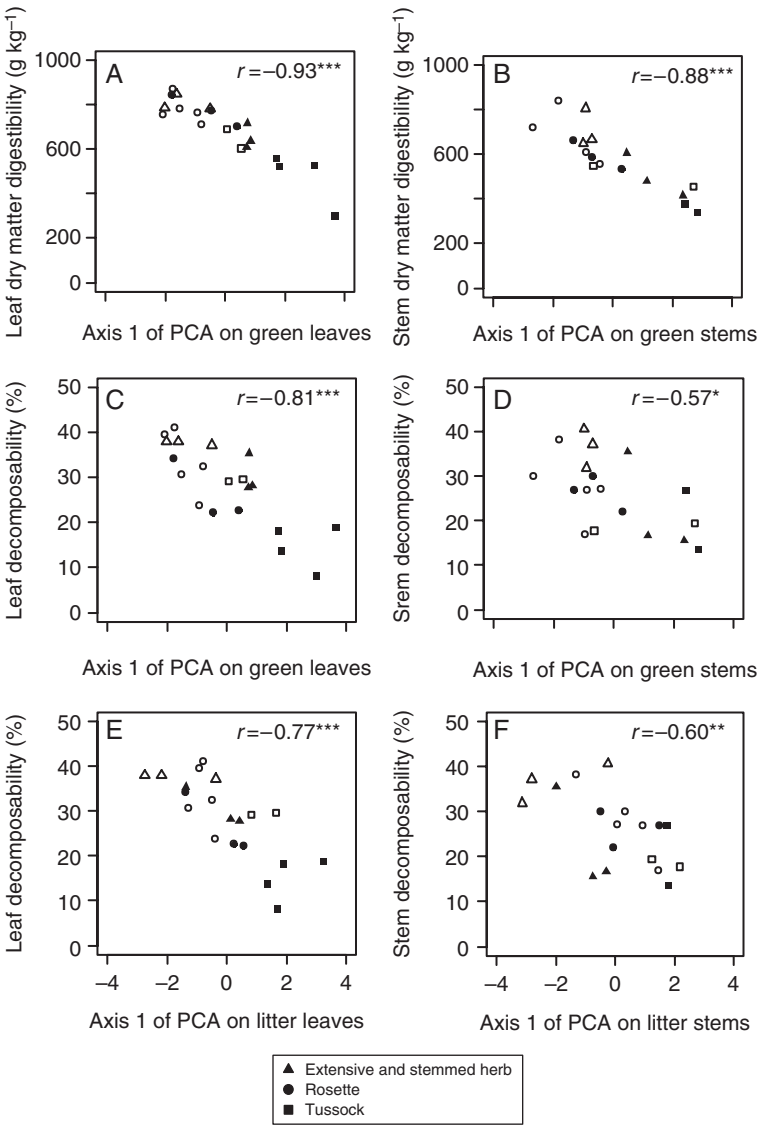

FIG. 5. Relationships between axis 1 of the PCA combining traits of living organs and (A) leaf dry matter digestibility, (B) stem dry matter digestibility, (C) leaf decomposability, (D) stem decomposability, and between axis 1 of PCA combining traits of litter and (E) leaf decomposability and (F) stem decomposability. Open symbols correspond to the G++F+ treatment and solid symbols to the G+F− treatment. The *r* values are Pearson correlation coefficients for all data. Relationships between individual traits and digestibility and litter decomposability are given in Supplementary Data Appendix 3. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Extensive and stemmed herb: see Table 1.

Relative influence of traits on digestibility and decomposability

The regression model showed that the digestibility of leaves was mainly explained by NDF (negative relationship) and nitrogen concentration (positive relationship) (Table 2). NDF was also the most important variable explaining the digestibility of stems, with marginal effects of NC (Table 2). For the two organs, DMC was excluded from the regression models due to the collinearity with several other traits, in particular NDF (Fig. 4, and see Discussion section).

For litter, the regression model showed that nitrogen was the most important variable explaining the decomposability of leaves (Table 2). When traits of living leaves were considered to explain the decomposability of the corresponding litter, PC, NDF and nitrogen presented higher relative importance than DMC (Table 2). Decomposability of stems was more tightly related to NDF in both regression models considering the traits of litter or living organs (Table 2).

DISCUSSION

Digestibility–decomposability relationships

The first objective of our study was to test the afterlife effect hypothesis (Grime and Anderson, 1986) across different organs for rangeland species found in contrasted management regimes. The strong positive relationship between the two degradation processes supported this hypothesis, which postulates that highly defended leaves have low digestibility and produce more

TABLE 2. *Effects of functional traits on dry matter digestibility and decomposability for leaves and stems. The table shows* t *values from multiple linear regression models, and with their relative importance in dry matter digestibility or decomposability variation (%). The* R^2 *adjusted results shown are from best model after traits selection.*

		Dry matter digestibility				Decomposability			
		Leaf		Stem		Leaf		Stem	
		Effect	Relative importance	Effect	Relative importance	Effect	Relative importance	Effect	Relative importance
Green trait	DMC					2.80*	17.1		
	N	3.23**	32.8			4.23***	26.6		
	NDF	−7.77***	67.2	−3.03**	92.5	−4.37***	27.4	−4.12***	100
	P					4.65	28.8		
	Adjusted R^2	0.91		0.42		0.85		0.48	
Litter trait	N								
	NDF					3.65**	100	−4.12***	100
	P								
	Tannins								
	Adjusted R^2					0.42		0.50	

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant.

recalcitrant litter, leading to low decomposition rates (Melillo *et al.*, 1982; Grime *et al.*, 1996; Cornelissen *et al.*, 1999, 2004; Hättenschwiler and Vitousek, 2000). This hypothesis had only been tested in two studies in the context of vertebrate herbivores (Cornelissen *et al.*, 2004; White *et al.*, 2004) and only on leaves. Here we found a single general relationship between the two processes for both leaves and stems, and across management regimes, suggesting that afterlife effects are maintained whatever the source of variation inducing differences in the quality of plant material.

The range of variation observed in our study conducted on 16 species from Mediterranean rangelands was comparable to that observed in the study by Cornelissen *et al.* (2004) on 32 subarctic plant species (≈300–872 versus 350–800 g kg^{-1} for leaf digestibility, respectively; ≈8.13–41.3 % in microcosm versus 20–80 % *in situ* for leaf litter mass loss, respectively). Our results were in line with previous studies showing that dicotyledons, composed mainly of rosettes and extensive and stemmed herbs, presented higher digestibility (Duru, 1997) and decomposability than monocotyledons (Cornelissen *et al.*, 1999, 2004; Pérez-Harguindeguy *et al.*, 2000; Cornwell *et al.*, 2008; Pálková and Lepš, 2008). In a simultaneous multi-species comparison of decomposition rates of leaf litters in a temperate flora, graminoid monocots showed on average lower litter decomposition rates than herbaceous dicots (Cornelissen, 1996). Graminoid monocots have generally physically tougher leaves with greater silicon contents than herbaceous dicots, and this corresponds with the lesser decomposability of the former (Cornelissen and Thompson, 1997).

Our results showed that digestibility of leaves was 1.2 times higher than that of stems, in agreement with previous studies (Duru, 1997; Bidlack *et al.*, 1999; Arzani *et al.*, 2004; Karn *et al.*, 2006; Duru *et al.*, 2008; Beecher *et al.*, 2013): leaves are rich in nitrogen and soluble compounds that are easily degradable, while stems contained more fibre and structural tissue (Poorter and Bergkotte, 1992; Duru *et al.*, 2000; Duru, 2003). However, decomposability was similar for both organs (8.13–41.3 % for leaves and 13.5–35.2 % for stems), whereas we expected leaves to decompose faster than stems (Semmartin and Ghersa, 2006; Freschet *et al.*, 2012, 2013). In stems, there is an increase in cell-wall concentration and a decrease in soluble cells during development (Buxton, 1996), making basal segments more digestible than upper segments (Pritchard *et al.*, 1963).

High grazing and fertilization in the G++F+ treatment induced a modification in species composition over time in these rangelands (Chollet *et al.*, 2014), resulting in species with higher digestibility and decomposability in the G++F+ than in the G+F− treatment. We hypothesized that more intensive management practices hastened plant development in relation to faster growth and in relation to changes in chemical composition, such as an increase in nitrogen concentration (Pontes *et al.*, 2007; Carrère *et al.*, 2010; Lavorel and Grigulis, 2012) and a decrease in fibre content (Duru and Ducrocq, 2002; Carrère *et al.*, 2010; Lavorel and Grigulis, 2012). This hypothesis was confirmed here: the G++F+ treatment was dominated by exploitative species with high nitrogen concentration and low fibre content, likely to allow compensatory growth after grazing (Herms and Mattson, 1992); conversely, the G+F− treatment was dominated by conservative species with high leaf DMC (see also Pontes *et al.*, 2007), tannin concentration and fibre content (see also De Bello *et al.*, 2010), protecting plants from herbivory via efficient defence mechanisms (Herms and Mattson, 1992). Thus, the response of plant traits to grazing and fertilization induced changes both in the digestibility of living organs and in the decomposability of litter whose quality was dependent on these traits.

Traits related to digestibility or decomposability

The second objective of this study was to determine which traits explained variations in digestibility and decomposability. Dry matter content was the trait contributing most to explaining variation of the first axis of the PCA analysis for living leaves and stems. This axis was strongly correlated to both digestibility and decomposability. However, DMC was excluded from the regression model as it was strongly correlated to NDF. Among

the measured traits, NC and NDF had strong explanatory power: high NC represents high nutritional quality and has been shown to influence digestibility (Al Haj Khaled *et al.*, 2006; Karn *et al.*, 2006; Pontes *et al.*, 2007; Lavorel and Grigulis, 2012) and decomposability (Pérez-Harguindeguy *et al.*, 2000; Bakker *et al.*, 2011; Dias *et al.*, 2013); conversely, NDF is effective for anti-herbivore defence (Coley, 1988), affecting negatively both digestibility (Al Haj Khaled *et al.*, 2006; Bumb *et al.*, 2016) and decomposability (Cobo *et al.*, 2002; Wardle *et al.*, 2002; White *et al.*, 2004). Digestibility was more strongly related to traits of living leaves (NC, PC and NDF) than decomposability, which was expected since digestibility is a property of living material. Changes in the properties of plant material during the senescence process (Supplementary Data Appendix S2) probably explain the weaker relationships with decomposability. Surprisingly, we also found that the decomposability of litter was more tightly related to the traits of living leaves (NDF, nitrogen and phosphorus concentrations) than to those of litter, which was unexpected. We currently do not have any convincing explanation for this, especially since NDF of litter would logically have a more direct effect on litter decomposition (Supplementary Data Appendix S2). A possible explanation for this may be that decomposition is affected by traits of living organs, as these traits control the phyllosphere/microbiota on organs and hence control, at least, the beginning of decomposition.

Dry matter content as a functional marker of degradation processes

Traits related to the morpho-anatomy of living organs, such as NDF and DMC, were tightly correlated and were good predictors of differences in digestibility and decomposability. Other studies further confirm the good association between NDF and DMC in a range of temperate grassland species (Al Haj Khaled *et al.*, 2006; Pontes *et al.*, 2007) (Fig. 6). However, in our analysis DMC of plant organs was excluded from the regression model used to assess the relative effects of the different traits on the two degradation processes studied, due to the collinearity between DMC and several other traits, including NDF.

A high DMC of leaves indicates a high proportion of dense tissues (sclerenchyma and vascular bundles) in the leaf volume (Garnier and Laurent, 1994), which is probably the reason for the low digestibility and decomposability of such leaves. Leaf DMC has recurrently been found to relate to digestibility on the one hand (Al Haj Khaled *et al.*, 2006; Pontes *et al.*, 2007; Ansquer *et al.*, 2009; Gardarin *et al.*, 2014) and to decomposability on the other hand (Cornelissen *et al.*, 2004; Kazakou *et al.*, 2006), both at the species and the community level. Therefore, and even if NDF exerts a strong control on digestibility and decomposability, we suggest that DMC, which is far easier and quicker to determine, can safely be used as an efficient marker of both degradation processes.

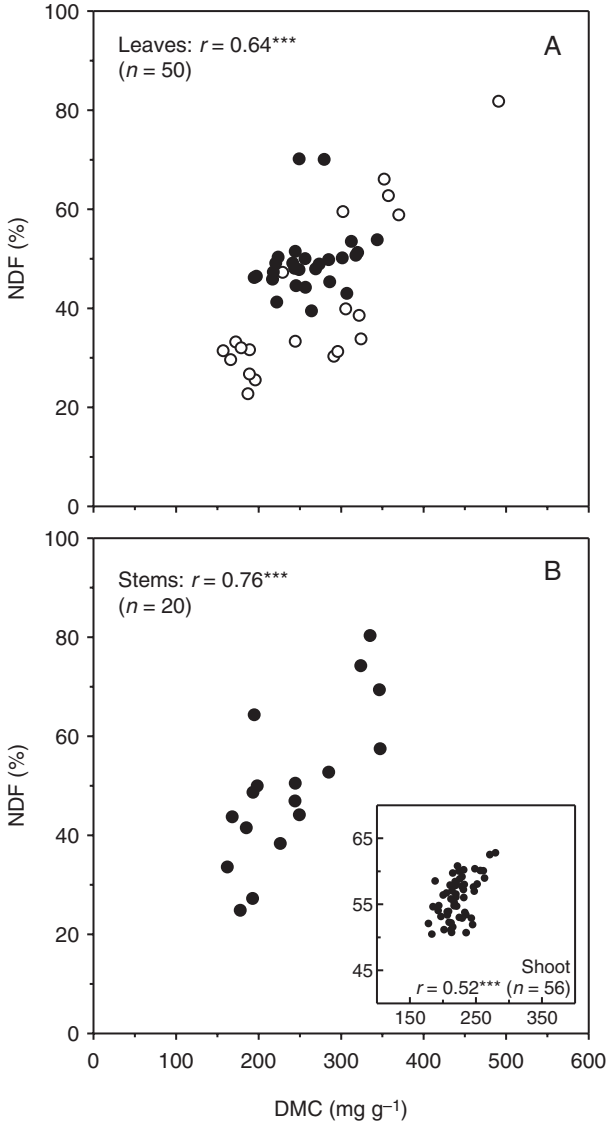

FIG. 6. Relationship between dry matter content and fibre concentration of (A) leaves – combination of data from this study (open circles) and from Al Haj Khaled *et al.* (2006) obtained for 15 grass species from temperate permanent grasslands grown under two nitrogen levels (solid circles) – and (B) stems (this study). The inset in (B) shows the relationship between dry matter content measured on leaves and fibre concentration measured on whole shoots for 14 grass species from temperate permanent grasslands grown under two nitrogen levels and cutting frequencies (Pontes *et al.* 2007). The number of data points (n) and the values of Pearson's correlation coefficients (r) are given for each relationship. ***$P < 0.001$.

CONCLUSIONS

The broad scope of this work allows us to demonstrate a strong positive relationship between digestibility and decomposability across species, organs and management regimes, thereby widening the generality of the afterlife effects hypothesis. Our findings provide further support for the key role played by foliar traits in the link between plant and soil via the decomposition pathway. Traits measured on living organs can be used to predict both digestibility and decomposability, and these two degradation processes are governed by similar traits describing the structural composition of organs, fibres in particular. We also demonstrated the importance of stems for nutrient and carbon cycling and thus ecosystem functioning. Finally, we showed that fertile habitats support high herbivory and cause positive feedbacks in such ecosystems because species typical of such habitats have high crude protein content, are more digestible and produce litter that decomposes faster. Infertile habitats support low herbivory and cause negative feedbacks because

species typical of such infertile habitats have high amounts of structural carbohydrates and more persistent and less digestible organs, and produce recalcitrant litters with low decomposition.

SUPPLEMENTARY DATA

Supplementary data are available at https://academic.oup.com/aob and consist of the following. Supplementary Data Appendix S1: correlations between dry matter digestibility and decomposability with functional traits (dry matter, nitrogen, phosphorus, NDF and tannin contents) measured on green and litter material. Appendix S2: Spearman ranking coefficients between green and litter traits (for nitrogen and phosphorus concentrations, NDF and tannin concentration) measured on leaves and stems. An empty cell indicates that the correlation was not tested due to absence of measurements on green or litter plant material. Appendix S3: relationships between dry matter digestibility and (A) green NDF, (D) green DMC, (F) green nitrogen content and (I) green phosphorus content; between decomposability and (B) green NDF, (E) green DMC, (G) green nitrogen content and (J) green phosphorus content; and between decomposability and (C) litter NDF, (H) litter nitrogen content and (K) litter phosphorus content. The *r* values are Pearson or Spearman correlation coefficients for all data. Appendix S4: PCA between functional traits (NDF and dry matter, nitrogen, phosphorus and tannin concentrations) measured (A) on living leaves, (B) leaf litter, (C) living stems and (D) stem litter.

ACKNOWLEDGEMENTS

We thank J. Richarte for his help in the field and laboratory. All digestibility measurements were conducted in collaboration with D. Bastianelli, and with technical help from L. Bonnal and E. Baby (UMR SELMET, CIRAD of Montpellier). Chemical analyses were conducted at the Plateforme d'Analyses Chimiques en Ecologie (PACE) and the decomposition experiment was carried out in the Terrain d'Expériences (TE) facilities, both at the Centre d'Ecologie Fonctionnelle et Evolutive (CEFE-CNRS), Montpellier, France. J. Devaux helped prepare the microcosms and conduct the controlled chamber decomposition experiment. We thank the technical staff of the experimental station INRA La Fage for access to facilities. This work was funded by the EC2CO CASCADE (INSU CNRS) project. I.B. was supported by a doctoral fellowship from Montpellier Supagro.

LITERATURE CITED

Aerts R, **Chapin FS III**. **2000**. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research* **30**: 1–67.

Al Haj Khaled R, **Duru M**, **Decruyenaere V**, **Jouany C**, **Cruz P. 2006**. Using leaf traits to rank native grasses according to their nutritive value. *Rangeland Ecological Management* **59**: 648–654.

Andrés S, **Giráldez JF**, **López S**, **Mantecón ÁR**, **Calleja A. 2005**. Nutritive evaluation of herbage from permanent meadows by near-infrared reflectance spectroscopy: 1. Prediction of chemical composition and *in vitro* digestibility. *Journal of the Science of Food and Agriculture* **85**: 1564–1571.

Ansquer P, **Duru M**, **Theau JP**, **Cruz P. 2009**. Functional traits as indicators of fodder provision over a short time scale in species-rich grasslands. *Annals of Botany* **103**: 117–126.

Arzani H, **Zohdi M**, **Fish E**, ***et al.*** **2004**. Phenological effects on forage quality of five grass species. *Journal of Range Management* **57**: 624–629.

Aufrère J, **Graviou D**, **Demarquilly C**, **Perez JM**, **Andrieu J. 1996**. Near infrared reflectance spectroscopy to predict energy value of compound feeds for swine and ruminants. *Animal Feed Science and Technology* **62**: 77–90.

Aufrère J, **Baumont R**, **Delaby L**, ***et al.*** **2007**. Prévision de la digestibilité des fourrages par la méthode pepsine-cellulase. Le point sur les équations proposées. *INRA Productions Animales* **20**: 129–136.

Bakker MA, **Carreño-Rocabado G**, **Poorter L. 2011**. Leaf economics traits predict litter decomposition of tropical plants and differ among land use types. *Functional Ecology* **25**: 473–483.

Barkaoui K, **Bernard-Verdier M**, **Navas M-L. 2013**. Questioning the reliability of the point intercept method for assessing community functional structure in low-productive and highly diverse Mediterranean grasslands. *Folia Geobotanica* **48**: 393–414.

Bates D, **Mächler M**, **Bolker B**, **Walker S. 2015**. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.

Bradstreet RB. 1954. Kjeldahl method for organic nitrogen. *Analytical Chemistry* **26**: 185–187.

Beecher M, **Hennessy D**, **Boland TM**, **McEvoy M**, **O'Donovan M**, **Lewis E. 2013**. The variation in morphology of perennial ryegrass cultivars throughout the grazing season and effects on organic matter digestibility. *Grass and Forage Science* **70**: 19–29.

De Bello F, **Lavorel S**, **Díaz S**, ***et al.*** **2010**. Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity and Conservation* **19**: 2873–2893.

Berendse F. 1994. Competition between plant populations at low and high nutrient supplies. *Oikos* **71**: 253–260.

Bernard C. 1996. *Flore des causses hautes terres, gorges, valées et vallons (Aveyron, Lozère, Hérault et Gard)*. Saint-Sulpice-de-Royan: Société Botanique du Centre-Ouest.

Bernard-Verdier M, **Navas M-L**, **Vellend M**, **Violle C**, **Fayolle A**, **Garnier E. 2012**. Community assembly along a soil depth gradient: contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. *Journal of Ecology* **100**: 1422–1433.

Bidlack JE, **Buxton DR. 1992**. Content and deposition rates of cellulose, hemicellulose, and lignin during regrowth of forage grasses and legumes. *Canadian Journal of Plant Science* **72**: 809–818.

Bidlack JE, **Vaughan JE**, **Dewald CL 1999**. Forage quality of 10 eastern gamagrass [*Tripsacum dactyloides* (L.) L.] genotypes. *Journal of Range Management* **52**: 661–665.

Birth GS, **Hecht HG. 1987**. The physics of near infrared reflectance In: Williams P, Norris K. eds. *Near-infrared technology in agricultural and food industry*. St Paul, MN: American Association of Cereal Chemists, 1–6.

Birouste M, **Kazakou E**, **Blanchard A**, **Roumet C. 2012**. Plant traits and decomposition: are the relationships for roots comparable to those for leaves? *Annals of Botany* **109**: 463–472.

Bryant JP, **Provenza FD**, **Pastor J**, **Reichard PB**, **Clausen TP**, **Dutoit JT. 1991**. Interactions between woody plants and browsing mammals mediated by secondary metabolites. *Annual Review of Ecological Systems* **22**: 431–446.

Bumb I, **Garnier E**, **Bastianelli D**, **Richarte J**, **Bonnal L**, **Kazakou E. 2016**. Influence of management regime and harvest date on the herbage quality of species from Mediterranean rangelands: the importance of dry matter content. *AoB Plants* **8**: doi: 10.1093/aobpla/plw045.

Buxton DR. 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology* **59**: 37–49.

Carrère P, **Pontes L. da S**, **Andueza D**, ***et al.*** **2010**. Evolution de la valeur nutritive de graminées prairiales au cours de leur cycle de développement. *Fourrages* **201**: 27–35.

Chollet S, **Rambal S**, **Fayolle A**, **Hubert D**, **Foulquié D**, **Garnier E. 2014**. Combined effects of climate, resource availability, and plant traits on biomass produced in a Mediterranean rangeland. *Ecology* **95**: 737–748.

Cobo JG, **Barrios E**, **Kass DCL**, **Thomas RJ. 2002**. Decomposition and nutrient release by green manures in a tropical hillside agroecosystem. *Plant and Soil* **240**: 331–342.

Coley PD. 1988. Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia* **74**: 531–536.

Cornelissen JHC. 1996. An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. *Journal of Ecology* **84**: 573–582.

Cornelissen JHC, **Thompson K. 1997**. Functional leaf attributes predict litter decomposition rate in herbaceous plants. *New Phytologist* **135**: 109–114.

Cornelissen JHC, **Pérez-Harguindeguy N**, **Diaz S**, ***et al.* 1999**. Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. *New Phytologist* **143**: 191–200.

Cornelissen JHC, **Quested HM**, **Gwynn-Jones D**, ***et al.* 2004**. Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. *Functional Ecology* **18**: 779–786.

Cornwell WK, **Cornelissen JHC**, **Amatangelo K**, ***et al.* 2008**. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* **11**: 1065–71.

Cortez J, **Garnier E**, **Pérez-Harguindeguy N**, **Debussche M**, **Gillon D. 2007**. Plant traits, litter quality and decomposition in a Mediterranean old-field succession. *Plant and Soil* **296**: 19–34.

Dias T, **Oakley S**, **Alarcón-Gutiérrez E**, ***et al.* 2013**. N-driven changes in a plant community affect leaf-litter traits and may delay organic matter decomposition in a Mediterranean maquis. *Soil Biology and Biochemistry* **58**: 163–171.

Dray S, **Dufour AB. 2007**. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* **22**: 1–20.

Duru M. 1997. Leaf and stem *in vitro* digestibility for grasses and dicotyledons of meadow plant communities in spring. *Journal of the Science of Food and Agriculture* **74**: 175–185.

Duru M. 2003. Effect of nitrogen fertiliser rates and defoliation regimes on the vertical structure and composition (crude protein content and digestibility) of a grass sward. *Journal of the Science of Food and Agriculture* **83**: 1469–1479.

Duru M, **Ducrocq H. 2002**. A model of lamina digestibility of orchardgrass as influenced by nitrogen and defoliation. *Crop Science* **42**: 214–223.

Duru M, **Delprat V**, **Fabre C**, **Feuillerac E. 2000**. Effect of nitrogen fertiliser supply and winter cutting on morphological composition and herbage digestibility of a *Dactylis glomerata* L sward in spring. *Journal of the Science of Food and Agriculture* **80**: 33–42.

Duru M, **Cruz P**, **Theau J-P. 2008**. Un modèle générique de digestibilité des graminées des prairies semées et permanentes pour raisonner les pratiques agricoles. *Fourrages* **193**: 79–102.

Freschet GT, **Cornelissen JHC**, **van Logtestijn RSP**, **Aerts R. 2010**. Substantial nutrient resorption from leaves, stems and roots in a subarctic flora: what is the link with other resource economics traits? *New Phytologist* **186**: 879–889.

Freschet GT, **Aerts R**, **Cornelissen JHC. 2012**. A plant economics spectrum of litter decomposability. *Functional Ecology* **26**: 56–65.

Freschet GT, **Cornwell WK**, **Wardle DA**, ***et al.* 2013**. Linking litter decomposition of above- and below-ground organs to plant-soil feedbacks worldwide. *Journal of Ecology* **101**: 943–952.

Gardarin A, **Garnier E**, **Carrère P**, ***et al.* 2014**. Plant trait-digestibility relationships across management and climate gradients in permanent grasslands. *Journal of Applied Ecology* **51**: 1207–1217.

Garnier E, **Laurent G. 1994**. Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. *New Phytologist* **128**: 725–736.

Garnier E, **Shipley B**, **Roumet C**, **Laurent G. 2001**. A standardized protocol for the determination of specific leaf area and leaf dry matter content. *Functional Ecology* **15**: 688–695.

Garnier E, **Navas M-L**, **Grigulis K. 2016**. *Plant functional diversity. Organism traits, community structure, and ecosystem properties*. Oxford: Oxford University Press.

Grime JP. 1979. *Plant strategies and vegetation processes*. John Wiley & Sons: Chichester.

Grime JP, **Anderson JM. 1986**. Environmental controls over organism activity. Forest ecosystems in Alaskan taiga: a synthesis of structure and function. In: Van Cleve K, Chapin FS III, Flanagan PW, Vierek LA, Dyrness CT. eds. *Ecological studies*. Berlin: Springer, 89–95.

Grime PJ, **Cornelissen JHC**, **Thompson K**, **Hodgson JG. 1996**. Evidence of a causal connection between anti-herbivore defence and the decomposition rate of leaves. *Oikos* **77**: 489–494.

Grimshaw HM, **Allen SE**, **Parkinson JA. 1989**. Nutrient elements. In: Allen SE. ed. *Chemical analysis of ecological materials*. Oxford: Blackwell, 81–159.

Halekoh U, **Højsgaard S. 2014**. A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models. The R package pbkrtest. *Journal of Statistical Software* **59**: 1–32.

Hättenschwiler S, **Vitousek PM. 2000**. The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology & Evolution* **15**: 238–243.

Herms DA, **Mattson WJ. 1992**. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* **67**: 283–335.

Hobbie SE. 1992. Effects of plant species on nutrient cycling. *Trends in Ecology & Evolution* **7**: 336–9.

Karn JF, **Berdahl JD**, **Frank AB. 2006**. Nutritive quality of four perennial grasses as affected by species, cultivar, maturity, and plant tissue. *Agronomy Journal* **98**: 1400–1409.

Kazakou E, **Vile D**, **Shipley B**, **Gallet C**, **Garnier E. 2006**. Co-variations in litter decomposition, leaf traits and plant growth in species from a Mediterranean old-field succession. *Functional Ecology* **20**: 21–30.

Kazakou E, **Violle C**, **Roumet C**, **Pintor C**, **Gimenez O**, **Garnier E. 2009**. Litter quality and decomposability of species from a Mediterranean succession depend on leaf traits but not on nitrogen supply. *Annals of Botany* **104**: 1151–1161.

Kutner M, **Nachtsheim C**, **Neter J. 2004**. *Applied linear regression models*. New York: McGraw-Hill.

Lavorel S, **Grigulis K. 2012**. How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. *Journal of Ecology* **100**: 128–140.

Louault F, **Pillar VD**, **Aufrère J**, **Garnier E**, **Soussana J-F. 2005**. Plant traits and functional types in response to reduced disturbance in a semi-natural grassland. *Journal of Vegetation Science* **16**: 151–160.

Melillo JM, **Aber JD**, **Muratore JF. 1982**. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* **63**: 621–626.

Onoda Y, **Westoby M**, **Adler PB**, ***et al.* 2011**. Global patterns of leaf mechanical properties. *Ecology Letters* **14**: 301–12.

Pálková K, **Lepš J. 2008**. Positive relationship between plant palatability and litter decomposition in meadow plants. *Community Ecology* **9**: 17–27.

Pérez-Harguindeguy N, **Diaz S**, **Cornelissen JHC**, ***et al.* 2000**. Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. *Plant and Soil* **218**: 21–30.

Pérez-Harguindeguy N, **Díaz S**, **Garnier E**, ***et al.* 2013**. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* **61**: 167–234.

Pontes L da S, **Soussana J-F**, **Louault F**, **Andueza D**, **Carrère P. 2007**. Leaf traits affect the above-ground productivity and quality of pasture grasses. *Functional Ecology* **21**: 844–853.

Poorter H, **Bergkotte M. 1992**. Chemical composition of 24 wild species differing in relative growth rate. *Plant, Cell & Environment* **15**: 221–229.

Pritchard GI, **Folkins LP**, **Pigden WJ. 1963**. The *in vitro* digestibility of whole grasses and their parts at progressive stages of maturity. *Canadian Journal of Plant Science* **43**: 79–87.

R Development Core Team. **2010**. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Semmartin M, **Ghersa CM. 2006**. Intraspecific changes in plant morphology, associated with grazing, and effects on litter quality, carbon and nutrient dynamics during decomposition. *Austral Ecology* **31**: 99–105.

Stuth J, **Jama A**, **Tolleson DG. 2003**. Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. *Field Crops Research* **84**: 45–56.

Van Soest PJ, **Robertson JB**, **Lewis BA. 1991**. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–3597.

Taylor B, **Parkinson D. 1988**. A new microcosm approach to litter decomposition studies. *Canadian Journal of Botany* **66**: 1933–1939.

Wardle DA, **Barker GM**, **Bonner KI**, **Nicholson KS. 1998**. Can comparative approaches based on plant ecophysiological traits predict the nature of biotic interactions and individual plant species effects in ecosystems? *Journal of Ecology* **86**: 405–420.

Wardle DA, **Bonner KI**, **Barker GM. 2002**. Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. *Functional Ecology* **16**: 585–595.

Wardle DA, **Bardgett RD**, **Klironomos JN**, **Setälä H**, **Van Der Putten WH. 2004**. Ecological linkages between aboveground and belowground biota. *Science* **304**: 1629–1633.

Waterman PG, **Mole S. 1994**. *Analysis of phenolic plant metabolites*. Oxford: Blackwell.

White TA, **Barker DJ**, **Moore KJ. 2004**. Vegetation diversity, growth, quality and decomposition in managed grasslands. *Agriculture, Ecosystems & Environment* **101**: 73–84.