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RESEARCH ARTICLE

Responses of leaf biomechanics and underlying traits to rangeland management differ between graminoids and forbs

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Abstract

Question: Although leaf biomechanical properties have been identified as critical traits for plant-herbivore interactions, their responses to grazing pressure have been poorly investigated. Intensification of rangeland management, associated with fertilization and an increase in grazing pressure, has been shown to favour fast-growing species that can compensate for biomass losses due to grazing. According to the postulated trade-off between resource acquisition and defence, it is often expected that acquisitive traits should be associated with low leaf mechanical resistance. Here we investigated the responses of two leaf biomechanical traits, and their underlying traits, to management intensification.

Location: We used a long-term experiment in a rangeland located in the Mediterranean region of Southern France, in which three treatments corresponding to different fertilizer inputs and sheep grazing pressures were established.

Methods: We sampled 24 abundant graminoid and forb species. The responses of work to shear and force to tear to the treatments were tested together with those of growth-related leaf traits (leaf mass per area, dry matter content). To better understand the observed patterns, we tested whether the difference between species' leaf biomechanics could be explained by morpho-anatomical characteristics such as leaf thickness and density.

Results: Consistent with the acquisition–defence trade-off hypothesis, we found that graminoids from fertilized and intensely grazed areas had lower leaf resistance than those in ungrazed areas. However, no difference in leaf biomechanics was found in forbs despite a significant decrease in leaf mass per area and leaf dry matter content with management intensification. Consistent with this, we found no significant effect of morpho-anatomical traits on either biomechanical trait in forbs.

Conclusions: Our results suggest that the observed responses in graminoids result from phenotypic constraints between resource acquisition and biomechanical defence. However, these phenotypic constraints appeared to be released in forbs, questioning the idea of a universal relationship between these two functions.

KEYWORDS
fertilization, growth–defence trade-off, leaf biomechanics, leaf toughness, plant–herbivore interactions, rangeland management, sheep grazing, trait responses

1 | INTRODUCTION

Grazing and fertilization are known to strongly affect the functional composition of grasslands (Díaz et al., 2007; Garnier et al., 2016 and references therein). In plants, trait combinations selected under grazing conditions (or resistance strategies sensu Belsky et al., 1993) are expected to vary depending on grazing intensity and frequency, herbivore selectivity and resource availability (Noy-Meir et al., 1989; Vesk & Westoby, 2001; Garcia et al., 2003; Pakeman, 2004). Whereas high grazing intensity – particularly under low resource availability – often promotes defence strategies involving chemical or morphological traits that deter herbivores (Dyksterhuis, 1949; Tobler et al., 2003), extensive grazing mostly favours avoidance or tolerance strategies associated with a small stature or a high rate of regrowth, respectively (Díaz et al., 2001). Under sufficient resource availability, a few studies have shown that species favoured by grazing tend to have high leaf nutrient content, and low leaf mass per area (LMA) and leaf dry matter content (LDMC) (Díaz et al., 2001; Cingolani et al., 2005; Bumb et al., 2016; Garnier et al., 2018) typical of fast resource-acquisition strategies. It suggests that tolerance strategies are prevalent in these systems providing a beneficial feedback loop on forage quality: grazing favours tolerant species against herbivores that are nutritionally valuable for grazers (Coley et al., 1985; Duru et al., 2014; Bumb et al., 2016). This concept has been sometimes referred to as "grazing (or browsing) lawns" (McNaughton, 1984; Fornara & Du Toit, 2007).

Leaf biomechanics has been identified as a critical effect trait for plant–herbivore interactions (Feeny, 1976; Coley, 1983; Choong, 1996) because tough leaves tend to be less palatable (Massey et al., 2007; see Pérez-Harguindeguy et al., 2003 for rangeland species). Although conceptually integrated within the framework of resistance strategies to herbivory (Belsky et al., 1993; Boege & Marquis, 2005), the response of leaf biomechanics to grazing has been poorly investigated compared with other leaf traits (but see Cingolani et al., 2005; Díaz et al., 2001; Gorné & Díaz, 2022; Streit et al., 2022). Two distinct pathways are likely to link leaf biomechanics to resistance strategies against herbivory: (1) defence against herbivory should be associated with high leaf toughness, whereas (2) given sufficient nutrient availability, tolerance should be associated with low leaf toughness because of the well-established trade-off, at the leaf level, between fast rates of resource acquisition (associated with low LMA, LDMC and high leaf nitrogen content) and investment in structural tissue (Coley, 1988; Herms & Mattson, 1992; Coley & Barone, 1996; Züst & Agrawal, 2017).

It is often assumed that morpho-anatomical traits, such as LMA and LDMC, are good surrogates for leaf biomechanics. Notably, LMA is used as a simple sclerophylly index for practical reasons despite leaf biomechanical properties being more directly related to the sclerophylly syndrome (Cowling & Campbell, 1983; Salleo & Nardini, 2000). Leaf biomechanical properties depend on leaf thickness (LT) and the material properties of the leaf (Onoda et al., 2011). Because LMA can be written as the product of LT and leaf tissue density (Vile et al., 2005), it appears as an integrative trait that is expected to relate positively to leaf toughness. By contrast, LDMC is a good surrogate for leaf tissue density (Garnier & Laurent, 1994; Nadal et al., 2023) and is expected to relate to leaf material properties (Onoda et al., 2011). In a global analysis, Onoda et al. (2011) confirmed these relationships by showing significant effects of LMA and LT on three leaf biomechanical traits, and LDMC on the corresponding material properties. However, this analysis also revealed that a given mechanical resistance can be associated with a wide range of LMA or LDMC. For example, a force to tear of ca. 1 kN/m has been measured on many species across taxonomic and functional groups, spanning a range of LMA from 20 to more than 200 g/m^2 (Onoda et al., 2011). Therefore, one can expect that the responses of morpho-anatomical traits (e.g., LMA, LT and LDMC) to management practices can be decoupled from leaf biomechanics, especially when integrating species from various taxonomic groups (grasses and forbs in the case of rangeland).

Here, we investigated the response of two leaf biomechanical traits in herbaceous species growing under various intensities of grazing and fertilization in a Mediterranean rangeland. We used two traits commonly used to assess leaf biomechanics, namely work to shear (WS) and force to tear (FT) (Onoda et al., 2011). Henry et al. (1996) suggested that these traits relate to two contrasted defence mechanisms against grazers: whereas a high FT may be important during the prehension of the leaf, shearing resistance may be important during chewing. Interestingly, a worldwide compilation of these traits revealed a strong bias in the choice of mechanical tests depending on plant growth forms (Onoda et al., 2011). Tearing tests have been commonly used on herbaceous species, unlike shearing tests, which have been conducted mainly on shrubs and trees. Although the correlation between tearing and shearing traits has been tested in woody species (Read & Sanson, 2003; Caldwell et al., 2016; Enrico et al., 2016), to our knowledge, our study is among the first to compare the two biomechanical traits in herbaceous species. It allowed us to assess whether their individual responses to rangeland management are consistent and how they covary.

Using a permanent rangeland that has traditionally been extensively grazed, Bumb et al. (2016) has shown how management intensification (associated with fertilization and an increase in grazing pressure) tended to favour species with tolerance strategies (high leaf nitrogen content and low LDMC and fibre content). It was further demonstrated that LMA was a good predictor of species' demographic parameters in response to intensification (Garnier et al., 2018). Here, from the same study site, we compared the responses of LMA and LDMC to management intensification with those of leaf biomechanics to address the following questions: Are tolerance strategies against grazing resulting from management intensification associated with lower leaf toughness, in agreement with the acquisition–defence trade-off? Are results consistent for both leaf biomechanical traits, and among taxonomic groups? How do leaf biomechanical traits relate to morpho-anatomical traits in this grazed system?

We expect a decrease in LMA and LDMC with management intensification due to the selection of tolerant species to herbivory. We expect WS and FT to be correlated with LMA and LDMC, and therefore to also decrease with management intensification. However, because abundant species in our study site belong to various functional and taxonomic groups, we can also expect no correlation or a weak correlation between leaf biomechanics and resource–economics traits. The latter would result in decoupled responses of leaf biomechanics and resource–economics traits to the treatments.

2 | METHODS

2.1 | Study site, species selection and sampling

The study was conducted in the Mediterranean rangeland of the INRA "La Fage" experimental station (43°55′ N, 3°05′ E, 800 m above sea level). Since 1972, this 280-ha rangeland has been grazed by a sheep flock (Romane breed) raised outdoors year-round for meat production. Traditionally, it has been extensively grazed (corresponding to removal of ca. 20% of the total above-ground productivity) without fertilization (treatment GU, hereafter). In 1978, 18 ha were converted into three fertilized (65 kg/ha of nitrogen every year and 40 kg/ha of phosphorus every 3 years) and intensively grazed (removal of ca. 60% of the total productivity) paddocks (treatment G+F, hereafter). Finally, in 1987, 3.8 ha were fenced to exclude sheep grazing in a non-fertilized paddock (treatment UU, hereafter). The long-term surveys of permanent plots located in each treatment demonstrated that plant communities progressively diverged from close species compositions after the establishment of the treatments, especially in G+F (Appendix S1 for more details, and Garnier et al., 2018). Differences in trait values observed in the current study can thus be interpreted as a consequence of species changes induced by differences in management regimes, although the effects of minor differences in initial species composition and environmental characteristics (topography, soil type, etc.) cannot be completely ruled out.

Based on vegetation surveys conducted in 2014 (cf. Bumb et al., 2016; Kazakou et al., 2022), 24 species were selected among the most abundant graminoids and forbs in each treatment. Together these species formed between 80 and 93% of the total abundance depending on the treatment (Appendix S2). All selected species have been shown to be positively affected by the treatment (using long-term demographic analyses from Garnier et al., 2018). This allowed us to avoid species maladapted to local conditions but still present because of the inertia of community dynamics. The species list (Table 1) includes graminoids (8 Poaceae and 1 Cyperaceae), which dominate plant communities, and forbs (15 Eudicots species belonging to 7 botanical families). Because some species were selected in different treatments, a total of 38 populations (species × treatment) were harvested (Table 1 and Appendix S2).

Plant individuals were harvested between 18 May and 22 June 2015, at the peak of vegetation of the corresponding communities and before sheep grazing. Eight individuals per species were selected across a large area within each of the three treatments (ca. 5 ha for UU, ca. 16 ha for GU and ca. 7 ha for G+F). The sampling areas correspond to three paddocks located ca. 800 m from each other. Each selected individual was healthy and had at least two young, but fully developed and undamaged leaves (Pérez-Harguindeguy et al., 2013). Harvested plants were immediately put in test tubes with water and placed in a cooling box. They were then stored at 4°C for at least 12 h to ensure full leaf rehydration (Garnier et al., 2001).

2.2 | Measurements of leaf biomechanical properties

From each harvested individual, two successive, mature and undamaged leaves were selected to perform the mechanical tests. The first leaf was used for a shearing test, whereas the second was used for a tearing test. All measurements were conducted at the AMAP laboratory (Montpellier, France) within two days following plant harvest. For species with compound leaves, mechanical tests were performed on the largest leaflet.

Biomechanical properties are integrated measures that relate to the overall leaf properties. In our study, we quantified two leaf biomechanical properties, namely WS (J/m) and FT (kN/m), expressed per fracture length. They are expected to depend mainly on LMA and LT. By contrast, material properties correspond to biomechanical properties standardized by the leaf thickness, namely specific work to shear (SWS, kJ/m^2) and specific force to tear (SFT, MN/m^2) in our case. They are expressed per section area and are expected to be mainly determined by LDMC.

WS was measured using a universal testing machine (Instron 5940; Instron, Canton, MA, USA), following a protocol adapted from Ang et al. (2008). Each leaf was manually clamped horizontally to 4-mm spaced supports and cut perpendicularly to the midrib using a razor blade. The razor blade was set at 20° to the horizontal allowing to cut the leaf by a vertical displacement of the blade. An incomplete cut (from the leaf edge to the edge of the midrib), was performed at a rate of 10 mm/min. A second pass (blank pass) was subsequently performed to measure the work done against friction and to account for the strain energy stored within the specimen at the end of cutting during the first pass (Figure 1a). The work done in cutting (WS) is the difference in work between the actual and blank passes (Figure 1a). Hence, WS includes both the energy required to initiate the cut and propagate the crack in the leaf. SWS was then calculated by dividing WS by the leaf thickness.

TABLE 1 Description and sampling details of the 24 species used in the study

Species names	Family	Life cycle	Taxonomic group	UU (*n* = 10)	GU (*n* = 14)	G+F (*n* = 14)
Anisantha sterilis	Poaceae	Annual	Graminoid			x
Anthyllis vulneraria	Fabaceae	Annual	Forb	x	x	
Brachypodium pinnatum	Poaceae	Perennial	Graminoid	x	x	
Bromopsis erecta	Poaceae	Perennial	Graminoid	x	x	x
Bromus hordeaceus	Poaceae	Annual	Graminoid			x
Capsella bursa-pastoris	Brassicaceae	Annual	Forb			x
Carex humilis	Cyperaceae	Perennial	Graminoid	x	x	
Coronilla minima	Fabaceae	Perennial	Forb		x	
Erodium cicutarium	Geraniaceae	Annual	Forb			x
Festuca christiani-bernardii	Poaceae	Perennial	Graminoid	x	x	
Geranium molle	Geraniaceae	Annual	Forb			x
Helianthemum apenninum	Cistaceae	Perennial	Forb		x	
Helianthemum canum	Cistaceae	Perennial	Forb	x	x	
Hippocrepis comosa	Fabaceae	Perennial	Forb	x	x	
Lotus corniculatus	Fabaceae	Perennial	Forb		x	x
Pilosella officinarum	Asteraceae	Perennial	Forb		x	x
Plantago lanceolata	Plantaginaceae	Perennial	Forb			x
Poa bulbosa	Poaceae	Perennial	Graminoid			x
Potentilla verna	Rosaceae	Perennial	Forb	x	x	x
Poterium sanguisorba	Rosaceae	Perennial	Forb	x	x	
Stipa pennata	Poaceae	Perennial	Graminoid	x	x	
Trifolium repens	Fabaceae	Perennial	Forb			x
Veronica arvensis	Plantaginaceae	Annual	Forb			x
Vulpia myuros	Poaceae	Annual	Graminoid			x

Note: Across all treatments, 38 populations were harvested, including 15 graminoid populations and 23 forbs populations.

Abbreviations: GU, grazed and unfertilized; G+F, intensely grazed and fertilized; UU, ungrazed and unfertilized. Numbers in brackets indicate the number of species sampled in each management regime.

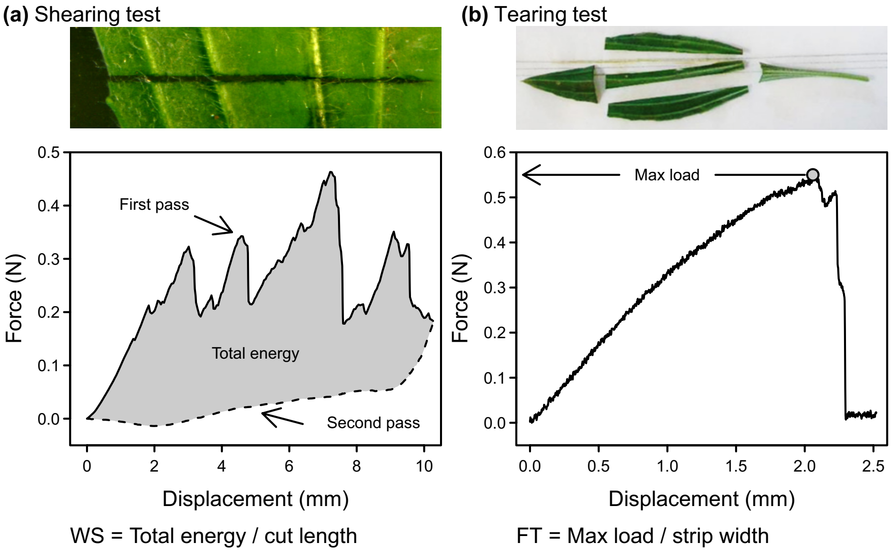


FIGURE 1 Example of (a) shearing and (b) tearing tests on the leaves of *Plantago lanceolata*. (a) The leaf section cut during the test. The image is aligned to the *x*-axis of the plot below it to show the correspondence between anatomical structures (veins and mesophyll) and the force-displacement curve. Work to shear (WS) is the difference in energy (J) between the first and second passes standardized by fracture length (per m). (b) Image showing how the 20 mm × 4 mm leaf strip used for the tearing test was cut from the leaf. Force to tear (FT) is the max load (N) standardized by strip width (m)

FT was measured using a MicroTester (Instron) according to a protocol adapted from Pérez-Harguindeguy et al. (2013). Leaves were cut into a ca. 4-mm-wide strip including the midrib in its long axis (Figure 1b). The sample was then manually clamped to 20-mm spaced supports (reduced to 5 mm for the small leaves) and tested under tension at a crosshead speed of 6 mm/min until a fracture surface had propagated across the entire leaf strip. FT (kN/m) is the highest force value recorded during the test standardized by the strip width (measurement under a microscope before the test) (Figure 1b). SFT was then calculated by dividing FT by the leaf thickness.

2.3 | Measurements of other leaf traits

Leaf water-saturated fresh mass and leaf area were also measured before mechanical tests on the same leaves. Petioles and rachis were removed before the measurements. LT was measured using a linear variable displacement transducer. Leaf area was estimated using 1,200 dpi scans of the leaf samples. Once the mechanical tests were conducted, leaf samples were dried at 60°C for 72 h and weighed to measure the dry mass. LMA and LDMC were computed using these measurements (Garnier et al., 2001).

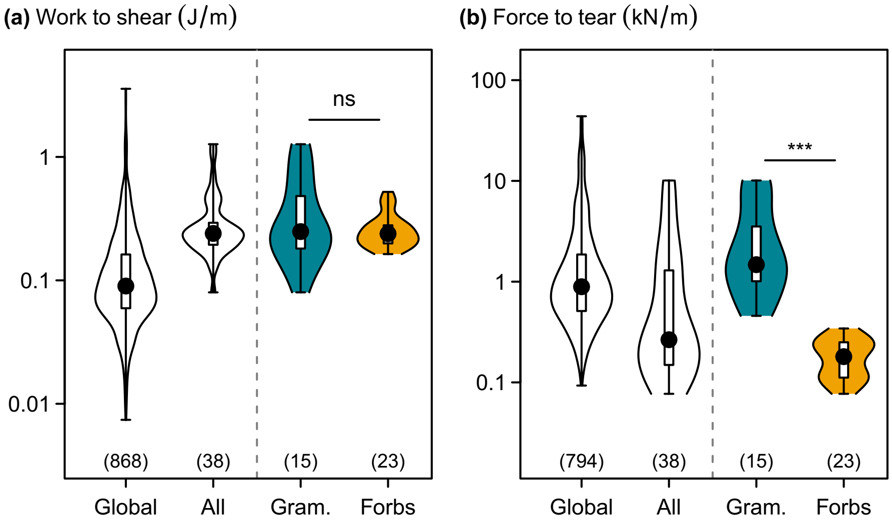
2.4 | Statistical analyses

All traits were log-transformed before statistical analyses to fulfil normality assumptions and avoid heteroscedasticity. Differences in trait values between taxonomic groups were tested using Wilcoxon rank sum tests because of the strong differences in trait variance between groups. Differences in trait values between treatments were tested using analyses of variance (ANOVAs) and post-hoc Tukey's tests (R package *multcomp* [R Core Team, R Foundation for Statistical Computing, Vienna, AT], Hothorn et al., 2008). The effects of LMA, LT and LDMC on biomechanical traits were tested using linear mixed models including the species identity as a random factor. The latter allows us to account for the fact that some species were sampled in several treatments. The correlation between biomechanical traits was performed using Pearson's product–moment correlation coefficient for each taxonomic group separately. We compared the slopes and intercepts of the correlations using standardized major axis estimations (SMA) with the R package *smatr* ([R Core Team, R Foundation for Statistical Computing, Vienna, AT]; Warton et al., 2012). Confidence intervals for SMA were constructed using a cross-validation procedure by recalculating the SMA coefficients for 1,000 random sampling with replacement of the data set. To our knowledge, SMA does not allow for random effects. Hence these analyses were conducted using trait values both at the population (including several values for some species) and at the species levels (based on species mean values).

3 | RESULTS

LMA ranged from 22.8 g/m^2 (*Anisantha sterilis* sampled in G+F) to 253.9 g/m^2 (*Stipa pennata* in UU) and LDMC from 112.6 g/kg (*Capsella bursa-pastoris* in G+F) to 635.7 g/kg (*S. pennata* in UU). WS values were relatively high (see Figure 2a for a comparison with a global data set) and ranged from 0.05 J/m (*Vulpia myuros* in G+F) to 2.24 J/m (*S. pennata* in UU). FT covered a high range of values (Figure 2b) from 0.03 kN/m (*Helianthemum canum* in UU) to 31.65 kN/m (*S. pennata* in UU). LMA and WS were not significantly different between forbs and graminoids (Wilcoxon rank sum tests: $W=63$, $p=0.815$ and $W=57$, $p=0.558$, respectively), whereas LDMC and FT were significantly lower in forbs compared with graminoids (Wilcoxon rank sum test: $W=118$, $p=0.002$ and $W=135$, $p<<0.001$, respectively).

In agreement with our hypotheses, we found that LMA (ANOVA: $F_{2,35}=19.9$, $p<<0.001$) and LDMC (ANOVA: $F_{2,35}=10.6$, $p<0.001$) of abundant species decreased with management intensification. Results were qualitatively similar when accounting for species relative abundances (Appendix S3). These trends were strengthened


(a) Work to shear (J/m)

(b) Force to tear (kN/m)

FIGURE 2 Distribution of leaf biomechanical traits (a, work to shear; b, force to tear). Each panel is divided in two parts: the left part represents a comparison between the trait distribution for the Onoda et al. (2011) worldwide data set (global) and the 38 plant populations harvested in our study (All), whereas the right part represents the trait distribution for forbs (blue) and graminoid (yellow) of the study separately. Boxplots, representing the quantiles of the distributions (0%, 25%, 50%, 75% and 100%) are added to each violin. Significance levels of Wilcoxon rank sum tests are ns: $p>0.05$; ***$p<0.001$

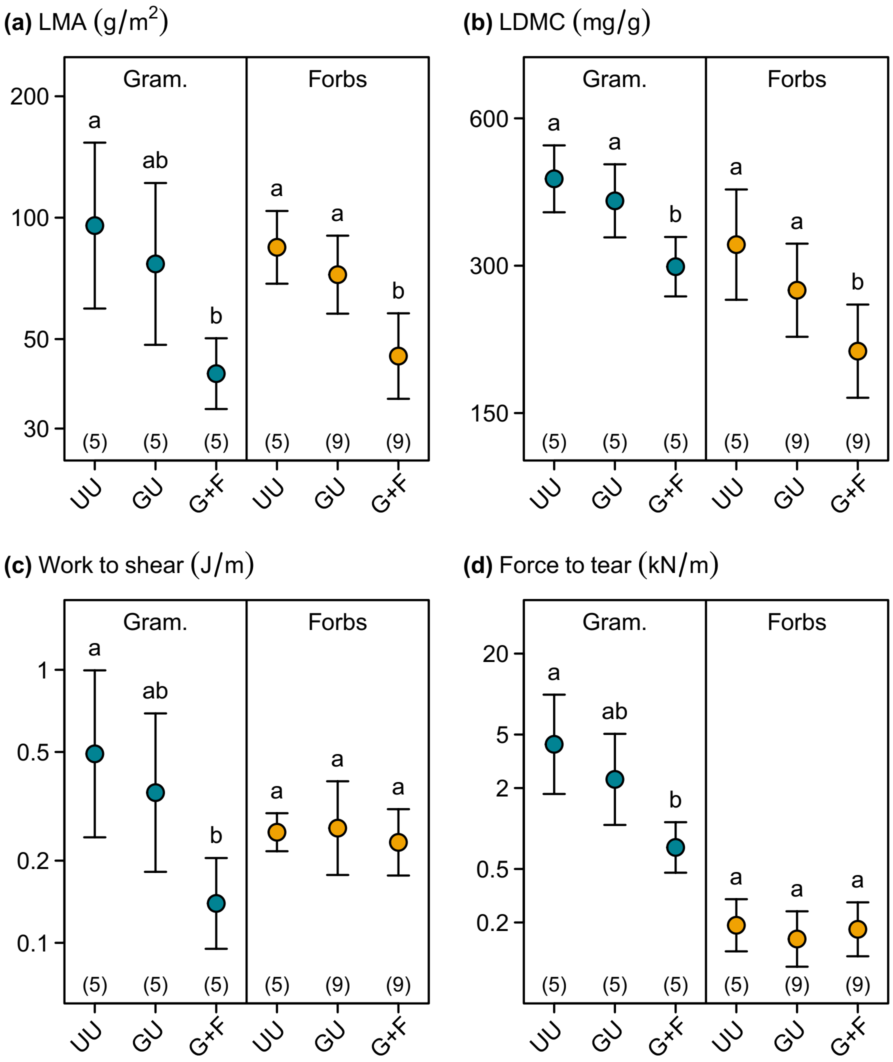


FIGURE 3 Leaf traits of the most abundant species in each management treatment (see Table 1): (a) leaf mass per area, (b) leaf dry matter content, (c) work to shear and (d) force to tear. For each treatment, graminoids and forbs were analysed separately. Points are the mean value of all sampled species and error bars represent standard deviations (the corresponding *n* are given in parentheses). Points that share the same letter are not significantly different: post-hoc Tukey test, $p > 0.05$. UU, ungrazed and unfertilized; GU, grazed and unfertilized; and G+F, intensely grazed and fertilized

when graminoids and forbs were analysed separately (Figure 3a,b). By contrast, leaf biomechanical traits showed a lower (WS, ANOVA: $F_{2,35} = 4.8$, $p = 0.015$) or a non-significant (FT, ANOVA: $F_{2,35} = 2$, $p = 0.147$) response to the treatments. Again, results were qualitatively similar when accounting for species relative abundances (Appendix S3). Separate analyses for each taxonomic group revealed that only graminoids showed a significant decrease in WS and FT with management intensification, whereas forbs showed no significant response for any of these traits (Figure 3c,d).

LMA was positively related to both WS and FT, whereas LT had a significant effect on WS only (Table 2). LDMC had a positive effect on both the SWS and SFT (Table 2). These effects were found to be significant in graminoids when taxonomic groups were analysed separately (Table 2, Figure 4). Although all relationships were significant in graminoids (Table 2, Figure 4), biomechanical traits were more strongly affected by LMA and LDMC than by LT. Conversely, none of the morpho-anatomical traits had a significant effect on biomechanical traits in forbs, except for a weak relationship between LMA and WS (Table 2, Figure 4).

We found a positive correlation between WS and FT ($r^2 = 0.31$, $p << 0.001$; Figure 5). When analysed for both taxonomic groups separately, the relationships between WS and FT shared the same slope (common slope = 1.29, slope comparison test, $p = 0.46$) but with a shift in intercept (forbs, 0.01; graminoids, 0.98; intercept comparison test, $p << 0.001$), indicating that at a given WS value, graminoids had 9.3-fold higher FT than forbs (Figure 5). However, r^2 values indicate a much tighter relationship between WS and FT in graminoids ($r^2 = 0.95$) than in forbs ($r^2 = 0.22$). When the same analysis was run at the species level (using the species means across treatments), the results remain qualitatively similar (common slope = 1.26 and intercepts are 0.01 in forbs and 0.96 in graminoids).

4 | DISCUSSION

4.1 | Leaf trait responses to management intensification

Grazing intensification under high resource availability has been found to favour tolerance strategies against herbivores associated with fast resource acquisition to compensate for biomass losses (Díaz et al., 2007; Bumb et al., 2016). Consistent with this, we found

TABLE 2 Linear regression tests (χ^2, Wald's test statistical value) on the effects of leaf morpho-anatomical traits (LMA, Leaf thickness, LDMC) on biomechanical traits (WS, FT, SWS and SFT)

	χ^2	df	*p* value	χ^2	df	*p* value
	LMA on WS			**LMA on FT**		
All	34.2	1, 36	**<<0.001*****	18.7	1, 36	**<<0.001*****
Graminoids	40.5	1, 13	**<<0.001*****	46.1	1, 13	**<<0.001*****
Forbs	3.9	1, 21	**0.049***	0.7	1, 21	0.389
	Leaf thickness on WS			**Leaf thickness on FT**		
All	14.2	1, 36	**<0.001*****	0.3	1, 36	0.575
Graminoids	12.2	1, 13	**<0.001*****	9.6	1,13	**0.002****
Forbs	3.4	1, 21	0.065	0.1	1, 21	0.728
	LDMC on SWS			**LDMC on SFT**		
All	15.2	1, 36	**<<0.001*****	29.4	1, 36	**<<0.001*****
Graminoids	17.9	1, 13	**<<0.001*****	45.7	1, 13	**<<0.001*****
Forbs	1.1	1, 21	0.283	0.3	1, 21	0.561

Note: The analyses were run for all populations and for graminoids and forbs separately. Each test includes the species identity as a random factor to account for species sampled in several treatments. Bold values indicate significant tests. Significance levels are: $^*p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

that LMA and LDMC of abundant species (both in graminoids and forbs) decreased with increasing grazing pressure and soil fertility. In agreement with the hypothesis of an acquisition–defence trade-off (Coley, 1988; Coley & Barone, 1996; Züst & Agrawal, 2017), these trait responses were associated with a decrease in leaf toughness in graminoids (as assessed by both WS and FT). Similar results were found on species sets from temperate, subhumid, upland grasslands of central Argentina and northern Israel (Díaz et al., 2001). In grasses, it has been shown that growth rate relates negatively to leaf tissue density which, in turn, depends on the proportion of non-veinal sclerenchymatic cells (Van Arendonk & Poorter, 1994), or total cell wall area (Garnier & Laurent, 1994) per unit leaf volume. We can therefore assume that the decrease in leaf toughness found in graminoids is due to a smaller proportion of supporting tissues in the leaves of fast-growing species. By contrast, LT is less related to plant growth strategies and appeared to have a weak influence on biomechanical properties. Other leaf characteristics can be further investigated in future studies. For example, a high growth rate has been related to lower leaf silicon concentration in grasses (Massey et al., 2007) which has been shown to affect leaf biomechanics in a small number of species (Simpson et al., 2017 for FT; Johnson et al., 2019 for WS).

However, no response in leaf biomechanical properties was found in forbs: abundant forbs in the intensively grazed and fertilized treatment showed similar leaf biomechanical properties to those from ungrazed areas, despite having much lower LDMC on average (200.7 g/kg in G+F against 331.2 g/kg in UU). Interestingly, the contrasting responses between taxonomic groups inverted the ranking of forbs and graminoids based on their WS: forbs had lower WS than graminoids in ungrazed and traditionally grazed treatments, whereas the reverse was found in the intensively grazed and fertilized treatment. Whether this switch in ranking can modify plant–herbivore interactions and, hence, affect the community composition, remains to be tested. Indeed, one can expect this pattern to affect grazer selectivity, and thus modify the herbivory pressure experienced by each species group.

Our results question the idea of universal phenotypic constraints between resource-acquisition capacity and biomechanical properties of leaves. Although morpho-anatomical and biomechanical traits were tightly related in graminoids, LMA, LT and LDMC had surprisingly weak effects on the leaf biomechanics of forbs (most relationships were not significant). This suggests that other leaf traits poorly related to resource acquisition determine leaf biomechanical properties in this species group. The spatial arrangement of veins and vascular bundles appears as a good candidate to explain this decoupling (Lucas et al., 2000; Roth-Nebelsick et al., 2001). Indeed, vein patterns and orientations create material heterogeneity along the propagation of cracks, affecting leaf biomechanical properties independently from tissue properties (Roth-Nebelsick et al., 2001). Graminoids are characterized by parallel venation patterns with relatively low structural variation between species. Indeed, efficient light interception in graminoids relies on the fibre architecture of their leaves for basic mechanical support to keep leaf blades upright (Vincent, 1982). This is entirely different for forbs in which stems and petioles fulfil this essential function. It results in lower constraints in fibre orientation within the leaf blade and allows a larger diversity of leaf forms and venation architecture in this species group. Consistent with this idea, the abundant forbs selected in our study spanned a wide range of taxonomic families with diverse venation architectures. Another interesting result is that the intraspecific trait responses of six species found in the three treatments revealed that most species – including forbs – showed a decrease in LMA, LDMC and FT with management intensification (Appendix S4). This result corroborates a stronger relationship between leaf biomechanics and LMA and LDMC at the intraspecific level because of the low variation in leaf shape and venation network between individuals of the same species. A clear quantification of the effect of the venation architecture (Blonder et al., 2018) on the leaf biomechanical properties remains to be tested, however, and is a promising avenue for future research (Sack & Scoffoni, 2013).

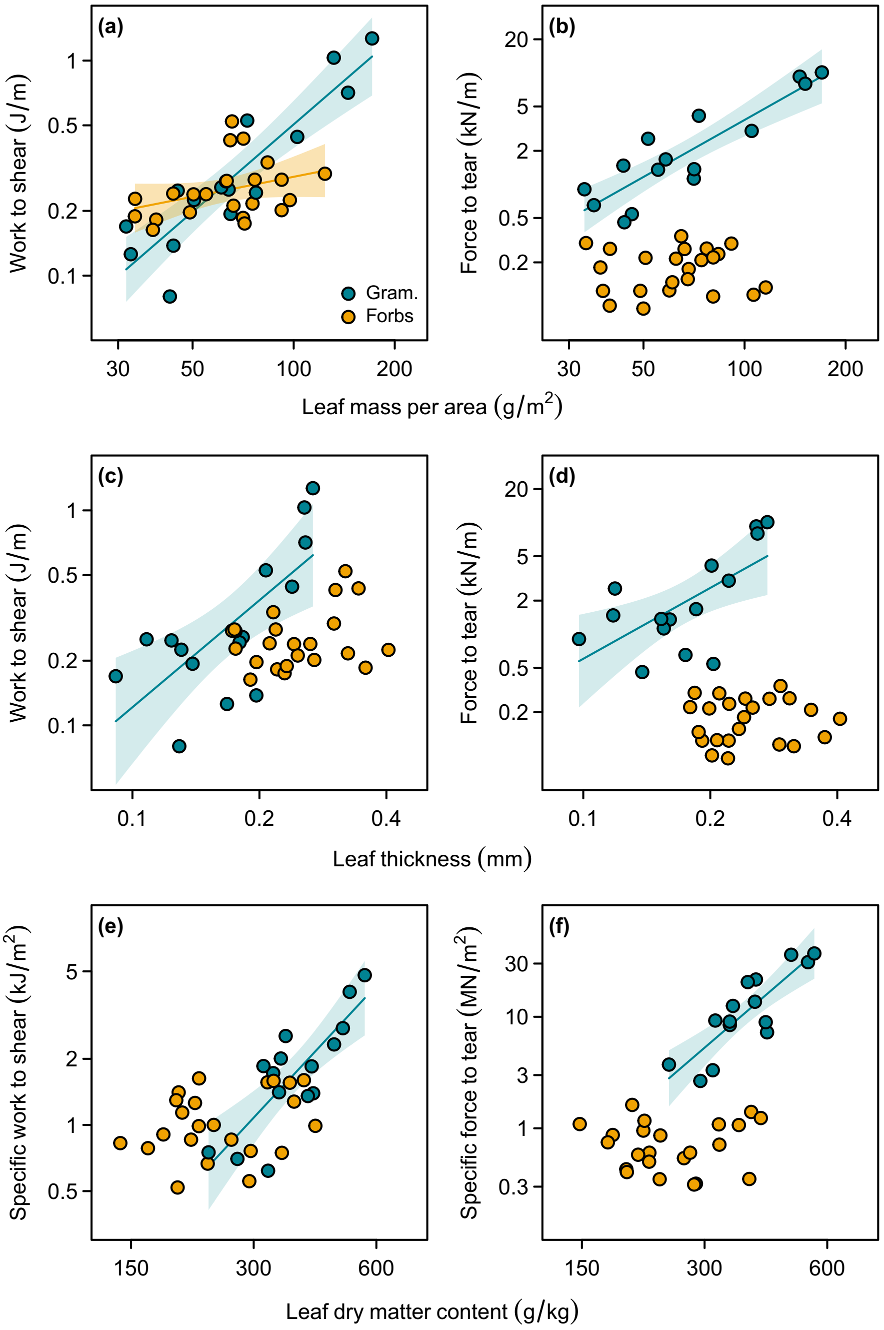


FIGURE 4 Effects of morpho-anatomical traits on leaf biomechanics. Each point represents the mean value of a species population ($n=15$ for graminoids, in blue, and $n=23$ for forbs, in yellow). The effects of leaf mass per area (a, b) and leaf thickness (c, d) on work to shear (a, c) and force to tear (b, d) were tested. Similarly, the effects of leaf dry matter content (e, f) on specific work to shear (e) and specific force to tear (f) were tested. Significant results ($p<0.05$) are represented by regression lines and confidence intervals. See Table 2 for statistics.

To our knowledge, this study is one of the first to show decoupled responses of acquisition traits and biomechanical traits to management intensification in forbs. Whether the same conclusions apply in other systems, especially where forbs have a higher contribution to the plant community, remains an open question. Nevertheless, our study focuses on leaf mechanical defences only and further investigation is needed to assess whether the more general concept of acquisition–defence trade-off holds across taxonomic groups. The latter would require considering other aspects of plant defence such as defensive structures (e.g., trichomes, thorns) or secondary compounds (e.g., tannins and other phenolic compounds). In particular, forbs are often considered to invest more in chemical defence than graminoids, and the lack of response found in forbs' mechanical defence does not exclude a trade-off between resource acquisition and defence based on leaf chemical compounds. Further studies should thus consider this aspect of defence in addition to biomechanics, which, considering the wide variety of secondary compounds produced by forbs to deter herbivores (e.g., glucosinolates in Brassicaceae, or tropane alkaloids in Solanales) constitutes a real challenge.

4.2 | WS and FT capture two different leaf biomechanical properties

The relationship between WS and FT was clearly structured by taxonomic groups, with graminoids having almost a tenfold higher FT than forbs at any given WS. Comparable results were found on shrubs and trees where, at a given WS, FT differed according to the species'

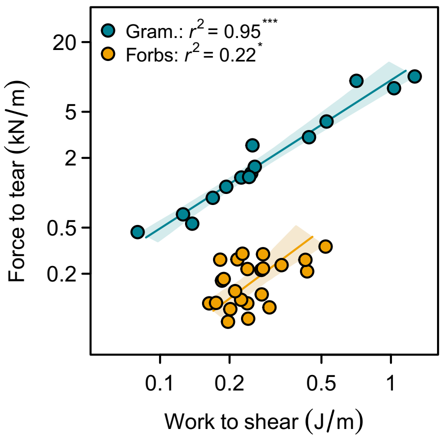


FIGURE 5 Correlation between work to shear and force to tear. Each point represents the mean value of a species population ($n = 15$ for graminoids and $n = 23$ for forbs). The r^2 and significance of the correlation is given for each taxonomic group separately. Lines and coloured areas represent standardized major axis (SMA) regression lines and confidence intervals respectively. Significance levels are: $^*p < 0.05$ and $^{***}p < 0.001$.

venation patterns in the following order: terete leaves > parallel venation > reticulate venation (Enrico et al., 2016). The tearing test, which applies mechanical constraints along the longitudinal axis of the leaf, was mostly influenced by the orientation of the vein network (also reported in Onoda et al., 2011) although cutting tests did not discriminate venation types. Moreover, we found the two traits to be strongly correlated in graminoids although the correlation was weak in forbs. It suggests that the two traits could respond independently to environmental factors in forbs. Again, venation architecture is probably the major factor explaining these differences. In graminoids, both tests apply mechanical constraints along a vein network of consistent orientation across species. Conversely, in forbs, the tearing test is probably mostly driven by the midrib properties, whereas the spatial arrangement of secondary veins, which strongly differs between species, may have a stronger influence on the shearing test.

The functional significance of high mechanical resistance remains controversial and several hypotheses, which are not mutually exclusive, have been proposed to explain its adaptive significance (Turner, 1994; Read & Sanson, 2003). These include: (1) anti-herbivore defence; (2) an adaptation to seasonal water deficits; and (3) longer conservation of nutrients, through the enhancement of leaf longevity and the reduction of leaf decomposition rate (Pérez-Harguindeguy et al., 2000; see Edwards et al., 2000 and references therein). Despite sclerophylly being defined in terms of biomechanical properties, little research has been undertaken on the biomechanical properties of sclerophyllous leaves (Edwards et al., 2000; Read & Sanson, 2003). Our study reveals that the methodological choice for mechanical measurements can lead to very different species rankings, suggesting contrasted functional significance of WS and FT. Future studies evaluating the adaptive value of leaf toughness should therefore consider several mechanical measurements to ensure a clear interpretation of plant responses to selection pressures.

5 | CONCLUSIONS

We demonstrated a decrease in LMA and LDMC in both graminoids and forbs with management intensification in the studied Mediterranean rangeland. This result is consistent with the idea that management intensification selects species with a tolerance strategy against herbivores, associated with a fast growth rate to compensate for biomass losses. In agreement with the acquisition–defence trade-off, we found these trait variations to be associated with lower leaf toughness in graminoids. However, no difference in leaf biomechanics was found in forbs despite large differences in their resource-acquisition strategies. This decoupled response between resource acquisition and leaf biomechanics was probably because of the larger variation in leaf shapes and venation networks in this species group. Whether other aspects of the plant defence against herbivores (such as chemical defences) follow the same trends remains an open question. Finally, our study calls for a better understanding of the underlying determinants of leaf mechanical resistance in different groups of species. Further investigation is also needed to clarify the functional significance of leaf mechanical resistance, demonstrating the adaptive value of a high leaf mechanical resistance in various environmental conditions.

AUTHOR CONTRIBUTIONS

JS, EG, EK and NPR conceived the ideas and designed the methodology. JS, KS, JR, SC, EK and EG collected the data. JS analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors state that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study is openly available in the InDoRES repository (https://doi.org/10.48579/PRO/TFQABR).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Analysis of the temporal changes in species composition induced by the management regimes.

Appendix S2. Relative abundance of the 24 species used in the study.

Appendix S3. Community weighted means (CWM) of leaf traits measured in each management regime.

Appendix S4. Intraspecific trait responses to management intensification.

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