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Intra-specific multi-trait approach reveals scarce ability in the variation of resource exploitation strategies for a dominant tall-grass under intense disturbance

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Abstract

Mowing is a suitable practice to restore the grassland coenological composition after invasion of tall-grasses. However, how it affects the tall-grass intraspecific variation is still unknown. We investigated if mowing decreases the competitive ability of the tall-grass *Brachypodium rupestre*, and if this reduction is due to its poor ability to adapt to the new conditions by reducing resource investment in leaf and flower production, resource storage in belowground organs, increasing seed output and reducing seed mass.

In 2017, we measured vegetative and reproductive traits of *B. rupestre* in two fenced areas in central Apennines (Italy). Half of each area had been mown twice a year since 2010 and half remained unmown. To investigate the effect of mowing on *B. rupestre*'s functional strategies, we used linear mixed-effects modelling and correlation analysis.

Mowing significantly reduced the trait values related to competitive ability / resource acquisition (vegetative height and leaf traits), resource storage (mature hypogeogenous rhizome), and sexual reproduction (mean seed mass, number of flowers, reproductive height), but increased seed output. We did not find significant variation of dry matter content in epigeogenous rhizomes and in the shoot number.

B. rupestre is not prone to endure a prolonged disturbance, not being able to reallocate resources from acquisitive and retentive to reproductive structures. However, it is able to guarantee the presence of individuals to a certain degree by changing the reproductive strategies, such as decreasing the seed mass and increasing the seed output.

Keywords: *Brachypodium rupestre*, grassland restoration, plant height, recurrent mowing, specific leaf area, plant traits

1. Introduction

Semi-natural European grasslands are biodiversity hot-spots (Dengler et al., 2014) currently endangered by land abandonment, which started after the Second World War and persisted over time (Lasanta-Martínez et al., 2005). Abandonment or improper management trigger dynamic processes (Biondi et al. 2006, 2016) towards forest ecosystems (Bracchetti et al., 2012; Malavasi et al., 2018). In the first phases of such dynamic processes a common phenomenon is the spread of dominant tall-grasses (see Grime 2001, Louault et al., 2002; Peco et al., 2006; Malatesta et al., 2019), which may lead the community to a new stable condition that does not evolve to shrubland and forest (Canals et al., 2017). In Europe, there are several species that could play this role, including *Deschampsia cespitosa* (Jendrišáková et al., 2011), *Calamagrostis epigejos* and *C. villosa* (Alps, Niedrist et al., 2009), *Molinia caerulea* (Lepš, 2014), *Bothriochloa ischaemum* (Szentés et al., 2012) and *Brachypodium pinnatum* (Canals et al., 2017). Consequently, knowing the processes that regulate the responses of such species to disturbance is a wide-ranging question and is mandatory in defining grassland restoration plans (Suding and Hobbs, 2009).

Throughout the Italian peninsula, *Brachypodium rupestre* (Host) Roem. & Schult. is one of the most widespread dominant tall-grasses in the sub-montane landscapes. *B. rupestre* is a West Eurasian species (Catalán et al., 2016), indigenous in Italy, where is widespread in the sub-Mediterranean mountains, below 1200-1300 m a.s.l., in all regions except Sardinia (Bartolucci et al., 2018). It is a late-flowering perennial grass, with long rhizomes forming loose and spaced tussocks (Lucchese, 1987; Camiz et al., 1991). *Brachypodium* perennial species show competitive-stress tolerant performances (CS *sensu* Grime, 2001) and, since they have silica-rich and hairy leaves, are highly unpalatable (see Catorci et al., 2014). The dominance ability of such species is provided by a wide set of traits, such as high tiller density and branching frequency (Pottier and Evette, 2010), clonal growth and clonal integration strategy (de Kroon and Bobbink, 1997). In addition, these species also have a high intra-specific variability of leaf traits (e.g. specific leaf area in *Brachypodium genuense*), suggesting the ability to change their strategies of resource exploitation (from acquisitive to conservative and vice versa), according to the variations of environmental productivity (Tardella et

al., 2017). These characteristics allow the rapid spread of *Brachypodium* sp. pl. clonal tussocks, which often form nearly mono-dominant stands in the final phases of invasion of abandoned or improperly managed grasslands (de Kroon and Bobbink, 1997).

Mowing is a non-selective disturbance considered one of the most appropriate management types for ensuring the high conservation value of grasslands (Tälle et al., 2014). Several studies have shown that mowing fosters resource allocation to leaves or underground storage organs (e.g. Klimeš and Klimešová, 2002; Craine, 2005). Because of this and since it reduces aboveground phytomass and prevents litter accumulation (when hay is removed), mowing promotes the survival of light-demanding seedlings and low-growth species (Ilmarinen and Mikola, 2009), but reduces the dominance ability of dominant/invasive tall-grasses (Grime, 2001), mostly because it changes the resource foraging, use and storage strategies of such plants (Canals et al., 2017). In fact, Bonanomi et al. (2006) demonstrated that recurrent once-a-year mowing events reduce plant height, aboveground phytomass and cover value of *B. rupestre* in central Apennines. Moreover, Bobbink and Willems (1991) suggested the adequacy of late mowing to avoid reallocation of nutrients to storage organs, while Socher et al. (2013) proved the effectiveness of management based on twice-a-year mowing in biodiversity conservation. However, how twice-a-year mowing reflects on the strategies of resource exploitation and the reproductive performances of tall-grasses in general and *Brachypodium* species, is still largely unknown. To fill this gap, from 2010 to 2017 we applied mowing twice a year, at the end of June, to avoid the completion of *B. rupestre* reproductive cycle, and at the end of October, to remove the phytomass regrowth. We measured some traits to detect changes in the main functional dimensions that affect *B. rupestre*'s ecological strategies, namely, competitive ability (plant height and specific leaf area, seed mass), response to disturbance, i.e. mowing tolerance (leaf senescence, rhizome dry matter content), mowing avoidance (plant height and leaf senescence), and resource allocation to sexual reproduction (flower traits, seed mass, and seed output) (Grime, 2001; Lavorel et al., 2007; Garnier et al., 2016).

In detail, vegetative plant height is related to competition for light, indicating the resource investment for stem biomass construction (Pérez-Harguindeguy et al., 2013). It is also an indicator of

plant fitness and response to environmental constraints, since it is associated with the ability to tolerate environmental stresses (Lavorel and Garnier, 2002; Bricca et al., 2019), in addition to disturbances, such as mowing (Lepš, 2014).

Specific leaf area (SLA) is a good proxy of the fast-slow continuum strategy in the leaf economic spectrum (i.e. the acquisition/conservation resource gradient; Wright et al., 2004). Higher SLA values are associated to shorter leaf life span, higher leaf nitrogen concentration, higher relative growth rate and photosynthetic rate, increasing the species' competitive ability. Lower SLA values instead, are associated to relatively tough leaves, higher resistance to decomposition, and lower relative growth rate and photosynthetic rate, thus to a lower competitive ability (Westoby et al., 2002).

As recurrent mowing proved to lead to a depletion of the stored resources (Klimeš and Klimešová, 2002), weakening the overall tolerance of plant to mowing, we assessed the variations in mowing tolerance ability using the belowground dry matter content for the epigeogenous and hypogeogenous rhizome of *B. rupestre* as a proxy of resource conservation. In fact, dry matter content is highly correlated with tissue density (Shipley and Vu, 2002; Birouste et al., 2014) and high-density tissues are linked to a slow-growing and nutrient conservation strategy (Ryser, 1996; Craine et al., 2002), while low-density tissues allow rapid resource acquisition and growth (Ryser and Lambers, 1995; Ryser, 1996). We considered plant height and the occurrence of leaf senescence symptoms as indicators of a mowing-avoidance strategy. In fact, small size is fostered under mowing, because minimizes the loss of plant biomass (Lepš, 2014), while an earlier reproductive cycle may avoid the mowing-induced loss of phytomass (e.g. Bricca et al., 2020). Early leaf senescence is also a strategy used by grasses to overcome resource shortage like summer drought (Volaire et al., 2014). Being correlated with the remobilization of protein nitrogen from leaves under severe defoliation (Thomas et al., 2002), it is considered an adaptation in the plant's nitrogen economy (Feller and Fischer, 1994) and, thus, part of a mowing-tolerance strategy.

Trait measurements of sexual reproductive structures (flower and seed traits) have been used to compare resource allocation patterns to reproductive and vegetative structures depending on the availability of resources and in disturbed vs undisturbed conditions (Fu et al., 2010). In particular,

mowing changes the reproductive strategies of dominant species fostering the production of small seeds (Grime, 2001; Bricca et al., 2020), because of limitation of time available for growth due to disturbance events. It should be highlighted that competitive performance of seedlings was associated to heavy seeds (Leishman and Westoby, 1994; Moles and Westoby, 2006), so that variation of seed size might indicate a variation of the dominance behavior of the considered species.

In the light of these considerations, we hypothesized that *B. rupestre* adapted its strategies to twice-a-year mowing with different mechanisms: i) reduced investment in aboveground vegetative structures (decrease in plant height and SLA), resulting in a lower rate of resource acquisition and use and lower competitive ability; ii) diminished tolerance to disturbance by decreasing resource retention in belowground structures, but increased resort to avoidance strategies, such as earlier leaf senescence and lower plant height; iii) reduced investment in reproductive structures and shift of sexual reproduction towards a more ruderal strategy, namely a higher number of lighter seeds, to ensure the ability to persist under intense disturbance conditions.

2. Methods

2.1 Study area and experimental settings

Study site (Italy, central Apennines) encompasses north-facing slopes, with limestone bedrock (337398.00 m E, 4757200.00 m N, UTM coordinate system). Climate is sub-Mediterranean (Rivas-Martínez et al., 2011), characterized by the alternation of winter and summer drought stress. Annual rainfall is about 1,000 mm, and the average annual temperature is 10-11°C. Such characteristics, generally linked with shallow soils, are the source of low aboveground productivity (170 to 190 g m⁻² of dry matter in not invaded grasslands – Catorci et al., 2009).

The study area has a centuries-old history of extensive grazing. Traditionally, grasslands are grazed by flocks between late spring and early autumn and, in the least steep slopes, hay is mown in early summer (Catorci et al., 2009). However, during the last 30 years, vast grassland areas have been undergrazed and are undergoing dynamic processes (Chelli et al., 2019).

The experimental site (2 ha) was established and fenced in February 2010. The site has homogeneous altitude (1,200-1,300 m a.s.l.), aspect (west–northwest facing slope) and slope angle (20-25°). Soil is neutral-subacidic, 30 to 40 cm deep, with a texture characterized mainly by sand (8-10%), silt (32-35%) and clay (50-60%). Skeleton amount ranges from 45 to 50%, while total nitrogen from 6 to 7 g Kg⁻¹ (Catorci et al., 2012). To reduce the variability due to the different management histories, the experimental site was selected inside the same ownership.

The experimental site was invaded by *B. rupestre*, which had an average cover value that exceeded 60%. The surface was divided into two areas (blocks) of 1 ha each (100 m × 100 m). Half of each block (0.5 ha each) was mown with litter removal twice a year, starting from 2010. The mowing was applied at the end of June, just before the *B. rupestre* flowering, and at the end of October. The sward was cut around 2 cm above the soil level, using a grass trimmer. The other halves of the two blocks (0.5 ha each) were used as control areas; they were left unmown and litter was not removed. We chose mowing as a disturbance type, since it is considered a non-selective disturbance (Kohler et al., 2005), and since summer mowing proved to avoid the reallocation of nutrients to storage organs of dominant species (Willems, 2001).

2.2 Sampling design and data collection

For the aim of this work we used a multi-trait approach, following Klimešová et al. (2008).

In 2017, we planned the data collection in two phases: (1) early summer (end of June-early July), corresponding to the flowering peak of *B. rupestre* and the peak of resource use, where we recorded data on plant height, leaf traits and reproductive traits; (2) late summer (end of August-early September, i.e. during the peak of resource storage) corresponding to seed maturation and dispersion, where we recorded data on reproductive traits (seed traits, reproductive plant height) and belowground traits. To ensure the data collection, the managed areas were not mown in June 2017.

2.3 First sampling phase

Using the “random points” tool of the QGIS software, we overlaid the experimental site with a 5 x 5 m grid and randomly selected, at the crosses of the grid, 20 points per block in the mown areas (40 in all) and 20 points per block in the unmown areas (40 in all). The minimum distance between two points was 5m, to limit the issue of spatial autocorrelation. We avoided taking random points within a buffer of 5 m adjacent to the fences and the edges between contiguous blocks. We selected the nearest *B. rupestris* tussock within the range of 0.5 m from the point, if any.

For each tussock, we counted the number of reproductive and vegetative shoots. We measured plant height (m), namely, the shortest distance between the upper boundary of the photosynthetic tissues (i.e. the top of the general canopy of the plant excluding inflorescences) and the ground level, for each shoot of each tussock. For each measurement, we recorded whether it was taken from a reproductive or vegetative shoot.

We collected all the leaves in each shoot of each tussock, except not fully expanded leaves and leaves with symptoms of pathogens. In order to preserve the leaves, we followed the protocols of Pérez-Harguindeguy et al. (2013). The leaf area (mm^2 , hereafter LA), was obtained with the use of a desktop scanner and the “Leaf Area Measurement” software, University of Sheffield (UK) version 1.3. The leaves were then dried for at least 72 hours at 80 °C in order to determine the leaf dry mass (mg), hereafter LDM. SLA was calculated for each individual leaf, as the ratio of LA to LDM ($\text{mm}^2 \text{mg}^{-1}$). To account for differences in the start of the leaf senescence process, for each leaf we evaluated whether it was completely green or with symptoms of senescence (binary variable, i.e. presence/absence of senescence symptoms). Finally, for each leaf, we recorded whether it was taken from a reproductive or a vegetative shoot.

In the spike of each flowering shoot of each selected tussock, we collected the total number of spikelets and the number of spikelets in full bloom (at least the 75% of flowers completely open, whose stamens hang outside the flower). In all, we collected 302 leaves from 170 shoots, and 624 spikelets from 109 spikes (i.e., flowering shoots).

2.4 Second sampling phase

Using the abovementioned procedure, we selected 35 tussocks per block in the mown areas (70 in all), and 35 tussocks per block in the unmown areas (70 in all); then, we randomly selected one spike per tussock. In this second phase we avoided the tussocks that in the previous phase were selected. We measured reproductive plant height as the distance of the highest point of each selected spike from the ground, the spike length and the length of its spikelets, and counted the number of flowers (which either underwent fruiting or not) in each spikelet and their total number in the spike. We counted the total number of seeds in each spike; then, we oven-dried the seeds for at least 72 hours at 80 °C; finally, we measured the overall seed mass and calculated the mean seed mass per spike. In all, we collected 850 spikelets and 2151 seeds.

Furthermore, with the same procedure, we excavated 10 tussocks per block (20 in all) in the mown areas and 10 tussocks per block (20 in all) in the control areas. We placed each tussock inside a plastic bag, which was immersed in water for 24 h; thereafter, the tussock was washed out in the laboratory and all the rhizomes were cleaned. We arranged rhizomes according to their morphology into two main categories: epigeogenous and hypogeogenous rhizomes (Klimeš and Klimešová 2002). We classified the hypogeogenous rhizomes in two age categories: (1) young, whose distal part is still in development, of white color and without roots; (2) mature, of brown color, with scale leaves close to the stem. For each tussock, we collected two rhizomes for each category (epigeogenous, young hypogeogenous, and mature hypogeogenous rhizome), for a total of 240 samples. We weighed each sample to measure its fresh mass and, then, oven-dried each sample at 75 °C for 96 hours, to achieve constant weight and then re-weighed it, to measure its dry mass. We expressed the rhizome dry matter content ($RDMC_{sat}$; mg g^{-1}) as the ratio between dry mass (mg) and water-saturated fresh mass (g) and then we averaged the values of this index across each tussock and for each rhizome type.

2.5 Statistical analyses

We calculated mean and standard deviation of the quantitative response variables. We used mixed-effects modelling to understand how traits were affected by the treatment (two-level categorical

variable, i.e. mown vs unmown). We generated models for normally distributed quantitative response variables, using the Gaussian distribution of errors, by means of the `lme` function of the *nlme* R-package. Non-normally distributed quantitative variables were transformed using logarithmic, squared root or exponential transformations, tested for normality and then, fitted in the models using the `lme` function.

We accounted for the sampling design using as random intercepts: i) the shoot identity nested inside tussock identity, nested inside block identity for leaf traits (first sampling period); ii) the tussock identity, nested inside block identity for plant height, total number of spikelets per spike, and number of flowering spikelets per spike (first sampling period); iii) the block identity for number of shoots per tussock (first sampling period), reproductive and belowground traits (second sampling period).

For the leaf traits (SLA, LA, LDM, and leaf senescence), plant height and number of shoots per tussock were measured on both flowering and vegetative shoots. Firstly, we tested whether the phenological status of the plant influenced the traits of *B. rupestre*. Thus, prior to all the other analyses, using an Akaike information criterion (AIC)-based model selection procedure applied to the full model, fitted using the `lme` function and the maximum likelihood (ML) method (including treatment, phenological status and their interaction), we selected the best fitting model (i.e. that with the lowest AIC) for each trait. To assess the quality of the selected model, we calculated also the AIC weight. This model selection was performed using the `dredge` function of the *MuMIn* R-package (version 1.15.6). For all the other traits we considered only treatment as fixed effect. Following Bitomsky et al. (2019) and Tordoni et al. (2019), to reduce the bias due to the potential effects of spatial autocorrelation in the response variables, we repeated the model selection including different correlation structures of the data. The structures taken into accounts were: the compound symmetry structure corresponding to a constant correlation, exponential spatial correlation, autoregressive structure of order 1, Gaussian spatial correlation, and rational quadratics spatial correlation, all available in the *nlme* library (Pinheiro et al., 2019). Among the best models we chose the one with the lowest AIC (Bitomsky et al., 2019). Then, we compared the selected model with the null model

(i.e. without fixed effects) to assess whether the best fitting model was statistically significant and, if so, we refitted it with the restricted maximum likelihood (REML) method to obtain more precise and unbiased parameter's estimates (Zuur et al., 2009). After the analyses, we checked the model linearity assumptions.

We used the `rsquared` function of the *piecewiseSEM* R-package (v. 2.0.2) to calculate the R^2 of linear mixed-effects model, and the R^2 (marginal and conditional) of generalized linear mixed-effects models (method = "trigamma"). Conditional R^2 accounts for the explanatory power of both fixed and random effects, whereas marginal R^2 only accounts for fixed effects (Nakagawa and Schielzeth, 2013).

As some of the response variables are counts (number of shoots, number of spikelets, number of flowering spikelets, number of fertilized / unfertilized flowers, seed output) or binary variables (occurrence of leaf senescence symptoms), and the only package that can fit models with the Poisson or binomial error distribution and with correlation structures is the *MASS* R-package (v. 7.3-47), through the `glmmPQL` function (Bolker et al., 2009), we could not perform an AIC-based model selection. Thus, we compared the fitted models based on a generalized explained variance approach (Jaeger et al., 2016). Firstly, we calculated R^2 of all the possible models (fitted for each combination of fixed effects and their interaction term, if there was more than one fixed effect) without correlation structure and with each of the aforementioned correlation structures, using the `r2beta` function (*r2glmm* R-package, version 0.1.2). Then, using the `r2dt` function of the same package, within each set of possible models with the same (or without) correlation structure, we selected the model whose generalized explained variance (R^2) was significantly higher than that of the other ones. Then, using the same procedure, we compared the selected models to find the one with the highest generalized explained variance. If there was one model which fitted with one of the correlation structures, which explained a significantly higher variance than the model fitted without correlation structure, we extracted the slope estimates and their significance values from that model, refitted using penalized quasi-likelihood method (`glmmPQL` function, *MASS* package). If variance explained was not significantly higher, we fitted the model without correlation structure with the `glmer` function

(Poisson error distribution, link = log or binomial error distribution, link = logit) of the lme4 R-package for generalized linear mixed-effects models.

A summary of statistical analyses, R-package and R-function used, sampling period, observation unit, fixed and random effects for each response variable, are indicated in Supplementary Table S1. The R scripts of the best models are included in Supplementary Table S2; data matrices are in Supplement S3.

To highlight the existence of possible trade-offs among traits, we performed non-parametric correlations between quantitative variables within each sample (leaf traits / plant height in the first sampling phase; reproductive traits in the second sampling period; belowground traits in the second sampling period) using the Spearman's correlation coefficient (*stats* R-package, method = "spearman").

3. Results

We found a significant negative influence of recurrent mowing on LA (decreasing values of 56% in mown conditions), LDM (-61%), and plant height (-40%), but not on SLA (-2%); while we found a not significant positive effect (+37%) on the number of shoots per tussock (Tables 1 and 2). SLA, LA, and LDM were significantly higher in vegetative than in reproductive shoots (+15%, +62%, and +55%, respectively), while plant height had opposite trend (-22%) (Tables 1 and 2). The onset of leaf senescence was fostered by mowing (+21% of leaves with senescence symptoms), especially in reproductive shoots (+45%) (Tables 1 and 2). Concerning the reproductive traits, mowing decreased the number of spikelets (-35%) and flowering spikelets (-10%) at the flowering peak, reproductive plant height (-19%), mean length of spikes (-30%) and spikelets (-7%), number of fertilized/unfertilized flowers (-45%), and mean seed mass (-33%) in the phase of seed maturation and dispersion, but increased the seed output (+11%) (Tables 1 and 2). In addition, we detected negative effect of mowing on the RDMC values of mature hypogeogenous rhizome (-10%), but not on those of epigeogenous (-1%) and young hypogeogenous rhizomes (-6%) (Tables 1 and 2).

Finally, we found strong positive correlations both in mown and in unmown areas, between LA and LDM, as well as between mean number of flowers per spikelet, their number per spike and spikelet length, and between seed output and total seed mass per spike. In the unmown areas seed output and total seed mass per spike were mostly uncorrelated with the other variables, whereas in mown areas they showed weak, but significant correlations (Table 3).

4. Discussion

4.1 Effect of twice-a-year mowing on competitive ability

It was proved that disturbance leads to the reduction of *B. rupestris* aboveground phytomass and cover value (Bonanomi et al., 2006), following the Grime's prediction (2001) regarding the response to disturbance of competitive stress-tolerant species. Consistently, the observed reduction of several aboveground vegetative trait values can be understood as a decreasing dominance ability of the individuals, which has a great effect on plant community composition (Bricca et al., 2020).

In particular, the reduction in plant height means lower resource investment of individuals for shoot biomass construction (Pérez-Harguindeguy et al., 2013), reflecting a worsened plant fitness (Lavorel and Garnier, 2002).

Contrarily to our hypothesis, SLA did not show a significant variation in response to mowing, confirming previous findings obtained using sheep grazing (Catorci et al., 2014). SLA is considered as an indicator of species position along resource gradients (Wright et al., 2004), and the variation of local resources has been proven to drive SLA intra-specific changes (Messier et al., 2010). Therefore, the absence of variation could be interpreted as the ineffectiveness of *B. rupestris* in modifying its resource acquisition strategies in relation to disturbance. Having a non-modifiable resource acquisition system, individuals suffer a decrease of aboveground phytomass and cover value (Bricca et al., 2020) as indicated by the reduction of both dry mass and leaf area. Hence, it is likely that *B. rupestris* utilize only one strategy even when the balance between the resources available in the environment or in the storage organs, and the resources necessary for the replacement of the leaf tissues becomes negative.

4.2 Effect of twice-a-year mowing on resistance to disturbance

The significant reduction of the mature hypogeogenous RDMC_{sat} in the mown areas is consistent with de Bello et al. (2012). Although this trait has been poorly investigated, it is conceivable that the observed variations might underline a decrease of non-structural carbohydrates concentration, hence of stored resources. Indeed, Klimeš and Klimešová (2002) found a decrease of total non-structural carbohydrates concentration in *Calamagrostis epigejos* under two recurrent mowing events per year. As storage of carbon seems to be an important strategy for re-occupancy of the canopy after mowing (Klimeš and Klimešová, 2002), it is presumable that such process is the driving force of the whole set of observed results. However, such relationship has to be further investigated in more detail.

Moreover, leaves underwent senescence earlier in disturbed conditions, as observed in *Lolium perenne* subjected to defoliation (Cullen et al., 2006). In Mediterranean climate the early senescence of leaves has been interpreted as a strategy to overcome the lack of resources during the dry summer period (Catorci et al., 2012; Volaire et al., 2014). As leaf senescence plays an important role in the plant's nitrogen economy (Feller and Fischer, 1994) and is correlated with the remobilization of protein nitrogen from leaves (Thomas et al., 2002), our results allow to infer that this strategy was suitable also reduce the costs of leaf loss and re-growth, also thanks to the smaller plant size.

4.3 Effect of twice-a-year mowing on resource allocation to sexual reproduction

Campbell and Grime (1992) found that higher disturbance intensity determines lower vegetative and reproductive performances in grass species. In particular, it was stated that disturbance tends to reduce the number of shoots bearing flowers and seeds (Jantunen et al., 2007), fostering vegetative propagation (Klejin and Steinger, 2002). Consistently, we observed decreasing values of spike length, number of spikelets per spike, number of flowers per spike, mean number of flowers per spikelet. However, we did not detect significant effects on the number of flowering shoots.

Another effect of disturbance is related to the seed mass, as we found a decreasing trend in disturbed conditions. This is consistent with Aarssen and Jordan (2001), who showed positive relation

between plant size and the mass of propagules because of limitation of time available for growth due to disturbance events. However, the reduction in seed mass from unmown to mown conditions reflects the increase of the number of seeds, resulting in a positive correlation with most of the other reproductive traits. In fact, the proportion of flowers that underwent fertilization and seed maturation increased under disturbance (not attributable to a different impact of external factors, given that the data were collected in the same period of the year). As the total seed mass per spike did not change significantly, such results likely underline that, under disturbance, the same amount of resources was devoted to produce a higher number of small seeds. It could be interesting to note that small seeds can be advantageous under reduced competition (Leishman et al., 2000). Contrariwise, larger seeds have been interpreted as a successful trait under dense cover, where light may be limiting (Bernard-Verdier et al., 2012), and associated to competitive performance of seedlings (Leishman and Westoby, 1994; Moles and Westoby, 2006). From this point of view, it seems that the strategy of the individuals fits well with the new condition of the community, which under disturbance regimes will not be affected by these dominant plants (i.e. competitive exclusion) in driving the species assemblage (Bricca et al., 2020).

5. Conclusion

Our results indicate that *B. rupestre* is not a species prone to endure prolonged disturbances, not being able to change the resource acquisition strategy and to reallocate resources from acquisitive / retentive to reproductive structures. Instead, individuals change the reproductive strategies decreasing the seed mass and increasing the number of seeds. This picture of our results could suggest that, even if the species has no longer the ability to dominate the system, it is able to guarantee the presence of individuals to a certain degree changing the sexual reproduction towards a more ruderal strategy, thanks to the dispersion of a greater number of seeds suited to the new conditions of the community; however, more research is needed to clarify this aspect.

Author contributions

Andrea Catorci: Conceptualization, Investigation, Supervision, Writing-Original Draft

Federico Maria Tardella: Investigation, Methodology, Visualization, Formal Analysis, Writing-Original Draft

Alessandro Bricca: Investigation, Writing-Original Draft

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Supplementary materials

Table S1. List of plant traits collected, with their respective variable type, sampling time, observation unit on which the measurement was taken, error distribution family, fixed and random effects included in the mixed-effects models, R-package, and R-function used.

Table S2. R script of the best model. B – Block; B/T – Tussock nested inside block; B/T/S – Shoot nested inside tussock, nested inside block.

Supplement S3. Data matrices used for statistical elaborations.

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2 **Table 1.** Descriptive statistics (mean \pm standard deviation) for plant traits collected at maximum
 3 flowering and at seed maturation time in the unmown and mown areas.
 4

Plant traits	Stem type	Treatment	
		Unmown	Mown
Number of shoots	Vegetative / reproductive	1.30 \pm 0.59	1.78 \pm 1.16
	Vegetative	1.17 \pm 0.39	1.96 \pm 0.69
	Reproductive	1.35 \pm 0.65	1.67 \pm 1.36
Plant height (m)	Vegetative / reproductive	0.52 \pm 0.12	0.31 \pm 0.11
	Vegetative	0.43 \pm 0.11	0.20 \pm 0.05
	Reproductive	0.55 \pm 0.10	0.38 \pm 0.08
Specific leaf area (mm ² mg ⁻¹)	Vegetative / reproductive	18.65 \pm 12.20	18.33 \pm 11.95
	Vegetative	20.62 \pm 12.53	20.00 \pm 6.31
	Reproductive	17.97 \pm 12.08	17.58 \pm 13.69
Leaf area (mm ²)	Vegetative / reproductive	510.01 \pm 357.49	225.16 \pm 168.14
	Vegetative	712.40 \pm 322.22	325.35 \pm 168.78
	Reproductive	439.51 \pm 343.46	180.63 \pm 148.02
Leaf dry mass (mg)	Vegetative / reproductive	31.71 \pm 22.81	12.26 \pm 7.14
	Vegetative	43.05 \pm 25.15	16.36 \pm 6.54
	Reproductive	27.75 \pm 20.66	10.44 \pm 6.64
Relative frequency of leaf senescence symptoms	Vegetative / reproductive	0.39	0.47
	Vegetative	0.32	0.18
	Reproductive	0.42	0.61
Total number of spikelets	Reproductive	7.2 \pm 1.4	4.7 \pm 1.4
Number of flowering spikelets	Reproductive	2.0 \pm 2.3	1.8 \pm 1.9
Spike length (mm)	Reproductive	84.6 \pm 21.1	59.3 \pm 20.0
Mean spikelet length (mm)	Reproductive	23.22 \pm 4.94	21.55 \pm 4.81
Mean number of flowers per spikelet	Reproductive	10.57 \pm 3.28	8.13 \pm 2.82
Number of flowers	Reproductive	75.47 \pm 30.30	41.49 \pm 21.45
Seed output	Reproductive	14.57 \pm 9.04	16.16 \pm 10.92
Total seed mass (mg)	Reproductive	0.05 \pm 0.03	0.04 \pm 0.03
Mean seed mass (mg)	Reproductive	0.003 \pm 0.001	0.002 \pm 0.001
Reproductive plant height (m)	Reproductive	0.70 \pm 0.11	0.57 \pm 0.08
Rhizome dry matter content (mg ⁻¹ g)	Epigeogenous	452.0 \pm 62.4	445.7 \pm 26.9
	Mature hypogeogenous	472.5 \pm 57.6	426.0 \pm 49.7
	Young hypogeogenous	336.0 \pm 77.5	314.8 \pm 48.6

5
6

7 **Table 2.** Effect of treatment, shoot phenology and their interaction, on whole plant traits and leaf traits, and of
8 treatment on reproductive and belowground traits, as performed using generalized linear models, linear mixed-
9 effects models or generalized linear mixed-effects models. The R function used, the error distribution family,
10 the random effect structure, the AIC (Akaike Information Criterion) value, the weighted AIC (AICw), and the
11 R^2 values (in parentheses, the conditional R^2 value, in the case of mixed-effects models) are reported. RDMC_{sat}:
12 rhizome dry matter content. ^a Rational quadratics spatial correlation; ^b Compound symmetry structure
13 corresponding to a constant correlation; ^c Gaussian spatial correlation; ^d Autoregressive process of order 1. +
14 log-transformed; ++ cube root transformed; +++ square root transformed; § fifth-root transformed; § raised to the
15 third power. * $p < 0.05$, *** $p < 0.001$, n.s. not significant

16

		Fixed effects estimate					
Plant traits	u.m.	Interaction			AIC	AICw	R^2
		Treatment Mown	Shoot phenology Vegetative	Treatment Mown: Shoot phenology Vegetative			
Number of shoots		0.310 ^{n.s.}	-	-	302.9	0.454	4.6 (4.6)
Plant height	m	-0.174 ^{***}	-0.116 ^{***}	-0.064 [*]	-346.4	0.748	70.4 (76.4)
+Specific leaf area ^b	mm ² mg ⁻¹	-	0.150 [*]	-	477.3	0.574	1.4 (25.0)
++Leaf area ^d	mm ²	-2.098 ^{***}	1.451 ^{***}	-	1144.8	0.714	34.5 (41.0)
§Leaf dry mass ^d	mg	-0.830 ^{***}	0.453 ^{***}	-	21.2	0.729	36.2 (38.8)
Leaf senescence symptoms (presence / absence) ^b		1.126 ^{***}	-0.726 ^{n.s.}	-1.478 [*]	-	-	13.7
Total number of spikelets ^a		-0.412 ^{***}	-	-	-	-	73.8
Number of flowering spikelets ^b		-0.410 ^{***}	-	-	-	-	39.3
Spike length ^c	mm	-25.627 ^{***}	-	-	1249.4	-	28.0 (28.0)
+Mean spikelet length ^b	mm	-0.077 [*]	-	-	-32.4	-	3.2 (3.9)
+Mean number of flowers per spikelet		-0.261 ^{***}	-	-	74.7	-	15.2 (15.2)
Number of flowers		-0.598 ^{***}	-	-	2292.4	-	83.9 (83.9)
+++Reproductive plant height ^a	m	-0.884 ^{***}	-	-	258.4	-	34.8 (35.8)
Seed output		0.103 [*]	-	-	1411.7	-	3.8 (3.8)
+Total seed mass ^b	mg	-0.049 ^{n.s.}	-	-	332.7	-	0.002 (0.002)
Mean seed mass ^a	mg	-0.001 ^{***}	-	-	-	-	13.3 (13.6)
§Epigeogenous RDMC _{sat} ^b	mg g ⁻¹	-	-	-	1420.2	-	2.1 (3.0)

Young hypogeogenous RDMC _{sat}	mg g ⁻¹	-21.165 ^{n.s.}	-	-	441.4	-	2.7 (2.7)
Mature hypogeogenous RDMC _{sat} ^b	mg g ⁻¹	-46.397 [*]	-	-	437.9	-	16.3 (16.7)

17

18 **Table 3.** Spearman's ρ coefficients of pairwise correlations between leaf traits, reproductive traits and belowground traits in the mown (above the diagonal) and unmown (below the diagonal) areas.

Trait	SLA	LA	LDM	Mean number of flowers per spikelet	Number of flowers per spike	Reproductive height	Spike length	Mean spikelet length	Seed output	Mean seed mass	Total seed mass	Epigeogenous RDMC _{sat}	Young hypogeogenous RDMC _{sat}	Mature hypogeogenous RDMC _{sat}
SLA	1	0.68***	0.24**
LA	0.48***	1	0.84***
LDM	-0.06 ^{n.s.}	0.71***	1
Mean number of flowers per spikelet	.	.	.	1	0.79***	0.39***	0.48***	0.72***	0.31**	-0.10 ^{n.s.}	0.23 ^{n.s.}	.	.	.
Number of flowers per spike	.	.	.	0.77***	1	0.57***	0.76***	0.55**	0.52***	0.06 ^{n.s.}	0.49***	.	.	.
Reproductive height	.	.	.	0.37**	0.57***	1	0.60*	0.29*	0.34**	0.29*	0.42***	.	.	.
Spike length	.	.	.	0.36**	0.63***	0.67***	1	0.36**	0.44***	0.09 ^{n.s.}	0.41***	.	.	.
Mean spikelet length	.	.	.	0.81***	0.67***	0.32**	0.44***	1	0.37**	0.00 ^{n.s.}	0.36**	.	.	.
Seed output	.	.	.	0.22 ^{n.s.}	0.31*	0.08 ^{n.s.}	0.21 ^{n.s.}	0.18 ^{n.s.}	1	0.04 ^{n.s.}	0.83***	.	.	.
Mean seed mass	.	.	.	0.08 ^{n.s.}	0.15 ^{n.s.}	0.32**	0.21 ^{n.s.}	0.12 ^{n.s.}	0.35**	1	0.52***	.	.	.
Total seed mass	.	.	.	0.18 ^{n.s.}	0.26*	0.14 ^{n.s.}	0.18 ^{n.s.}	0.14 ^{n.s.}	0.96***	0.54***	1	.	.	.
Epigeogenous RDMC _{sat}	1	-0.14 ^{n.s.}	0.37 ^{n.s.}
Young hypogeogenous RDMC _{sat}	0.15 ^{n.s.}	1	0.16 ^{n.s.}
Mature hypogeogenous RDMC _{sat}	0.08 ^{n.s.}	0.20 ^{n.s.}	1

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21 LA – Leaf area; LDM – Leaf dry matter; RDMC_{sat} - Rhizome dry matter content; SLA – Specific leaf area

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23 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. not significant

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