Functional group composition and defoliation intensity affect differentially community invasibility according to exotic species ontogenetic stage

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ABSTRACT

Functional group composition can positively influence plant community invasibility through niche vacancy when the resident community does not sufficiently overlap with the invaders. Phenology plays a key role in determining functional group composition, as it defines the temporal scale on which niche overlap occurs. Defoliation may regulate the effect of functional group composition by releasing resources, resulting in interactive effects. In a manipulative experiment, we assembled native communities with different functional group compositions: perennial warm- and cool-season grasses growing either separately or together. These communities were subjected to two defoliation regimes to test the hypothesis that niche overlap determines the invasion success of *Lolium multiflorum*, an annual grass belonging to a functional group under-represented in the native flora of the region. We also hypothesized that higher defoliation intensity would increase community invasibility, promoting invasion and reducing the differences observed between communities under lower defoliation intensity. We evaluated this in two ontogenetic stages of the invader by counting seedlings and measuring above-ground biomass at the end of the growing season. The evidence shows that community invasibility is mediated by functional group composition through phenological overlap, although priority effects were also important. Defoliation

intensity promoted seedling establishment similarly across all communities but did not affect the resulting above-ground biomass at the end of the growing season. Instead, it negatively impacted the performance of the invader growing in monoculture. Considering the variable effects on each ontogenetic stage is important for developing targeted management strategies at the appropriate temporal scale.

INTRODUCTION

A central question in invasion ecology is how community composition prevents or reduces exotic species invasion (Mack et al. 2000; Lockwood et al. 2007). Species composition can influence community invasibility through mechanisms such as niche vacancy and fitness differences (Shea and Chesson 2002; MacDougall et al. 2009). Niche vacancy may arise in low-diversity communities, where resident species use resources less completely, making the community more invasible. In contrast, species-rich communities are expected to be more resistant to invasion, as a higher diversity increases the likelihood of niche overlap between resident and invasive species, a concept known as the diversity-resistance hypothesis (Elton 1958; Tilman 1997; Naeem et al. 2000; Kennedy et al. 2002; Stachowicz and Byrnes 2006). Beyond species richness, the specific trait composition of a community may also determine the extent of niche overlap with potential invaders, and thus the degree of invasion resistance. This phenomenon is referred to as the "identity effect" (Crawley et al. 1999; Emery and Gross 2006, 2007). However, when an invader has a fitness advantage over resident species, it may still establish and spread despite high niche overlap. Therefore, community invasibility is thought to depend on the balance between niche overlap and the relative fitness of resident *versus* invasive species (MacDougall et al. 2009).

Beyond species composition, the functional group composition of a community may be a good predictor of its invasibility. Functional group overlap not only influences resistance to invasion but also provides insight into potential impacts of exotic species on ecosystem functioning (Longo et al. 2013; Byun et al. 2013). Functional groups are defined by shared traits—such as growth form, photosynthetic pathway, or phenology—which determine how species acquire resources across space and time. As such, overlap in these traits is expected to enhance invasion resistance by limiting available niches (Lavorel et al. 1997; Díaz and Cabido 2001; Fargione et al. 2003; Wolkovich and Cleland 2011; Byun et al. 2013). In addition, differences in life cycle strategies between resident and invasive functional groups, may determine which resources are most limiting to the invader at different ontogenetic stages. For instance, germination may be constrained by factors such as light, temperature, and humidity, whereas juvenile and adult stages may be more influenced by soil resource availability (Bhatla and Lal 2018). Thus, the outcome of interspecific or interguild competition is shaped by the functional traits of both resident and exotic species throughout their life cycles. However, how community resistance to invasion shifts across the ontogeny of exotic species remains understudied in plant invasion ecology (Lockwood et al. 2007). Investigating how functional group composition and defoliation affect exotic species performance across life stages may provide more targeted insights for managing invasions, particularly of annual species.

Disturbances are widely recognized as drivers of invasion, primarily by creating niche vacancies (Hobbs and Huenneke 1992). At a local scale, biomass removal can reduce community resource uptake and increase light availability, thereby creating opportunities for exotic species to colonize (Davis et al. 2000;

Levine 2000; Shea and Chesson 2002) or for subordinate species to expand its range (Grime 1998). Following such disturbance, invasion success is influenced by propagule pressure, species' tolerance to environmental stress and the capacity of invaders to preempt resource uptake or outcompete resident species (Chaneton et al. 1988; Burke and Grime 1996; Belyea and Lancaster 1999; Symstad and Tilman 2001). In temperate ecosystems, community-level resource uptake fluctuates seasonally and is closely tied to species' phenological traits. Consequently, biomass removal is likely to interact with functional group composition in determining invasion success, especially when invaders exhibit strong seasonal growth patterns (Wolkovich and Cleland 2011). Moreover, disturbances affect the relative fitness of species and therefore, different disturbances regimes (*e. g.* defoliation intensity or frequency) may modulate community's resistance to invasion.

Flooding Pampa grasslands have been subject to exotic plant invasions since the introduction of cattle, with the process intensifying during the 20th century (Soriano et al. 1992).These grasslands are primarily dominated by two phenologically distinct groups of native perennial grasses: warm-season and cool-season species. In contrast, most annual grasses and forbs, including many exotic species, are associated with the cool-season (Sala et al. 1986; Chaneton et al. 1988; Perelman et al. 2001). Approximately 25% of the flora in this ecosystem is composed of exotic species, the majority of which are annual grasses and forbs (Soriano et al. 1992; Chaneton et al. 2002). One of the most prominent of these is *Lolium multiflorum*, a highly productive annual winter grass that was intentionally introduced as forage (later on also referred as Lolium). It has since become widespread, significantly altering ecosystem functions such as nutrient cycling (Omacini et al. 2004), soil biota (Casas et al. 2011), and potentially reducing native plant biodiversity (Perelman et al. 2007).

Observational studies have found a negative correlation between native warm-season grass richness and Lolium cover (Perelman et al. 2003, 2007), though causality could not be established. Concomitantly, a functional group removal experiment showed that only the removal of warm-season grasses exerted a temporary increase in Lolium invasion and this effect was different from randomly removing equal amount of biomass (Longo et al. 2013), therefore evidencing a functional identity effect on community invasibility. As heavy grazing pressure have historically degraded cool-season grasses, this limitation hindered the ability to properly test the role of phenological overlap as a resistance mechanism under comparable conditions to the warm-season grass removal treatment. Improving our understanding of how community composition influences resistance to invasion could inform grazing management strategies—such as targeted seasonal herbivory—that aim to enhance grassland productivity while conserving native biodiversity.

We evaluated how phenological overlap of native functional groups interacts with defoliation intensity during the establishment and the growing period of the invader *Lolium multiflorum*. To address this, we conducted a community assembly experiment using varying combinations of native functional groups of a typically Flooding Pampa grasslands, exposed to two contrasting defoliation regimes. Within this framework, we tested the following two hypotheses:

1 First, community resistance to invasion is mediated by functional groups composition through phenological overlap. Therefore, the community that offers the greatest

phenological overlap during the whole exotic species life cycle will be the most resistant to invasion (e.g. cool and warm-season species). If resistance to invasion takes place in a particular ontogenetic stage, a single functional group will be responsible for community resistance for that particular stage. In this case we expect that native warm-season grasses will particularly affect Lolium establishment while native cool-season grasses will be better in reducing Lolium above-ground biomass.

2 Second, defoliation intensity regulates functional group composition effects on community resistance to invasion, given that it creates microsites favorable to invasion by reducing interspecific competition. According to this, the above predictions will take place under low defoliation intensity. Under high defoliation intensity we predict more invasion and it will be similar between communities with different functional group composition (interaction effect *Composition* x *Defoliation intensity*).

METHODS

Study site and experimental design

The experiment run in an open common garden placed in the School of Agriculture of the University of Buenos Aires, Argentina (34°35'32,46" S; 58°28'44,55" O). Climatic conditions were similar to those that characterize field climate (see in Supplementary Information: Environmental pots conditions). Native communities representing vegetation patches of a humid mesophytic meadow of the central Flooding Pampa (Perelman et al. 2001) were assembled in 40 x 40 x 23cm pots (4 species/pot) in December 2009 and later invaded with an annual exotic species, *Lolium multiflorum*. We established a complete factorial design with two factors: functional group composition (4 levels) and defoliation regime (2 levels). Functional group composition included native dominant grasses grouped by phenology as described below (Chaneton et al. 1988; Perelman et al. 2001, 2007), resulting in four treatments represented by warm-season grasses (WS), cool-season grasses (CS), a mixed community with warm and cool-season grasses (WCS) and a control treatment where the invader grew in monoculture (Control). Defoliation treatments consisted in clipping above-ground biomass in all pots at two different canopy height: 7cm or 20cm, to represent grassland structure in situations of higher or lower grazing intensity respectively (Sala et al. 1986). These 8 treatments were replicated in five random blocks (n=40). The experiment lasted two years, enabling us to capture two life cycles of the invader. Canopy structure and species composition were maintained during the two years of the experiment.

Since native grasses differ in traits beyond phenology, we used a pool of 15 species, nine warm-season grasses and six cool-season grasses, to capture within functional group heterogeneity. In a blocked design, from the pool of 15 species we randomly selected for each block 4 warm-season grasses for WS, 4 cool-season grasses for CS and from these eight species we randomly selected 2WS+2CS for WCS, the mixed community (see in Supplementary Information: Figure 1). The resulting species composition was repeated for both defoliation regimes within the block. This random blocking procedure enabled us to include within functional group heterogeneity while avoiding confusion due to variation in species identity

between replicates (Tilman 1997). The same procedure was repeated for each of the five blocks, with the only restriction of having all 15 species present in the experiment. Finally, Control treatment was made up of the same soil covering it with litter to avoid extreme micro environmental conditions for exotic seeds. All 40 pots were later seeded with the exotic species (+E) *Lolium multiflorum* resulting in four functional group composition treatments: WS_{+E}, CS_{+E}, WCS_{+E} and Control (more details are described in Supplementary Information: Materials and experimental procedures).

Finally, to be able to describe exotic species *vs.* native functional groups patterns of resource uptake as well as phenology overlap, we assembled homologous native communities for each block under both defoliation regimes (n=30), but without the addition of the exotic species (WS_{-E}, CS_{-E} and WCS_{-E}). This approach provided insights into the potential mechanisms acting in invasion resistance (*e.g.* Does Lolium invade more in communities that leave more soil resources?).

Variables measured and Statistical analysis

To describe initial community conditions and account for initial differences among pots, we measured aerial vegetation cover and soil variables including organic carbon, available nitrogen (nitrate and ammonium) and phosphorus, pH and electric conductivity (more details and results in Supplementary Information: Environmental pot conditions). Only initial soil NO_3^- differed significantly among communities and thus it was included as a covariate in statistical analysis for invasion success. We also measured intercepted photosynthetic active radiation (PAR) for the first and second, to evaluate changes in light quality perceived by seeds (see Appendix).

We measured Lolium invasion success during two experimental years in two ontogenetic stages, during emergence and at the end of the growing period. Each year, seedlings were counted when most of them had at least three leaves, in order to differentiate Lolium from other species. To account for spatial heterogeneity, we centered a frame of 16 cells (7 x 7 cm each) and chose randomly four cells with the restriction that each cell was placed next to each of the four species present in the pot (more details in Supplementary Information: Figure 2b.), making a total sample area of 196 cm^2 per pot. Biomass sampling was different for first and second year. At the end of the first growing period, we harvested all above-ground biomass (7 cm above ground level) within the former frame centered in each pot, allowing a sample area equal to 784 cm^2. We didn't reach ground level to prevent a drastic effect on performance of communities during the following year. At the end of the second growing period, we placed two frames of 20 x 5 cm at ground level (see Supplementary Information: Figure 2c) and harvested all above-ground biomass, allowing us to capture within pot heterogeneity due to different species' growing form. Each time, biomass was sorted by species (Lolium *vs.* native functional groups) dried at 60 °C for 48 h and weighed (0.01 g). Seedlings and biomass data were squared transformed and analyzed with a linear mixed effect model using *nlme* package (Pinheiro and Bates 2000; Pinheiro et al. 2023) including functional group composition, defoliation intensity, experimental year and its interactions as fixed factors and pots nested within blocks (species composition within functional group) as a random model structure. Initial soil NO_3^- was included as a covariate due to variations from first to second year among treatments. When interaction was significant, we compared treatments with different functional group composition within each level of defoliation intensity, using *predictmeans* package (Luo et al. 2002). Also, we explored Pearson correlation

between seedlings and above-ground biomass for each year, to see the relationship between ontogenetic stages. Finally, we explored Pearson correlation between the above-ground biomass of native functional groups and exotic species, to compare with the patterns previously observed in the field (Perelman et al. 2007).

To characterize phenological overlap, we measured normalized difference vegetation index (NDVI) as a proxy of community phenology and productivity (Pettorelli et al. 2005) in uninvaded communities and in Lolium monocultures during the second year of invasion. The NDVI was estimated for seven dates covering all four seasons, always approximately two weeks after applying defoliation treatment. We used a manual radiometer (Skye instruments, England) to measure incident and reflected radiation of red and infrared spectral bands, within an area of ~30 cm of diameter (~707 cm^2) centered in each pot. Measurements were made at midday without clouds in the sky. To compare phenology patterns we plotted NDVI values for each type of community (WS_{-E}, CS_{-E}, WCS_{-E}) and in Lolium monoculture under both defoliation regimes.

To describe soil resources uptake patterns of native functional groups and Lolium, we sampled available nitrogen (nitrate and ammonium) and phosphorus in uninvaded communities at the end of the second year (more details in Supplementary Information: Environmental pot conditions). We assumed that differences in final soil resources in the pots were mainly due to community uptake. Nutrients were analyzed with a linear mixed-effect model including functional group composition, defoliation intensity and its interactions as fixed factors and pots within blocks (species composition within functional group) as a random effect. To remove the effect of initial soil's nutrients concentrations over final soil's nutrients concentration, we considered the former as a covariable. All statistical analyses and data wrangling were performed using R software (R Core Team 2023).

RESULTS

[SEEDLINGS and PAR] Functional group composition affected exotic's seedling establishment in both consecutive years irrespective of defoliation regime (Table 1). Warm- and cool-season grasses reduced community invasibility only when growing separately; when growing together this resistance to invasion declined (Figure 1a). In average, pots showed more seedlings the second year than first one. A higher defoliation intensity had a marginal effect on Lolium's seedlings (Table 1), despite strong differences observed in photosynthetic active radiation (PAR) intercepted by the canopy (see Appendix, Table 2). During Lolium's germination, low-defoliation communities intercepted 50% of PAR both years, while high-defoliation communities intercepted 25% in the first year and 13% in the second, irrespective of native functional group composition.

[BIOMASS] Functional group composition affected Lolium's community invasibility depending on defoliation intensity, irrespective of the year observed (Table 1). Under low defoliation intensity, all native communities (*i.e.* WS_{+E}, CS_{+E} and WCS_{+E}) reduced invasion similarly; instead, under high defoliation intensity only WS_{+E} and CS_{+E} reduced invasion. Lolium above-ground biomass was negatively affected by a high defoliation intensity only when growing alone (see Control treatments in Figure 1b and Figure 2).

235 There was a positive relationship between Lolium's seedlings and above-ground biomass, especially
236 during the first year (Figure 2). This relationship was lower the second year (ρ = 0.57 and ρ = 0.42 the first
237 and second year respectively). Finally, we found a negative correlation between Lolium and native grasses
238 biomass when considering both years (ρ = -0.48), although this correlation was higher on warm (ρ = -0.37)
239 rather than cool-season grasses (ρ = -0.19).

240 [NDVI] In native non invaded communities, NDVI showed phenological variations according to
241 species functional groups, with some variation depending on defoliation intensity (Figure 3). Under high
242 defoliation intensity, cool-season grasses had the major NDVI values in middle winter (August) and at the
243 beginning of spring, while warm-season grasses had their major values in middle spring and summer
244 (November to March), showing a strong decrease in winter. Communities with both functional groups,
245 showed a more stable NDVI along the year with its peak in spring and summer, similar to warm-season
246 grasses growing alone. In general, high defoliation intensity slightly increased NDVI during the growing
247 period, being it more notorious in cool-season grasses communities, which turned from showing lower
248 NDVI values respect to WS_{-E} and CWS_{-E} under low defoliation, to higher values than other communities
249 during winter and spring. Lolium had on average greater NDVI values in both defoliation treatments.

250 [SOIL RESOURCES] After two growing periods, soil P, but not N, changed between Lolium
251 monocultures and native non invaded communities (-E), particularly for cool-season grasses (Table 3).
252 Lolium monoculture left less phosphorus in the soil than warm and especially cool-season grasses, while
253 mixed communities were more heterogeneous and left intermediate values respect to CS_{-E} and WS_{-E}. In
254 contrast, NO_3^-, NH_4^+ and total Nitrogen were similar between treatments, regardless of defoliation regime.

255 **TABLES AND FIGURES**

Table 1. Univariate ANOVAs for the effect of functional group composition, defoliation intensity and experimental year on Lolium's seedling establishment (n=40) and above-ground biomass (n=37) collected at the end of each growing cycle. The intercept shows significance of the model.

			Seedlings		**Biomass**		
	Num d.f.	Den. d.f.	*F*	*P*	Den d.f.*	*F*	*P*
(Intercept)	1	31	957.43	<0.0001	31	50.83	<0.0001
Functional group composition	3	28	15.76	**<0.0001**	25	20.91	**<0.0001**
Defoliation intensity	1	31	5.76	**0.023**	31	10.03	**0.003**
Year	1	28	4.53	**0.042**	25	0.00	0.981
NO3- ppm t0	1	28	3.56	0.069	25	0.24	0.630
FG comp. x Def. Intensity	3	31	1.15	0.345	31	4.98	**0.006**
FG comp. x Year	3	28	0.50	0.682	25	1.34	0.284
Def. Intensity x Year	1	28	1.39	0.248	25	0.00	1.000
FG comp. x Def. Intensity x Year	3	28	1.19	0.332	25	0.16	0.924

256 *For above-ground biomass three replicates were missed.

Table 3. Univariate ANOVAs of soil's nutrients content at the end of the second year of the experiment in uninvaded communities (-E) with different functional group composition and Lolium monoculture, under both defoliation regimes (n=40). Note that initial soil nutrient differences (Nutrient at t0) did not affect final results. The intercept shows significance of the model.

			NO_3^-		**NH_4^-**		**N**		**P***	
	Num d.f.	*Den. d.f.*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
(Intercept)	1	27	1068	<0.0001	1063	<0.0001	1217	<0.0001	2720	<0.0001
Functional group composition	3	27	2.1	0.1210	0.7	0.5528	2.4	0.0932	32.3	**<0.0001**
Defoliation intensity	1	27	0.2	0.6475	2.1	0.1571	0.0	0.9757	1.3	0.2663
NO3- ppm t0	1	27	0.3	0.5684	0.0	0.9983	0.2	0.6374	0.2	0.6643
FG comp. x Def. Intensity	3	27	0.1	0.9499	0.8	0.5185	0.1	0.9514	2.0	0.1412

257

258 * For P, mean values with its 95% confidence interval [lower bound, upper bound] were as follows. Control:
259 4.1ppm [3.9, 4.3], WS_{-E}: 4.6ppm [4.4, 4.9]; $CS_{-E:}$ 5.1 [4.8, 5.4], WCS_{-E}: 4.9ppm [4.4, 5.3].

260

Figure 1. Invasion by *Lolium multiflorum* in communities assembled with native warm-season grasses (WS+E), cool-season grasses (CS+E), both functional groups growing together (WCS+E) and no resident community (Control) in two consecutive years, for two ontogenetic stages: (a) seedlings and (b) above-ground biomass at the end of the growing season. Different lowercase letters above the bars, show significant differences between treatments (Tukey test $P < 0.05$) after considering interacting factors (See Table 1). Bars represent mean ±SE (n=80 and n=77 for panel a and b respectively).

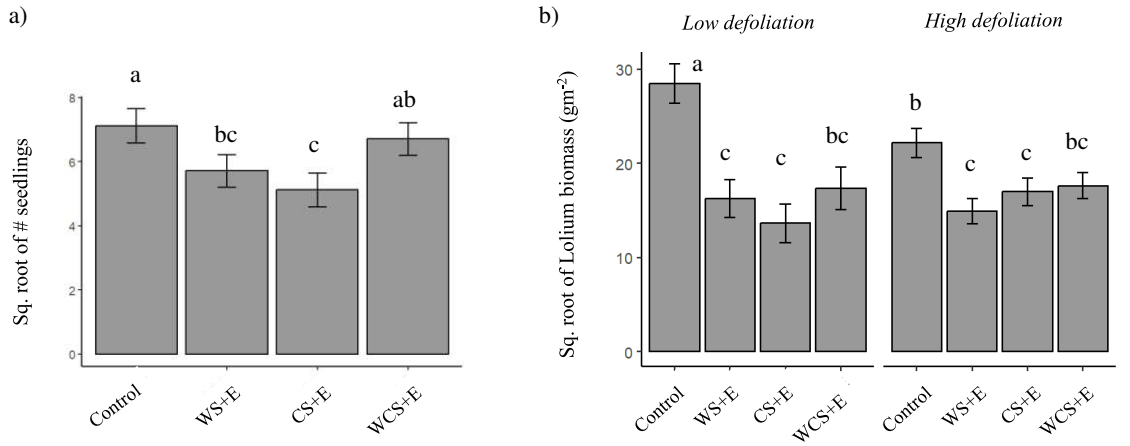

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Figure 2. Pearson correlation between Lolium multiflorum seedlings and above-ground biomass in communities assembled with cool-season grasses (CS_{+E}), warm-season grasses (WS_{+E}), both functional groups growing together (CWS_{+E}) and no resident community (Control) for the first year of invasion. Filled and empty circles represent Low or High defoliation intensity treatment respectively.

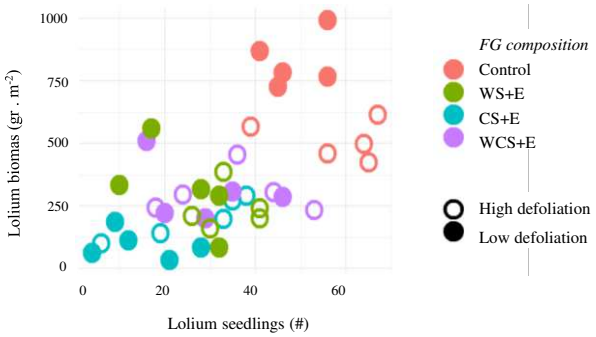

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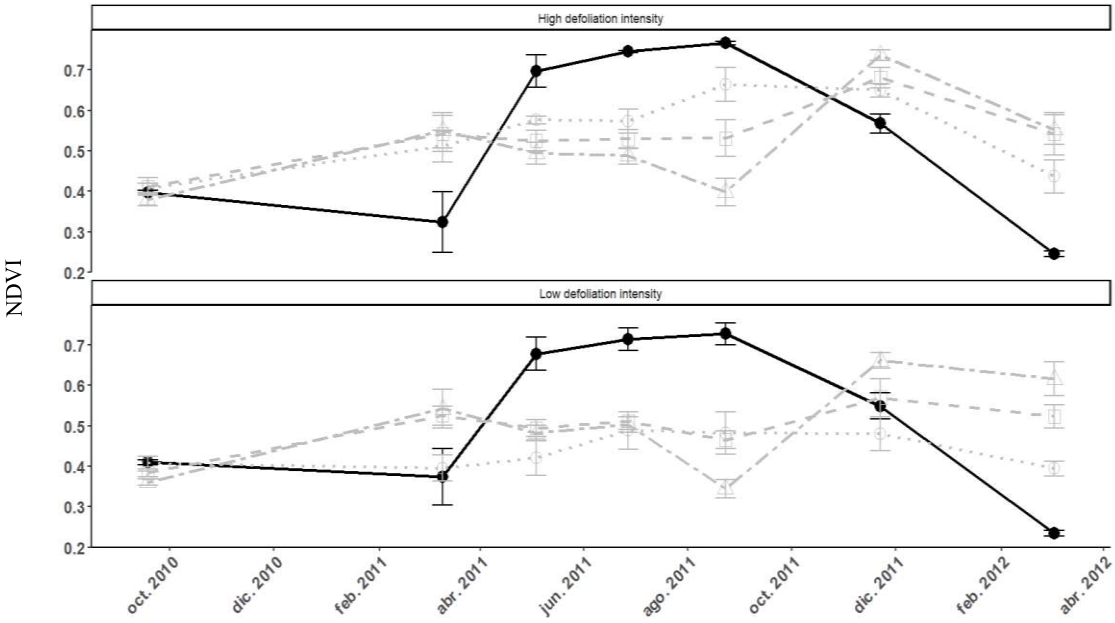
Figure 3. Normalized difference vegetation index (NDVI) describing phenology of *Lolium multiflorum* monocultures and native functional group growing without invasion, in communities exposed to high (upper panel) and low (lower panel) defoliation intensity. Symbols represent Control (●), CS-E (○), WS-E (Δ), WCS-E (□). Acronyms as in Figure 1, only here without the presence of the invader (-E).



DISCUSSION

Community invasibility was influenced by functional group composition through phenological overlap between the resident community and the invader. Along with defoliation intensity, this contributed to community resistance to invasion, although effects varied by the invader's ontogenetic stage (Figure 1a and 1b). Each functional group exhibited phenological overlap at different ontogenetic stages of Lolium (Figure 3). Cool-season grasses (CS) showed the greatest phenological overlap throughout the invader's life cycle. In contrast, warm-season grasses (WS) showed temporal separation, exerting priority effects. These results suggest that the underlying mechanisms differed across the invader's ontogenetic stages. In the second year, greater invasion was observed at the seedling stage across all treatments, a pattern not maintained at the adult stage (Table 1, $P_{Year} = 0.042$ vs. $P_{Year} = 0.981$ respectively). Although seedling abundance was positively related to final above-ground biomass (Figure 2), the underlying mechanisms likely differed across stages.

Seeds respond primarily to light, temperature, and moisture during germination (Bhatla and Lal 2018). In our experiment, temperature and moisture were not expected to vary between treatments with different functional group compositions due to continuous watering and homogeneous environmental conditions at the site (see Supplementary Information). Instead, photosynthetically active radiation (PAR) measured near the soil surface (~2 cm above-ground) was reduced by resident communities in both years but did not differ significantly between functional group compositions (see Results and Appendix, Table 2). This suggests that the amount of light reaching the soil was similar across the three assembled communities (WS, CS, and WCS).

This could explain the comparable resistance to invasion observed in WS and CS communities but does not account for the antagonistic effect between native functional groups suggested by the greater invasion in mixed communities (Figure 1a). One possible explanation is that the spectral properties perceived by seeds and seedlings are not fully captured by PAR measurements. While PAR is known to influence plant productivity and affect community invasibility (Renne et al. 2006; Gomez et al. 2022), it does not necessarily change in proportion to far-red light (Smith 1982), which is a better descriptor for light quality during germination (Toole 1963; Casal 2000). Light scattering depends on vegetation structure (Gates et al. 1965; Ollinger 2011), which in turn is influenced by species composition, thereby altering the community's spectral properties within the canopy (Gubsch et al. 2010; Zhao et al. 2021; Kothari et al. 2023). Therefore, the greater invasion observed in WCS at this stage may be evidence of light quality differences between communities at the soil level that are not reflected in PAR measurements.

Seedlings responded only marginally to the defoliation treatments (Table 1), whereas previous field and laboratory studies showed a stronger promotion of *Lolium* seedlings under high defoliation treatments (Deregibus et al. 1994; Jacobo et al. 2000). The light differences generated by our treatments (7 cm vs. 20 cm) may not have been as substantial as those in previous studies, where major differences were observed when stubble height was below 5 cm compared to heights around 5–10 cm, 10–15 cm, or 10–20cm (Deregibus et al. 1994). If the phytochromes involved in *Lolium* germination and seedling establishment do not respond linearly to light -as has been demonstrated in other species (Blaauw-Jansen and Blaauw 1975; Lambers and Oliveira 2019)- it is possible that our treatments did not reach the minimum stubble height at which the light environment elicits a stronger phytochrome response.

During the juvenile-adult stage, the effect of functional group composition on community invasibility depended on defoliation intensity (Table 1). Communities composed of CS or WS were equally resistant to invasion under both defoliation regimes (Figure 1b). The community composed of WS was almost as efficient as *Lolium* at uptaking phosphorus from the soil, regardless of defoliation intensity (Table 3, see footnote). In similar grasslands of the Flooding Pampa, it was found that warm-season grasses were not limited by phosphorus, while *Lolium multiflorum* was (Mendoza et al. 1983; Collantes et al. 1998; Rodríguez et al. 2007). In other systems, Lolium has been shown to respond strongly to P-rich environments during spring (Abe and Ozaki 1998), suggesting that phosphorus could be a limiting resource for its growth. Therefore, it is possible that in a P-poor system such as the Flooding Pampa grasslands (Collantes et al. 1998), WS may exert invasion resistance to Lolium through this mechanism, especially under defoliation regimes where phosphorus reserves are needed for regrowth (Oyarzabal and Oesterheld 2009). In contrast, CS showed the greatest phenological overlap with Lolium throughout the growing season, indicating that competition for light could be the principal mechanism of resistance to invasion exerted by this functional group. The apparent lack of resistance to invasion from CS grasses in the field may simply reflect their historically low biomass. Clearly, *Lolium*'s fitness overrides community resistance to invasion, making it a strong competitor both in this experiment and in the field. This shifts the balance between niche overlap and fitness in favor of the invader (MacDougall et al. 2009). This is evidenced by the higher NDVI values of the invader during the growing season.

In this and other grasslands, C4 species have been identified as responsible for resistance to invasion (Fargione and Tilman 2005; Perelman et al. 2007; Hooper and Dukes 2010; Longo et al. 2013; Bresciano et al. 2014). In particular, in a Flooding Pampa grassland, a functional group identity effect was observed after controlling for the warm-season (WS) biomass, which is composed predominantly of C4 species (Longo et al. 2013). Conversely, in this experiment, we also found invasion resistance in cool-season (CS) grasses—an underrepresented functional group in the field composed predominantly of C3 species. Both niche overlap and priority effects have been proposed as mechanisms responsible for invasion resistance (Fargione and Tilman 2005; Wolkovich and Cleland 2011; Wainwright et al. 2012), which could be interpreted as contrasting results. This apparent contradiction is addressed by the hypothesis that community invasibility is determined by the balance between niche overlap and fitness differences (Ortega and Pearson 2005; MacDougall et al. 2009). Our evidence supports this latter hypothesis: when the greatest temporal niche overlap occurred (e.g., in CS), *Lolium*'s fitness determined species coexistence. In contrast, when the temporal niches of the resident community and the invader differed, priority effects determined both invasion resistance and species coexistence. This is an important consideration for management strategies (Hess et al. 2019).

A separate explanation is needed for the interaction between functional group composition and defoliation intensity during this ontogenetic stage of Lolium. Mixed communities (WCS) were resistant to invasion under low defoliation intensity but were not resistant under high defoliation intensity compared to the control treatment (Table 1 and Figure 1b). This could be interpreted as an antagonistic effect similar to that observed during seedling establishment; however, in this case, the change is driven by Lolium's performance in monoculture. While Lolium reached similar invasion levels in native communities under both defoliation regimes, its growth was negatively affected by defoliation intensity when growing alone (Figure 1b). Defoliation may not only improve the quantity and quality of light reaching lower vegetation strata but also affect physiological mechanisms involved in regrowth (McNaughton 1979; McNaughton and Chapin 1985; Oesterheld and McNaughton 1991; Oyarzabal and Oesterheld 2009). It is possible that when growing alone, intraspecific competition is strong enough to be influenced by defoliation intensity, whereas in mixed communities, interspecific competition reduces Lolium populations more than defoliation intensity does, thus having no effect on community invasibility for this ontogenetic stage.

Our main conclusions are, first, experimental evidences support~~s~~ the hypothesis that community resistance to invasion is mediated by functional group composition through phenological overlap, although priority effects were equally important in this experiment. This explains the lack of support for our predictions, as all communities were similarly invaded throughout the growing period. This result is consistent with field studies, where native cool-season grasses are underrepresented and warm-season grasses dominate grasslands, indicating a functional group identity effect. Second, predictions derived from the fluctuating resource theory (Davis et al. 2000) did not fully apply to our system, as our second hypothesis assumed. Instead, invasion patterns depended on the ontogenetic stage of the invader. Defoliation intensity promoted invasion during seedling establishment by enriching the light environment, although light quality (e.g., the R:FR ratio) still requires further investigation under different functional group compositions. In contrast, defoliation intensity did not affect invasion levels in native communities. This is likely because defoliation not only frees up resources but also negatively impacts the invader. Davis

et al. (2000) stated that a community's susceptibility to invasion increases with the introduction of grazers, particularly in nutrient-rich systems. In nutrient-poor systems like those used in our experiment, however, limited resource availability constrains regrowth after grazing (Proulx and Mazumder 1998), a mechanism that likely influenced our results. And third, the differing mechanisms acting at each ontogenetic stage of the invader must be considered when identifying which stage of the life cycle is most sensitive to invasion drivers. This understanding is essential for developing targeted management strategies at the appropriate temporal scale.

372 **APPENDIX**

373 Photosinthetic active radiation (PAR: µmol m-2 s-1) was measured with a bar radiometer
374 (Cavadevices, Buenos Aires). The bar was located above the canopy for PAR incidence and at 2 cm above-
375 ground level (PAR under the canopy) in two distant parallel positions within each plot, separated 10 cm
376 from the center axis of the plot. This allowed us to capture within plot heterogeneity due to different species'
377 growing form. Each measure covered 30 cm long. We then estimated the percentage of PAR intercepted
378 by the canopy (PAR under the canopy/PAR incidence) and used for statistical analysis the media of both
379 observations. Higher levels of PAR intercepted meant more light absorbed or reflected by the canopy,
380 leaving less light for exotic seeds or seedlings. Data was analyzed with a linear mixed effect model using
381 *nlme* package (Pinheiro and Bates 2000; Pinheiro et al. 2023) including functional group composition,
382 defoliation intensity, experimental year and its interactions as fixed factors and pots nested within blocks
383 (species composition within functional group) as a random model structure. When interaction was
384 significant, we compared treatments with different functional group composition within each level of
385 defoliation intensity, using *predictmeans* package (Luo et al. 2002). See Table 2 for results.

Table 2. Univariate ANOVAs for the effect of functional group composition, defoliation intensity and experimental year on PAR (n=40).

	Num d.f.	Den. d.f.	*F*	*P*
(Intercept)	1	32	798.43	**<.0001**
Functional group composition	3	28	70.73	**<.0001**
Defoliation intensity	1	28	1.38	0.248
Year	1	32	2.61	0.115
FG comp. x Def. Intensity	3	28	16.15	**<.0001**
FG comp. x Year	3	32	0.38	0.763
Def. Intensity x Year	1	32	10.31	**0.003**
FG comp. x Def. Intensity x Year	3	32	0.64	0.589

386
387 The interaction observed between functional group composition and defoliation intensity as well
388 as the principal effect of the former, was due to the fact that Control treatment did not have plants at the
389 moment of Lolium germination (a monoculture); therefore, there was not light interception in those pots
390 (comparisons not shown). Nevertheless, defoliation intensity did interact with experimental year, a result
391 described in the main text.
392

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STATEMENTS AND DECLARATIONS

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DATA ACCESSIBILITY

571 Data would be accessible upon gently request.

572

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