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Trampling enhances the dominance of graminoids over forbs in flooded grassland mesocosms

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Keywords
Community structure; Flooding; Grassland recovery; Grazing; *Lotus tenuis*; Trampling

Abbreviations
MRPP = Multi-response permutation procedure

Nomenclature
sensu Cabrera & Zardini (1978)

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Abstract

Questions: What are the interactive effects of flooding and cattle trampling upon the structural attributes and the floristic composition of a plant community? Do the effects on the plant community persist over an extended recovery period?

Location: Flooding Pampa grasslands, Argentina (36°30′ S, 58°30′ W).

Methods: We assessed the effects of 40-d of flooding, trampling and the combination thereof on plant cover and biomass, vertical distribution of foliage and floristic composition in lowland grassland mesocosms. We considered a 120-d recovery period to evaluate the persistence of flooding and trampling effects on the plant community.

Results: Flooding, with or without trampling, increased cover and biomass of the graminoid species, especially marsh grasses, which developed a taller canopy, whereas most of the forb species were negatively affected. This was enhanced by trampling, as the aerial biomass of the dominant legume *Lotus tenuis* decreased by 90%, while three major forb species disappeared. Trampling under flooding conditions did not reduce the total above-ground biomass production, as the growth enhancement of graminoids was enough to compensate for the breakdown of the forbs. Below-ground biomass was lower when both perturbations occurred simultaneously. After 120-d of recovery, graminoids continued to be dominant while the remaining forbs (including *L. tenuis*) recovered only partially. Below-ground biomass recovered fully at the end of the growing season.

Conclusions: The combination of flooding and trampling shifts the community co-dominance of graminoids and forbs towards a persistent dominance of graminoid species. When both perturbations are combined, the above-ground production of the grassland is unaffected and root biomass is rapidly recovered. However, the loss of the legume *L. tenuis* deserves attention because this is the unique nitrogen-fixing species of the ecosystem, which improves the forage quality for livestock production.

Introduction

Trampling and flooding are two major co-occurring perturbations in many humid grassland ecosystems subjected to livestock grazing (Soriano 1991; Milchunas & Lauenroth 1993; Kohler et al. 2004; Altesor et al. 2006). Those perturbations exert antagonistic effects upon the structural attributes and the floristic composition of the plant community. Trampling modifies the community structure by flattening out the vertical distribution of the canopy (Edmond 1964; Pellerin et al. 2006; Van Uytvanck et al. 2010), and changes the floristic composition by favouring prostrate stoloniferous graminoids and rosette forbs (Sala et al. 1986; Sun & Liddle 1993; Cole 1995a; Kobayashi et al. 1997). In contrast, flooding modifies the community structure by promoting the location of foliage in upper layers of the canopy (Pehrsson 1988; Insausti et al. 1999; Striker et al. 2005) and subordinating species with rosette and creeping growth forms, which remain mostly submerged and shaded during the flooding period (Insausti et al. 1999; Van Uytvanck et al. 2010). To date, the effects of

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both perturbations on grassland vegetation have been studied separately. For the first time, this work explores the interactive effects of flooding and trampling on plant community structure and the floristic composition of a lowland temperate grassland. The study should contribute to predicting changes in the vegetation dynamics of humid grassland ecosystems for the purposes of conservation and management.

Graminoids and forbs are co-dominant in many humid grasslands ecosystems of the world, such as the North American prairies (Fahnestock & Detling 2002), floodplains in the Rhine River (Lenssen et al. 2004) and South American grasslands (Soriano 1991; Altesor et al. 2006). Such co-dominance between both life forms also occurs in grazed grasslands of the Flooding Pampa (Argentina), the study site of this work. Here, native graminoids (grasses and sedges) are co-dominant with forb species, mainly exotic weeds (Sala et al. 1986; Perelman et al. 2007), while in some cases, the legume *Lotus tenuis* is an important forage species in terms of sustaining livestock production (Quinos et al. 1998; Striker et al. 2008). Continuous cattle grazing enhances the selective defoliation of palatable species (i.e. grasses and *L. tenuis*), which leads to the opening of the canopy, the relaxation of competitive interactions and, therefore, the recurrent invasion of the grassland by weed forbs (Sala et al. 1986; Oesterheld & Sala 1990; Insausti & Grimoldi 2006). Conversely, flooding rapidly reverts the grazing effects on the plant community by promoting the abundance of flood-tolerant graminoids and reducing the presence of weed forbs (Insausti et al. 1999), most of which are flood-sensitive species (Grimoldi et al. 2005). The legume *L. tenuis* also persists because of its higher flooding tolerance under conditions that do not involve trampling (Striker et al. 2005, 2006). Recently, we identified the grass *Paspalum dilatatum* as tolerant and some coexisting dicot species (i.e. weed forbs and the legume *L. tenuis*) as sensitive to the combination of flooding and trampling (Striker et al. 2006, 2007). We demonstrated that the generation of aerenchyma tissue induced by flooding increases the root weakness of the forb species but has no effect on the strength of graminoid roots (Striker et al. 2006, 2007). Using this framework, we scaled up to the grassland level by testing the hypothesis that the combination of flooding and trampling shifts the co-dominance between life forms towards a substantial dominance of graminoid species.

Most studies of flooding effects on grassland vegetation relied on plant community descriptions before and after the flooding event (Chaneton et al. 1988). This is for two different reasons: (1) it is not feasible to simulate flooding in field conditions, and (3) when large natural floods occur, it is impossible to create comparable non-flooded control plots. Thus, the interpretation of the results is constrained by the difficulty in replicating treatments or having adequate controls (non-flooded plots at the same place and time). The use of mesocosms (as model systems) is an effective way to analyse the effect of flooding on the structural attributes of the plant community, provided that the experimental results are compared with the patterns observed under field conditions (Fraser & Keddy 1997; Insausti et al. 1999; Striker 2008). In addition, many flooding studies do not take into account the recovery period after the stress; as a result, the long-term effects on vegetation remain unclear (Davies et al. 2000; but see Beltman et al. 2007). In this regard, many studies on trampling effects have pointed out that, in order to understand plant community dynamics, it is crucial to evaluate grassland recovery after trampling damage instead of focusing only on their immediate effects (Cole 1995a, b; Roovers et al. 2004). For these reasons, we evaluated the effects of flooding and trampling using a mesocosm experimental approach and considered a period for the recovery of the grassland as part of the experimental protocol.

We conducted an outdoor experiment in which grassland mesocosms were subjected to 40 d of flooding (hereafter referred to as 40-d) in conjunction with simulated cattle trampling. The interactive effects on the plant community were evaluated at two different times – at the end of the 40-d flooding period, and after a recovery period of 120 d – under well-drained soil conditions in order to evaluate the persisting effects of treatments until the end of the growing season. Changes in plant cover, vertical canopy structure, above-ground and below-ground biomass and the floristic composition of the plant community were assessed as a function of time. We specifically addressed the following questions: (1) What are the interactive effects of flooding and cattle trampling upon the structural attributes and the floristic composition of the plant community? (2) Do the effects on the plant community significantly persist over an extended recovery period?

Materials and Methods

Study site and grassland mesocosms collection

In August 2006, 20 homogeneous patches of vegetation were selected within an extensive stand (> 100 ha; see also Table 1 in Aragón & Oesterheld 2008) of lowland grassland located in the Department of Pila, Province of Buenos Aires, Argentina (36°30′ S, 58°30′ W). The stand corresponds to the most widespread plant community of these grasslands within the vegetation units of humid mesophytic meadows (*sensu* Perelman et al. 2001), phytosociologically defined as *Piptochaetium montevidensis*,

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Table 1. Repeated measures ANOVA results for the effects of flooding (F), trampling (T) and the combination thereof ($F \times T$) on plant cover in grassland mesocosms. F-values of the rmANOVAs are presented. Degrees of freedom for each source of variation are: 1 (Flooding), 1 (Trampling), 1 ($F \times T$), 2 (time), 16 (Error of F, T & $F \times T$) and 32 (Error of time, $F \times$ time, $T \times$ time, $F \times T \times$ time). Significant differences: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns $P > 0.05$.

Plant cover	Main effects			Interactions			
	F	T	Time	$F \times T$	$F \times$ time	$T \times$ time	$F \times T \times$ time
Total	10.82**	6.91*	252.8***	0.01 ns	32.2**	17.7**	0.40 ns
Graminoids	35.7***	0.01 ns	187.1***	0.19 ns	65.8***	2.31 ns	1.31 ns
Forbs	11.10**	19.7***	59.9***	0.90 ns	36.9***	38.75***	14.35***

Ambrosia tenuifolia, *Eclipta bellidioides* and *Mentha pulegium* (Burkart et al. 1990). This plant community is found in flat areas associated with typical Natraquol soils characterized by an A_1 horizon with 3.3% organic carbon (C), and by a natric B_{2t} horizon 17 cm deep with 53.3% clay content. It is frequently exposed to flooding events of varying intensity and duration, resulting from heavy rainfall, the flat topography and the poor draining conditions of the soil (Soriano 1991). Vegetation patches were selected based on their similarity in terms of floristic composition, ground cover and vertical structure of vegetation. The similarity of the composition of species at the start of the study was calculated using Sorensen's similarity index (Greig-Smith 1983), which yielded an overall mean similarity of 75 ± 8% ($n = 180$ pairwise comparisons). Mean species richness of the patches was 18 ± 3, which constitutes a typical representation of the plant diversity in these grasslands (*sensu* Chaneton & Facelli 1991; Perelman et al. 2001, 2007).

At the centre of each selected patch, a plot of 0.8 m × 0.5 m was marked and split in two equal subplots (0.4 m × 0.5 m) to facilitate the extraction of soil monoliths to support intact vegetation (= mesocosms). Mesocosms were carefully dug out to a depth of 0.35 m, ensuring the collection of most below-ground biomass of the grassland as is known that more than 85% of root biomass are concentrated at the first 0.2 m soil depth (Taboada & Lavado 1988; Soriano 1991). Once extracted, grassland mesocosms were put into plastic containers and transported to the Faculty of Agronomy at the University of Buenos Aires. There, a grid with five columns and four rows was drawn on the ground of the experimental garden ($n = 20$), leaving a 1-m walkway, and the mesocosms were randomly assigned to each position. All mesocosms were watered daily until the beginning of the experiment.

Experimental design

In early spring, after one month of acclimatization, mesocosms were subjected to flooding and trampling treatments following a completely randomized design (2 × 2) with five replicates. Flooding treatment was applied for 40 d at a water level of 6 cm above soil surface, followed by a recovery period of 120 d until the end of the summer. Flooding duration and intensity simulated a typical natural flooding occurring at these grasslands (Paruelo & Sala 1990). Trampling was applied on day 20 by covering ca. 52% of soil surface in accordance with the average trampled surface observed under field conditions. The actual proportion of the trampled surface in field conditions was previously estimated over 10 randomly placed 5-m long transects located in homologous stands of the grazed grassland. The trampling treatment consisted of 25 hoof impacts randomly assigned in a rectangular grid of 40 points over each mesocosm. Trampling was simulated using a real cow hoof (surface of 82 cm^2) filled with plumb and epoxy resin attached to a metal handle (0.5 m long, total weight of the system was ca. 22.5 kg.). In order to simulate the hoof impact of a treading cow, we measured the soil bulk density in continuously grazed sites and used these reference values for our system. After preliminary tests, we determined that the hoof should be vertically dropped from a height of 0.45 m to compact the soil (at soil water contents close to field capacity) to simulate the steps of cattle under field conditions (1.24 ± 0.12 vs $1.27 \pm 0.10\ \mathrm{g\,cm^{-3}}$; $n = 4$; $t_6 = 0.19$; $P = 0.85$; soil bulk density at non-trampled sites was $1.02 \pm 0.03\ \mathrm{g\,cm^{-3}}$). This procedure was validated in a previous experiment in which we studied flooding and trampling effects on the individual plant performance of *P. dilatatum* and *L. tenuis* (Striker et al. 2006).

Canopy cover and vertical distribution of foliage

Changes in the plant community structure as a consequence of treatments were evaluated through non-destructive measurements of plant cover. The plant cover of species was estimated using the point-quadrat method (Mueller-Dombois & Ellenberg 1974; Greig-Smith 1983) on three dates: at the beginning of the experiment (day 0), at the end of flooding (day 40) and after the recovery period (day 120). Measurements were done using a wooden frame that supported 10 steel rods measuring

0.4 m in length and spaced by 2.5 cm. The plant cover was recorded in 20 point-quadrats systematically arranged at the centre of the mesocosm in two rows within a 0.6 m × 0.3 m grid, for a total of 100 sampling points per treatment/date. The sampling intensity was appropriate for monitoring fine-grained vegetation at this scale (Greig-Smith 1983). Each pin was lowered vertically through the canopy, registering the height and the species in every contact. The number of hits per species was grouped to obtain an estimate of the cover of graminoids and forbs. In addition, on day 40 (the end of the flooding period) and on day 160 (the end of recovery period) the number of contacts of graminoids and forbs were grouped into sets of 6 cm (height) to characterize the vertical distribution of foliage by life form.

Above-ground and below-ground grassland biomass

Above-ground biomass was harvested on two dates: at the end of flooding (half mesocosm) and after the recovery period (the other half mesocosm). This biomass was separated into green and dead material. Green biomass was sorted by life form (graminoids and forbs) and by main species (Cornelissen et al. 2003). Below-ground biomass was estimated on the same dates by extracting two soil cores per mesocosm (0.1 m diameter × 0.3 m depth). This biomass was carefully separated from the soil under water pressure and separated into roots and reserve organs (i.e. rhizomes, tap roots and bulbs). In all cases, the material was weighed after oven drying for 72 h at 70°C.

Statistical analyses

The treatment effects on the total cover of graminoids and forbs were evaluated by repeated measures analysis of variance (rmANOVA), with flooding and trampling as the main effects between-subject; time was the within-subject factor. Before analyses, cover data was transformed through ($\sqrt{x}+0.5$) to ensure the homogeneity of variances and checked to confirm the hypothesis of sphericity of the covariance matrices (Greenhouse–Geisser) (Von Ende 1993). On day 40, the vertical distribution of the foliage of graminoids and forbs on the different layers of canopy was analysed by MANOVA. Above-ground and below-ground biomasses were studied using two-way ANOVAS. The aerial biomass of individual plant species was not normally distributed. In consequence, the overall effects of treatments on the biomass of the major plant species within life forms was examined using the multi-response permutation procedure (MRPP), a robust non-parametric multivariate test (Mielke 1984). In the MRPP analysis, samples were grouped by treatment and pair-wise differences among treatments were tested using Sorensen (Bray–Curtis) as a measure of distance. These analyses were performed using PC-ORD 4.0 for Windows (MjM Software, Gleneden Beach, OR, USA). Data analyses involving biomass were performed separately for flooding and recovery periods. All results are presented as untransformed means of five replicates± standard error.

Results

Dynamics of plant cover and canopy architecture as affected by flooding and trampling

During the first 40-d of experiment, flooding increased total plant cover by 55% because of a high promotion of graminoids and a slight decrease in forbs cover (Fig. 1, Table 1). In contrast, trampling led to a decrease of 21% in total plant cover by equally damaging graminoids and forbs (18% and 21%, respectively) (Fig. 1, Table 1). The combination of flooding and trampling caused a drastic

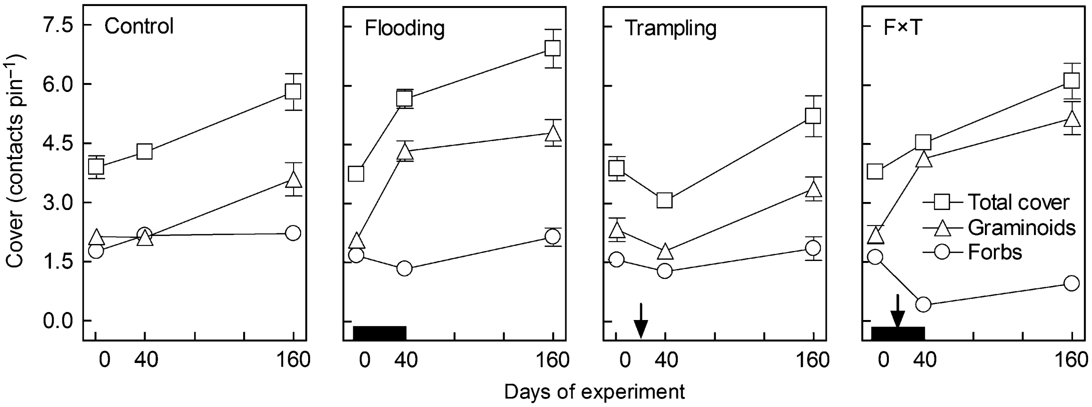

Fig. 1. Dynamics of plant cover (total, graminoids and forbs) in grasslands mesocosms under different treatments: control, flooding, trampling and flooding × trampling ($F \times T$). Horizontal closed bars indicate the duration of the experimental flooding (40 d). Arrows indicate the application of the simulated trampling (day 20). A recovery period of 120 d after flooding was allowed to evaluate the persistence of the treatments within the same growing season. Values are means ± SE of five replicates.

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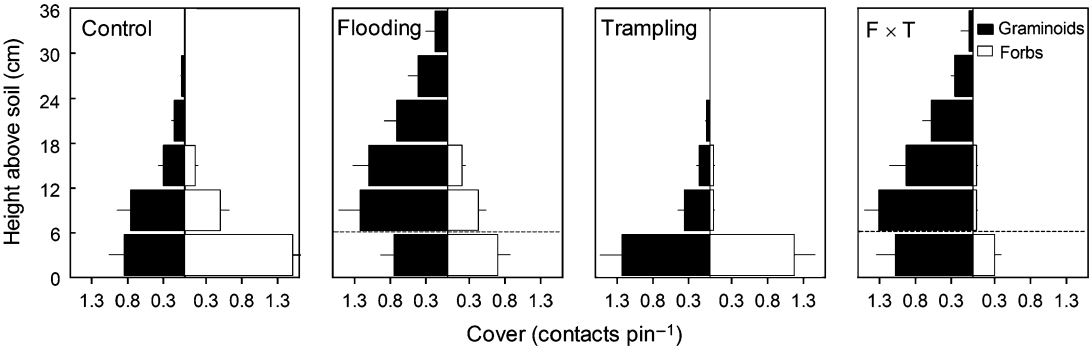

Fig. 2. Vertical distribution of plant cover of graminoids and forbs in grasslands mesocosms under different treatments: control, flooding, trampling and flooding × trampling (*F* × *T*) at the end of flooding period (day 40). Dashed lines indicate the flooding level (6 cm). Values are means ± SE of five replicates.

decrease (76%) in the cover of forb species, which was fully counterbalanced by the enhancement of graminoids. On day 40, the total cover of the mesocosms subjected to the combination of flooding and trampling was similar to control conditions, but the graminoid group proved to be exclusively dominant (Fig. 1, Table 1). During the recovery period, this dominance continued: forb cover in flooded and trampled mesocosms reached less than half of the values of all other conditions (compare 40 versus 160-days in Fig. 1). Therefore, a recovery period of duration three times longer than the flooding event was not enough to allow the forb group to recover. In contrast, mesocosms previously subjected to flooding or trampling (separately) showed an important increase in forb cover that was enough to reach values similar to the controls (Fig. 1).

For both life forms, flooding and trampling shifted the vertical distribution of plant cover in a contrasting way (MANOVA: Wilk's $\lambda = 0.09$ and 0.07; $F_{5,96} = 203.3$ and 245.5; $P < 0.0001$ for graminoids and forbs, respectively). As expected, flooding determined the location of plant foliage at the upper layers (graminoids: $F_{5,96} = 55.8$, $P < 0.001$; forbs: $F_{5,96} = 40.5$, $P < 0.001$), with a much more pronounced response for graminoids than for the other species (Fig. 2). In contrast, trampling provoked a concentration of plant cover at the lower layers for both groups (graminoids: $F_{5,96} = 12.1$, $P < 0.001$; forbs: $F_{5,96} = 18.3$, $P < 0.001$; Fig. 2). When flooding and trampling were combined, the vertical distribution of graminoids changed in the same way for plots subjected only to flooding conditions (74-82% cover above water; $F_{5,96} = 0.2$, $P = 0.85$), while forbs remained fully submerged with their leaves located close to the soil surface ($F_{5,96} = 8.2$, $P < 0.001$). In particular, the legume *L. tenuis* was unable to emerge from the water when flooding and trampling were combined, with almost all stems below the water level. Remarkably, for each life form, differences in the vertical distribution of the foliage persisted until the end of the recovery period (see the Supporting Information, Appendix S1).

Above-ground and below-ground biomass responses induced by flooding and trampling

Flooding, with or without trampling, determined a higher total above-ground biomass of grassland mesocosms; this response was strongly associated with the intense promotion of the graminoid group (Fig. 3, $F_{1,16} = 21.4$, $P < 0.001$). In contrast, the biomass of forbs was 22–33% lower under trampling and flooding conditions ($F_{1,16} =$ 29.9 and 53.3, respectively; in both cases $P < 0.001$). In the case of those treatments, the reduction in the biomass of forbs was comparable to the registered amount of dead biomass, suggesting that this life form was the main source of dead biomass in different treatments (Fig. 3). Importantly, the enhancement of total biomass production under flooding conditions was not affected by the application of the trampling treatment; meanwhile, this combination of treatments reduced the biomass accumulation of the forb group (interaction $F \times T$: $F_{1,16} = 21.5$, $P < 0.01$; Fig. 3) more than proportionally. Differences in the total biomass among treatments persisted until the end of recovery period; the biomass of forbs was still lower in the plots that were subjected to the combination of flooding and trampling (Fig. 3, $F_{1,16} > 11.9$, $P < 0.01$).

Similar to above-ground biomass, the application of flooding or trampling, as a unique factor, did not decrease the total below-ground biomass in comparison with the control mesocosms (compare Fig. 3 and Table 2; $F_{1,16} = 0.3$, $P = 0.45$). It was not feasible to separate below-ground biomass among different species; instead, we separated functional compartments as roots from reserve organs (Table 2). Interestingly, flooding mesocosms showed a higher proportion of root biomass that compensated for the lessened biomass of reserve organs ($F_{1,16} = 5.1$, $P < 0.05$), making total below-ground biomass similar to that attained under control conditions (Table 2). As expected, the combination of flooding and trampling provoked a significant reduction of total below-ground biomass (roots and reserves organs) in relation to

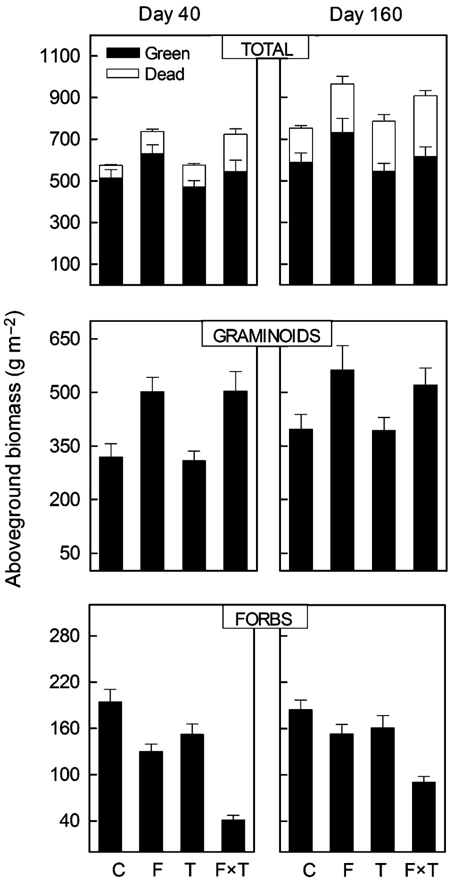

Fig. 3. Above-ground biomass in grassland mesocosms under different treatments: control (*C*), flooding (*F*), trampling (*T*) and flooding × trampling ($F \times T$). Flooding lasted 40 d. Trampling was applied at day 20 of experiment. A recovery period of 120 d after flooding was allowed to evaluate the persistence of the treatments within the same growing season. Values are means ± SE of five replicates.

the other treatments (Table 2, interaction $F \times T$: $F_{1,16} = 6.7$, $P < 0.05$). At the end of the recovery period, there were no differences in the total below-ground biomass of the plants subjected to different treatments, indicating an important level of recovery of this parameter after the applied perturbations (Table 2; $F_{1,16} = 0.2$, 2.6 and 0.3 for *F*, *T* and $F \times T$, respectively; in all cases $P > 0.05$). However, there is still a trend towards a lower biomass of reserve organs in the mesocosms that were subjected simultaneously to flooding and trampling conditions (Table 2; $P = 0.07$).

Changes in floristic composition

Analyses by MRPP showed that both perturbations, flooding and trampling, significantly affected the floristic composition of the grasslands (Table 3, Appendix S2). In the graminoid group, flooding more than doubled the above-ground biomass of three marsh grasses, namely *Leersia hexandra*, *Panicum gouinii* and *Paspalidium geminatum* and the sedge *Eleocharis viridans* with respect to that obtained under control conditions (63-68% versus 28-35%; Table 3). In these species, the enhancement of growth produced by flooding was not reduced when trampling was applied simultaneously (compare *F* versus $F \times T$ in Table 3). In contrast, the combination of flooding and trampling reduced the above-ground biomass (90%) of the dominant legume *L. tenuis* more than proportionally. This combination also provoked deleterious effects on most of the other major forb species, in particular *Leontodon taraxacoides*, *M. pulegium* and *Phylla canescens*, with no aboveground biomass detected at the end of the flooding period (Table 3). Additionally, the biomass of *L. tenuis* was greatly reduced along with its dominance among the forbs: the legume comprised only 19% of the above-ground biomass of forbs when flooding and trampling were combined, in comparison with *ca*. 50% under all other treatments (Table 3). It should be noted that flooding or trampling produced slight reductions of *L. tenuis* biomass (21-25% in relation to controls) separately, but in no case did it lead to the disappearance of any forb species (Table 3).

After the 120-d recovery period, the dominance of the aforementioned marsh grasses and the sedge species still persisted in both treatments subjected to flooding conditions (Table 3, Appendix S2); even though the warm-season grass *P. dilatatum* showed a peak of biomass accumulation, the dominance of the grasslands was shared in all experimental treatments. As expected, no biomass accumulation was recorded for *L. multiflorum* (a cool-season annual bunchgrass) towards the end of its growing season (Table 3). Remarkably, in the legume *L. tenuis*, the negative impact of the combined perturbations was still highly visible: the species achieved only a quarter of above-ground biomass in the mesocosms that were formerly subjected to flooding and trampling in relation to all other treatments (Table 3). Finally, the forb species with negligible above-ground biomass on day 40 under flooding and trampling conditions reappeared in the grasslands, attaining slow and partial recovery until the end of the growing season (Table 3).

Discussion

The effects of flooding and trampling on plant community structure

The combination of flooding and trampling shifts the initial community co-dominance of graminoids and forbs towards an exclusive and persistent dominance of the graminoid species until the end of the growing season. We recorded contrasting responses of these life forms in terms of plant cover, biomass production and floristic

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Table 2. Below-ground biomass (g m^{-2}) in grassland mesocosms subjected to control, flooding, trampling and flooding × trampling ($F \times T$) conditions. Flooding lasted 40 d. Trampling was applied at day 20 of experiment. A recovery period of 120 d after flooding was allowed to evaluate the persistence of the treatments within the same growing season. Values are means ± SE of five replicates.

Belowground biomass	Control	Flooding	Trampling	$F \times T$
Day 40				
Roots	496.5 ± 28.7	599.7 ± 17.4	477.4 ± 18.2	327.2 ± 25.5
Reserve organs	183.4 ± 42.0	115.9 ± 17.8	176.9 ± 22.9	118.0 ± 23.3
Total	679.9 ± 70.0	715.6 ± 30.6	654.3 ± 30.6	445.2 ± 45.8
Day 160				
Roots	611.2 ± 39.7	655.1 ± 30.4	566.7 ± 33.2	579.4 ± 27.9
Reserve organs	210.1 ± 21.0	197.4 ± 18.9	184.6 ± 27.1	133.7 ± 18.4
Total	821.3 ± 79.1	852.5 ± 59.2	751.3 ± 66.4	713.1 ± 51.1

Table 3. Above-ground biomass of major species (g m^{-2}) harvested in grassland mesocosms under different treatments: control (C), flooding (F), trampling (T) and flooding × trampling ($F \times T$). Species were chosen to represent more than 90% of the total biomass and included in the MRPP analyses to test differences in plant composition (see Appendix S2). All species are perennial, except *L. multiflorum*, which is annual. Flooding lasted 40 d. Trampling was applied at day 20 of experiment. A recovery period of 120 d after flooding was allowed to evaluate the persistence of the treatments within the same growing season. nd = Not detected biomass. Values are means ± SE of five replicates.

Above-ground biomass	Day 40 (Flooding period)				Day 160 (After recovery period)			
	C	F	T	$F \times T$	C	F	T	$F \times T$
Graminoids								
Lolium multiflorum	97 ± 26	57 ± 9	129 ± 15	81 ± 20	7 ± 2	4 ± 2	5 ± 2	3 ± 1
Paspalum dilatatum	76 ± 14	99 ± 15	61 ± 14	101 ± 33	172 ± 35	184 ± 44	159 ± 19	173 ± 18
Leersia hexandra	59 ± 8	177 ± 31	49 ± 14	164 ± 26	66 ± 9	195 ± 39	68 ± 20	183 ± 37
Panicum gouinii	37 ± 18	72 ± 14	38 ± 10	85 ± 22	49 ± 17	122 ± 28	62 ± 23	95 ± 18
Paspalidium geminatum	9 ± 3	52 ± 8	12 ± 2	54 ± 13	11 ± 3	24 ± 14	10 ± 2	16 ± 6
Eleocharis viridans	5 ± 2	17 ± 4	7 ± 2	15 ± 2	23 ± 7	25 ± 7	17 ± 4	37 ± 11
Forbs								
Lotus tenuis	95 ± 11	71 ± 6	75 ± 4	8 ± 1	111 ± 11	92 ± 5	74 ± 8	24 ± 7
Leontodon taraxacoides	11 ± 3	3 ±	20 ± 11	nd	17 ± 2	8 ± 2	13 ± 4	4 ± 1
Eryngium ebracteatum	18 ± 5	20 ± 6	18 ± 3	13 ± 1	17 ± 3	25 ± 4	25 ± 9	23 ± 5
Mentha pulegium	13 ± 5	5 ± 1	2 ± 1	nd	12 ± 3	8 ± 2	7 ± 1	7 ± 4
Phylla canescens	10 ± 2	5 ± 1	9 ± 2	nd	14 ± 2	2 ± 1	11 ± 4	5 ± 2
Aster squamatus	7 ± 2	8 ± 1	9 ± 2	14 ± 6	5 ± 1	17 ± 10	13 ± 5	16 ± 11
Hydrocotile bonariensis	7 ± 3	13 ± 3	5 ± 1	3 ± 1	4 ± 1	9 ± 2	14 ± 6	10 ± 3

composition of the grasslands when flooding and trampling were applied simultaneously. The results showed that flooding, with or without trampling, invariably promotes the dominance of marsh grass species (Table 3) while producing a negative impact on the biomass accumulation of the forb group (see also Insausti et al. 1999; Lenssen et al. 2004). This was drastically intensified by occurrence of trampling (Figs. 1 and 3). Trampling, as an individual factor, slightly reduced the biomass accumulation of the forbs group but not that of the graminoids, a finding that concurred with many previous reports showing a higher trampling tolerance of graminoid species (Sun & Liddle 1993; Cole 1995b; Kobayashi et al. 1997; Kohler et al. 2004; Striker et al. 2006). Remarkably, the combination of flooding and trampling did not affect above-ground grassland productivity as the enhancement of graminoids during flooding counterbalanced the damage to the forb group. In sown pastures, cattle trampling (i.e. damage by cow hooves) has been reported to often decrease pasture productivity under increasing soil water contents (Edmond 1964; Menneer et al. 2005; see review by Drewry et al. 2008). Comparatively, our results highlight the great tolerance of the Flooding Pampa grasslands to deal with trampling under extremely wet soil conditions.

As expected, flooding and trampling had the opposite effect on the vertical distribution of plant cover. The canopy of flooded grasslands was taller, with most leaves surpassing the water level (Pehrsson 1988; Insausti et al. 1999), while the canopy of trampled grassland was concentrated in the first height layers (Kobayashi et al. 1997; Roovers et al. 2004;Fig. 2). The combination of flooding and trampling led to a canopy fully dominated by tall graminoid plants, with the remaining forbs located at subordinated positions of the canopy and their leaves were consequently shaded and submerged. Undoubtedly, this differential vertical distribution of the canopy has major implications for the biomass accumulation of each life form under flooding conditions. The increment of plant height and thus the restoration of contact with the atmospheric environment are crucial to allowing the gas exchange which defines plant growth under flooding conditions (Colmer 2003; Colmer & Voesenek 2009). When this occurs, the emergent graminoids plants can sustain aerial C fixation (Zimmerman 2006) and oxygen capture to allow plant growth and the aeration of the submerged tissues (Laan et al. 1990; Voesenek et al. 2006). In contrast, forbs were flattened by trampling and remained completely submerged; therefore, they cannot sustain such crucial processes. Therefore, the capacity of each life form to maintain a positive growth rate under the combination of flooding and trampling not only depends on its tolerance to mechanical damage by hooves (Cole 1995b) but also on the ability to rapidly emerge from water after a trampling disturbance. It should be noted that this positive response of graminoids (increases in plant height) exposes their foliage to defoliation by grazers (Oesterheld & McNaughton 1991), so that the potential benefit of graminoids emerging from water would also be dependent on the grazing intensity.

Changes in species composition as induced by trampling on flooded soil

The combination of flooding and trampling caused a lower biomass accumulation of most forbs and the disappearance of the aerial part of three major species of this group: *L. taraxacoides*, *M. pulegium* and *P. canescens* (Table 3). These species possess rosette or creeping growth habits, which lead to full submersion and oxygen deprivation under the combined treatments. Comparatively, dominant graminoids (i.e. *L. hexandra*, *P. dilatatum*, *P. gouinii* and *P. geminatum*) were able to enhance plant height by increasing their tiller insertion angle and leaf elongation (Kirkman & Sharitz 1993; Insausti et al. 2001; Mollard et al. 2008; Striker et al. 2008) even under trampling plus flooded conditions (Fig. 2). Although prostrated growth has usually been regarded as a trait that favours trampling tolerance (Sun & Liddle 1993; Cole 1995a; Kobayashi et al. 1997), the erect form proved to be an important feature for plant survival in this humid grassland ecosystem under the combination of flooding and trampling. Graminoids and forbs have important differences in root traits in terms of the aeration capacity of the submerged organs (i.e. generation of root aerenchyma tissue) and in terms of their resistance to compression to withstand trampling events. The major graminoids in our mesocosms have highly constitutive root aerenchyma and great plasticity, allowing them to increase the proportion of aerenchyma without losing root strength (for *P. dilatatum* and *P. geminatum* see Striker et al. 2006, 2007). By contrast, most forb species have roots with low constitutive aerenchyma (for *L. taraxacoides* and *M. pulegium* see Grimoldi et al. 1999, 2005), which presents a disadvantage. In these species, the increase in root aerenchyma induced by flooding favours the structural weakness of the root system (Striker et al. 2007). Consequently, it appears that the reduction of grassland root biomass provoked by trampling under flooding conditions (Table 2) was more likely caused by forb roots being more damaged than the graminoids.

The way in which the biomass of the legume *L. tenuis* responded to the application of the different treatments corresponded to its reputation as a flood-tolerant species (Striker et al. 2005; Manzur et al. 2009), though one susceptible to trampling on flooded soil (Striker et al. 2006). In our experiment, the lower biomass of this species during the combined perturbations appeared to be the reason for the death of some plants (i.e. heavily trampled; see also Striker et al. 2006) and for the decreased growth of the less affected plants (i.e. slightly trampled). The depressed growth of the surviving plants could be associated with the slow emergence from water of the trampled shoots and with a low ability to compete for sunlight under a taller graminoid canopy. It is important to note that the Flooding Pampa grasslands lack significant native legumes in their flora (Burkart et al. 1990); therefore, the loss of the unique naturalized legume species deserves special attention. The presence of legume species in a plant community has been shown to modify its functioning because of the addition of nitrogen (N) through symbiotic fixation and through their low demand for available soil N (Vitousek et al. 1987; Temperton et al. 2007). For our study site, the work of Quinos et al. (1998) particularly exemplifies this fact, demonstrating that the presence of *L. tenuis* enhanced the growth of neighbouring grasses as competition for this nutrient decreases. Indeed, a recent study on fertilization reaffirmed that N constitutes an important limitation for the productivity of these grasslands (Semmartin et al. 2007). Thus, two viewpoints can be considered in terms of the loss of the unique and important N-fixing species of the system. First, the decline of this legume would affect the N budget (by less atmospheric incorporation) and recycling (slow mineralization related to higher C:N ratio of the litter) (Chaneton et al. 1996; Temperton et al. 2007). Second, in the context of grazing, the quality of the available forage to cattle would be worse because the component with the higher protein content is severely reduced in the diet (Jacobo et al. 2006).

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Grassland recovery after trampling under flooding conditions

The dominance of graminoids persisted until the end of the experiment on the mesocosms subjected to the combination of flooding and trampling. This was confirmed in terms of plant cover, the vertical distribution of the canopy, the above-ground biomass and the floristic composition of the grasslands (Figs 1 and 3, Table 3, Appendix S1). The dominance was observed after a recovery period (120-d) three times longer than the duration of the experimental treatments and which continued until the end of the growing season of the Flooding Pampa grasslands. Trampling-induced floristic changes provoking persistent graminoid dominance after flooding could be altered in the presence of defoliation by grazers, as the opening of the canopy and the selectivity for the more palatable grass species (Sala et al. 1986) could benefit their coexistence with unpalatable grasses and forbs (Jacobo et al. 2006). When the recovery period was analysed, the effect of the treatments on the structural properties of these humid grasslands persisted, though without decreasing the above-ground or below-ground productivity of the system during the growing season (see also Milchunas & Lauenroth 1993; Cingolani et al. 2005). We could not disentangle the composition of the root structures among different plant species, but the total below-ground biomass had fully recovered at the end of the recovery period. This suggests that the below-ground system of these grasslands is highly resilient to disturbances (*sensu* Lavorel 1999), a fact with major implications for the functioning of the grasslands in terms of soil spatial exploration, water and nutrient uptake (i.e. water balance, nutrient and carbon fluxes; Stanton 1988; Craine et al. 2002). Owing to its importance, this topic deserves further experimental work.

Despite the persistent dominance of the graminoids in the grasslands, it is important to note that the remaining forbs (including *L. tenuis*) attained partial recuperation at the end of the flooding period, and lost species started to reappear through sprouting from reserve organs. This denoted the ability of some forb species to regrow soon after flooding (beneath a closed canopy) from the reserve organs of pre-existent individuals (tap roots of *L. taraxacoides*, rhizomes of *M. pulegium* and stolons of *P. canescens*; see also Grimoldi et al. 2005). These observations concur with the proportional lower values of reserve organs biomass at the end of the recovery period (Table 2). As trampling can occur with some amount of defoliation over graminoids, the recovery of forbs probably would be facilitated by grazers because of a relaxation in the competitive interactions between plants (Sala et al. 1986). Alternatively, previous studies have explained the recolonization of forb species after disturbances by seedling recruitment on canopy gaps associated with continuous grazing (Facelli 1988; Oesterheld & Sala 1990; Insausti et al. 1995; Insausti & Grimoldi 2006). Our results supplement this information by indicating that, in the short term (i.e. during the same growing season), grasslands could also be recolonized through resprouting from reserve organs, and that both strategies have to be taken into account to understand the vegetation dynamics of this system.

Grassland management implications

The results obtained in this experiment provide significant information in terms of the management of humid grasslands ecosystems. In the flooded grassland, the relative abundance of graminoids, weeds and the forage legume *L. tenuis* should be taken into account when assessing the potential impact of trampling by cattle. Trampling of the grassland when *L. tenuis* is infrequent (but undesirable forbs are abundant) can be regarded as having positive effects on the natural forage resource because of the decline of the weed species. Thus, as trampling does not affect the growth promotion of graminoids induced by flooding, both perturbations could rapidly revert poor grassland conditions by displacing weeds and promoting native palatable grasses (*L. hexandra, P. geminatum* and *P. dilatatum*; see Chaneton et al. 1988 for species forage values). In contrast, when *L. tenuis* is an abundant component of the grasslands, trampling during flooded should be avoided as it negatively affects forage quality by immediately reducing the biomass of the legume component. Furthermore, the recovery of *L. tenuis* from the combination of flooding and trampling appears to be very slow if competition with taller graminoids is not reduced by grazing when the flooding subsides (Table 3). Therefore, if grassland species are considered a forage resource for livestock, the changes in species composition induced by trampling on flooded soil could be positive or negative depending on the initial condition of the grasslands.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. Vertical distribution of plant cover of graminoids and forbs in grasslands mesocosms under different treatments: control, flooding, trampling and flooding × trampling ($F \times T$) at the end of recovery period (day 160). MANOVA: Wilk's $\Lambda = 0.08$ and 0.09; $F_{5,96} = 114.2$ and 143.4; $P < 0.0001$ for graminoids and forbs, respectively. Values are means ± SE of five replicates.

Appendix S2. Summary statistics for multi-response permutation procedure (MRPP) results corresponding to all possible pairwise comparisons between treatments: control (C), flooding (F), trampling (T) and flooding × trampling ($F \times T$).

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