Lotus tenuis and *Schedonorus arundinaceus* co-culture exposed to defoliation and water stress

Co-cultivo de *Lotus tenuis* y *Schedonorus arundinaceus* ante defoliación y estrés hídrico

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Originales: *Recepción:* 23/05/2019 - *Aceptación:* 03/06/2021

Abstract

The present study aimed to investigate the effect of defoliation frequency (low and high) and water stress (excess or deficit) on biomass production, P and N nutrition, and symbiosis with native soil microorganisms on a *Lotus tenuis* and *Schedonorus arundinaceus* co-culture in a pot experiment. Combined effects of defoliation frequency and water stress affected plant accumulated shoot biomass. *L. tenuis* root biomass decreased in response to defoliation and water stress, while *S. arundinaceus* root biomass was similar between non-defoliated and defoliated plants, at all water levels. Low and high frequencies of defoliation in a waterlogged soil can be considered the most stressful scenario for *L. tenuis* and *S. arundinaceus* co-culture. Colonization of arbuscular mycorrhizal fungi in *L. tenuis* roots and dark septate endophytes colonization in *S. arundinaceus* roots were affected by both factors, whereas arbuscular mycorrhizal colonization in *S. arundinaceus* was affected only by water stress. Both plants tolerated defoliation and water stress due to the interaction between the translocation of nutrients and carbon compounds from roots to shoots, and P and N absorption (plus N_2 fixation in *L. tenuis*).

Keywords
Lotus tenuis - *Schedonorus arundinaceus* co-culture • P and N nutrition • water stress • defoliation • native soil microorganisms

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Resumen

El presente estudio tuvo como objetivo investigar el efecto de la frecuencia de defoliación (baja y alta) y el estrés hídrico (exceso y déficit) sobre la producción de biomasa, la nutrición P y N y la simbiosis con microorganismos edáficos nativos en un co-cultivo de *Lotus tenuis* y *Schedonorus arundinaceus* en un ensayo en macetas. Los efectos combinados de la frecuencia de defoliación y el estrés hídrico afectaron la biomasa acumulada del vástago. La biomasa radical de *L. tenuis* disminuyó en respuesta a la defoliación y estrés hídrico, mientras que en *S. arundinaceus* fue similar entre no defoliadas y defoliadas en todos los niveles hídricos. La defoliación (baja o alta frecuencia) en un suelo inundado puede ser considerada el escenario con mayor estrés para el co-cultivo de *L. tenuis* y *S. arundinaceus*. La colonización micorrícica arbuscular en las raíces de *L. tenuis* y por endofitos septados oscuros en las raíces de *S. arundinaceus* fueron afectadas por ambos factores, mientras que la colonización micorrícica arbuscular en *S. arundinaceus* fue solo afectada por el estrés hídrico. Ambas especies toleraron defoliación y estrés hídrico debido a la interacción entre la traslocación de nutrientes y compuestos carbonados desde la raíz hacia el vástago y la absorción de P y N (más el N_2 fijado en *L. tenuis*).

Palabras clave

Co-cultivo *Lotus tenuis* - *Schedonorus arundinaceus* • nutrición P y N • estrés hídrico • defoliación • microorganismos edáficos nativos

Introduction

The performance of legume-grass mixtures has been evaluated in several grassland ecosystems. This plant combination is especially advantageous under conditions of stress and low availability of environmental resources (4, 7). Legumes can contribute to increasing grass forage yield, substituting added inorganic N-fertilizers through symbiotic N_2 fixation, enhancing environmental stress tolerance, and increasing its nutritional value (4, 21).

In the pampas grasslands of the Province of Buenos Aires (Argentina), lowlands occupy a significant proportion of the total production area for dairy cattle. These grasslands are dominated by a range of perennial native grasses (6, 24), with lack of legumes and subjected to periodic droughts or floods. In addition, their soils show high levels of salinity and sodicity (9). In these areas, species persistence within the plant community depends on their tolerance to a combination of stressful conditions, on their ability to absorb and mobilize nutrients, and on soil water content, the latter of which modifies nutrient availability for plant growth (11).

Lotus tenuis Waldst. & Kit. is a much-appreciated legume given its high forage nutritional value for beef and dairy cattle in nutrient-deficient soils (5). In addition, *L. tenuis* is tolerant to water stress (10, 29) and to defoliation in saline-sodic soils (11). *Schedonorus arundinaceus* (Schreb.) Dumort is a grass that tolerates saline conditions and grazing (1). *Lotus tenuis* and *S. arundinaceus* co-culture has been reported to be a sustainable alternative for forage quality improvement (21). However, little is known about its performance under the combined effect of defoliation and water stress. These stressful conditions may allow evaluating tolerance mechanisms and persistence of the *L. tenuis* - *S. arundinaceus* co-culture under adverse conditions. The impact of high-defoliation frequencies on both plants under soil water deficit or soil water excess is expected to be different from that under well-watered conditions.

As known, removal of photosynthetic biomass, by either grazing or defoliation, alters plant carbon balance, forcing physiological changes within the plant. The production of new plant tissue depends on the availability of nutrient reserves and on plant ability to efficiently absorb soil nutrients (19, 28). During postdefoliation regrowth, rapid carbohydrate degradation and resynthesis occurs in roots (18). In addition, 40 to 60% of root total N is mobilized to meet shoot N demand during early regrowth (18). In relation to P nutrition, a previous study has demonstrated that grass defoliation tolerance depends on P reserves and that P reutilization and remobilization provide tolerance to defoliation by promoting

compensatory growth under P-deficient conditions (23). This pattern suggests that the effect of nutritional reserves on grass regrowth depends on the level of those reserves, as shown for N reserves by Hamilton *et al.* (14). With respect to legumes in particular, a previous study has shown that *L. tenuis* plants defoliated up to 75% of their aerial biomass are able to compensate total absorbed P and N by non-defoliated plants after a 30-day recovery period (11). This is associated with higher rates of shoot growth in defoliated plants compared to non-defoliated ones, along with a sufficient level of absorbed nutrients necessary to sustain shoot regrowth (11). It is necessary to emphasize that, to estimate changes in forage quality in a legume-grass co-culture under stress, studying P and N metabolism in conjunction, is important.

It is known that conditions that alter root growth, reduce plant photosynthetic capacity, and modify soil environment, may also affect the symbiotic relationship between plants and soil microorganisms (3). In addition, defoliation and soil-water content may influence arbuscular mycorrhizal (AM) fungal colonization, and AM fungi may, in turn and under stress conditions, alter plant competitive ability to improve plant nutrition and water status. Dark septate endophytic (DSE) fungi not only may function as pathogens or saprophytes, but also may form mutualistic associations similar to those of mycorrhizae (17). Several studies have shown that DSE fungi improve plant growth and N and P nutrition (17, 22). DSE fungal colonization has been shown to respond to herbivory, nutrient availability, and habitat in a contrasting manner compared to the pattern observed with AM fungi (12, 26). Although several studies have described the effect of defoliation by cutting-either under natural or controlled conditions-on the growth of different plant species in monoculture, few investigations have evaluated the ability of legume-grass mixtures to associate with AM or DSE fungi. Such an approach to evaluate the dynamics of symbiosis with native soil microorganisms, especially under abiotic-stress conditions, is highly desirable.

Based on all the above, this study aimed to investigate the combined effect of different defoliation frequencies and water stresses on the performance of *L. tenuis* and *S. arundinaceus* grown in co-culture in a saline-sodic soil. The hypotheses were that, at high defoliation frequency plus water stress conditions, (i) both plant species are not able to compensate clipped shoot biomass, and (ii) AM fungal colonization in both plant species decreases and DSE fungal colonization in *S. arundinaceus* roots increases. The prediction was that, under these stress conditions, regrowth would be low, as a result of a drastic reduction of root biomass, which decreases nutrient absorption and carbon reserves, and affects the symbiotic relationship between plants and native soil microorganisms.

Materials and methods

Soil characteristics and experimental setup

The soil was a Typic Natraqualf from San Vicente (Buenos Aires, Argentina). The plant community in the sampling site was dominated by *L. tenuis*, *Distichlis spicata* (L.) Greene, *Paspalum vaginatum* Swartz, *Eleocharis viridans* Kük. ex Oken, and *Cynodon dactylon* (L.) Pers. The soil is characterized by: pH 9.53, electrical conductivity 10.10 dS/m, Na^+ 19.45 $cmol_c$/kg, available P 8.72 mg/kg (Bray I), total carbon 9.10 g/kg, and total N 1.02 g/kg (9). The soil was air-dried and sieved through a 2-mm mesh screen.

Seeds of *L. tenuis* cv. Esmeralda and *S. arundinaceus* cv. Arizona were surface-sterilized and pregerminated under sterile conditions. Fifty closed-bottom 1.6-L pots were filled with 950 g of air-dried soil. Three seedlings of each plant species were planted together in each pot and the soil surface was covered with 1 cm of sterilized sand to conserve moisture. During the experimental period, pots were placed in a greenhouse at a mean relative humidity of 65 ± 15%, mean temperature of 30 ± 5°C and 20 ± 3°C during days and nights, a photoperiod ranging from 10 to 12 h, and a photon-flux density at midday of 900-1,300 µmol $m^{-2}s^{-1}$. Pots were randomized and daily rotated to minimize potential gradient effects.

After 62 days of growth in soil at 80% of field capacity (46.10% w/w, 33 kPa), five pots were harvested (initial plants) and the remaining pots were subjected to a combination of two defoliation frequencies and two water stress conditions (water excess and deficit). Defoliation treatments comprised removing all plant material 4 cm above ground level at

different time intervals over a 28-day period, starting with defoliation of all plants, except controls (non-defoliated plants under all irrigation conditions), at the beginning of this period. *L. tenuis* and *S. arundinaceus* were defoliated by clipping at a frequency of 2 and 4 times representing intervals of 2 and 1 weeks between defoliations, respectively. The 2-time frequency is here referred to as low defoliation frequency (LF) and the 4-time frequency as high defoliation frequency (HF). Water-stress treatments consisted in subjecting plants to water excess (WE), water deficit (WD), or 80% of field capacity (WW- well watered plants) for a 28-day period. The WE treatment consisted in maintaining a water level of 1 cm above the soil surface, whereas the WD treatment consisted in keeping soil moisture at 24.50 w/w (1,488 kPa), near the permanent-wilting-point level of soil hydration (1,500 kPa). Control pots contained non-defoliated plants maintained at the three different water levels (WW, WE, and WD). Five replicates were assigned to each combination of defoliation frequency and water treatment. The water status of pots was examined daily by weighing them and the amount of water lost was replaced. At the end of the 28 days of treatment, all plants were harvested (final plants).

Plant yield and analytical determinations in plant tissue and soil

After each harvest (initial and final plants), *L. tenuis* and *S. arundinaceus* biomasses were separated into shoots and roots. All shoot biomasses of each plant species removed during the defoliation and water treatments were dried and weighed to be later included in the shoot fraction of the corresponding plant at the end of the experiment, determining accumulated shoot dry weight. Shoots and roots of each plant species were oven-dried at 70°C for 48 h and weighed. A portion of fresh root was used to measure AM fungal colonization in both plant species and number of rhizobia nodules in *L. tenuis* roots. For each species, defoliation or stress-tolerance efficiency (STE) for each water level was calculated using the following equation:

STE (%) = (dry biomass of defoliated plant/ dry biomass of non-defoliated plant) x 100

Shoots and roots of each species were digested separately in sulfuric acid to determine P content by a modification of the Murphy Riley method and N content by the Kjeldahl method (16). Total absorbed N per nodule of rhizobia was calculated (10). Soil P availability was measured by Bray and Kurtz. The root fraction colonized by AM fungi (mycorrhizal colonization index) and the root fraction colonized by DSE fungi (DSE colonization index) were assessed following McGonigle *et al.* (1990). Septate, melanized or hyaline hyphae and microsclerotia were classified as DSE fungi (2). Rhizobia nodules were counted in whole fresh root system under a binocular stereomicroscope (7.5x). Results were analyzed by ANOVA with Tukey's mean separation test ($P \leq 0.05$) using InfoStat statistical software (8).

Results and Discussion

Plant growth

Under each combination of treatments (water level+defoliation) studied in a saline-sodic soil, *L. tenuis* contributed near 80% of the total biomass produced by the co-culture (figure 1a, page 104). *Lotus tenuis* defoliated plants (low and high frequency) were not able to compensate for the shoot biomass produced by non-defoliated plants under well watered conditions through tissue regrowth (defoliation frequency mean value of STE: 59.7%) (figure 1a, page 104 and table 1, page 104). Compensation of removed shoot tissue, especially after frequent defoliation, requires large amounts of energy investment, which is derived from reallocating energy stored in the remnant shoot and root tissue (18), resulting in reduced shoot biomass and root growth (figure 1b, page 104 and table 1, page 104). Unlike *L. tenuis*, *S. arundinaceus* produced similar amounts of accumulated shoot biomass in defoliated and non-defoliated plants under well watered conditions (figure 1a, page 104 and table 1, page 104).

Different letters indicate significant differences (*P< 0.05*) for each plant species among treatments according to Tukey's test.

Letras diferentes indican diferencias significativas (*P< 0,05*) para cada especie entre tratamientos según test de Tukey.

DW, dry weight; ND, no defoliation; LF, low defoliation frequency; HF, high defoliation frequency; WW, well watered; WE, water excess; WD, water deficit. Mean (N=5)± SE.

DW, peso seco; ND, no defoliada; LF, baja frecuencia de defoliación; HF, alta frecuencia de defoliación; WW, bien regada; WE, exceso hídrico; WD, déficit hídrico. Media (N=5)± EE.

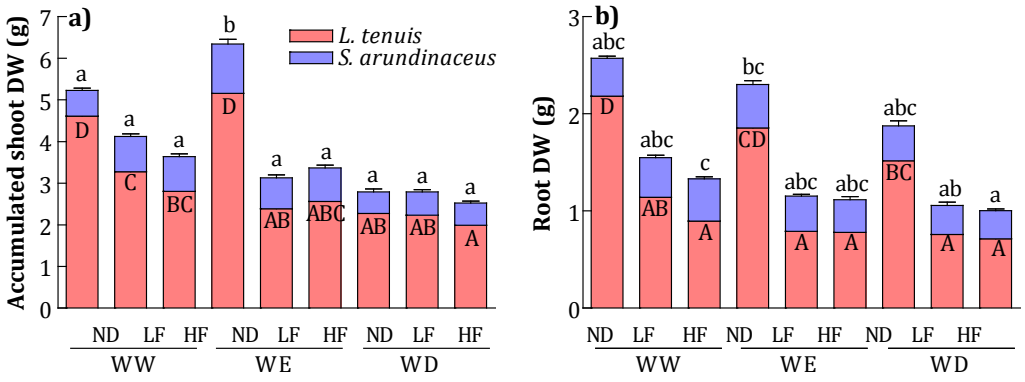


Figure 1. Biomass (a, b) of *Lotus tenuis and Schedonorus arundinaceus* grown in co-culture under a combination of different defoliation frequencies and water stress.

Figura 1. Biomasa (a, b) de *Lotus tenuis* y *Schedonorus arundinaceus* crecidas en co-cultivo ante la combinación de diferentes frecuencias de defoliación y estrés hídrico.

Table 1. Results of the two-way ANOVA used to evaluate the effects of defoliation frequencies (D) and water treatments (W) on different variables.

Tabla 1. Resultados del ANOVA de dos vías usado para evaluar el efecto de la frecuencia de defoliación (D) y estrés hídrico (W) sobre diferentes variables.

Variable	L. tenuis						S. arundinaceus					
	D		W		D xW		D		W		D xW	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Accumulated shoot DW	35.3	***	40.5	***	8.0	***	1.3	ns	18.5	***	7.54	***
Root DW	104.9	***	13.3	***	1.8	ns	1.5	ns	7.9	**	2.1	ns
STE plant	2.3	ns	22.8	***	1.3	ns	0.01	ns	27.1	***	0.2	ns
Plant P content	120.0	***	209.4	***	96.0	***	15.3	***	133.2	***	45.5	***
Plant N content	18.1	***	27.5	***	0.4	ns	20.9	***	6.9	***	6.4	***
N total/nodules	3.7	*	29.5	***	5.2	**						
MC index	25.5	***	69.9	***	9.8	***	2.0	ns	99.6	***	0.2	ns
DSE colonization index							27.7	***	61.9	***	25.6	***
Number of nodules/FW	0.9	ns	13.6	***	0.5	ns						

F values and significant differences: *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001; ns, *P* >0.05.

DW, dry weight; FW, fresh weight; MC index, mycorrhizal colonization index.

Valores de *F* y diferencias significativas: *, *P*<0,05; **, *P*<0,01; ***, *P*<0,001; ns, *P* >0,05. DW, peso seco; FW, peso fresco; MC index, índice de colonización micorrícica.

Since *S. arundinaceus* was able to compensate for biomass clipping by increasing the allocated biomass below the cutting height (data not shown) and maintaining root growth relative to that of non-defoliated plants, defoliation at the two frequencies actually resulted in enhanced plant growth (figure 1a, b and table 1). These results are consistent with the highest values of STE recorded for this species (defoliation frequency mean value of 126.5%), which showed high tolerance to frequent defoliation under well watered conditions.

Under the water excess treatment, *L. tenuis* and *S. arundinaceus* non-defoliated plants recorded the highest accumulated shoot biomass (5.16 g for *L. tenuis* and 1.18 g for *S. arundinaceus*) (figure 1a and table 1). In agreement with that previously described by several authors (11, 27), the adaptation of both species to water excess conditions may be due to an increased soil nutrient availability (11) along with the consequent reduction in soil salinity and sodicity as a result of soil water excess (11). In the present study, the soil of

non-defoliated plants under water excess showed a significant increase in available P with regard to the well watered condition (9.72 mg/kg and 12.23 mg/kg for well watered and water excess, respectively). The combination of water excess plus defoliation treatments resulted in a decrease in accumulated shoot and root biomasses of both plant species. These results were associated with lowest efficiencies in tolerating stress (figure 1 a, b, page 104). Mean defoliation frequency of STE was 46.5% for *L. tenuis* and 68.9% for *S. arundinaceus* (table 1, page 104). Both low and high defoliation frequencies in a waterlogged soil can be considered as the most stressful scenario for a *L. tenuis* and *S. arundinaceus* co-culture. The low STE of both species may be associated with a marked loss of nutrients, which affected plant ability to recover the defoliated biomass when defoliation frequency increased, or with decreases in root growth and soil P availability (defoliation frequency mean value of 10.43 mg/kg).

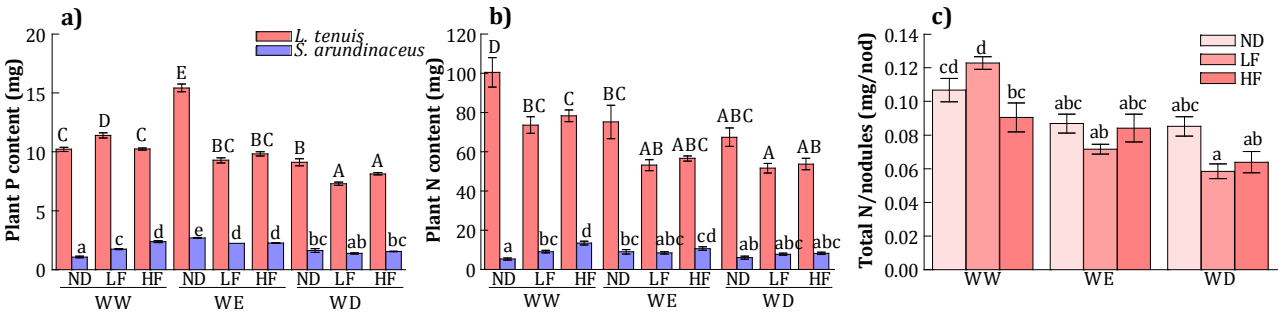
Under water deficit, non-defoliated and defoliated plants of both species produced similar quantities of shoot biomass (figure 1 a, b and table 1, page 104). A reduction in soil moisture resulted in a decrease in soil P availability (mean value for the combination of defoliation and water treatment of 8.39 mg/kg), affecting plant growth and P absorption. Defoliated plants growing under water deficit, however, have an adaptive advantage because a lower proportion of aerial biomass leads to an improvement in plant water balance and an avoidance of excessive losses of water through transpiration compared to non-defoliated plants. In these species, defoliation may alleviate the deleterious effect of water deficit on shoot biomass accumulation, enabling defoliated plants to compensate for the quantity of shoot biomass produced by control plants under drought, whose response becomes manifest in a high STE percentage (figure 1a, page 104). Mean defoliation frequency of STE was 75.2% for *L. tenuis* and 95.5% for *S. arundinaceus* (table 1, page 104). Loss of apical dominance after defoliation and basal meristem activation may stimulate such clipped stems to develop more biomass (15). Moreover, an increase in the photochemical efficiency and photosynthetic capability of remnant leaves below a cutting induces a compensatory growth response through an increase in photosynthates (27).

Defoliation generally results in reduced root growth (15). Root biomass loss in *L. tenuis* in response to defoliation frequency, especially in combination with water stress, would be attributable to such a decrease in root growth, a diversion of assimilated substances, and a mobilization of P and N from roots to regrowing shoot tissues (figure 1b and table 1, page 104). In contrast, the strategy of defoliated *S. arundinaceus* plants was to maintain root growth after low and high defoliation frequencies independently of water status, similar to the respective non-defoliated ones (figure 1b and table 1, page 104).

P and N nutrition

Lotus tenuis defoliated plants under well watered conditions were able to compensate total absorbed P by non-defoliated plants (figure 2a, page 106 and table 1, page 104). This occurred in association with an internal recycling of nutrients from necrotic roots and/or an additional amount of absorbed soil P satisfying shoot regrowth demands. Under water stress plus defoliation, defoliated plants were not able to compensate total absorbed P by non-defoliated plants (figure 2a, page 106 and table 1, page 104). In this case, the plant strategy focuses on preserving the root system through increasing P allocation to roots while limiting P transport to shoots. This strategy was reflected by a decrease in shoot regrowth and plant P content under water stress and defoliation.

Defoliation induces a reduction in the N uptake and N_2 fixation capacity of forage legumes (14) until carbon supply to roots is restored. In the present study, plant N content in *L. tenuis* under defoliation decreased regardless of water status (figure 2b, page 106 and table 1, page 104). Total absorbed N per nodule and number of nodules in *L. tenuis* roots was similar under defoliation in all irrigation conditions (figure 2c, 3c, page 106 and table 1, page 104). These results indicate that the N_2-fixing efficiency of roots of defoliated plants was similar to that of non-defoliated plants. In this situation, defoliated plants mobilized N from roots to shoots to sustain the growth of the latter.



Treatment symbols and statistical notes are the same as those for figure 1 (page 104).
Los símbolos de tratamientos y notas estadísticas son iguales a los de la figura 1 (pág. 104).

Figure 2. Plant P (a) and N (b) content in *Lotus tenuis and Schedonorus arundinaceus* grown in co-culture under a combination of different defoliation frequencies and water stress. Figure c shows total N per nodule of *L. tenuis*.

Figura 2. Contenido de P y N en las plantas de *Lotus tenuis* y *Schedonorus arundinaceus* crecidas en co-cultivo ante la combinación de diferentes frecuencias de defoliación y estrés hídrico en un suelo salino-sódico. La figura c muestra el N total por nódulo de *L. tenuis*.

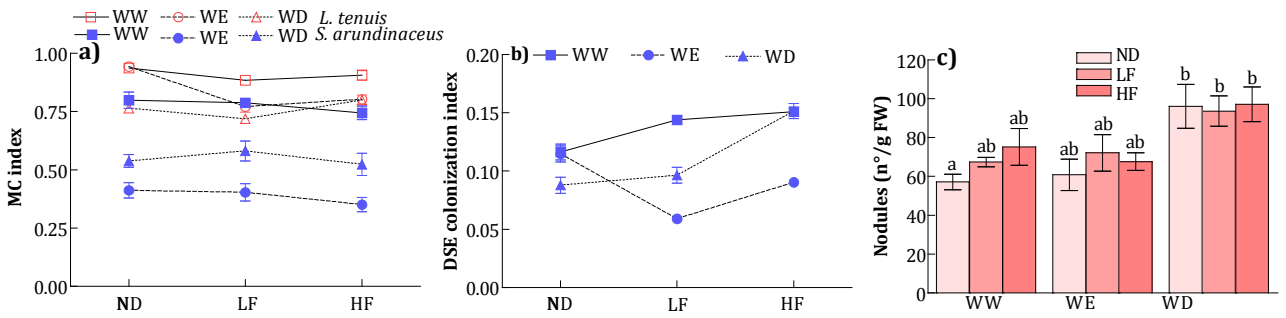
Schedonorus arundinaceus exhibited a similar pattern of plant P and N content in all water treatments (figure 2 a, b and table 1, page 104). In this species, root growth maintenance after defoliation allowed plants to maintain or increase soil P and N uptake in response to increased shoot demands. Iqbal *et al.* (2012) reported that P and N reallocation from roots to shoots would appear to be an essential component of *S. arundinaceus* tolerance to stressful conditions.

The results of plant growth of both species at high defoliation frequency and water excess are consistent with the proposed hypothesis, but responses to water deficit are inconsistent. In fact, both plant species showed higher STE after the combination of defoliation and water deficit than after that of defoliation and water excess.

Fungal colonization and root nodulation

Defoliation may influence plant function through changes in AM fungal colonization (3). Variations in the mycorrhizal response to the loss of above-ground biomass have been suggested to be due to the amount of tissue removed and root growth rate (13). Despite the decrease here observed in root biomass of *L. tenuis* defoliated plants (figure 1b, page 104), defoliation frequency increased the AM colonization index in plant roots under well watered and water deficit conditions (figure 3a, page 107 and table 1, page 104). This result contrasts the proposed hypothesis that defoliation decreases AM colonization. A positive effect on AM colonization may be a consequence of increased nutrient demand by defoliated plants (25). In contrast, the AM colonization in *L. tenuis* roots was affected by the defoliation plus water excess treatment (figure 3a, page 107), where the combination of stresses seemed to be the most stressful scenario for *L. tenuis* growth (figure 1b, page 104). The decrease in AM colonization in defoliated plants was in line with a reduction in root biomass. AM colonization in *S. arundinaceus* roots under water stress and defoliation was minimal (figure 3a, page 107 and table 1, page 104), and similar quantities of root biomass were observed in the three water treatments evaluated (figure 1b, page 104). Barto and Rilling (2010) found similar results and proposed that root biomass resilience to defoliation may explain the lack of effect on AM colonization of grasses.

In the present study, *S. arundinaceus* roots were co-colonized by AM fungi and DSE (figure 3b, page 107 and table 1, page 104). In agreement with the proposed hypothesis, DSE colonization was affected by defoliation frequency, water stress, and the combination of both stresses. Root DSE colonization in defoliated plants grown on well watered and water-deficit soils increased compared to non-defoliated plants, whereas that under water excess, decreased. Since DSE colonization reached lower values than AM colonization, it may be that AM colonization limits DSE colonization due to competition for host carbohydrates. In a previous field study, *S. arundinaceus* roots were much more extensively colonized by AM fungi when grown with *L. tenuis* in co-culture than when grown in monoculture (21).



Treatment symbols and statistical notes are the same as those for figure 1 (page 104).
Red symbols correspond to *L. tenuis* and blue ones to *S. arundinaceus*.
Los símbolos de tratamientos y notas estadísticas son iguales a los de la figura 1 (pág. 104).
Los símbolos rojos corresponden a *L. tenuis* y los azules a *S. arundinaceus*.

Figure 3. Mycorrhizal colonization (MC) index (a) in both plant species, DSE colonization index in *Schedonorus arundinaceus* (b) and number of nodules in *Lotus tenuis* roots (c). FW, fresh weight.

Figura 3. Índice de colonización micorrícica (MC) (a) en las raíces de ambas especies, índice de colonización de DSE en las raíces de *Schedonorus arundinaceus* (b) y número de nódulos en las raíces de *Lotus tenuis* (c). FW, peso fresco.

The presence of *L. tenuis* in mixed communities is crucial to improve not only grassland quality for beef production (5), but also to maintain the AM fungal community, especially under water-stress conditions (9, 11). The high percent of AM colonization in *L. tenuis* roots may be another advantage over the less extensively colonized *S. arundinaceus* roots, accessing soil moisture and nutrients for plant regrowth, mainly under water deficit, given that *S. arundinaceus* is somewhat resistant to drought (1).

Conclusions

These results showed that *L. tenuis* and *S. arundinaceus* tolerated defoliation+water stress in a saline-sodic soil through reutilization and remobilization of P and N reserves from roots to shoots, the concomitant soil P and N uptake capacity, and the additional N fixed by rhizobia in *L. tenuis* roots. The presence of *L. tenuis* in mixtures is crucial to maintain AM fungal communities, especially under water-stress conditions. Promoting the presence of *L. tenuis* through low defoliation frequency would improve forage yield and quality with the maintenance of AM symbiosis in legume–grass communities. The present work is a first step for future studies under field conditions promoting better management recommendations of forage production in saline-sodic soils of lowlands in the pampas grasslands.

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Acknowledgements

The author appreciates the financial support by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina) (PIP 0950). The author also thanks María Victoria Gonzalez Eusevi for revising the English of the manuscript.