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The effects of submergence on anatomical, morphological and biomass allocation responses of tropical grasses *Chloris gayana* and *Panicum coloratum* at seedling stage

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Abstract. Submergence is a major factor affecting seedling recruitment in lowland grassland ecosystems. Our aim was to evaluate the tolerance to increasing flooding intensity of the seedlings of tropical grasses *Chloris gayana* K. and *Panicum coloratum* L., whose use as a forage species is increasing in humid grasslands. For this purpose, 2-week-old seedlings of *C. gayana* and *P. coloratum* were subjected to control, partial submergence (PS) and complete submergence (CS) in clear water for 14 days and allowed to grow for a subsequent 12-day period to assess their recovery. The following responses were assessed: generation of root aerenchyma, morphological changes and emergence from water, biomass allocation in relation to plant size, and biomass accumulation. Results showed that constitutive root aerenchyma was high in both species. Under PS and CS, root aerenchyma increased by up to 50–55% in *C. gayana* and up to 40–48% in *P. coloratum.* Under PS, the increase in seedling height for both species was the same as for controls. Under CS, *C. gayana* further increased its height and emerged more quickly from water; *P. coloratum* was not able to increase its height, and therefore the seedlings always remained underwater. The escape-from-water response of *C. gayana* was associated with preferential biomass allocation towards shoots and with a marked lengthening of leaf blades. By contrast, there was no change in allocation in *P. coloratum*, and its leaves were shorter under CS. The final biomass of *C. gayana* under CS was similar to that under PS, and equivalent to 54% of its controls. In *P. coloratum*, biomass under PS and CS were 64 and 21% of its controls (respectively), which indicates that injury caused by CS persisted during the post-submergence period. In conclusion, both species are tolerant to PS at the seedling stage. However, when flood depth increases by submerging the seedlings, *C. gayana* is able to escape from water while *P. coloratum* is not, thus strongly affecting its recovery. Therefore, *C. gayana* appears to be a more promising species for cultivation in lowland grasslands prone to flooding of unpredictable intensity.

Additional keywords: allometry, *Chloris gayana*, *Panicum coloratum*, root aerenchyma, seedling height, submergence.

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Introduction

Flooding is a natural disturbance occurring in many humid grasslands devoted to livestock production worldwide (Soriano 1991; van Eck *et al*. 2004). Forage grasses are major components of these ecosystems and their tolerance to water excess is crucial in determining their recruitment, production and persistence (Crawford 2003; Striker *et al*. 2006, 2011*a*). Nowadays, as a result of global warming, winters can be less restrictive for plant growth in some grassland areas (especially at higher latitudes) as a consequence of higher minimal temperatures and shorter freezing periods (De Boeck *et al*. 2008; Kreyling 2010; Chapman *et al*. 2012). In this scenario, some grasses of tropical and subtropical origin with high yielding potential are being incorporated in lowland areas of temperate humid grasslands, which suffer from flooding of variable intensity. These types of grasses are empirically regarded as flood-tolerant in general, but in fact, few works have examined their tolerance to water excess in experiments (Anderson 1972; Baruch 1994). In this respect, *Chloris gayana* K. and *Panicum coloratum* L. are promising species for temperate lowlands, as both grow well at mild temperatures and are able to withstand soil waterlogging (i.e. roots flooded by 10–15 days); *C. gayana* proved to be more tolerant to this stress than *P. coloratum* when both were compared in terms of biomass (Anderson 1972; Boschma *et al*. 2008). Despite this, the performance of both species at increasing water depth, which compromises shoot submergence is unknown. Therefore, in this paper we address this issue by comparatively evaluating the tolerance to increasing flooding intensity – provoking partial and complete submergence – of *C. gayana* and *P. coloratum* at the seedling stage. This ontogenetic phase is critical in determining species recruitment under field conditions, as seedlings can be easily covered by water (partially or completely) during periods of water excess, which can severely affect their growth and survival. This is the case of the Flooding Pampa grasslands (Argentina) where the sowing of these species, during spring season, coincides with the common episodes of flooding (Soriano 1991).

Under partial submergence conditions, plants have to cope with oxygen shortage as a result of the much slower oxygen diffusion in water than in air (i.e. 10^{-4} slower diffusion; Visser and Voesenek 2004; Colmer and Voesenek 2009). It is well known that flood-tolerant plants develop anatomical and morphological responses in order to alleviate the adverse effects of soil anaerobiosis (Colmer 2003). At the anatomical level, plants can promote their potential for oxygen conduction by generating constitutive aerenchyma in the root cortex, and even more through lysigeny or an increase in root diameter (Justin and Armstrong 1987; Grimoldi *et al*. 2005*a*; Striker *et al*. 2007). The aerenchymatic tissue gives plants a lower resistance pathway for oxygen transport through diffusive mechanisms from air-exposed shoots towards the submerged tissues (Colmer 2003; Colmer and Voesenek 2009). At the morphological level, flooded plants increase their height, usually through petiole/leaf elongation, and as a result, they increase the leaf area exposed above the water level (Grimoldi *et al*. 1999; Insausti *et al*. 2001; Striker *et al*. 2011*b*). This allows plants to maintain aerial photosynthesis and facilitates the capture of oxygen by leaves for the oxygenation of submerged tissues through the aerenchyma system (Laan *et al*. 1990; Striker *et al*. 2005; Colmer and Voesenek 2009). In this study, we characterise the tolerance to partial submergence of *C. gayana* and *P. coloratum* by assessing their anatomical, morphological and biomass allocation responses to the anaerobiosis conditions of soil.

Complete submergence cause a much more stressful scenario for plants as all organs are covered by water and shoots suffer from shortages of carbon and oxygen imposed by slow gas diffusion through water. To cope with this stress, flood-tolerant plants can use two different strategies (*sensu*Bailey-Serres and Voesenek 2008, 2010). The first is an escape strategy by which plants promote shoot elongation in order to re-establish air contact rapidly. The second is a sit-and-wait strategy based on maintaining a steady conservation of plant energy without shoot elongation (quiescence) (Bailey-Serres and Voesenek 2008, 2010; Hattori *et al*. 2011). The escape strategy seems to be beneficial in shallow floodwater where shoot elongation is quite plausible. The quiescence strategy seems to be advantageous in deep floodwater where it is unlikely that plants will reach the water surface through shoot elongation; the saving of energy and carbohydrates thus improves their chances for survival, and facilitates the generation of new tissues after de-submergence (Bailey-Serres and Voesenek 2008; Colmer and Voesenek 2009). Both strategies have been found in different cultivars of the graminaceous *Oriza sativa* (Setter and Laureles 1996; Hattori *et al*. 2011) and dicotyledonous species of the *Rumex* genus (Voesenek *et al*. 1990; Chen *et al*. 2009, 2011; Pierik *et al*. 2009), ecotypes of *Ranunculus repens* (Lynn and Waldren 2003), and forage species of the genus *Lotus* (Manzur *et al*. 2009; Striker *et al*. 2012) and *Rorippa* (Akman *et al*. 2012). In tropical grass species, there is only a single record and it corresponds to adult plants of *Hemarthria altissima*, which showed quiescence when dealing with 20 days of complete submergence (Luo *et al*. 2011). However, no comparable studies for seedlings of forage tropical grasses have yet to address the tolerance to submergence in terms of escape or quiescence strategies. Furthermore, in the cases where graminaceous seedlings/plants increase their height by 'escaping' from water, it is still unclear whether this response is mainly due to a preferential allocation of biomass towards shoots, to an elongation of leaf blades and/or leaf sheaths (without major changes in biomass allocation), or to a combination of both responses (for rice: Kawano *et al*. 2009; but also Parlanti *et al*. 2011). In this paper, we examined allometric relationships between shoot and root biomass (Poorter and Nagel 2000; Poorter *et al*. 2012), and between leaf blades and leaf sheaths (biomass and length) in order to shed light on these issues.

The objective of this paper was to evaluate the tolerance to increasing flooding intensity of seedlings of the tropical grasses *C. gayana* and *P. coloratum* in order to assess their potential as a forage species in humid temperate grasslands. For this purpose, 2-week-old seedlings of these species were subjected to partial and complete submergence in clear water for 14 days. The following main responses were assessed: root aerenchyma, blade and sheath lengths, seedling height, biomass allocation in relation to plant size and biomass accumulation. In addition, recovery of each species was also determined 12 days after the water subsided, as this recovery is related to seedling recruitment in natural environments (Lenssen *et al*. 2004). To the best of our knowledge, this is the first study to address the submergence effects on tropical forage grasses with potential for use in temperate prone-to-flooding grasslands, and the first to show that both types of responses – shifting in biomass allocation towards shoots as well as lengthening of leaves – are both required to develop an escape strategy in a grass species facing complete submergence, such as *C. gayana*.

Materials and methods

Species description

Chloris gayana Kunth (Rhodes grass) and *P. coloratum* L. var. *colorarum* (Klein grass) are major tropical grasses found worldwide. They are cultivated in the United States (Texas), Africa, Australia, Japan, South America and under irrigation in the Middle East (Bogdan 1969; Boschma *et al*. 2008). *Chloris gayana* is a stoloniferous and tufted, leafy perennial grass with ascending stems (0.5–1 m tall) and hairless leaves measuring 10–50 cm long. *Panicum coloratum* is a shortly rhizomatous, tufted perennial that sometimes displays long spreading stolons and has culms measuring 0.3–1.5 m at maturity. Foliage is variable with leaves glabrous to hairy (5–40 cm long) varying in colour from green to glaucous. Both species are utilised as cultivated pastures for direct grazing, to produce hay and silage and also to stabilise sites with erosion problems. In particular, these species are regarded as tolerant to soil salinity and drought (Bogdan 1969; Dear *et al*. 2008) but they are also introduced in areas suffering from water excess of variable intensity, causing not only soil waterlogging (see Boschma *et al*. 2008) but also plant submergence.

In the Flooding Pampa grasslands of Argentina both species are being incorporated in lowland areas suffering from intermittent flooding. In particular, these species are introduced in vegetation units named ‘humid halophytic steppes’ (*sensu* Perelman *et al*. 2001), which encompass the 25–30% of the grasslands area of this region. Such vegetation units are located in very flat areas with nil slope and typic Natraquoll and Natraqualf soils (Soriano 1991). Plant communities in these areas are typically dominated by *Distichlis spicata* and *D. scoparia* (saltgrasses), with relative low forage productivity, which ranges from 500 to 2000 kg DM ha^{-1} $year^{-1}$ (Deregibus and Cahuepé 1983). So, the successful introduction of the tropical grass species examined in this paper looks promising for enhancing the forage productivity of these areas as both are regarded to produce more than 4000 kg DM ha^{-1} $year^{-1}$ (Bogdan 1969; Boschma *et al*. 2008; Dear *et al*. 2008).

Experimental details

Seeds of *C. gayana* K. (cv. Fine cut) and *P. coloratum* L. (cv. Klein Verde) were germinated in an incubator (25°C) in polystyrene boxes containing absorbent white paper saturated with distilled water. After 2–3 days, germinated seeds were transplanted to 1-L plastic pots (three per pot) filled with sand and topsoil (1 : 1) from lowland grassland of the Flooding Pampa of Argentina (organic carbon 3.3%, see further details in Soriano 1991) and transferred to the experimental garden at INFIVE (the Plant Physiology Institute in La Plata, Buenos Aires, Argentina). In order to avoid nutrient limitation, pots were fertilised with diammonium phosphate (with a dose equivalent to 100 kg ha^{-1}) before transplanting. Seedlings were left to grow for 14 days and subsequently thinned to one per pot.

Seedlings of each species with three fully expanded leaves were subjected to three treatments for 14 days following a fully randomised design with 10 replicates: (*i*) control (C): watered daily to field capacity, (*ii*) partial submergence (PS): seedlings were immersed in clear water at a depth of 7 cm, with the intention of covering seedlings until they had reached half their initial height (14.0 ± 1.6 cm height), and (*iii*) complete submergence (CS): seedlings were submerged in clear water at a depth of 21 cm, which corresponded to a water column of 7 cm above the top of the seedlings (see Day 0 in Fig. 2). Submergence water was carefully replaced every 4 days. The photosynthetic photon flux density, which reached the seedlings in contact with atmospheric air (C and PS treatments) was $1800 \pm 200\,\mu mol\,m^{-2}\,s^{-1}$, while for submerged seedlings (CS treatment) it was $680 \pm 58\,\mu mol\,m^{-2}\,s^{-1}$ (LI-192 Underwater Quantum Sensor; Li-Cor Inc., Lincoln, NE, USA). The latter was a light environment that allows for the underwater photosynthesis of C_4 species (Colmer and Pedersen 2008). The dissolved oxygen of submergence water ranged between 0.40 and 0.75 mg oxygen L^{-1} (LT Lutron DO-5510 equipment; Taipei, Taiwan), indicating the hypoxic environment caused by the submergence treatments. After the submergence period, seedlings were allowed to grow under well drained conditions for 12 days to assess their recovery (Malik *et al*. 2002; Real *et al*. 2008; Striker 2008, 2012). Temperature during the experiment ranged between 18 and 28°C. The submergence duration and water depth applied were consistent with the submergence scenarios that the seedlings of these species can experience in the lowland areas of the Flooding Pampas after being sown in spring (see Manzur *et al*. 2009; Striker *et al*. 2011*a*).

Root aerenchyma and diameter

Root aerenchyma formation was measured using optical techniques. At the end of the submergence period, root samples were taken, carefully washed and then preserved in formalin-acetic alcohol until needed. For each species and treatment, five root sections per plant ($n = 10$) were randomly selected for anatomical observations and measurements. Segments of roots 2 cm from the tip (position with maximum aerenchyma values based on previous experiences) were dehydrated in a series of increasing ethanol concentrations and embedded in paraffin wax. Sections 15–20 µm thick were cut, stained with safranin and Fast Green and mounted in Canada balsam. The proportion of root aerenchyma per total cross-sectional and root diameter were determined using an optical microscope (Zeiss Axioplan, Zeiss; Oberkochen, Germany) connected to an image analyser (Imagenation Px, Imagenation Corp.; Breaveton, OR, USA).

Seedling height, leaf length and biomass

Seedling height was quantified every 2–3 days during the submergence period and every 4 days during the recovery phase. The leaf blade and sheath length of the two last fully expanded leaves (grown during submergence treatments) on each plant were recorded at the end of the submergence period (see also Kawano *et al*. 2009). These variables provide information on the strategy of each species during CS. Biomass harvests were carried out at the beginning of the treatments in additional randomly chosen individuals (Day 0, $n = 10$), at the end of submergence period (Day 14), and at the end of the recovery phase (Day 26). The biomass was dissected into leaf blades, leaf sheaths and roots. All plant material was weighed after oven drying for 72 h at 80°C.

Statistical analyses

Variations in seedling height during the experiment were evaluated by two-way repeated-measures ANOVA with ‘submergence’ and ‘species’ as the between-subject main factors, and time as the within-subject factor. Root aerenchyma and diameter, and biomass dataset were evaluated by two-way ANOVA. When significant interactions were detected, a subsequent Tukey test was applied to determine the effect of the treatments. In addition, orthogonal contrasts were performed to compare the data between Day 0 and Day 14 within species. Normality and the homogeneity of variances were previously verified. The variables involving proportions (i.e. root aerenchyma) were arcsine $\sqrt{x}$-transformed previous analyses. Biomass data were analysed separately for the submergence and recovery period. In addition, the relationships between (*i*) shoot and root biomass, (*ii*) blade and sheath biomass, and (*iii*) blade and sheath length were studied through linear regression using Ln-transformed data (see Poorter and Nagel 2000; Striker *et al*. 2012). Slope tests were done in order to compare these relationships among the treatments for each species. When slopes and intercepts among regressions did not differ, data were pooled and a single linear equation for species is presented. Statistical analyses were done using the STATISTICA package for Windows (StatSoft; Tulsa, OK, USA). All results are presented as non-transformed means of 10 replicates ± standard error.

Results

Root aerenchyma responses

The constitutive root aerenchyma was high in both species (~37%) due to the presence of an extensive system of aerenchyma tissue arranged radially in the root cortex, separated by rows of parenchymatic cells (or their remnant walls) and surrounded by a ring of sclerenchymatic cells in the exodermis (Fig. 1). Under PS and CS, root aerenchyma increased by up to 50–55% in *C. gayana* and up to 40–48% in *P. coloratum* (Fig. 1, Table 1). These rises in the proportion of aerenchyma responded to a further lysis of cells in the root cortex (see photographs in Fig. 1), and to an increase in root diameter, which was 11–18% higher under excess water conditions (Fig. 1). Both the increment in root aerenchyma and diameter are typical responses to oxygen deprivation in flooding-tolerant species (Fig. 1, Table 1).

Seedling height under PS and CS

Under PS, both species were able to increase seedling height the same as C until doubling their height in comparison to the beginning of the experiment (Fig. 2). Interestingly, during CS, the species responded in a different way (rmANOVA species × submergence: $P < 0.001$). Seedlings of *C. gayana* markedly increased their height, thus emerging faster from water a few days after the beginning of the submergence treatment. Contrarily, *P. coloratum* was not able to increase its height and remained underwater throughout the submergence period, maintaining the same height range as at the beginning of the experiment ($P = 0.19$, compare Day 14 with Day 0 in Fig. 2). During the recovery period, differences in seedling height for *C. gayana* were not more apparent ($P > 0.45$ for final seedling height). *P. coloratum* seedlings previously subjected to CS experienced a negligent increment in height, which indicates that the effects of anoxia injury due to submergence continue until the recovery period (Fig. 2).

Allometric relationships as affected by submergence treatments

In *C. gayana*, the relationship between shoot and root biomass (Ln of the transformed values) revealed a preferential biomass allocation towards shoot compartment during CS (detrimental to roots). This response was indicated by the higher slope of this relationship for submerged seedlings in comparison to seedlings growing under C conditions and PS (Fig. 3, upper panels). This was as expected in accordance to the escape strategy exhibited by this species from the seedling-height responses (Fig. 2). The relationship between leaf blade and leaf sheath biomass remained the same regardless of the treatment (slope tests: $P > 0.05$). This indicates that leaves maintained a constant proportion of mass assigned to blades and to sheaths, regardless of differences in shoot size when plants were grown under C or stress conditions (see middle panels of Fig. 3). Interestingly, this species responded to CS with a pronounced lengthening of the leaf blades (for a given leaf sheath length) with respect to the other treatments, as indicated by the higher slope for the blade to sheath length relationship (see lower panels of Fig. 3). This means that, for a given sheath length, completely submerged seedlings had longer leaf blades than partially submerged or C seedlings (see closed circles in the lower panel of Fig. 3). This response also favoured the development of the escape strategy used by *C. gayana* in order to achieve a faster emergence of leaves above water.

In *P. coloratum*, there was no evident relationship between the biomass of shoots and roots under CS ($P > 0.05$) and the allocation pattern (to shoot and to roots) was similar among seedlings subjected to C and PS in terms of the fitted parameters (slope and intercept: $P > 0.05$ in both cases). In this species, the relationship between leaf blade and sheath did not change among treatments either in terms of biomass or length ($P > 0.2$, see Fig. 3). As a result, only a reduction in leaf size was detected under progressively stressful conditions from C (the longest leaf blades and sheaths) to CS (the shortest leaf blades and sheaths, Fig. 3 lower panels).

Biomass responses

Submergence reduced the total biomass accumulation of seedlings of both species but to a different degree depending on the treatment (Fig. 4, Table 1). Under PS, seedlings of *C. gayana* and *P. coloratum* registered ~28% less total biomass in comparison to their respective C (Fig. 4, left panels for each species). This response was a result of a lower shoot and root biomass of 28–31% and 27–32% (respectively), a response similar for both species (Table 1). Interestingly, species were affected by CS in different ways. Seedlings of *C. gayana* attained 57% less total biomass while *P. coloratum* almost did not grow when completely underwater ($P > 0.11$, compare biomass on Day 0 and Day 14). Under this scenario, *C. gayana* prioritised shoot growth, which attained a biomass similar to that experienced during PS, and ceased root growth ($P = 0.49$, compare root mass on Day 0 and Day 14 in Fig. 4). This preferential allocation of biomass to shoot instead of roots (see Fig. 3) provided further evidence on the escape strategy used by this species to deal with CS.

After the submergence period, species experienced differential recovery in terms of biomass accumulation (Fig. 4, right panels for each species). Seedlings of *C. gayana* were able to resume vigorous growth after CS, roots in particular, which allowed them an almost full recovery with respect to PS seedlings (90%), and partial recovery (54%) in comparison to C (Fig. 4, right panels, Table 1). In *P. coloratum*, the negative effects of submergence on biomass accumulation could be seen until the end of the experiment, regardless of whether seedlings were partially or fully submerged. On that note, seedlings previously subjected to PS attained 64% of the biomass reached by C (Fig. 4, right panels, Table 1). Moreover, in the most stressful scenario provoked by CS, seedlings of *P. coloratum* were severely affected, attaining only 21% of biomass in comparison to C (Fig. 4, Table 1). This finding was

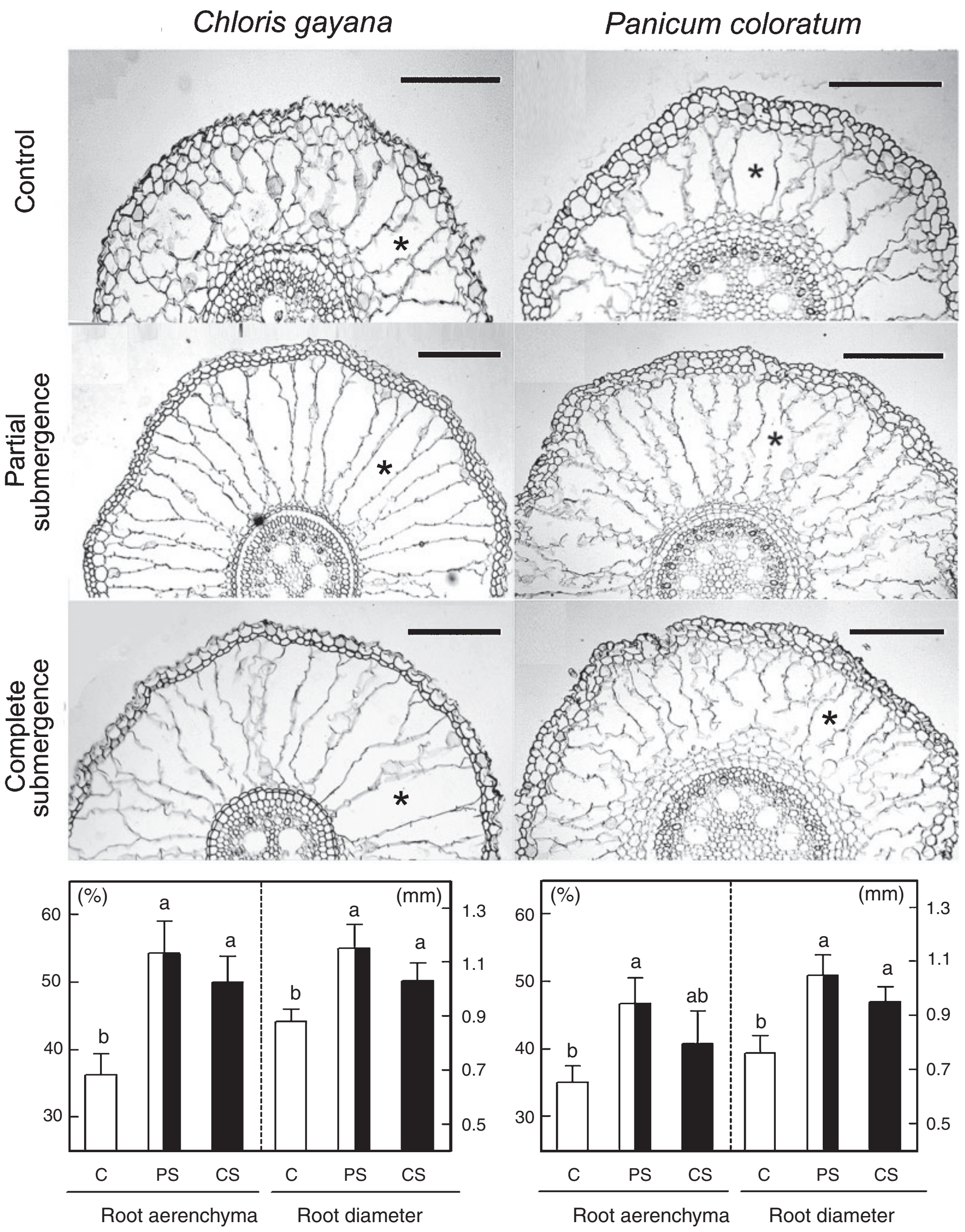

Fig. 1. Root anatomical traits of *Chloris gayana* and *Panicum coloratum* seedlings subjected to control (C), partial submergence (PS) and complete submergence (CS). Submergence period lasted 14 days. Asterisks indicate lysigenous aerenchyma lacunae. Scale bars represent 200 µm. Different letters indicate significant differences ($P < 0.05$) among treatments within each species based on Tukey's test. Values are means ± s.e. of 10 replicates.

in line with the very slight height increment registered for seedlings (Fig. 2).

Discussion

The research demonstrates that *C. gayana* has greater tolerance to water excess, which causes seedling submergence than *P. coloratum* for a period of up to 2 weeks. This differential tolerance was based on two main responses: the prioritisation of utilising biomass gained during submergence for shoot growth (see also Striker *et al.* 2012; for *Lotus* spp.), and further lengthening of leaf blades (our finding; see Discussion below). Both responses allow the seedlings of *C. gayana* to escape from water quickly. This de-submergence ability gives this species a better chance at seedling recruitment in grasslands prone to flooding of high intensity (i.e. water depth compromising seedling submergence) (see also Crawford 2003; Lenssen *et al.* 2004). On the other hand, *P. coloratum* performed

Table 1. Results of two-way ANOVA for the effects of 'Submergence levels' and 'Species' on root porosity, root diameter and biomass of *Chloris gayana* and *Panicum coloratum* seedlings

F-values of the ANOVA are presented. Degrees of freedom for each source of variation are: 2 (Submergence), 1 (Species), 2 (Submergence × Species) and 54 (Error). $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; n.s., $P>0.05$

Variable	Main effects Submergence	 Species	Interaction Sub × species
Root aerenchyma	23.49***	11.91**	1.93 n.s.
Root diameter	21.38***	10.53**	1.67 n.s.
Biomass			
After submergence (*Day 12*)			
Total	59.72***	84.91***	0.68 n.s.
Shoot	29.16***	52.50***	3.73*
Roots	106.48***	114.77***	16.67***
After recovery (*Day 26*)			
Total	93.61***	199.89***	11.65***
Shoot	74.09***	134.18***	9.96**
Roots	42.76***	128.00***	4.57*

adequately under PS but poorly under CS. In the latter case, seedlings experienced almost no growth and their leaves did not elongate. The fact that *P. coloratum* did not respond to CS should not be interpreted as a quiescence strategy. Species using quiescence have the capacity to slow their metabolism during submergence in order to save energy, and after water subsides, they are able to resume vigorous growth (Bailey-Serres and Voesenek 2008, 2010). In this case, the minimal recovery of biomass shown by *P. coloratum* 12 days after the water subsided indicates that this species was significantly stressed by CS (although all seedlings survived), which did not represent a quiescent strategy. Seedlings of *P. coloratum* are simply sensitive to this condition, in contrast to reported findings for seedlings of *Lotus tenuis*, a legume used in lowlands like in the Flooding Pampa grasslands, which adopts a real quiescence strategy as shown by its high recovery after submergence (Striker *et al.* 2012).

Both species were able to grow under PS, accumulating 70–80% biomass in comparison to C in both shoots and roots, as seen in others waterlogging-tolerant C_4 grasses like *Brachiaria mutica*, *Echinochloa polystachya* (Baruch 1994), *Panicum antidotale* (Ashraf 2003) and *Paspalum dilatatum* (Striker *et al.* 2006, 2008). In both cases, the relatively solid plant performance in this scenario was associated with two main responses, oxygen capture and transport, which facilitated the aeration of submerged tissue (Colmer 2003; Colmer and Voesenek 2009). First, both species were able to reach the same seedling height as C during the experiment (Fig. 2); the proportion of leaves above water level progressively increased, which promotes oxygen capture (Laan *et al.* 1990; Grimoldi *et al.* 1999; Striker *et al.* 2005). Second, oxygen transport to the roots through diffusive mechanisms appears to be facilitated by high initial constitutive aerenchyma, which was further increased by PS as a result of increases in root diameter and cell lysigeny in the root cortex (Fig. 1, see also Visser *et al.* 2000; Striker *et al.* 2007). The same type and magnitude of responses in front of PS have been registered previously in other species successfully inhabiting lowlands at the Flooding Pampa like the grass *P. dilatatum* (Insausti *et al.* 2001; Striker *et al.* 2006; Mollard *et al.* 2010) and the legume *L. tenuis* (Striker *et al.* 2008; Manzur *et al.* 2009). So, considering these results, it can be stated that both *C. gayana* and *P. coloratum* are promising species to incorporate into temperate areas suffering from water excess, ranging from water saturated soils to soils with a thin water layer above the surface (without leading to seedling submergence for *Panicum*).

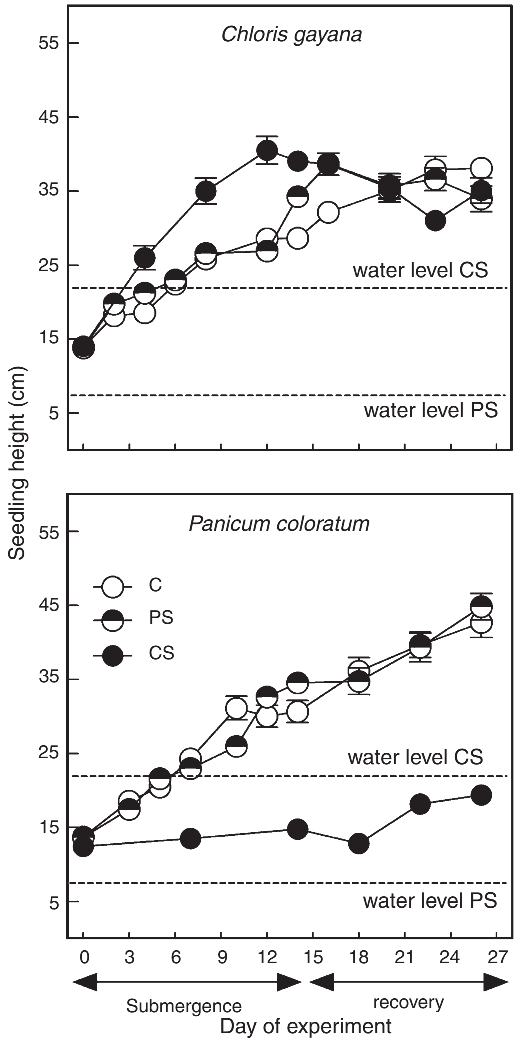

Fig. 2. Seedling height of *Chloris gayana* and *Panicum coloratum* subjected to control (C), partial submergence (PS) and complete submergence (CS) conditions. Submergence period lasted 14 days. Recovery period lasted 12 days. Dashed lines indicate water depth of the submergence treatments. Values are means ± s.e. of 10 replicates.

Poorter and Nagel (2000) highlighted the importance of incorporating allometric analysis in order to study the biomass allocation of plants under different stress conditions, as many morphological and biomass ratios change based on plant size. A recent meta-analysis by Poorter *et al.* (2012) has delved deeper into this topic by showing that under waterlogging and submergence, tolerant species increase biomass allocation to shoot (leaves and stems) and they can adjust their organs morphology more easily (i.e. longer leaves, higher specific leaf area). In our experiment, allometric analysis has provided further

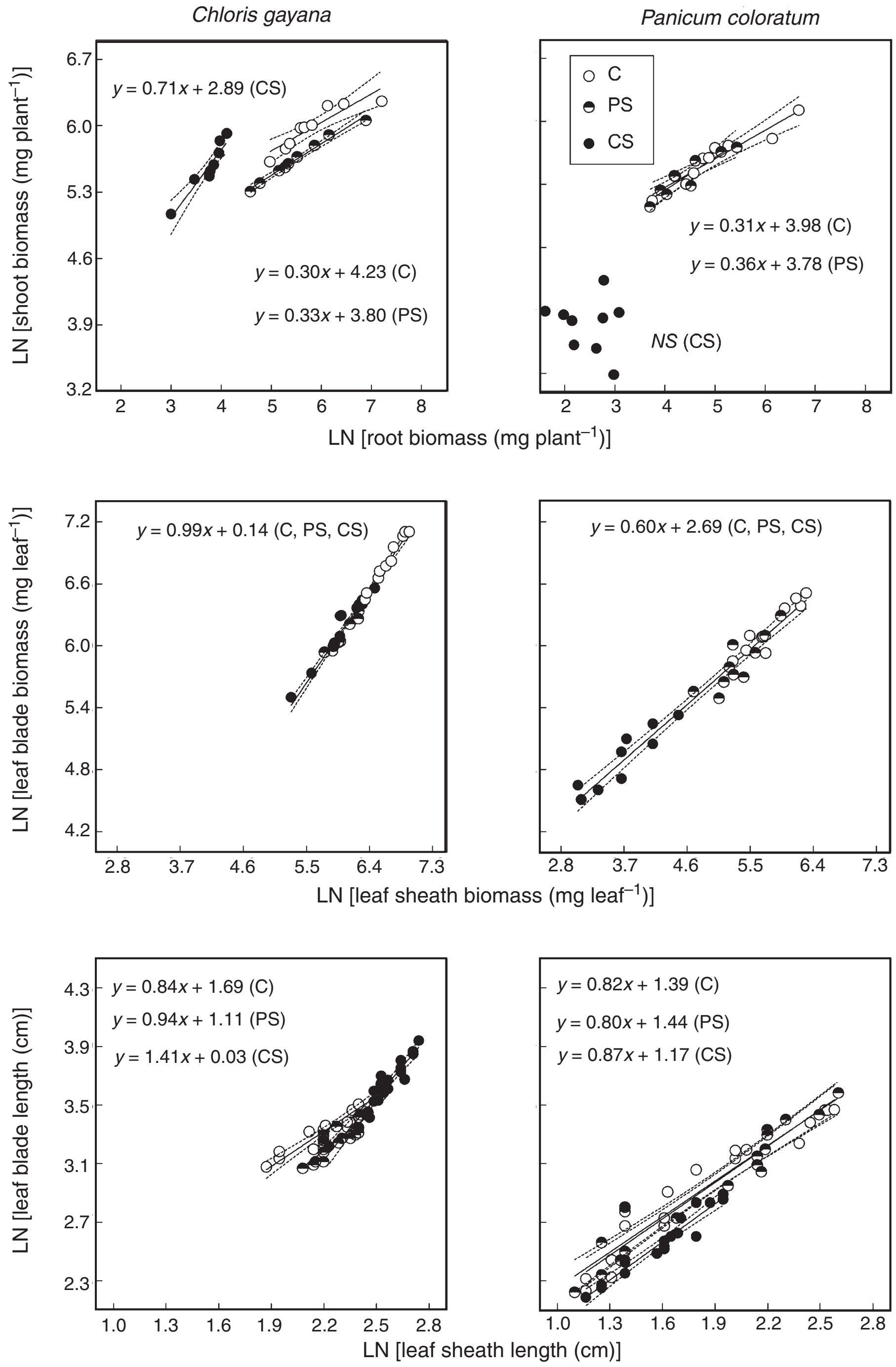

Fig. 3. Allometric relationships between (i) LN of shoot *v.* LN of root biomass, (ii) LN of leaf blade *v.* LN of leaf sheath biomass, and (iii) LN of leaf blade *v.* LN of leaf sheath length of *Chloris gayana* and *Panicum coloratum* seedlings subjected to control (C), partial submergence (PS) and complete submergence (CS). Note that when slopes and intercepts among fitted regressions did not differ, data were pooled and a single regression line for species is presented for brevity and clarity.

insight to show that, irrespective of plant size, the escape strategy developed by *C. gayana* under CS was achieved by both actively increasing biomass allocation towards shoots as well as by elongating leaf blades (Fig. 3). In this regard, both responses complement one other by helping plants to restore the contact of leaves with atmospheric air, thus ensuring submerged tissue oxygenation and aerial photosynthesis (see also Chen *et al*. 2009, 2011; Kawano *et al*. 2009; Pierik *et al*. 2009; Striker *et al*. 2012). Furthermore, biomass allocation between blades and sheaths resulted in a conservative trait (no treatments effect,

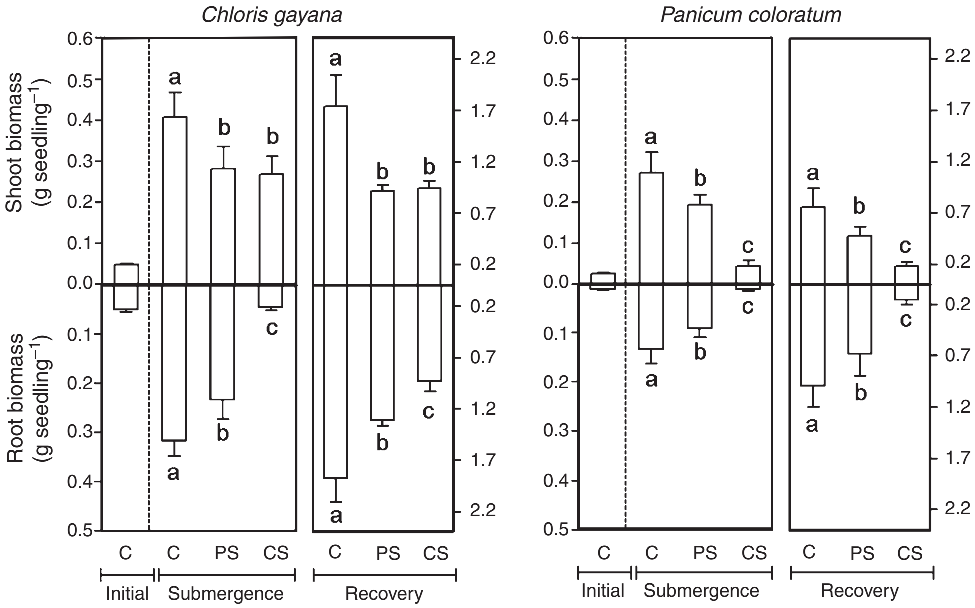

Fig. 4. Shoot and root dry mass of seedlings of *Chloris gayana* and *Panicum coloratum* subjected to control (C), partial submergence (PS) and complete submergence (CS) conditions. Graphs of each species are split into two panels: left panels show biomass at Day 0 (initial) and at Day 14 (submergence) while right panels show biomass at Day 26 (recovery). Note the different scales between left and right panels. Different letters indicate significant differences ($P < 0.05$) based on Tukey's test within each period. Values are means ± s.e. of 10 replicates.

see Duru and Ducrocq 2000; Grimoldi *et al.* 2005*b*), but leaf blades were proportionally larger than leaf sheaths (Fig. 3). Therefore, it can be inferred that completely submerged seedlings of *C. gayana* increased the specific leaf area as seen for dicots of the *Rumex* genus (Mommer and Visser 2005; Mommer *et al.* 2006). This means that these seedlings invested the same amount of carbon in obtaining larger leaf blades in order to facilitate their escape from water. It should be noticed that, as all seedlings started the experiment in a vertical position (cf. Cox *et al.* 2003; for *Rumex palustris*), leaf length was the main variable defining seedling height. For *P. coloratum*, the results of allometric analysis showed that treatments *per se* did not provoke any alteration between plant biomass and leaf length relationships (Fig. 3). It seems that submergence was very stressful for this species, and as plant growth was severely restricted, it was not possible for the plants to make changes in biomass allocation or leaf length (Poorter *et al.* 2012). Moreover, in this species, we were unable to establish a clear relationship between shoot and root mass for completely submerged seedlings. It is possible that the death rate of submerged tissues, particularly roots (compare Figs 3 and 4), differed among seedlings under this treatment to such an extent that the data registered became variable (see also Parent *et al.* 2008).

Species exhibited different levels of ability to recover during the phase following submergence, a crucial period to be considered in order to assess real specific tolerance to submergence (Malik *et al.* 2002; Real *et al.* 2008; Striker 2008, 2012). During the recovery phase, seedlings of *C. gayana* previously subjected to CS developed outstanding root growth while maintaining the same shoot growth as seedlings that had grown under PS. By contrast, *P. coloratum* was unable to resume vigorous growth during this period of either shoots or roots (Fig. 4). In this respect, recovery of both shoots and roots are critical for seedling establishment in field conditions (see also Striker *et al.* 2012; for *Lotus* species). On the one hand, if the species are sown in natural grasslands, the intensity of light competition with the established canopy is expected to increase after a disturbance such as submergence (Jutila and Grace 2002; Striker *et al.* 2011*a*). Thus, the better shoot recovery displayed by *C. gayana* gives it a greater chance at seedling establishment in the grasslands; while conversely, the poor shoot recovery displayed by *P. coloratum* could drastically constrain its possibilities for recruitment (see Crawford 2003; Lenssen *et al.* 2004). On the other hand, the preferential biomass allocation of *C. gayana* towards roots during its recovery allowed this species to re-establish a shoot to root biomass ratio more appropriate for facilitating water and nutrient supply under well drained soil. This response is in agreement with a 'functional equilibrium' theory (see Poorter and Nagel 2000) where now, after submergence, belowground resources (i.e. water and nutrients) can limit plant growth. By contrast, in *P. coloratum*, the poor root development resulting from CS clearly represents a disadvantage for seedling recruitment, even more if it is considered that spring floods are sometimes followed by drought periods (Fukao *et al.* 2011) during summer in natural grasslands, as it occurs in the Flooding Pampa of Argentina (Soriano 1991).

Conclusions

The results obtained in this experiment are significant for the ecophysiology and management of these cultivated forage species when seeking success in seedling recruitment in soils of grassland and pastures suffering from water excess. The strategic use of each species will depend on the water depth of the specific environment in which they are incorporated as part of the forage resources. In areas suffering from very shallow flooding events, such as plant communities located along intermediate topographical positions, the responses of both species (e.g. root aerenchyma increases and the location of most leaves above water) proved to be sufficient for maintaining substantial seedling growth. In these areas, the slightly higher tolerance to cold and freezing of *P. coloratum* with respect to *C. gayana* (Ludlow 1980) suggests that the former is a better option in temperate environments (prone to flooding), like the ones in which they are introduced nowadays. In contrast, in lowland areas in which seedlings may be subject to CS, *C. gayana* has proven capable of adjusting its allocation responses, promoting leaf elongation and prioritising shoot growth, as major responses of its 'escape from water' strategy. These complementary morphological and biomass allocation responses lead to fast de-submergence of leaves. When viewed from an allometric approach, this has not been reported before. Future work exploring the performance of other cultivars and genotypes of these species (see examples in Teakle *et al*. 2010; and Rogers *et al*. 2011; for *Lotus tenuis* and *Melilotus siculus*, respectively), would be interesting in order to assess the degree of intraspecific variation in the reported responses.

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