Recovery from short-term complete submergence in temperate pasture grasses

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Abstract. The ability to recover from the impact of short-term submergence was assessed on four widely used grasses in pastures: *Dactylis glomerata* L., *Bromus catharticus* Vahl., *Schedonorus arundinaceus* Schreb. (syn. *Festuca arundinacea*) and *Phalaris aquatica* L. Six-week-old plants were subjected to a 5-day complete submergence in clear water, followed by a 15-day recovery period. Dry mass after submergence, shoot and root growth, number of tillers per plant, leaf stomatal conductance and leaf greenness during recovery were assessed. *Dactylis glomerata* and *B. catharticus* were sensitive to submergence, showing very low relative growth rate (RGR) of shoots and roots during recovery (37–67% lower than controls) along with early leaf senescence and persistent partial stomatal closure. *Schedonorus arundinaceus* exhibited an intermediate tolerance, sustaining high RGR of shoots (similar to controls) and fully adjusting its leaf functionality during recovery despite being affected during submergence (40% decrease in dry mass and 37% in tiller number). *Phalaris aquatica* performed outstandingly, with dry mass unaffected by submergence, and unaltered stomatal conductance, leaf greenness, tillering and shoot growth during recovery. Therefore, in areas where flooding can often cause plant submergence, *P. aquatica* is recommended whereas the other species are not, because they may be outcompeted by flood-tolerant species.

Additional keywords: bulbous canary, cocksfoot, Harding grass, orchardgrass, prairie grass, tall fescue.

Introduction

Pasture grasses are increasingly subjected to stressful conditions, constraining plant growth and thereby forage production. Crop intensification worldwide has displaced pastures towards less favourable (i.e. suboptimal) areas (Wright and Wimberly 2013; Abraham *et al*. 2014), a significant proportion of which are lowlands suffering from flooding episodes. Thus, pasture species growing in these areas need to deal with temporary flooding as part of their environment. In addition, more frequent and intense flooding events are expected to occur from the ongoing effects of global climate change. This anticipates a higher proportion of pasture lands being flooded in the future, along with an increase in flooding intensity (i.e. water depth) of lowlands currently experiencing water excess (Hirabayashi *et al*. 2013). Submergence tolerance of grass species resulting from higher intensity flooding events has rarely been researched, except for Beard and Martin (1970), who evaluated the effects of prolonged submersion (5–60 days) on four turf-grass species, although only survival was scored.

Complete plant submergence associated with intense flooding is a very stressful scenario because a drastic reduction of irradiance and CO$_2$ for shoots is added to the shortage of oxygen for roots (Colmer and Voesenek 2009). Studies to date have provided insight into the strategies used by plants to deal with submergence, whereby plants are classified according to an 'escape strategy' (i.e. fast shoot-elongation to emerge from water, fueled by carbohydrate usage), or a 'quiescent strategy' (i.e. shoot elongation repressed, and carbohydrate consumption preserved). For review of these strategies, see Bailey-Serres and Voesenek (2008) and Striker (2012*a*) and references therein. However, real tolerance to submergence should be assessed after considering a growth period at the end of the stress, because the ability to recover from submergence can vary among species and accessions or cultivars within species (Striker 2012*b*; Striker *et al*. 2012). Despite its importance, recovery from submergence has been overlooked in most experiments dealing with flooding stress. For instance, more than 70% of studies based on pasture legumes used worldwide did not include a recovery period in their experimental design (review by Striker and Colmer 2017). Moreover, the international community of researchers leading studies on plant responses to flooding recently recognised and recommended a quantitative scoring of recovery after a stress period involving low oxygen (Sasidharan *et al*. 2017). Hence,

in the present experiment, particular attention was given to evaluating the performance of plants during a recovery period after submergence, by monitoring growth and certain leaf physiological parameters.

The ability of grasses to recover from submergence when water subsides can be related to the initiation of new tillers to resume growth (for sensitive IR42 rice, Singh *et al*. 2009), the maintenance of physiologically active leaves of current tillers without differences in tillering (for the tolerant grass *Paspalum dilatatum*, Mollard *et al*. 2010), or a combination of both. Soon after submergence subsides, the re-aeration of aerial tissues leads to the rapid formation of reactive oxygen species (ROS) (Steffens *et al*. 2013), which, in sensitive species, triggers chlorophyll degradation and early leaf senescence (in accessions of the tropical grass *Brachiaria humidicola*, Cardoso *et al*. 2013). By contrast, in submergence-tolerant species, the maintenance of green photosynthetically active leaves is associated with a post-submergence improvement in recovery ability (for the wetland grass *Hemarthria altissima*, Luo *et al*. 2009; for the subtropical grass *Chloris gayana*, Striker *et al*. 2017). In addition, the ability to restore stomatal conductance after certain stresses such as submergence is directly associated with the reduction of diffusive resistance of CO_2 into leaves, and therefore it is a positive indicator of leaf photosynthetic activity potential (Lambers *et al*. 2008). Therefore, monitoring leaf physiological parameters positively related to plant growth after submergence, such as leaf greenness and stomatal conductance, might improve understanding of the traits related to recovery from water excess in forage grasses.

This study was designed to assess the recovery ability of four commonly used pasture grasses from a 5-day complete submergence, a likely scenario under field conditions with intense rainfall. Individual plants of the widely used temperate pasture grasses *Dactylis glomerata* L., *Bromus catharticus* Vahl., *Schedonorus arundinaceus* Schreb. (syn. *Festuca arundinacea*) and *Phalaris aquatic* L. were exposed to a 5-day submergence-shock scenario and were allowed to recover for a 15-day period. Recovery was examined in terms of growth (dry mass accumulation and relative growth rate (RGR)), effect on number of tillers per plant, and post submergence dynamics of leaf stomatal conductance and greenness in old and young leaves (as a surrogate for leaf nitrogen status). Plant dry mass accumulations of shoots and roots were assessed as indicators of the immediate effects of the applied stress, and plant tillering was quantified as a variable of potential constraint for future–long-term growth. This study presents the first comparison of submergence tolerance among these four widely used pastures; previous literature presented only information on waterlogging tolerance (Humphries 1962; Jung *et al*. 2009; Martina and von Ende 2013; Ploschuk *et al*. 2017).

Materials and methods

Species description

Four temperate grass species that are used worldwide as pastures were selected for the study. *Dactylis glomerata* (common names cocksfoot and orchardgrass) is a tufted, deep-rooted, cool-season perennial grass with erect and glabrous culms up to 60–150 cm height. It is tolerant of shade and mild drought but sensitive to waterlogging (Ploschuk *et al*. 2017). *Bromus catharticus* (common name prairie grass) is a tufted, short-lived perennial or biennial grass with erect culms (up to 55–84 cm tall), unbranched, and widely distributed in temperate regions of the world (Jatimliansky *et al*. 1997). It is considered sensitive to excess soil water (Ploschuk *et al*. 2017). *Schedonorus arundinaceus* (common name tall fescue) is a long-lived, perennial, cool-season bunchgrass, with culms usually erect, stout and smooth. It is reputed to be tolerant to waterlogging (Gibson and Newman 2001). *Phalaris aquatica* (common names bulbous canary or Harding grass) is an erect, waist-high, deep-rooted and rhizomatous, stout perennial bunchgrass with hollow stems, also proven tolerant to waterlogging (Boschma *et al*. 2008; Ploschuk *et al*. 2017).

Experimental design

Seeds from *D. glomerata* cv. Omea, *B. catharticus* cv. Jerónimo, *S. arundinaceus* cv. Malma and *P. aquatica* cv. Mate were provided by Gentos (Buenos Aires). They were germinated in Petri dishes on filter paper moistened with distilled water in an incubator at 25°C, in darkness. Germinated seeds were transplanted to 1-L pots wholly filled with sand and topsoil (1 : 1 v/v, 1.2 kg substrate per pot) from natural grassland (3.1% organic carbon). The substrate was supplemented with Triple 15 Plus fertiliser (Ciudad Floral, Escobar, Argentina), comprising nitrogen, phosphorus and potassium (15% N, 15% P, 15% K), and micronutrients (0.1% zinc, 0.01% molybdenum, 0.01% iron, 0.05% copper and 0.1% boron). Fertiliser dose was 3 g per pot to avoid potential interferences of nutrient deficiencies with the plant responses to the treatments, especially during the recovery period. Pots were placed in a glasshouse under natural light conditions (October–November in Buenos Aires) and seedlings were thinned to one per pot during the first two weeks after transplanting.

After a 6-week growing period, two treatments were imposed following a completely randomised design with eight replicates: control and complete submergence. Plants assigned to the control treatment were watered to field capacity daily and allowed to drain freely. Completely submerged plants were immersed in water inside plastic containers (i.e. tanks) for 5 days, emulating a possible submergence scenario occurring under natural conditions, due to heavy rainfall in lowlands with limited runoff (i.e. flat areas of the landscape) (Di Bella *et al*. 2016; Striker *et al*. 2017). There were eight tanks, each containing 16 plants, i.e. two replicates per species per tank, with one replicate harvested at the end of submergence, and the remaining replicate at the end of recovery (see harvesting procedure below). As a reference, the oxygen diffusion rate in the substrate used can drop from 82.3×10^{-8} g cm^{-2} min^{-1} to 4.5×10^{-8} g cm^{-2} min^{-1} during the first 2 days of waterlogging (see Striker *et al*. 2011), indicating rapid anaerobic conditions established by water excess. Submergence was achieved by fully covering the plants for 5 days with artificial, clear floodwater made of distilled water plus 0.50 mM $CaSO_4$, 0.25 mM $MgSO_4$ and 1 mM $KHCO_3$ (see Pedersen *et al*. 2009). The water column was set at ~1.3 times plant height, which resulted in a range of water depths of 24–30 cm due to differences in plant height. The photosynthetic photon flux

density at the top of the submerged plants was $320 \pm 32\,\mu mol\,m^{-2}$ s^{-1} (LI-192 Underwater Quantum Sensor; LI-COR Biosciences, Lincoln, NE, USA), a light environment allowing underwater photosynthesis of C_3 temperate grasses (Colmer and Pedersen 2008). The temperature of submergence water ranged between 22.4°C and 23.2°C, and it was similar ($P = 0.41$) among tanks. Dissolved oxygen in the submergence water at midday ranged from 3.8 to $4.1\,mg\,L^{-1}$ (vs $7.4\,mg\,L^{-1}$ in air), measured by a DO-5510 dissolved oxygen meter (LT Lutron Electronic Enterprise, Taipei). Submergence was followed by a 15-day period under well-drained growing conditions to examine the ability of each species to recover from submergence.

During the post-submergence period, stomatal conductance of upper young expanded leaves was measured every 3 days by using a leaf porometer (model SC-1; Decagon Devices, Pullman, WA, USA) to evaluate the facilitation of CO_2 diffusion into leaves for photosynthesis. On the same dates, leaf greenness was measured in young (apical) and old (basal) fully expanded leaves by using a portable chlorophyll meter (SPAD-502; Konica Minolta Sensing, Osaka, Japan). This detected the effects of submergence on induced leaf yellowing as indicative of chlorophyll degradation, nitrogen remobilisation, and, lastly, anticipated leaf senescence, compared with leaves from control plants of similar age and position. Therefore, both parameters assessed are positively linked to the potential for carbon fixation of plants during the recovery post-stress period. In all cases, only leaves recovering from submergence were measured, there being no emergent leaves to consider in any species.

Air temperature and relative humidity of the greenhouse were monitored with a HUMICAP H temperature and relative humidity probe (Vaisala, Helsinki) and used to calculate air vapour pressure deficit (VPD_{air}) as the difference between saturated vapour pressure (i.e. Clausius–Clapeyron equation) and real air vapour pressure, which was considered indicative of the atmospheric evaporative demand. The VPD_{air} during the experiment ranged between 1.6 and 2.4 kPa.

Plants were harvested at the end of submergence ($n = 8$) and the end of the recovery period ($n = 8$), and the number of live tillers per plant (tillers with at least one green–functional leaf) was counted to determine whether there was submergence-induced death of tillers. Tillering is an important variable that allows projection of potential plant growth in a longer term exceeding the experimental period. Shoots were carefully separated from the roots, and dry masses were obtained after oven drying at 80°C for 72 h. The RGR during recovery for each combination of species and treatment was calculated for shoots and roots. Given that dry-mass harvests are destructive procedures, initial values were not paired with final values, so RGR values (of shoots and roots) were calculated as the average of all possible combinations of values between initial and final harvests of the recovery period, among plants of each species × treatment combination (as in Ploschuk *et al.* 2017).

Statistical analyses

Dry-mass responses and numbers of tillers per plant were analysed by 2-way analysis of variance (ANOVA) with species and submergence treatment as main factors. Stomatal conductance and leaf greenness were analysed by 3-way ANOVA with species, treatment and time as main factors. In all cases, *a posteriori* comparisons of means were performed with Tukey's tests. Normality and homogeneity of variances of the dataset were checked before performing ANOVAs. Statistical analyses were performed in GraphPad Prism 5 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Results

Plant growth and tillering as affected by complete submergence and subsequent recovery

All plants of the evaluated species remained underwater for the 5-day submergence period without leaf emergence. The short-term submergence had a negative impact on dry mass in three of the four species, namely *D. glomerata*, *B. catharticus* and *S. arundinaceus* (Fig. 1*a*) and there was a significant species × treatment interaction for shoot dry mass ($P < 0.01$, Table 1). The magnitude of the reduction in growth was species-specific. In *B. catharticus* and *D. glomerata*, the dry masses of shoots and roots of submerged plants corresponded to 37–51% and 52–68% of their controls, respectively (Fig. 1*a*). In *S. arundinaceus*, plant immersion for 5 days had a slightly less severe impact on dry mass of shoots and roots, equivalent to 62% and 58% of the well-drained control (Fig. 1*a*). By contrast, *P. aquatica* showed great tolerance in terms of dry mass, with no differences between treatments detected for either shoots or roots by the end of submergence (Fig. 1*a*, $P = 0.18$ by Tukey's test).

After submergence, species showed different ability to recover in terms of dry mass and RGR (Fig. 1*b, c*) and there was a species × treatment interaction for shoot dry mass ($P < 0.01$) and root dry mass ($P < 0.05$) (Table 1). The most sensitive species was *B. catharticus*, which registered only 29% and 15% of shoot and root dry mass, respectively, compared with the control (Fig. 1*b*). This reduced dry mass after submergence was a consequence of a 67% lower shoot RGR than in the control, along with the death of root tissues, as revealed by a negative RGR for roots during recovery (Fig. 1*c*). In *D. glomerata*, shoot and root RGR of previously submerged plants were 37% and 70% lower than those of control plants; consequently, the final dry mass of previously submerged plants represented 45% and 40% of the control for the shoot and root compartments (Fig. 1*b*). The case of *S. arundinaceus* was interesting because the differences in dry mass of shoots and roots relative to the control were within the same range by the end of the recovery period as they were after submergence. This was a result of the high RGR sustained for shoots (similar to the control) and, to a lesser extent, for roots (RGR 72% of the control) during recovery (Fig. 1*c*). The tolerant *P. aquatica* continued growing at similar shoot RGR, irrespective of submergence, and thereby attained similar final shoot dry mass between treatments (Fig. 1*c*). The root RGR of previously submerged plants of *P. aquatica* was 63% that of control plants during recovery, which resulted in final root dry mass 71% that of non-stressed control plants (Fig. 1*b*).
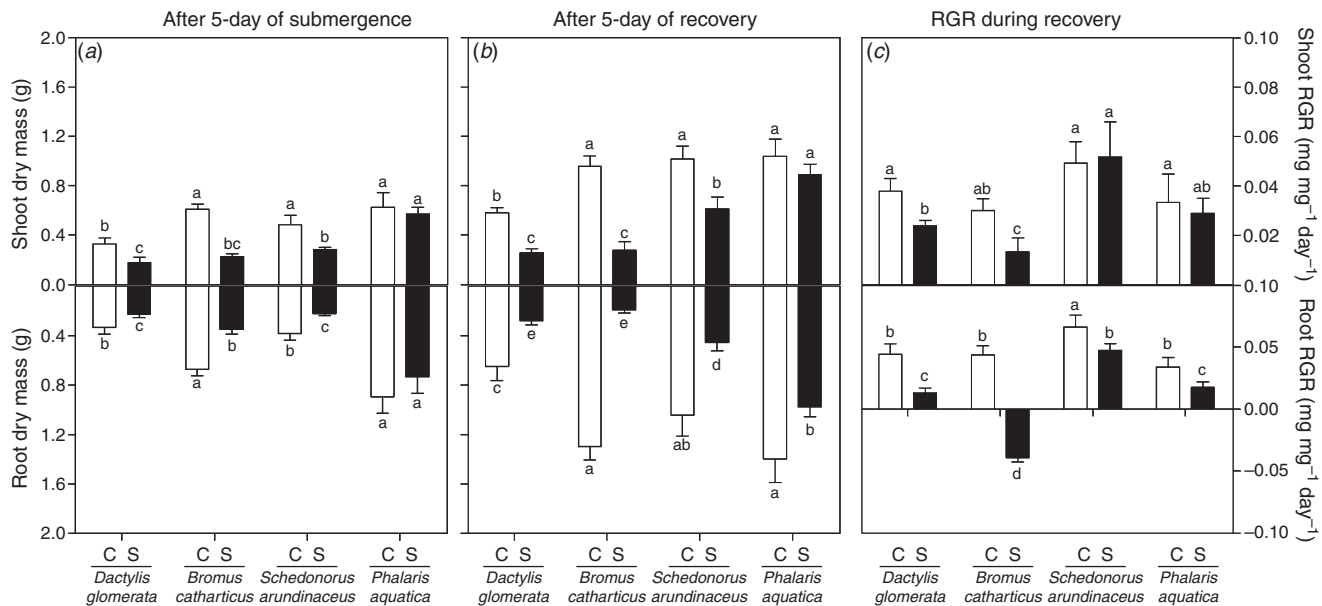
Fig. 1. Shoot and root dry mass (g plant^{-1}) of 6-week-old plants of *Dactylis glomerata*, *Bromus catharticus*, *Schedonorus arundinaceus* and *Phalaris aquatica* (*a*) after being subjected to complete submergence in clear water for 5 days, and (*b*) following a subsequent recovery of 15 days under well-drained conditions; (*c*) relative growth rate (RGR) of shoots and roots (mg mg^{-1} day^{-1}) during the recovery period after submergence of each species. For each period and parameter, means with the same letter are not significantly different (Tukey's tests at $P = 0.05$) between treatments and species. Values are means ± standard errors of eight replicates. Two-way ANOVA results are presented in Table 1.

Table 1. ***F*-values of two-way ANOVA for dry mass responses and tillers per plant of 6-week-old plants of *Dactylis glomerata*, *Bromus catharticus*, *Schedonorus arundinaceus* and *Phalaris aquatica* subjected to a 5-day complete submergence followed by a 15-day recovery under well-drained conditions**

Factors are species (Sp) and treatment (Tr). Degrees of freedom for each source of variation: species, 3; treatment, 1; Sp × T, 3; error, 56. Analyses were performed separately for the submergence and the recovery periods. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., $P > 0.05$

Plant responses	Submergence period			Recovery period		
	Species	Treatment	Sp × Tr	Species	Treatment	Sp × Tr
Shoot	10.49***	110.3***	6.66**	19.77***	108.6***	7.75**
Root	21.60***	12.49**	0.79n.s.	13.08***	57.24***	4.41*
No. of tillers per plant	46.98***	0.01n.s.	0.16n.s.	32.07***	4.91*	2.99*

The number of live tillers per plant recorded immediately after submergence did not change in any species, as expected from a relatively short stress period of submergence (Fig. 2*a–d*; no treatment or species × treatment interaction effects, Table 1). However, a differential response among species and treatments combinations for this variable was observed during recovery (significant treatment and species × treatment interaction effects, Table 1). *Bromus catharticus* showed a large decrease in the number of live tillers per plant during the period after submergence ($P < 0.001$ by Tukey's test), and previously submerged plants finished the experiment with only the main tiller alive, compared with 3.6 tillers plant^{-1} in the control (Fig. 2*b*). Such a negative impact due to tiller death was in line with the poor growth performance already noted for this species. In *S. arundinaceus*, the final number of tillers in previously submerged plants was also reduced (6.6 vs 10.6 tillers plant^{-1} in the control, Fig. 2*c*), although this grass sustained a shoot RGR similar to the control because of the high dry-mass accumulation of the remaining live tillers (Fig. 1*b, c*). In *D. glomerata* and *P. aquatica*, there were no

changes in final number of tillers per plant between treatments ($P = 0.28$). However, *P. aquatica* showed a marginal increase in this parameter during post-submergence recovery ($P = 0.065$ for control vs previously submerged plants, by Tukey's test), not observed in *D. glomerata* (cf. Fig. 2*a, d*).

Dynamics of stomatal conductance and leaf greenness after submergence

Stomatal conductance varied among species during the recovery period depending on the applied treatments (significant main and interaction effects, Table 2). *Dactylis glomerata* and *B. catharticus*, the species most sensitive to submergence in terms of recovery growth, showed large reductions in stomatal conductance soon after de-submergence ($P < 0.05$ in all cases, based on Tukey's tests), whereas *S. arundinaceus* and *P. aquatica* were able to maintain stomatal conductance at control levels ($P = 0.15$, based on Tukey's test) (Fig. 3*a–d*). The reduction in stomatal conductance ranged from 38% to 58% in *D. glomerata* (Fig. 3*a*) and from 51% to 69% in *B. catharticus* (Fig. 3*b*).

Leaf greenness (quantified in SPAD units) was reduced with time post-submergence in *D. glomerata* and *B. catharticus*, particularly in old leaves (Fig. 4*a*, *b*), indicating early leaf
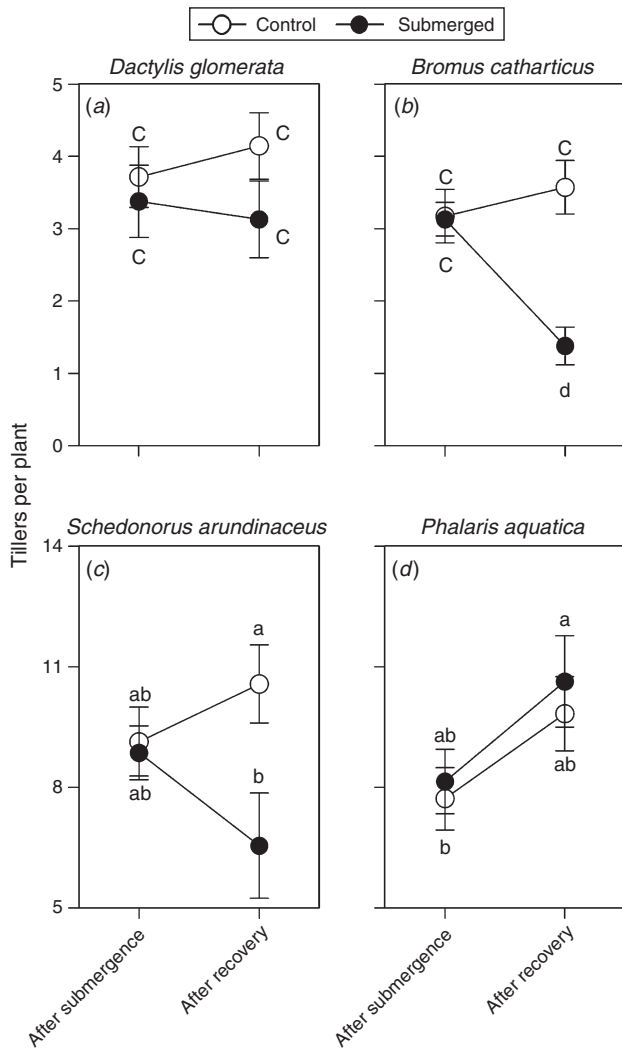
senescence in both sensitive species. The final values of old-leaf greenness of previously submerged plants in these two sensitive species were 33% to 39% lower than for old leaves from the well-drained control plants, and were similar between species (Fig. 4*a*, *b*). In young leaves, greenness was affected only in *D. glomerata*, early during the recovery period (reductions of 18–27%). However, by the end of the experiment this parameter had fully recovered in previously submerged relative to control plants (Fig. 4*a*). In *S. arundinaceus* and *P. aquatica*, there were no significant differences in young-leaf greenness among treatments (Fig. 4*c–d*), except in old leaves of *S. arundinaceus*, where slightly lower leaf greenness (23%) was observed at the end of the experiment compared with the control. The same pattern of responses with anticipated yellowing (i.e. low SPAD values) could be seen in the sensitive *D. glomerata* and *B. catharticus* if values were averaged for both leaf ages measured (see Supplementary materials fig. S1, available at the journal's website).

Discussion

This research presents evidence of the differential recovery ability from a 5-day complete submergence of four temperate grasses used in pastures worldwide. The ranking of tolerance to short-term submergence as assessed after a 15-day recovery period (see Striker 2012*b*) was as follows: *B. catharticus* and *D. glomerata* (as sensitive species) < *Schedonorus arundinaceus* (intermediate) < *Phalaris aquatica* (tolerant). Sensitivity to submergence manifested during recovery in the first two species was related to a very low RGR of shoots and roots (negative in *B. catharticus* roots, indicating loss or death of tissues) associated with reduced plant carbon fixation potential due to early leaf senescence (in old leaves) and persistent partial stomatal closure (in young leaves). The species *S. arundinaceus* exhibited an intermediate tolerance, sustaining a high shoot RGR and adjusting its leaf functionality during recovery after being negatively affected in terms of growth and tillering during submergence. The performance of *P. aquatica* was outstanding because it sustained unaffected shoot and root mass accumulation during submergence, and was able to maintain leaf functionality (greenness and stomatal conductance), tillering and shoot growth during recovery.

Fully submerged plants must deal with a stressful environment that is different from waterlogging, where shoots are (at least partially) in contact with the atmospheric air. Tolerances established for the latter situation do not apply to the same extent to submergence as imposed in this experiment. For example, cultivar IR42 of rice (a graminaceous crop)

Fig. 2. Numbers of tillers per plant of 6-week-old individuals of (*a*) *Dactylis glomerata*, (*b*) *Bromus catharticus*, (*c*) *Schedonorus arundinaceus* and (*d*) *Phalaris aquatica* at the end of a 5-day complete submergence and at the end of a 15-day recovery period. Means with the same letter are not significantly different (Tukey's tests at *P* = 0.05). Values are means ± standard errors of eight replicates. Note: parts (*a*) and (*b*) have a different *y*-axis scale from parts (*c*) and (*d*). Two-way ANOVA results are presented in Table 1.

Table 2.   *F*-values of three-way ANOVA for leaf greenness and stomatal conductance evolution of 6-week-old plants of *Dactylis glomerata*, *Bromus catharticus*, *Schedonorus arundinaceus* and *Phalaris aquatica* during the 15-day recovery period that followed a 5-day complete submergence
Factors are species (Sp), treatment (Tr) and time (T). Degrees of freedom for each source of variation: species, 3; treatment, 1; time, 4; Sp × Tr, 3; Sp × T, 12; Tr × T, 4; Sp × Tr × T, 12; error, 191. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., $P > 0.05$

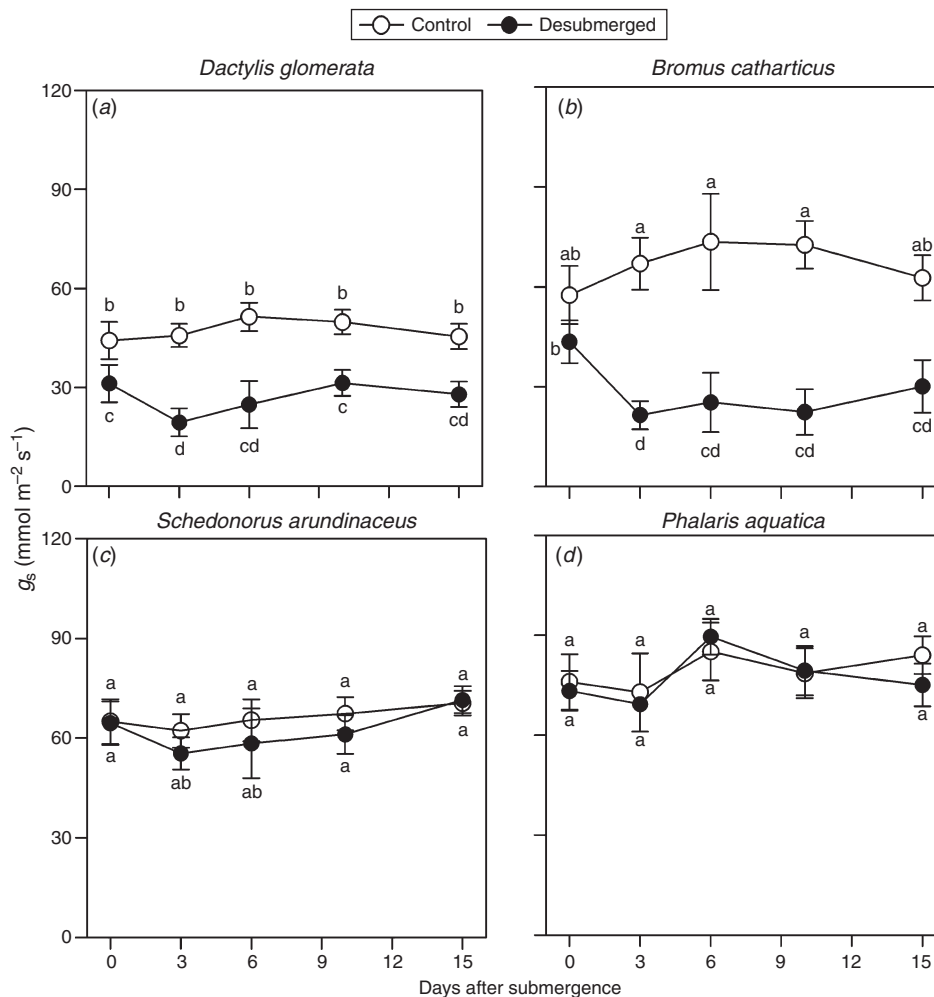| Leaf functioning after submergence	Source of variation						
	Species	Treatment	Time	Sp × Tr	Sp × T	Tr × T	Sp × Tr × T
Stomatal conductance	51.42***	29.97***	39.88***	9.8***	4.9***	2.65*	1.89*
Greenness of young leaves	24.57***	13.86***	1.54n.s.	5.40***	1.53n.s.	4.08**	0.88n.s.
Greenness of old leaves	89.39***	65.74***	6.66***	6.32***	1.87*	7.82***	1.88*

Fig. 3. Evolution of stomatal conductance (g_s) of fully expanded young leaves of 6-week-old plants of (*a*) *Dactylis glomerata*, (*b*) *Bromus catharticus*, (*c*) *Schedonorus arundinaceus* and (*d*) *Phalaris aquatica* during 15 days of growing under well-drained conditions, following a 5-day complete submergence compared with control conditions. Means with the same letter are not significantly different (Tukey's tests at *P* = 0.05). Values are means ± standard errors of eight replicates. ANOVA results are presented in Table 2.

tolerates waterlogging but it can perish if plants are completely submerged for a week (Atwell *et al*. 1982). The reduction in plant size in terms of dry mass and number of tillers exemplifies this point for the species used here when comparing the results of this experiment with those reported earlier for waterlogged (non-submerged) plants (Humphries 1962; Jung *et al*. 2009; Martina and von Ende 2013; Ploschuk *et al*. 2017). Plants of *B. catharticus* and *S. arundinaceus*, of similar age, experienced a decrease in the number of live tillers per plant after a 5-day submergence in the present study (Fig. 2*b, c*), but showed no alteration in this parameter after a 15-day recovery from a 15-day waterlogging (see Ploschuk *et al*. 2017). In *B. catharticus*, *D. glomerata* and (to a lesser extent) *S. arundinaceus*, the smaller plants induced by submergence will likely have their growth constrained in the long term, through reductions in the number of growing buds (i.e. fewer tillering sites per plant; see Matthew *et al*. 2000) and in the competitive ability of those small plants in a typically dense pasture (Lenssen *et al*. 2004; Jung *et al*. 2009). By contrast, in *P. aquatica*, plant growth and

tillering were not altered either by a 5-day complete submergence (Figs 1, 2*d*) or by a 15-day waterlogging (Humphries 1962; Ploschuk *et al*. 2017) when assessed after a 2-week recovery, similar to its tolerant close relative *P. arundinacea* (Martina and von Ende 2013). Therefore, *P. aquatica* is unlikely to be affected by short-term submergence, and so it becomes a convenient forage option for inclusion in lowlands prone to flooding with deep water-columns of short duration.

Stomatal conductance and leaf greenness (a surrogate for leaf nitrogen status; Striker *et al*. 2014) are related to leaf carbon fixation and plant growth resumption when water recedes; both are therefore expected to be positively linked to plant recovery (Luo *et al*. 2009, 2011; Striker *et al*. 2017). In the present experiment, these leaf functional variables were in line with the RGR responses observed during recovery and they allowed separation of the sensitive species *B. catharticus* and *D. glomerata* from the intermediate *S. arundinaceus* and the tolerant *P. aquatica*. Because information on stomatal behaviour after submergence in grass species is scarce, opportunities
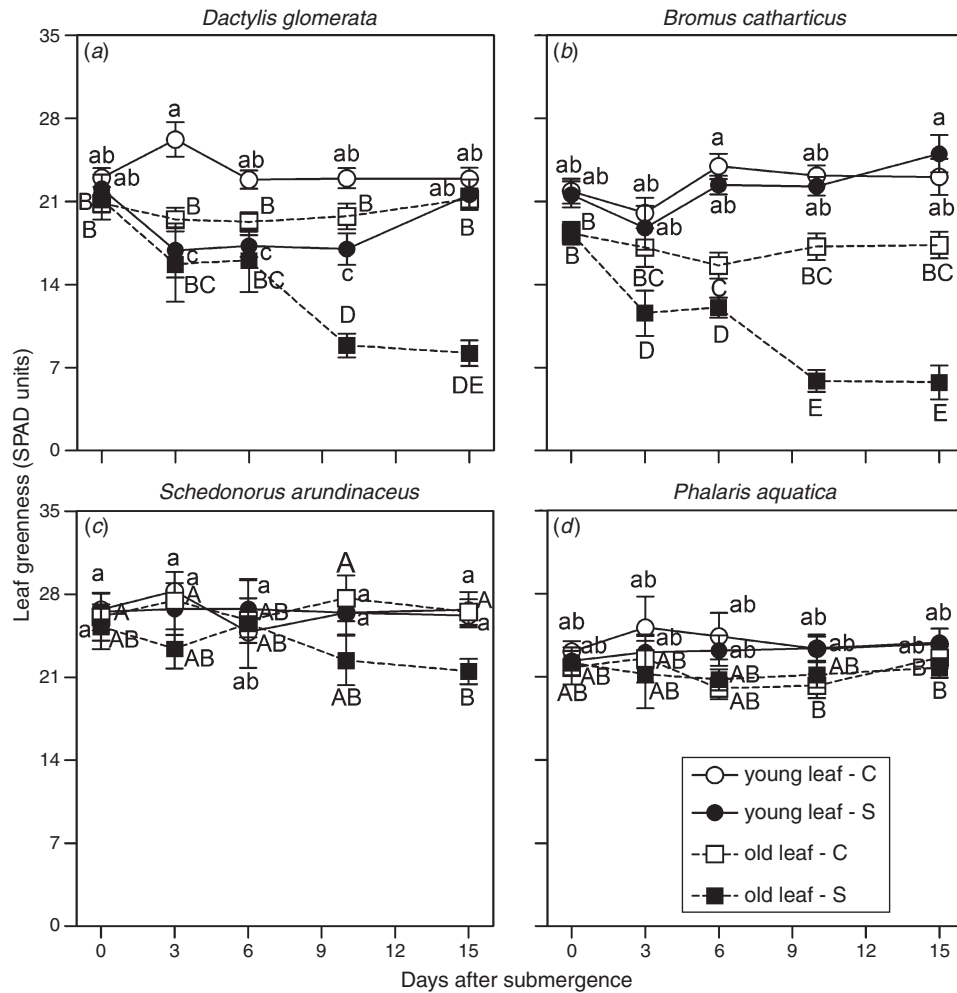
Fig. 4. Leaf greenness (SPAD units) of young apical and old basal leaves of 6-week-old plants of (*a*) *Dactylis glomerata*, (*b*) *Bromus catharticus*, (*c*) *Schedonorus arundinaceus* and (*d*) *Phalaris aquatica* during 15 days of growing under well-drained conditions, following a 5-day complete submergence (S) compared with control conditions (C). Means with the same letter are not significantly different (Tukey's tests at $P = 0.05$); lowercase letters compare young leaves, uppercase letters compare adult leaves. Values are means ± standard errors of eight replicates. ANOVA results are presented in Table 2.

for comparison are minimal (a single study in rice cv. IR42; Setter *et al*. 2010). Nevertheless, a low stomatal conductance as measured in both sensitive species is expected to be associated with higher resistance to CO_2 diffusion into leaves, partially explaining the limited shoot recovery after submergence. Also, *B. catharticus* and *D. glomerata* showed a rapid and progressive reduction in greenness of basal leaves with a likely low potential for carbon fixation, also contributing to the poor recovery in these species when scaling these leaf responses to plant level (for recovery from submergence–greenness relation in the grass *Chloris gayana*, see Striker *et al*. 2017). There are at least two potential, non-exclusive causes for the observed accelerated leaf yellowing. The first is a sudden accumulation of ROS upon re-aeration (Steffens *et al*. 2013). The second is a re-translocation of nitrogen to younger shoot tissues to cope with deficient nutrient uptake from small-sized root systems (Nakamura and Nakamura 2016; and see the low recovery of root RGR, negative in *B. catharticus* indicating tissue dead,

in Fig. 1*c*). These impaired leaf responses were not seen in *S. arundinaceus* and *P. aquatica*, in line with their less affected plant performance (RGR) during recovery. In *S. arundinaceus* (showing intermediate tolerance), the maintenance of leaf physiological functioning as in the control was likely at the expense of reducing the number of live tillers per plant. By contrast, *P. aquatica* showed no differences between control and submerged plants for either stomatal conductance or leaf greenness, as seen for leaf physiological parameters (greenness and chlorophyll fluorescence) in the wetland-invasive *Phragmites australis* when exposed to periods of 2–9 days underwater (Mauchamp and Méthy 2004). Therefore, *P. aquatica* also proved truly tolerant to short-term submergence in terms of its leaf physiological behaviour after the stress.

It is interesting that plant survival was not affected in any species, in agreement with Beard and Martin (1970) for the turf grasses *Agrostis palustris*, *Poa pratensis*, *P. annua* and *Festuca*

rubra after a 5-day submergence at 20°C water temperature (similar to this experiment). Longer submergence periods for the temperate grasses tested here are unlikely to occur during typical flooding in the fields where they are sown (Di Bella *et al.* 2016); hence, it is important to focus on their ability to recover from the stress. In this regard, the poor RGR of shoots and roots exhibited by individual plants of *B. catharticus* and *D. glomerata* during recovery could threaten their persistence. They might be outcompeted in pasture by more tolerant species (see e.g. Lenssen *et al.* 2004; Striker *et al.* 2011), which either have quicker recovery or are not severely affected by short-term submergence, such as *S. arundinaceus* and *P. aquatica*, respectively. Future works should focus on scaling up to long-term mesocosm experiments using a mixture of grasses including species of differential tolerance to submergence, to test the importance of competitive interactions after the stress in defining the recovery ability in the presence of natural neighbours in pasture canopies.

Conclusion

The results highlight that after considering a recovery period (*i*) *D. glomerata* and *B. catharticus* can be classified as sensitive to submergence owing to their diminished RGR, either of shoots or roots, whereas *S. arundinaceus* and *P. aquatica* can be regarded as intermediate and tolerant to submergence, respectively, owing to their maintained shoot RGR; and (*ii*) in the sensitive *D. glomerata* and *B. catharticus*, stomata remained partially closed even up to 15 days after recovery from submergence and basal (old) leaves rapidly started senescing, whereas in *S. arundinaceus* and *P. aquatica*, stomatal conductance and leaf greenness remained unaffected. Therefore, recovery from short-term submergence in these grasses appears to be related to leaf greenness and stomatal conductance maintenance, which might be critical to resume a rapid carbon fixation after the water subsides.

Conflicts of interest

The authors declare no conflicts of interest.

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