Growth during recovery evidences the waterlogging tolerance of forage grasses

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Abstract. Waterlogging is a stress of increasing importance for pastures as a consequence of global climate change. We evaluated the impact of waterlogging on four forage grasses with alleged differential tolerance, emphasising not only responses during the stress but also their reported ability to recover from it. To do this, 42-day plants of *Dactylis glomerata*, *Bromus catharticus*, *Festuca arundinacea* and *Phalaris aquatica* were subjected to 15-day waterlogging, followed by a subsequent 15-day recovery period. Shoot and root growth (i.e. RGR) during both periods, in addition to net photosynthesis and stomatal conductance rates during waterlogging were assessed. Sensitivity exhibited by *D. glomerata* and *B. catharticus* during waterlogging was related to growth arrest of roots – but not of shoots – along with a progressive fall in stomatal conductance and net photosynthesis. The injury during waterlogging preceded a negligible growth of shoots and roots, only evident during recovery in both species. By contrast, *P. aquatica* exhibited unaltered root RGR and promoted shoot RGR with no impact on leaf gas exchange during waterlogging; whereas *F. arundinacea* showed intermediate tolerance as root RGR was reduced during waterlogging, with stomatal conductance, net photosynthesis and shoot RGR remaining unaffected. These latter two species fully regained shoot and root RGR during recovery. So, *P. aquatica* and *F. arundinacea* seem more suitable for prone-to-flood lowlands, whereas to be conclusive about waterlogging tolerance, it is necessary to examine plant recovery as shown in *D. glomerata* and *B. catharticus*.

Additional keywords: flooding, hypoxia, pasture grasses, tillering.

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Introduction

Grasses are important components of pastures in temperate environments (Briske 1996). Waterlogging is a common stress for plants in pastures, as its occurrence is expected to increase in the next years as a result of global climatic change (Rosenzweig *et al.* 2002). The tolerance of grasses to hypoxia associated with waterlogging differs among species (Boschma *et al.* 2008). Despite the recognition of existing tolerance variability among grasses, most studies have focussed their attention on the immediate effects during waterlogging, whereas only a few works have addressed the waterlogging recovery ability of species with different reputation for dealing with this stress (Malik *et al.* 2002; Striker 2012). So, in this study we analysed plant growth (shoot and root separately) of four grasses widely used in temperate pastures with alleged differential waterlogging tolerance – *Dactylis glomerata* and *Bromus catharticus* as

sensitive species, and *Festuca arundinacea* and *Phalaris aquatica* as tolerant ones – during and after the stress.

Soon after soil is waterlogged, roots and microorganism respiration lead to oxygen depletion determining hypoxic stress for the roots (Colmer and Voesenek 2009; Sasidharan *et al.* 2017). The lack of soil oxygen induces a sequence of changes in plants that could include alterations of physiological processes along with morphological adjustments related to their survival under anaerobic conditions (Striker *et al.* 2005; Colmer and Voesenek 2009). At physiological level, waterlogging modifies water relations and carbon fixation of plants. Stomata closure, reduction of transpiration and a fall in net photosynthetic rate are common responses occurring in time and magnitude depending on the waterlogging tolerance of each species. Waterlogging-sensitive species can drastically reduce their physiological activity and die in a short time, whereas in

waterlogging-tolerant species, the same leaf variables, stomatal conductance, leaf transpiration and photosynthesis, can be maintained unaltered for several days (McFarlane *et al.* 2003; Striker *et al.* 2005). Therefore, in this experiment we monitored the changes in stomatal conductance, leaf transpiration and net photosynthesis in the above mentioned grass species with assumed differential waterlogging-induced hypoxia tolerance.

Plant morphology in grasses can be modified as a result of waterlogging stress (Mollard *et al.* 2010; Striker *et al.* 2011). Reductions in plant size due to changes in the number of tillers per plant and in the number of green (photosynthetic) leaves per tiller can occur, among other responses (e.g. length of leaf blades and sheaths; Striker *et al.* 2008), depending on the species tolerance and waterlogging intensity and duration (see Cardoso *et al.* 2013; for a comparison among *Brachiaria* species). Accelerated leaf senescence under waterlogging – a response associated with ethylene accumulation in tissues, nutrient deficiencies and/or toxicities (Sasidharan *et al.* 2017) – is often observed in sensitive grasses, like *Brachiaria brizantha* and *B. ruziziensis*, which leads to a reduction in the number of functional green leaves per tiller (Cardoso *et al.* 2013). This reduction in functional leaf area mainly represents a constraint for current carbon assimilation. In addition, if waterlogging persists in time, the number of tillers per plant can also be reduced, both by restriction in new tillers appearance or by death of previously formed tillers (Malik *et al.* 2002; Mollard *et al.* 2010). Hence, we assessed the effects on tiller number and green leaves per tiller as a consequence of 15-day waterlogging and how these morphological variables adjusted in species with differential tolerance during a subsequent recovery phase.

In this study, we aimed to provide a better understanding of forage grasses response to waterlogging by comparing four widely used species with differential tolerance. To do so, morphological variables and physiological responses, along with dry mass accumulation were monitored during 15-day waterlogging. Shoot and root growth were analysed not only during waterlogging, but also during a recovery period under well drained conditions. Our results highlight that (*i*) the physiological performance of sensitive grasses, *D. glomerata* and *B. catharticus*, was rapidly affected during waterlogging, whereas it remained unaffected in the tolerant *Festuca arundinacea* and *Phalaris aquatica*, (*ii*) growth arrest during waterlogging was evident only for roots in the sensitive species, but the reduction in shoot growth was only evident during the recovery period. So, plant growth assessment during a recovery period is recommended to define the grass species tolerance in order to cover potentially delayed effects caused by previous waterlogging.

Materials and methods

Species description

In this experiment four temperate forage grass species used worldwide with differential tolerance to waterlogging were selected to (*i*) analyse plant morpho-physiological variables affected by waterlogging and to (*ii*) assess plant growth (shoot and root) during and after the imposed stress. *Dactylis glomerata* L. (cocksfoot) is a strongly tufted, deep-rooted, long-lived cool-season perennial grass reaching a height of 60–150 cm with erect

and glabrous culms. It produces a continuous growth of young leaves and it is regarded as sensitive to waterlogging (Lolicato and Rumball 1994). *Bromus catharticus* Vahl. (prairie grass) is a tufted biennial (or shortly perennial) grass with erect culms, up to 55–84 cm tall, unbranched, with 2–6 nodes, widely distributed in temperate regions of the world (Puecher *et al.* 2001), also considered sensitive to soil water excess (Trebino *et al.* 1996). *Festuca arundinacea* Schreb. (tall fescue) is a perennial, cool-season bunchgrass up to 2 m tall, with culms which are usually erect, stout and smooth. It produces a large number of coarse, tough roots, generally presenting short rhizomes and it is reputed to be tolerant to waterlogging (Gibson and Newman 2001). *Phalaris aquatica* L. (bulbous canary-grass) is a tufted, deep-rooted, and rhizomatous perennial grass that can reach 1–2 m in height with erect and hollow stems, also presumed to be tolerant to waterlogging (Boschma *et al.* 2008).

Experimental design

Seeds from *D. glomerata* cv. Omea, *B. catharticus* cv. Jerónimo, *F. arundinacea* cv. Malma and *Phalaris aquatica* cv. Mate were germinated in an incubator at 25°C in Petri dishes covered by filter paper moistened with distilled water. Germinated seeds were transplanted to 1-L pots filled with sand and topsoil (3% organic carbon; 1 : 1 v/v; see further soil characteristics in Di Bella *et al.* 2016*a*) placed in a glasshouse under natural light conditions (September–October in Buenos Aires, Argentina). During the first two weeks, seedlings were thinned to one per pot.

After 6 weeks' growth, two treatments were imposed following a completely randomised design with 10 replicates on 40 similar-sized 42-day-old plants per species during 15 days: control and waterlogging. Control plants were daily watered to field capacity and allowed to drain freely. Waterlogged plants were subjected to a condition of flooded soil with tap water up to 1 cm above the soil surface for 15 days, emulating a possible scenario under natural conditions (Striker *et al.* 2011; Di Bella *et al.* 2016*a*). In order to impose waterlogging, pots were randomly placed into plastic containers (0.7 m × 0.5 m × 0.2 m depth), displaying a total of eight containers with 20 plants in each one; four containers were filled with water to reach the abovementioned level. The water in the different containers was periodically checked in order to verify that it was at similar temperature in all of them ($P > 0.36$ in all cases). After the waterlogging period, flood water was drained from the corresponding containers, initiating another 15-day period of growth under well drained conditions in order to assess the recovery ability of each one of the species (Striker 2012; Sasidharan *et al.* 2017).

Physiological measurements

Net photosynthesis (A), stomatal conductance (g_s) and transpiration (E) rates were measured in fully expanded leaf blades using a portable infrared gas analyser system model Li-Cor 6400 (Li-Cor Inc., Lincoln, NE, USA) under saturating light (1500 μmol m^{-2} s^{-1} PPFD) provided by the 6400-40 leaf chamber fluorometer, using a mix of 90% red and 10% blue light. Air flow, CO_2 concentration in the reference chamber and block temperature were automatically controlled by the equipment at 300 μmol s^{-1}, 400 μmol mol^{-1} (ppm) and 24°C,

respectively. Measurements were made at the beginning of the treatments (Day 0) and 2, 7 and 15 days after. At the same time, air temperature and relative humidity of the glasshouse were monitored (Temperature and RH Probe HUMICAP H, Vaisala, Finland). Both variables were used to calculate air vapour pressure deficit (VPD_{air}) as the difference between the saturated vapour pressure (i.e. Clausius–Clapeyron Equation) and the actual air vapour pressure. VPD_{air} was used as indicative of the atmospheric evaporative demand.

Morphological measurements and dry mass responses

The number of green leaves per tiller and the number of tillers per plant were periodically measured in seven plants of each species and treatment, during both the waterlogging and the recovery periods. The number of green leaves per tiller was calculated as the average of the plant mature tillers. Measurements were made at the beginning of the treatments (Day 0) and 5, 12, 19 and 26 days after. The first variable allows inferring current plant assimilation related to the number of functional green leaves (see also physiological measurements above), whereas the second variable allows projecting the potential plant growth in the long term.

Plants were harvested at the beginning of the treatments (Day 0), at the end of waterlogging (Day 15) and at the end of the recovery period (Day 30). Shoots were carefully separated from the roots and their dry masses were obtained after oven drying the tissues at 80°C for 72 h. The relative growth rate (RGR) for each combination of species and treatment was calculated for the entire plant, shoot and root, respectively. Given that dry mass harvests are destructive measures, initial values were not paired with final values for each period (waterlogging and recovery), so RGR values (of entire plants, shoot and root) were calculated as the average of all possible combinations of values between initial and final harvests, among plants of each species × treatment combination for each period (as in Di Bella *et al.* 2016*b*).

Statistical analyses

Dry mass responses were analysed by two-way ANOVA with 'species' and 'treatment' as main factors. Calculated RGR values (see above) were compared between treatments for each species by means of Student's *t*-test (degrees of freedom = 9). Morphological and physiological responses were analysed by three-way ANOVA with 'species', 'treatment' and 'time' as main factors. Additional contrasts were performed to detect differences between treatments within species. Assumptions regarding normality and homogeneity of variances of the dataset were previously checked before performing ANOVA. Statistical analyses were performed using InfoStat 2010 package for Windows (University of Córdoba, Córdoba, Argentina).

Results

Waterlogging impact on growth in four grass species

Dry mass responses to waterlogging differed according to each species (Table 1, see 'species' × 'treatment' interactions in Table 2). Shoot dry mass was not reduced in any species whereas waterlogging did affect root dry mass accumulation in three out of the four species examined (Table 1, Fig. 1). It is noteworthy that, shoot dry mass was promoted in *P. aquatica* as waterlogged plants reached 45% higher values than their controls for this variable (Table 1). Accordingly, there were no evident effects of waterlogging on shoot RGR during waterlogging in any species except for *P. aquatica* in which waterlogged plants showed nearly 46% higher values compared with the controls for this variable (Fig. 1*e*–*h*). Regarding roots, there were 29% and 50% reductions in RGR for *D. glomerata*, and *F. arundinacea* compared with their controls (Fig. 1*i, k*), which implied 35% and 47% lower root dry masses, respectively (Table 1), at the end of the waterlogging period. In *B. catharticus*, root RGR of waterlogged plants was negative indicating death of root tissues (Fig. 1*j*), which consequently derived in 27% lower dry mass than plants under control conditions (Table 1). Waterlogging did not affect root RGR of *P. aquatica* (Fig. 1*l*),

Table 1.   Dry mass (g) of *Dactilys glomerata*, *Bromus catharticus*, *Festuca arundinacea* and *Phalaris aquatica* at the beginning of the treatments (Day 0), after 15 days of waterlogging (1–2 cm water above soil; Day 15), and after a recovery period of 15 days at well drained conditions (Day 30)

C: control; WL: waterlogging. Values are means ± standard errors of 10 replicates. See statistical analyses in Table 2

Dry mass	*Dactilys glomerata*		*Bromus catharticus*		*Festuca arundinacea*		*Phalaris aquatica*	
	1. Initial (Day 0)						
Total	0.22 ± 0.03		2.16 ± 0.08		1.00 ± 0.10		1.42 ± 0.23	
Shoot	0.10 ± 0.02		0.82 ± 0.05		0.53 ± 0.06		0.64 ± 0.10	
Root	0.13 ± 0.02		1.33 ± 0.06		0.47 ± 0.07		0.78 ± 0.13	
	2. After waterlogging (Day 15)						
	C	WL	C	WL	C	WL	C	WL
Total	1.19 ± 0.16	0.93 ± 0.13	2.88 ± 0.18	2.51 ± 0.20	3.24 ± 0.33	2.52 ± 0.25	2.79 ± 0.34	3.76 ± 0.34
Shoot	0.54 ± 0.04	0.51 ± 0.06	1.47 ± 0.10	1.48 ± 0.13	1.56 ± 0.14	1.63 ± 0.15	1.42 ± 0.16	2.06 ± 0.09
Root	0.65 ± 0.12	0.42 ± 0.07	1.41 ± 0.09	1.03 ± 0.08	1.68 ± 0.19	0.89 ± 0.12	1.37 ± 0.19	1.70 ± 0.16
	3. After recovery (Day 30)						
	C	WL	C	WL	C	WL	C	WL
Total	1.44 ± 0.21	0.81 ± 0.06	3.49 ± 0.30	2.37 ± 0.20	5.82 ± 0.56	4.34 ± 0.26	4.59 ± 0.50	5.24 ± 0.29
Shoot	0.69 ± 0.07	0.53 ± 0.05	1.66 ± 0.14	1.42 ± 0.08	2.09 ± 0.09	2.35 ± 0.11	2.28 ± 0.15	2.86 ± 0.09
Root	0.75 ± 0.14	0.28 ± 0.02	1.83 ± 0.17	0.95 ± 0.12	3.73 ± 0.56	1.99 ± 0.21	2.31 ± 0.39	2.38 ± 0.21

Table 2. *F*-values of two-way ANOVA (factors: 'species' and 'treatment') for dry mass responses of 42-day-old plants of *Dactilys glomerata*, *Bromus catharticus*, *Festuca arundinacea* and *Phalaris aquatica* subjected to 15 days of waterlogging followed by a subsequent recovery of 15 days at well drained conditions[A]

Analyses were performed separately for the waterlogging and the recovery periods. n.s., $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Dry mass	Waterlogging period			Recovery period		
	Species (Sp)	Treatment (T)	Sp × T	Species (Sp)	Treatment (T)	Sp × T
Total	32.55***	0.08 n.s.	4.86**	60.64***	7.92**	2.86*
Shoot	32.22***	1.25 n.s.	2.77*	74.16***	0.22 n.s.	1.80 n.s.
Root	19.99***	6.64**	5.32**	29.97***	14.63***	3.46*

[A]Degrees of freedom for each source of variation were: 3 ('species'), 1 ('treatment'), 3 (Sp × T) and 66 ('error').
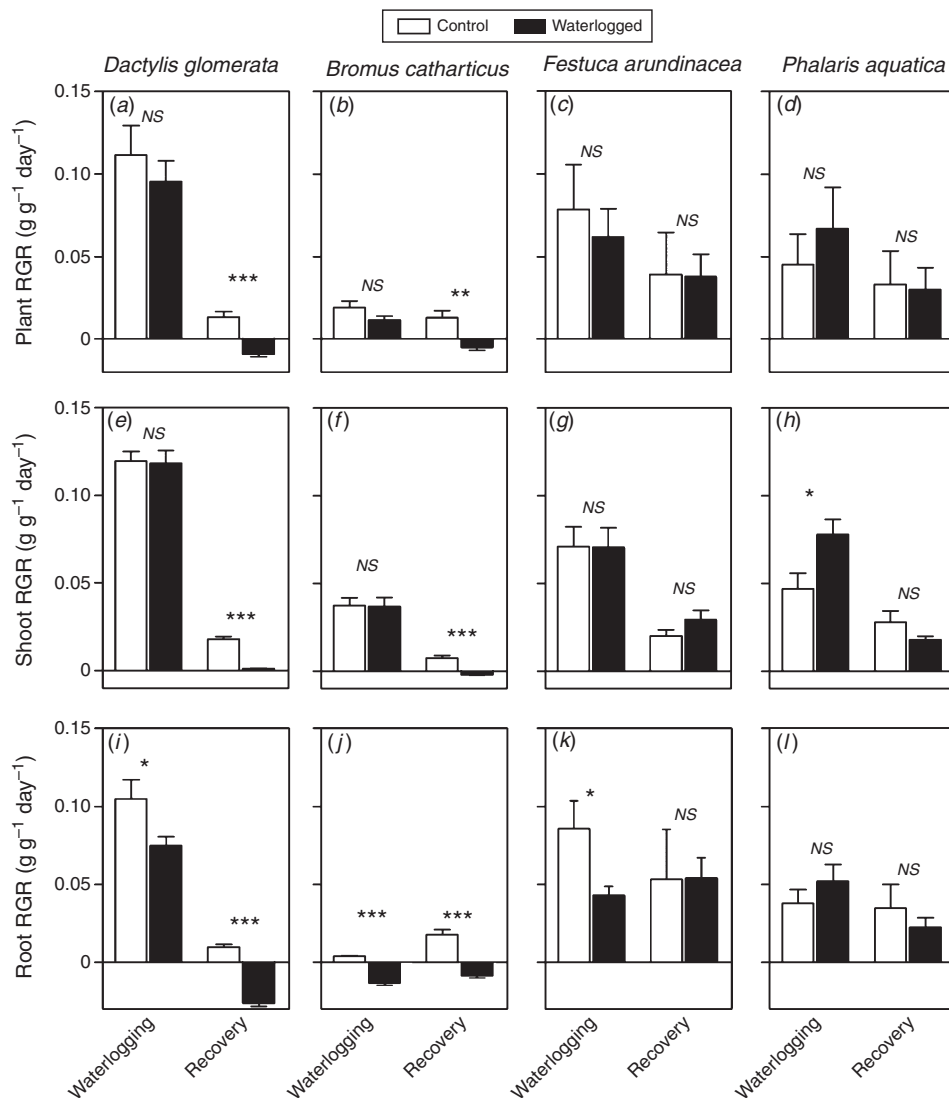
Fig. 1. Relative growth rate (RGR, g g^{-1} day^{-1}) of the entire plant (*a–d*), shoot (*e–h*) and root (*i–l*) of 42-day-old individuals of *Dactilys glomerata* (*a, e, i*), *Bromus catharticus* (*b, f, j*), *Festuca arundinacea* (*c, g, k*) and *Phalaris aquatica* (*d, h, l*) subjected to 15 days waterlogging (1–2 cm water above soil; i.e. '*waterlogging period*'), followed by a subsequent recovery of 15 days under well drained conditions (i.e. '*recovery period*'). Values are means ± standard errors of 10 replicates. Asterisks indicate significant differences between treatments for each species and period based on Student's *t*-tests (n.s., $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). See Table 1 for values of dry mass.

so no differences were found in root dry mass between treatments (Table 1).

Growth during recovery evidences species tolerance to waterlogging

Dry mass responses during recovery (i.e. after waterlogging) showed notorious differences among species, in contrast with the slight divergence registered during the waterlogging period (Table 1, see 'species' × 'treatment' interactions in Table 2). The fact that previously waterlogged *D. glomerata* and *B. catharticus* plants showed 44% and 32% lower final dry mass than their controls (Table 1) was the outcome of an almost negligible shoot RGR (i.e. 'zero' growth; Fig. 1*e, f*) and negative root RGR observed in both species during the recovery period (i.e. death of root tissues; Fig. 1*i, j*). Previously waterlogged plants of *F. arundinacea* registered 25% lower plant dry mass at the end of the experiment, not because of differences found in final shoot dry mass, but as a result of 47% lower final root dry mass compared with their controls (Tables 1 and 2). Interestingly, this species was able to sustain similar RGR for shoot and root regardless of the previous growing conditions of the plants (Fig. 1*c, g, k*), which suggests high ability to recover from 2 weeks of waterlogging. However, previously waterlogged plants of *P. aquatica* showed a slightly higher final shoot dry mass (*P* = 0.005) but similar final root dry mass compared with controls (Table 1). Higher shoot dry mass values were the result of differences achieved during waterlogging as both, shoot and root RGR, were the same between treatments during plant recovery (Fig. 1*d, h, l*).

Morphological and physiological responses

The number of tillers per plant varied along the experiment depending on the species (Fig. 2*a–d*; Table 3). Waterlogging did not substantially affect this attribute in any of the species, meaning that no tiller death was registered as a consequence of stress (Fig. 2*a–d*). In *D. glomerata* and *B. catharticus*, the number of tillers per plant remained unaltered along the whole experiment (Fig. 2*a, b*). In *F. arundinacea* and *P. aquatica* this variable increased at the same rate for both treatments despite the fact that, in both former species, a trend towards a higher number of tillers per plant was observed in waterlogged plants (Fig. 2*c, d*).

The number of green leaves per tiller along the experiment responded differently to treatments among species (Fig. 2*e–h*; see 'species × treatment' interaction in Table 3). *D. glomerata* showed a lower number of green leaves per tiller in waterlogged plants at the end of waterlogging and at the beginning of the recovery period as clear symptoms of stress (Fig. 2*e*). *B. catharticus*, *F. arundinacea* and *P. aquatica* did not present differences between treatments for this variable; the number of green leaves per tiller remained stable along the experiment (Fig. 2*f–h*).
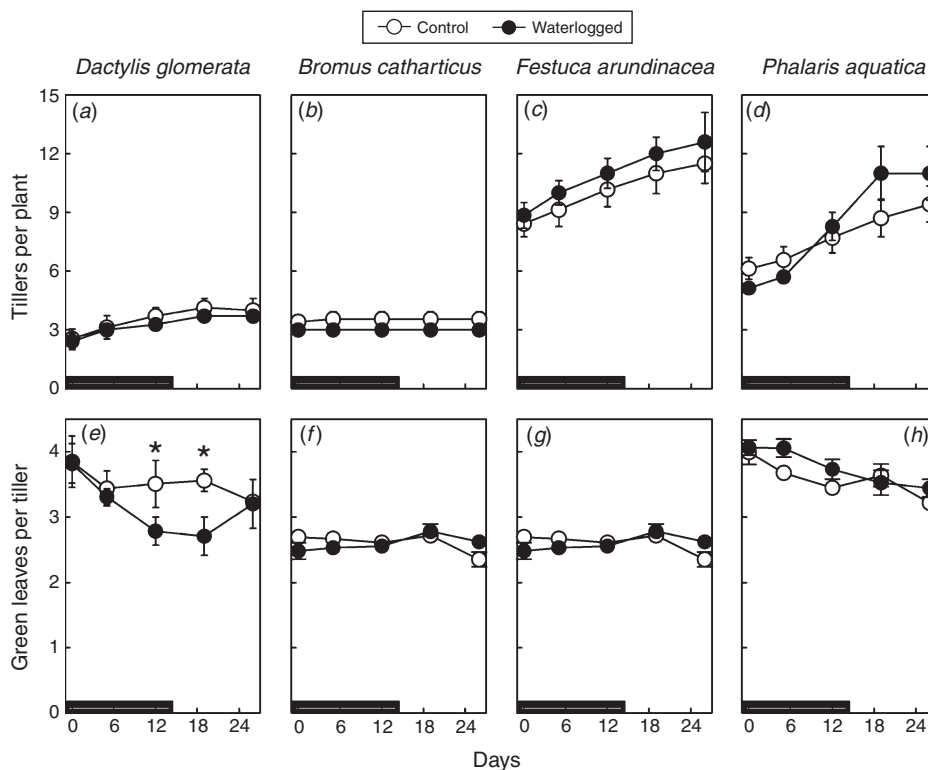
Fig. 2. Tillers per plant (*a–d*) and green leaves per tiller (*e–h*) of 42-day-old plants of *Dactilys glomerata* (*a, e*), *Bromus catharticus* (*b, f*), *Festuca arundinacea* (*c, g*) and *Phalaris aquatica* (*d, h*). Measurements were made at the beginning of the treatments (Day 0), and 5, 12, 19 and 26 days after. Black bars on the *x*-axis represent the 15-day waterlogging period. Values are means ± standard errors of 7 replicates. Asterisks denote significant differences (*P* < 0.05) between treatments for each species and measurement date.

Table 3. *F*-values of three-way ANOVA (factors 'species', 'treatment' and 'time') for tillers per plant and green leaves per tiller (morphological variables), and net photosynthesis, stomatal conductance and transpiration rates (physiological variables) of 42-day-old plants of *Dactylis glomerata*, *Bromus catharticus*, *Festuca arundinacea* and *Phalaris aquatica* subjected to 15 days of waterlogging followed by a subsequent recovery of 15 days at well drained conditions[A]

Analyses were performed separately for the waterlogging and the recovery periods when available data. n.s., $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Variables	Species (Sp)	Treatment (T)	time	Sp × T	Sp × time	T × time	Sp × T × time
1. *Morphological*			*Waterlogging period*				
Tillers per plant	193.55***	0.40 n.s.	12.46***	1.55 n.s.	2.11 n.s.	0.28 n.s.	0.40 n.s.
Green leaves per tiller	29.55***	4.69*	17.15***	0.58 n.s.	3.23**	0.65 n.s.	0.43 n.s.
			Recovery period				
Tillers per plant	111.73***	1.52 n.s.	0.33 n.s.	2.52 n.s.	0.17 n.s.	0.002 n.s.	0.02 n.s.
Green leaves per tiller	8.74***	4.67*	2.94 n.s.	3.62*	0.81 n.s.	17.44***	2.27 n.s.
2. *Physiological*			*Waterlogging period*				
Net photosynthesis	6.27***	18.69***	6.28**	2.04*	1.39 n.s.	4.07*	0.43 n.s.
Stomatal conductance	4.75**	11.30**	33.6***	2.75*	3.66**	5.75**	0.17 n.s.
Transpiration	4.74**	45.82***	9.26***	4.44**	2.35*	15.09***	0.37 n.s.

[A]Degrees of freedom for each source of variation were: 3 ('species'), 1 ('treatment'), 2–3 ('time' for morphological and physiological variables), 3 ('species × treatment'), 6 ('species × time' for morphological and physiological variables), 2 ('treatment' × 'time' for morphological and physiological variables), 6 ('species × treatment × time' for morphological and physiological variables) and 96–144 ('error' for morphological and physiological variables).

Leaf physiological variables were more sensitive to waterlogging than the morphological attributes mentioned above. The A, g_s and E rates varied along time between treatments depending on the species (see significant interactions for 'species × treatment', 'treatment × time' and 'species × time' in Table 3). In *D. glomerata* and *B. catharticus* waterlogging provoked progressive reductions in A, g_s and E reaching 64–80% (Fig. 3*a, b*), 62–71% (Fig. 3*e, f*) and 68–71% (Fig. 3*i, j*) lower values compared with controls on Day 15, respectively. In contrast, *F. arundinacea* and *P. aquatica* did not register any reduction in A (Fig. 3*c, d*) and g_s (Fig. 3*g, h*) due to waterlogging on any measurement date. In these species, 35–48% reduction in E due to waterlogging was observed at measurement on Day 7 in coincidence with the higher VPD_{air} date (2.4 kPa vs 1.7–1.9 kPa in the rest of measurement dates; see values in caption for Fig. 3). Both species were able to recover E values, same as controls by the end of waterlogging (Fig. 3*k, l*).

Discussion

This research presents four findings (*i*) the waterlogging sensitivity exhibited by *Dactylis glomerata* and *Bromus catharticus* was mainly related to root growth arrest (root RGR) during waterlogging, along with early leaf senescence (in *D. glomerata*) and progressive stomatal closure, in coincidence with a fall in net photosynthesis (both species); (*ii*) waterlogging tolerance of *Phalaris aquatica* was reflected in an unaltered root growth and promoted shoot growth with no impact on leaf physiological variables during the stress period; *Festuca arundinacea* showed an intermediate tolerance as root growth was reduced during waterlogging although shoot physiology and growth were not; (*iii*) in all cases the genuine tolerance of the species was established after assessing shoot and root growth during a recovery period after waterlogging (see also Malik *et al.* 2002; Striker 2012), where *D. glomerata* and *B. catharticus* showed negligible or negative (tissue death) growth values for both compartments (so they did not recover from the stress), and *F. arundinacea* and *P. aquatica* fully regained RGR for both,

shoots and roots, compared with their respective unstressed controls; and (*iv*) none of the effects induced on growth after 15 days' waterlogging were related to substantial changes in morphological traits, considering that there were no differences found in number of tillers per plant between treatments in any of the species analysed. In the following paragraphs, we integrate the discussion of these results highlighting the consequences for the forage use of these species in the context of future scenarios where soil waterlogging is becoming a frequent event.

Roots are the first organs to be affected by soil hypoxia as they are fully immersed in flood water (Colmer and Voesenek 2009). In this experiment, waterlogged plants of *B. catharticus* showed a negative root RGR indicating death of root tissues as seen in the waterlogging sensitive grass *Ehrharta calycina*, where 90% lower root dry mass (with respect to controls) was registered after 34 days of flooding (e.g. watertable at the soil surface; Humphries 1962). *D. glomerata*, a species rarely located on flooded soils (Winkel *et al.* 2016), and *F. arundinacea* (regarded as relatively tolerant; Blom *et al.* 1994) presented a restraint on root growth when waterlogged, similarly to what was found in *Cynodon dactylon*, which showed 22% lower root dry mass when subjected to 42 days of soil water excess (Ashraf and Yasmin 1991). Contrastingly, *P. aquatica* did not show any evidence of injury to roots as affected by waterlogging in the growth analysis made at the end of the stress period in agreement with facts reported for the waterlogging-tolerant *Aeluropus lagopoides* (no differences in this attribute between waterlogged and controls; Ashraf and Yasmin 1991). The high root porosity of *P. aquatica* (constitutive: 18.2%; waterlogging-induced: 33.6% see McDonald *et al.* 2002) allows expecting a facilitated internal tissue aeration through aerenchyma (Colmer and Voesenek 2009), which might explain unaffected root growth during waterlogging (Fig. 1*l*). In the case of *F. arundinacea* root porosity can reach 19–20% (Visser and Bögemann 2003), whereas in both sensitive species there are no values available for this important trait associated to tissue aeration.
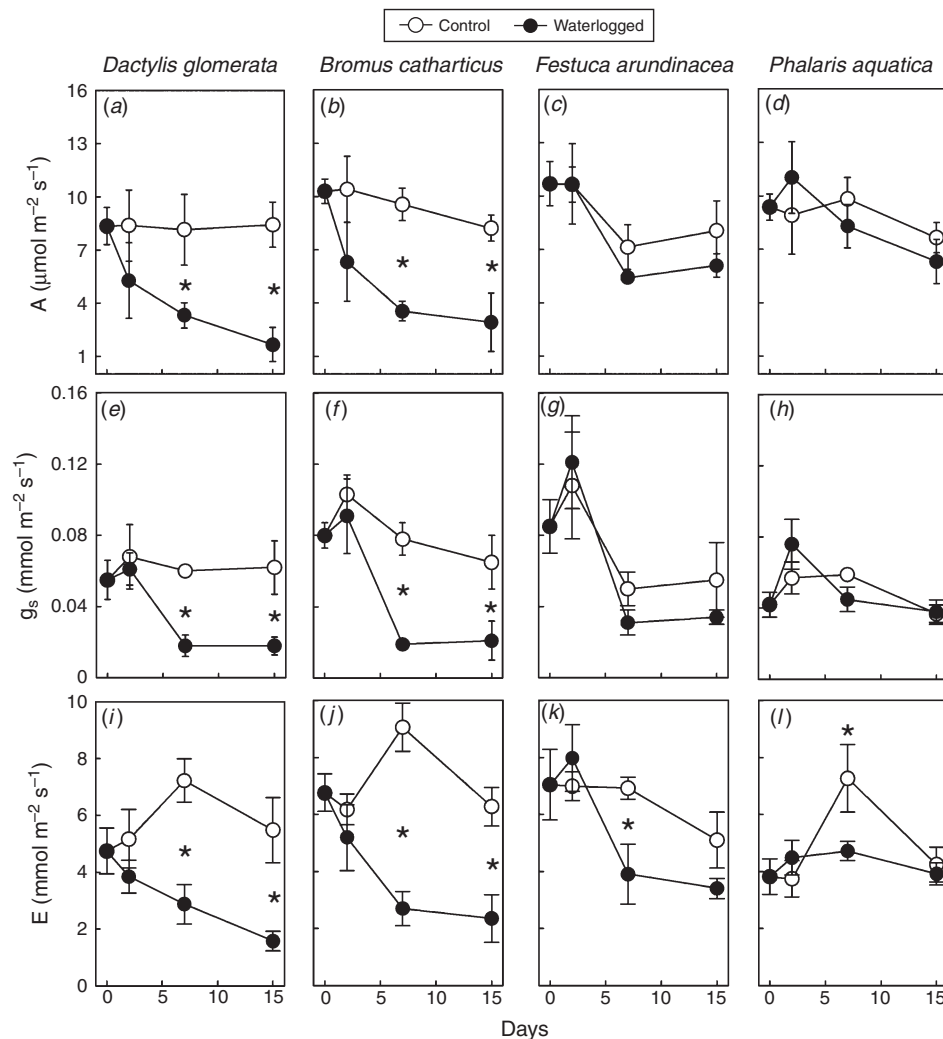
Fig. 3.   Net photosynthesis (A: *a–d*), stomatal conductance (g$_s$: *e–h*) and transpiration (E: *i–l*) rates of 42-day-old plants of *Dactilys glomerata* (*a, e, i*), *Bromus catharticus* (*b, f, j*), *Festuca arundinacea* (*c, g, k*) and *Phalaris aquatica* (*d, h, l*). VPD$_{air}$ was 1.7, 1.9, 2.4 and 1.8 kPa at Days 0, 2, 7 and 15 of treatment. Asterisks denote significant differences ($P < 0.05$) between treatments for each species and measurement date.

During waterlogging, stomatal conductance of the sensitive *D. glomerata* and *B. catharticus* was reduced along time, the same as net photosynthesis (Fig. 3) in a typical close relation between both variables in C$_3$ species, as seen in *Lolium perenne* subjected to 28 days of soil flooding (McFarlane *et al.* 2003). Stomatal closure could be related to an unbalance in water status due to a reduced capability for water uptake of injured (presumably hypoxic) roots combined with no changes in shoot transpiring mass (Table 1). Despite proposing stomatal closure as a response directed to prevent further loss of water by transpiration (Lange *et al.* 1971), it applies to species with capability to regulate their physiological behaviour (see *Lotus* spp. in Striker *et al.* 2005) whereas in our two sensitive grasses, it appears more likely to be a consequence (not a cause) of the applied stress (i.e. reductions in g$_s$, and thereby in A were progressive with no recovery on any date). Interestingly, in stressed plants of *D. glomerata*, the number of green leaves

per tiller was affected in waterlogged plants at the end of waterlogging (extended to the beginning of the recovery period), which is associated to their poor growth performance. The same kind of responses, in which this attribute is compromised when a stress is applied, has been registered in this same species when exposed to drought stress, after 80 days with soil moisture deficit of <25 mm (Volaire *et al.* 1998). In *F. arundinacea* and *P. aquatica* there were no major effects of waterlogging on g$_s$, E or A, suggesting the ability to maintain carbon assimilation despite the stress applied. Similar responses have been found in *Phalaris arundinacea*, in which 3-day waterlogged plants even presented slightly higher g$_s$, A and E rates compared with the controls (Caudle and Maricle 2012). It should be noticed that in *F. arundinacea*, the smaller root system (relative to the controls) attained by plants under waterlogging was enough to maintain certain level of root functioning (e.g. water uptake) as no effects on leaf physiology were recorded.

A similar physiological performance between treatments and higher shoot growth promoted under water excess reinforces the idea that in *P. aquatica*, as a wetland grass, waterlogging cannot be regarded as stressor but as a beneficial factor (Otte 2001). In this species, the promoted shoot growth during waterlogging could be related to a better plant water status due to a high water absorption by an active (submerged but) well aerated root system (tissue porosity up to 33.6%, McDonald *et al.* 2002) as seen in the grass *Paspalum dilatatum* (Insausti *et al.* 2001). By contrast to what typically occurs under well drained (control) conditions with air-filled macropores, in waterlogged soils the root contact surface with water is enlarged, so it might facilitate water absorption as soil macropores are filled with water.

Our results clearly show that the detrimental effects of waterlogging on sensitive species were more evident at the end of the recovery period than immediately after waterlogging (Fig. 1 and Table 1; see also van der Sman *et al.* 1993). However, information about growth of grasses with differential waterlogging tolerance during a recovery phase is not available, so opportunities for comparison are minimal. In this experiment, previously waterlogged plants from *D. glomerata* and *B. catharticus* suffered a restriction in plant dry mass production as a result of reductions in root growth during waterlogging combined with death of root tissues along the recovery. This limited ability to resume growth after the stress might add further limitations to individuals of these species in pasture mixtures, where they can be outcompeted by waterlogging-tolerant species (Jung *et al.* 2009). Contrastingly, despite presenting a reduction in root RGR during waterlogging, *F. arundinacea* successfully recovered from the stress, reaching similar RGR values between treatments for all the compartments. This indicates that the lower root and entire plant dry mass for previously waterlogged plants was a consequence of lower dry masses generated over the waterlogging period but not caused by a retraction during the recovery phase, inferring great capacity to recover from waterlogging. This attribute might help to explain the high ability of this species to colonise frequently disturbed sites (Scheneiter *et al.* 2016). *P. aquatica*, as a tolerant species, presented similar RGR between treatments during the recovery, as in the waterlogging period. In this species, it is possible to speculate that an additional benefit for its growth could be attained under field conditions due to the release of resources and some relaxation in competition among plants, expected from the death of plants of sensitive species, as reported in *P. arundinacea*, another wetland species of the same genus (Kercher and Zedler 2004).

Waterlogging did not affect the number of tillers per plant in any species either during the stress period, or the recovery period. In the case of the sensitive species *D. glomerata* and *B. catharticus* this lack of response in plant morphology is likely to be related to the relative short duration of the stress (2 weeks); an idea that is supported by findings by Etherington (1984), who showed a significant decrease in tiller number of *D. glomerata* plants after longer exposure to soil waterlogging (12 weeks). In *F. arundinacea* and *P. aquatica*, there was a clear trend towards an increase in tiller number along the experiment (despite plants having similar tiller number between treatments), which suggests good ability to continue with tiller development under soil

hypoxia as seen in the waterlogging-tolerant *P. arundinacea* (Martina and von Ende 2013).

In summary, the sensitive species *D. glomerata* and *B. catharticus* showed evident reductions in shoot growth only after the stress was removed despite injury over roots and on leaf physiology, detected during waterlogging. Therefore, evaluating tolerance in a recovery period is definitely relevant, as the ability to tolerate the stress by waterlogging or not is genuinely exhibited from analysis made on growth performances after removing the stress. Future work should advance aiming at unravelling the reasons for the major constraint for quick recovery. It may be associated to an immediate plant water unbalance after waterlogging resulting from a relatively less-affected transpiring shoot and a decayed root system unable to sustain water uptake adequately. Alternatively, it could be more related to carbon starvation due to accelerated leaf senescence triggered during waterlogging.

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