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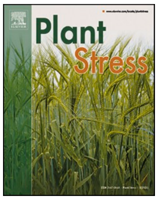

^{18}O isotopic labelling and soil water content fluctuations validate the hydraulic lift phenomena for C$_3$ grass species in drought conditions

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ABSTRACT

Hydraulic lift is a functional characteristic observed in some plant species, often associated with their ability to withstand drought conditions. It involves capturing water from deep soil layers and redistributing it to shallower soil layers through the plant's roots. *Bromus valdivianus* Phil., *Dactylis glomerata* L., and *Lolium perenne* L. may perform hydraulic lift at varying rates. Using both direct (isotopic labelling - δ^{18}O) and indirect (soil water content sensors) techniques, the study assessed and validated the hydraulic lift under extreme drought conditions on the soil top layer (below permanent wilting point), maintaining the bottom layer at high (20–25% filed capacity [FC]) and low (80–85% FC) levels of soil water restriction. Above- and below-ground biomass growth and morpho-physiological responses were evaluated. All species displayed some degree of hydraulic lift, with significant differences observed in the isotopic analysis and soil water content ($p > 0.05$). This illustrates that water was redistributed from the deep to shallower soil layer and validates that the hydraulic lift phenomenon is occurring in these C$_3$ grasses. *Bromus valdivianus* presented the highest δ^{18}O values (25.05‰) and highest increases in soil water content (μ=0.00626 m^3 m^{-3}; five events). *Bromus valdivianus* had a dry matter ratio of approximately 4:1 (0–20cm:20–40 cm). In contrast, L. *perenne* and *D. glomerata* had approximately 6:1 and 5:1, respectively. This difference in root morphology may explain the higher rate of hydraulic lift observed in *B. valdivianus* relative to L. *perenne* and *D. glomerata*. This paper validates the occurrence and provides initial insights into the hydraulic lift process occurrence of temperature grass species.

1. Introduction

Commonly associated with the drought tolerance of deep-rooting plant species, the hydraulic lift is a functional property that some species exhibit by capturing water from deep soil layers and releasing it into shallower soil layers via roots (Richards and Caldwell 1987; Caldwell and Richards 1989). The hydraulic lift phenomenon was initially described as water uptake and loss via roots (Shone and Flood 1980) and defined as either a water redistribution process via the soil-root continuum exhibited by vascular plants (Richards and Caldwell 1987; Caldwell and Richards 1989; Volpe et al., 2013). However, the demonstration of the functional importance of deep rooting and its relationship with soil water dynamics presents some inherent difficulties (Maeght et al., 2013; Pierret et al., 2016), mainly associated with the

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Abbreviations: AGDD, accumulated growing degree days; Bv, Bromus valdivianus Phil.; δ2H, deuterium stable isotope delta value; δ18O, oxygen-18 stable isotope delta value; Dg, Dactylis glomerata L.; DM, dry matter; FC, field capacity; HR, high level of water restriction; IRMS, isotope ratio mass spectrometry; Lp, Lolium perenne L.; LER, lamina elongation rate; LR, low level of water restriction; LS, leaf regrowth stage; MDA, leaf malondialdehyde concentration; NT, number of tillers; PWP, permanent wilting point; Sat, saturation point; Spp, species; SWC%, soil water content; TBA, thiobarbituric acid; TCA, trichloroacetic acid; VPD, vapour pressure deficit; WL, wavelengths; WR, levels of water restriction.

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necessity for non-destructive research to assess hydrological phenomena (Schymanski et al., 2008; Lux and Rost 2012; Hayat et al., 2020; Cai et al., 2022).

Techniques to reveal the hydraulic lift of plant species and its consequential water redistribution into different soil layers initially consisted of indirect measurements using micro tensiometers at different soil depths to assess the fluctuations in the soil water potential gradients (Ψ_S) (Vetterlein and Marschner 1993) and direct measurements using standard water relation methods associated to isotopic labelled water techniques (Dawson 1993). The phenomena have been modelled and demonstrated for forestry systems and savanna ecosystems due to the extensive root size of trees and shrubs. This facilitates the hydraulic lift validation, quantification up to complex soil-plant-atmosphere continuum models and predictions on commensalistic relationships of plants (Scholz et al., 2008; Domec et al., 2010; Yu and D'Odorico 2014; Ferreira et al., 2018; Bayala and Prieto 2019; Lee et al., 2021).

Even though crop and forage plants have a relatively smaller stature and a less extensive root system than trees, it is, directly and indirectly, proven that some crop and forage species can also exhibit hydraulic lift. Studies have assessed the hydraulic lift in cropping and foraging systems, for instance: *Medicago sativa* L. and *Zea mays* L. (Corak et al., 1987); *Cajanus cajan* L., *Sesbania sesban* L. and *Z. mays* (Sekiya and Yano 2004); *Lupinus angustifolius* L. (Doussan et al., 2006); *Oriza sativa* and *Z. mays* (Mahindawansha et al., 2019). Nonetheless, considerable uncertainty remains about its scale and possible applications to farming systems.

Regarding the potential positive outcomes on an ecosystem resulting from hydraulic lift, (Bogie et al., 2018) and, subsequently, (Singh et al., 2020) introduced the concept of 'bioirrigation' into the debate. These authors' hypothesis is based on the deliberate inclusion of deep-rooted species into monocultures, such that the redistributed water by the added species benefits the neighbouring plant species, enhancing their chance of survival or growth during drought periods. A study based on a soil water budget from a 15-year data set in a marine Mediterranean landscape found that *Olea europea* L. and *Quercus suber* L. trees subsidised deep water (below fractured-basalt layer) for neighbouring grass species during spring (Montaldo et al., 2021). In a study carried out on intercropped areas of *Panicum virgatum L.* (> ten years old) and adjacent shallow-rooted annual crops within the southern great plains of North America (climate ranging from continental humid to semi-arid), it was found that the soil under *P. virgatum* had consistently higher soil water content (margin of 15–100%) than nearby soil under shallow-rooted annual crops using stable hydrogen and oxygen isotopes (deuterium [δ^2H] and oxygen-18 [δ^{18}O] stable isotope delta values) (Oerter et al., 2021).

The hydraulic lift brings a new perspective on how the combination of functional species (e.g., different root system architecture) benefits the ecosystem, whereby commensalism allows one species to cope with periods of water restriction or drought that can negatively affect the ecosystem's stability. It is often conceptualised as the plant with the greater stature (tree or shrub) being the 'donator' and the smaller plant the 'recipient'; however, this does not encompass interactions in pasturelands, where the botanical composition of the ecosystems is based on small-sized plants (grasses, legumes, and herbs). Studies carried out on the hydraulic lift of *Bromus tectorum* L., *Lolium multiflorum* Lam., and *P. virgatum* proved that grasses are also capable of exhibiting hydraulic lift, to a certain extent defined by the aptitude of their root system (Leffler et al., 2005; Meunier et al., 2017; Oerter et al., 2021). The present study hypothesises that the temperate perennial grasses, *Bromus valdivianus* Phil. (Bv), *Dactylis glomerata* L. (Dg) and *Lolium perenne* L. (Lp), perform the hydraulic lift when subjected to water stress, and the phenomena occurrence is related to their root size and distribution. It was verified whether the three species exhibit the hydraulic lift or not, and if so, under which conditions of soil water restriction. The above- and below-ground biomass growth and morpho-physiological responses of the three species under two levels of water restriction were also evaluated to assess their relationship to the hydraulic lift phenomenon.

2. Material and methods

2.1. Environmental conditions and experiment setup

The study was conducted in a glasshouse at Massey University's Plant Growth Unit, Palmerston North, New Zealand, between October 2020 and April 2021. The glasshouse was fully exposed to natural light conditions, and when air temperature rose above 30 °C, inbuilt fans were activated for two hours, and air humidity regulators were activated for twenty minutes. The glasshouse environmental conditions were registered from March to April 2021, when the experimental data was collected (Fig. 1). The accumulated growing degree days were calculated according to Calvache et al. (2020), utilising a base temperature of 5 °C.

The study used twenty-four pots designed and built, as shown in Fig. 2. The pot had a mesh at the bottom, two irrigation pipes placed from outside, three windows sealed with translucent plastic to appreciate the root development and two gaps for soil water content (SWC%) sensors at the top and bottom layer. Each pot constituted one experimental unit. The experimental design was a randomised complete block design with three blocks ($n = 3$) and a 3×2 factorial arrangement of treatments). The main factors were the three grass species (Spp = Lp, Bv and Dg) and two levels of water restriction (WR) applied in the bottom soil layer (low level of water restriction [LR] – 600 mL/week, and high level of water restriction [HR] – 300 mL/week) for approximately 600 growing degree days (Calvache et al., 2020), equivalent to thirty-four days in the current glasshouse environment. One pot of each experimental treatment (Spp x WR) was randomly allocated within each of the three blocks (each block: 1 m x 0.8 m). The irrigation values were calculated to keep the bottom layers of the pots respectively at 90–100% field capacity (FC) and 20–30% FC, depending on the irrigation treatment, until the end of the trial. During the evaluation period, the top layer was never irrigated to simulate field conditions during the dry season. The bottom layer was irrigated through the outside pipes twice weekly according to the two water restriction levels.

The soil substrate used in the experimental pots was a mix of 50% allophanic soil (volcanic soil from Taranaki-Wanganui region in New Zealand) and 50% sand, fertilised with a combination of a slow-release (3–4 month Osmocote ®; 19% N, 9% P, 10% K + 2% MgO + trace), a fast-release fertiliser (8–10 week Osmoform ®; (22% N, 3% P, 11% K, 2% MgO + trace) and dolomite at a rate of 4 g L^{-1}, 0.5 g L^{-1} and 1.5 g L^{-1}, respectively. The soil physical attributes were determined at Landcare Research – Manaaki Whenua, Palmerston North, New Zealand, with six disturbed soil samples and nine undisturbed soil samples of 54.26 cm^3 cores ($h = 3.00$ cm; $\phi = 4.80$ cm). The sixteen cores were utilised to determine the water retention points (Ordóñez et al., 2018) at −330 hPa, −1000 hPa, −3000 and −15,000 hPa. With the addition of the calculated soil saturation, it was possible to parameterise the points and to generate the soil water retention curve (van Genuchten 1980; van Genuchten et al. 1991). The soil FC was assessed at −330 hPa, and the permanent wilting point (PWP) at −15,000 hPa (Fig. 3). Finally, the samples were dried in a forced ventilation oven at 106 °C and weighed again to determine other physical attributes. The soil had a dry bulk density of 1.34 g cm^{-3}, particle density of 2.65 g cm^{-3}, porosity of 49%, and was categorised as a sandy-loam soil texture.

On 19 November 2020, adult plants of L. *perenne* cv. Maxsyn, *B. valdivianus* cv. Bareno and *D. glomerata* cv. Greenly II were collected from a three-year-old pasture field and planted in spare pots to stimulate growth and tillering. Liquid fertiliser and defoliations down to 5 cm height were performed on 24 November 2020 and 19 December 2020. The fertilisation rate was based on a nitrogen (N) application equivalent to 20 kg N ha^{-1}, with water-soluble fertiliser (Peters Professional "Allrounder"; 20% N, 8.7% P, 16.6% K) plus trace elements (B, Cu, Fe, Mn, Mo, Zn).

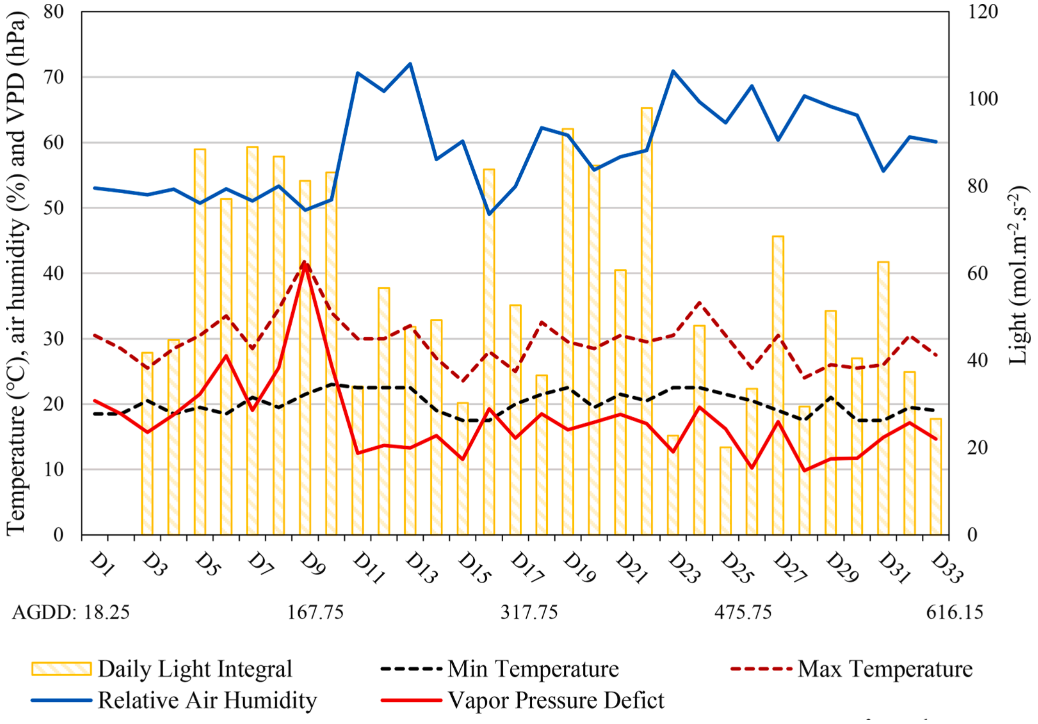

Fig. 1. Environmental conditions: daily light integral (moles of light per day – mol m^{-2} day^{-1}), minimum and maximum temperature (Celsius degree - ℃), relative air humidity (percentage -%), vapour pressure deficit (VPD – hPa) and in the glasshouse during 19 March 2021 and 20 April 2021 [day (D) 1 to D33]. Accumulated growing degree days (AGDD) on 19 March 2021 (D1), 27 March 2021 (D9), 04 April 2021 (D17), 12 April 2021 (D25) and 20 April 2021 (D33).

On 20 January 2021, fifteen tillers and roots of each grass species were transplanted into the experimental pots. Each group of fifteen joint tillers was detached from the original grass patch, and the roots were carefully washed. Each group of tillers was held with its roots hanging down in its final position, reaching the bottom layer of the pot. Then, the soil substrate (2.1 kg) was settled as the 15 cm bottom layer, followed by a mix of thick sand (1 to 2 mm) and thin gravel (10–20 mm) to complete the 10 cm capillary barrier, and another 15 cm soil substrate was added as the top layer. The SWC% sensors were placed at 10 and 30 cm depth. Six extra pots of bare soil were built up with SWC% sensors; the capillary barrier was present in three bare soil pots and not in the other three. Two bare soil pots of each type were added to each block. These pots were utilised to validate the barrier as a soil capillarity breaker, preventing the water rising flow (i.e., capillarity flow), thus “rewetting” the upper soil layer (Dawson 1993; Vetterlein and Marschner 1993).

The plants were irrigated once a day, ensuring no stress due to soil water restriction or soil waterlogging. On 01 February 2021, the plants were defoliated to 5 cm height and received the second application of water-soluble fertiliser to stimulate root growth. The experimental period started on 15 March 2021, when all pots presented roots in the bottom layer, visible through their windows (Fig. 2). For three days, the irrigation stopped to induce all the pots to reach the PWP in the top and bottom layer so that at the start of the experimental measurement period, all the pots had similar conditions below PWP. On 18 March 2021 (day 0), the plants were defoliated to 5 cm height, and the two levels of water restriction were imposed.

2.2. Below-ground measurements

Soil water content sensors (ECH2O series EC-5 VWC – METRE Group, Pullman, Washington, USA) were placed in all experimental pots (bare soil and with plants) at two depths to determine the SWC%: top layer (10 cm) and bottom layer (30 cm) (Fig. 2). These sensors were connected to a measurement and control data logger (CR1000 model – Campbell Scientific, Logan, Utah, USA) and a multiplexer (16/32B model – Campbell Sci., Logan, Utah, USA). The SWC% of plant and bare soil pots were assessed every five minutes, and an average was recorded every fifteen minutes. The data collected by the soil moisture sensors and registered in the data logger were calibrated and corrected according to the soil substrate’s physical properties and presented as volumetric water content (m^3 m^{-3}).

The hydraulic lift was assessed using enriched labelled water with stable isotopes of oxygen (Water-18O 10atom%, Tayo Nippon Sanso, Tokyo, JPN) diluted in tap water and presented as oxygen-18 stable isotope standard delta notation value (δ^{18}O). On 19 April 2021 (day 32), the labelled water enriched to 0.51% oxygen-18 (δ^{8}O = 400‰) was applied in the bottom layer at the same rates as the previous irrigations with tap water for the two water restriction treatments. The bare soil pots also received the ^{18}O-labelled water at the same level as the LR treatments. Two soil sub-samples across each top layer were collected the following day (day 33), between 11 AM and 2 PM, when the top SWC % sensors indicated that hydraulic lift was occurring. An auger was utilised to collect two 20 g samples per pot. The soil sub-samples were immediately placed in plastic containers and immersed in liquid nitrogen to stop root activity and release of exudates.

The sub-samples were utilised for isotopic abundance analysis, presented as in δ^{18}O values, which is the ratio of stable isotopes oxygen-18 and oxygen-16 samples. The soil sub-samples were placed inside a glove bag (Polyethylene glove bag, Thermo Scientific™, Waltham, Massachusetts, USA) filled with dry air, where the remaining small pieces of fine root material were thoroughly screened and manually removed. This process ensured that only the water from the soil material was extracted through cryogenic distillation. Approximately 0.30 mL of water was extracted from each sub-sample, and distillation was carried out until no more water residual was present in the soil samples. Later, the two sub-samples of each treatment were bulked to compose one water sample per pot, aiming for the minimum quantity of 0.50 mL necessary for the isotopic determination. A total of 24 samples were generated after the soil distillation – a factorial combination of Lp, Bv, Dg and 20–25% FC, 80–85% FC plus bare soil no-barrier and bare soil

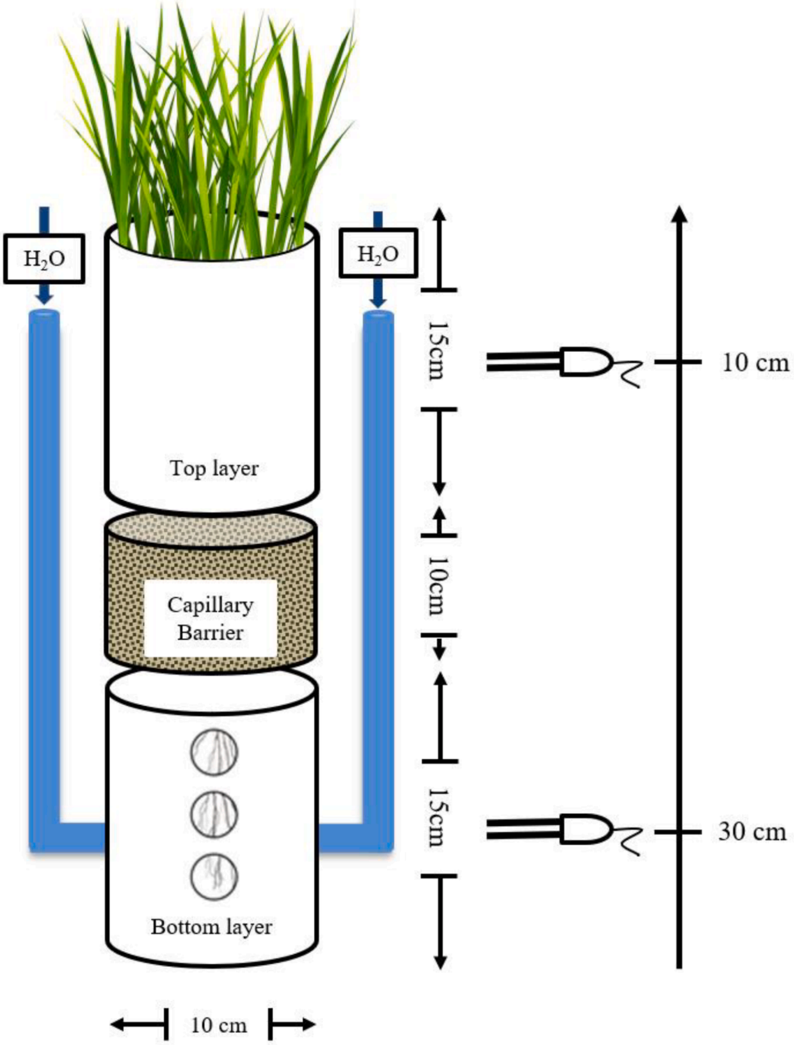

Fig. 2. Experimental pots diagram indicating pots' dimensions (10 cm x 40 cm), pots' layers heights (top layer - 15 cm; capillary barrier - 10 cm; bottom layer – 15 cm), irrigation tubes position from outside the pot, SWC% sensors position (at 10 cm and 30 cm depth) and windows position for root monitoring.

with-barrier pots samples, in three blocks.

Oxygen isotope ratios of water were determined by the method of equilibration with CO_2, the CO_2 being measured by isotope ratio mass spectrometry (IRMS) at the University of Otago's Chemistry Department - Isotrace NZ Ltd laboratories, Dunedin, New Zealand. A water sample of 0.5 mL was equilibrated with 12 mL of 0.3% CO_2 in helium on a Gasbench II (Thermo Scientific, Bremen, DEU) preparation unit for 18 h at 25.0 ± 0.1 °C. Ten repeated injections of the equilibrated gas were measured with a Thermo Advantage isotope ratio mass spectrometer in continuous-flow mode (CF-IRMS). The ten results were filtered by removing more than one standard deviation value from the average. The filtered average was corrected to the international VSMOW-SLAP isotope scale using a three-point calibration provided by three laboratory standards analysed before and after every batch of 18 samples. In addition, a control sample, chosen to be similar to the experimental samples, was measured at every 12th position to correct for instrumental drift. If applied, instrumental drift correction was calculated from a linear regression of the control sample result against time. Consensus values for the laboratory standards have been obtained from 6-year internal laboratory calibration records against primary reference materials, VSMOW, GISP and SLAP, external 6-member interlaboratory comparison exercise and by back-calculation from the ~170 members IAEA interlaboratory comparison exercise, WICO2012. The Isotrace NZ Ltd laboratories, Dunedin, New Zealand standards and their consensus values were as follows: ICE (18OVSMOW = −32.097 ± 0.075 ‰), TAP (18OVSMOW = −11.432 ± 0.038 ‰), SEA (18OVSMOW = −0.029 ± 0.036 ‰), and as reported also by Stokes et al. (2020).

The roots mass sampling was done on 20 April 2021 (day 33). The bottom meshes of the pots were removed; thus, the soil substrate contained in each pot could be entirely extracted and remain intact. Afterwards, they were split in the middle (0–20 cm and 20–40 cm depth) and manually washed off until the roots were completely clean. The roots were dried in a forced ventilation oven at 60 °C for 72 h and weighed as dry matter. Total biomass accumulation was the sum of above- and below-ground dry matter.

2.3. Above-ground measurements

The leaf water potential was assessed with a Scholander chamber and pressure bomb (Soil Moisture Equipment Crop, Santa Barbara, California, USA) on 14 April 2021 (day 27) from 10 AM to 03 PM. The newest fully expanded leaf from two tillers per pot was selected, and 10 cm from the tip towards the ligule of each leaf was cut and collected to be used separately in the Scholander chamber. Slowly increasing pressure flux was applied to each sample, and measurement was recorded once the sap was forced out and became visible through a magnifying lens (Turner 1981).

The leaf malondialdehyde concentration (MDA), a product of per-

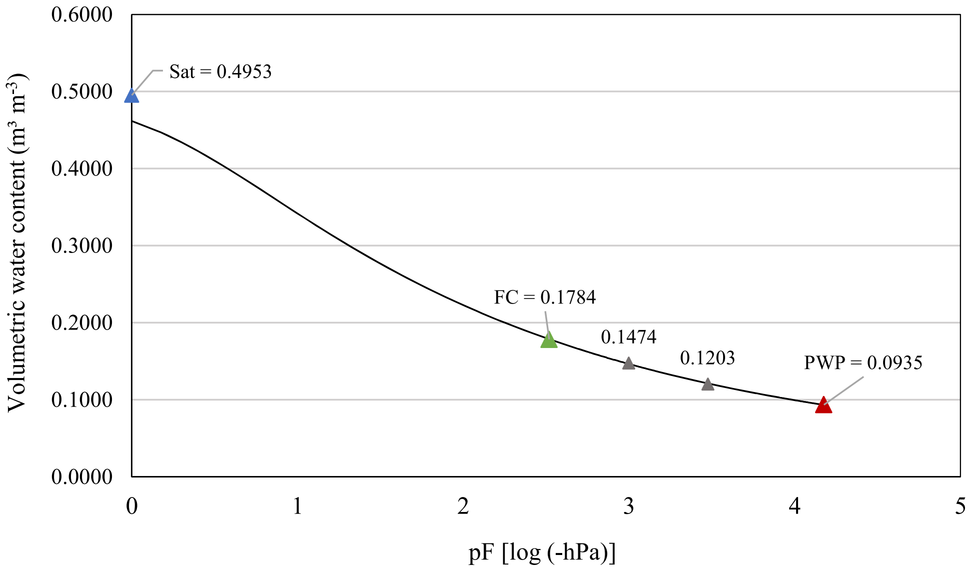

Fig. 3. Soil water retention curve: the relationship between volumetric water content ($m^3\ m^{-3}$) and log of the soil water matric potential (hPa) of the substrate utilised in the study. The curve indicates the saturation point (Sat in blue), field capacity (FC in green; −330 hPa), two intermediary points (−1000 hPa and −3000 hPa) and the permanent wilting point (PWP in red; −15,000 hPa).

oxidation of unsaturated acids and a great indicator to quantify the levels of drought stress on plant tissues, was determined according to Abid et al. (2018) and García-Favre et al. (2021b). On 19 April 2021 (day 32), the newest fully expanded leaf of two random tillers per pot was collected and instantly frozen in liquid nitrogen. The samples were stored in a freezer at −18 °C. In the laboratory, each sample separately of the leaf material was weighed and added to 2 mL of 5% trichloroacetic acid (TCA), grounded and centrifuged at 3000 rpm for 10 min. Subsequently, 1 mL of the supernatant obtained was added to 1 mL of 0.67% thiobarbituric acid (TBA), incubated in a boiling water bath for 30 min and centrifuged. The supernatant was displaced in a spectrometer to obtain the absorbence levels at 440, 532 and 660 nm wavelengths (WL). The absorbence levels at the three wavelengths of each sample were corrected according to their initial leaf material weight. The MDA concentration value was calculated by Eq. (1):

$$[MDA]\ \left(\mu mol\ L^{-1}\right) = 6.45 * (WL_{532} - WL_{600}) - 0.56 * WL_{440} \tag{1}$$

The plant material sampling and storing for plant osmotic potential were done simultaneously with the MDA, following the same sampling procedures. The lamina samples were taken to a laboratory to measure the plant osmotic potential with a Wescor HR-33T microvoltmeter and six C-52 sample chambers (WESCOR Inc, Logan, Utah, USA). The lamina was cut into a 25 mm^2 square to fit in the chamber holder cavity. The sample was placed flattened in the holder, sealed and allowed to equilibrate for 15 min until reaching pressure stability, and then the measurement was taken. Each chamber was calibrated against the water potential of five NaCl solutions (0.2, 0.4, 0.6, 0.8, 1.0 molal) at 15 °C (same room temperature as at the plant material measurements). The initial plant osmotic potential values obtained from the plant material were corrected due to the variations of each chamber in which they were specifically measured (Lang 1967).

The number of tillers per pot was assessed weekly (days 0, 8, 15, 22 and 29) from 19 March 2021 to 18 April 2021. To assess the lamina elongation rate, two individual tillers were marked per pot at the beginning of the study period (day 1) and continuously measured every three days (days 1, 4, 7, 10, 13, 16, 19, 22, 25, 28 and 31). A lamina was considered fully expanded when its ligule was visible and measured from the tip to the ligule. A new lamina was assessed when its tip was visible within the previous leaf sheath and measured from its tip to the previous leaf ligule (Poff et al., 2011). The lamina was considered senescing when its extremities began to decolour and dry, and only its remaining green part was measured. The daily lamina elongation rate was calculated as the difference between the accumulated lamina length per tiller of the current date (n) and the accumulated lamina length of the previous measurement date (n-3) divided by the date interval (three days).

The components of the yield, lamina area, and herbage mass were assessed on the final harvest (days 32 and 33). Five tillers per pot (the two marked tillers utilised for previous morphological measurements, plus three with similar leaf regrowth stage and size condition) were collected and fractioned into pseudo-stem, lamina, and dead material. The laminas were measured in an electronic integrator of leaf area (LI-3100C - LI-COR Biosciences, Lincoln, Nebraska, USA). All fractions were separately weighed as fresh, dried in a forced ventilation oven at 60 °C for 72 h and weighed again as the dry matter of each component. The remaining herbage masses in the pots were cut at soil surface level, and fractioned into pseudo-stem, lamina and dead material, separately weighed as fresh, dried in a forced ventilation oven at 60 °C for 72 h and weighed again as dry matter of each component. This process was repeated to define the percentage correlation of the tillers sample and the total herbage mass sample and, therefore, define the leaf area index, calculated as the lamina area (cm^2) over the pot surface area (cm^2). The plant morphological responses were measured and depicted as above-ground biomass accumulation of three components of the yield categories (lamina, sheath and dead material) per tiller and pot.

2.4. Statistical analysis

All statistical analyses were performed using SAS v 9.4 (SAS Institute Inc, Cary, NC, USA). The data were examined for normal distribution using the Shapiro-Wilk test, finding that all dependant variables followed a normal distribution.

Analyses of variance for the dependant variables that were recorded only once were performed using the GLIMMIX procedure with the following mixed model:

$$Y_{ijk} = \mu + Spp_i + WR_j + (Spp * WL)_{ij} + B_k + e_{ijk}$$

Where Spp_i is the fixed effect of i^{th} grass species (Lp, Bv and Dg), WR_j is the fixed effect of the j^{th} level of water restrictions (HR and LR), $Spp * WR_{ij}$ is the fixed effect of the interaction between the i^{th} grass

Table 1
Effect of three plant species (Spp = Bv, Dg and Lp), two water level restrictions (WR = LR and HR) and the interaction between treatments on total biomass (sum of above- and below-ground dry matter [DM]), above-ground biomass (total herbage DM) and below ground-biomass (total roots DM, top-layer DM and bottom-layer DM) at day 32.

	Total Biomass Above- and below-ground DM *(g)*	Above-ground biomass Total herbage DM *(g)*	Below-ground biomass Total root DM (0–40 cm) *(g)*	Root DM (0–20 cm) *(g)*	Root DM (20–40 cm) *(g)*
Spp					
Bromus valdivianus (Bv)	11.4^{b} (±1.0)	5.8 (±0.7)	5.6^{b} (±0.5)	4.5^{b} (±0.4)	1.1^{ab} (±0.1)
Dactylis glomerata (Dg)	14.7^{a} (±1.3)	5.7 (±0.8)	8.9^{a} (±0.7)	7.5^{a} (±0.7)	1.5^{a} (±0.2)
Lolium perenne (Lp)	12.1^{b} (±0.5)	6.9 (±0.4)	5.2^{b} (±0.5)	4.5^{b} (±0.5)	0.7^{b} (±0.2)
Significance	*	NS	***	**	*
WR					
Low Restriction (LR)	14.2^{a} (±0.8)	7.2^{a} (±0.4)	7.0 (±0.9)	5.9 (±0.7)	1.1 (±0.2)
High Restriction (HR)	11.3^{b} (±0.7)	5.1^{b} (±0.4)	6.2 (±0.6)	5.0 (±0.6)	1.1 (±0.1)
Significance	**	**	NS	NS	NS
Interaction Spp x WR					
BvLR	13.3 (±0.6)	7.3 (±0.4)	6.0 (±0.7)	4.8 (±0.7)	1.3 (±0.1)
BvHR	9.4 (±0.8)	4.3 (±0.2)	5.2 (±0.6)	4.1 (±0.4)	1.0 (±0.2)
DgLR	16.8 (±1.4)	6.8 (±1.2)	10.0 (±0.6)	8.4 (±0.5)	1.6 (±0.2)
DgHR	12.5 (±1.5)	4.7 (±0.6)	7.8 (±0.8)	6.5 (±1.2)	1.3 (±0.4)
LpLR	12.3 (±0.2)	7.4 (±0.5)	4.9 (±0.7)	4.5 (±0.7)	0.4 (±0.2)
LpHR	11.8 (±1.0)	6.3 (±0.4)	5.5 (±0.9)	4.5 (±0.8)	1.1 (±0.1)
Significance	NS	NS	NS	NS	NS

Superscripted letters that differ within the same columns and lines for the same fixed effect and/or interaction of fixed effects indicate statistically significant values at * $p \le 0.05$, ** $p \le 0.01$, *** $p \le 0.001$, **** $p = 0.0001$ and NS = non-significant. Following each least square mean is the (±) standard error of the mean.

species and the j^{th} level of water restriction, B_k is the random effect of the k^{th} block and e_{ijk} is the random residual assumed with mean zero and variance σ_e^2.

Analysis of variance for dependant variables that were repeatedly recorded over time on the same pot was performed using the GLIMMIX procedure with the following mixed model for repeated measures:

$$Y_{ijk} = \mu + \mathrm{Spp}_i + \mathrm{WL}_j + (\mathrm{Spp} * \mathrm{WR})_{ij} + B_k + T_m + (\mathrm{Spp} * T)_{im} + (\mathrm{WR} * T)_{jm} + (\mathrm{SPP} * \mathrm{WR} * T)_{ijm} + e_{ijkm}$$

Where all terms are as defined above, and T_m is the fixed effect of the m^{th} time. To account for repeated measures on the same pot through time, the best structure to model the variances and covariances of the random residuals was determined using the corrected Akaike Information Criteria Value (Wang and Goonewardene 2004). It was found that the best error structure was the Huynh–Feldt model.

F-values for the testing significant effects of fixed factors were calculated using the Satterthwaite approximation as suggested for split-plot design (Satterthwaite 1946). Least square means and standard errors for the fixed effects and combinations between the fixed effects were obtained and used for multiple mean comparisons using Fisher's least significant difference. Significant differences between the least squares means were declared at $p \le 0.05$.

The relationships between the measured variables were evaluated using multivariate statistical analyses with the CANDISC procedure applied to two different datasets. The first analysis explored the relationships amongst physiological responses, biomass accumulation and distribution, and the hydraulic lift resulting from soil water stress. The second analysis explored the relationships amongst general morpho-physiological variables as a result of species types (competitors and stress-tolerators) by Grime (1974). The canonical variate analysis (Jobson 1992; López et al., 2006) was performed to determine the extent to which variables explained most of the differences between species and soil water restriction levels.

3. Results

3.1. Biomass accumulation: above- and below-ground dry matter accumulation driven by species and levels of water restriction

There were no significant interactions between the three species and the two levels of water restriction (Spp x WR) for any of the biomass fractions ($p > 0.05$). The total biomass accumulation was significantly different for the Spp and WR; Dg accumulated 25.1% more total biomass than Lp and Bv ($p \le 0.05$) and LR pots accumulated 25.7% more total biomass than HR ($p \le 0.01$). Accordingly, the LR pots' above-ground biomass was 41.2% higher than the HR ($p \le 0.01$). The three species accumulated similar above-ground biomass ($p > 0.05$) (Table 1). The total below-ground biomass accumulation ($p \le 0.001$) and its distribution within the soil depth, as in the 0–20 cm soil layer ($p \le 0.01$) and the 20–40 cm soil layer ($p \le 0.05$) were significantly different between the Spp. Overall, Dg had the highest total below-ground biomass - 64.8% higher than Bv and Lp - and the highest root biomass in the 0–20 cm soil layer – 66.7% higher than Bv and Lp (Table 1). At the 20–40 cm soil layer, Bv had a similar root biomass as Dg.

3.2. Soil water dynamics and hydraulic lift: phenomena validation and characterisation

On 19 April at 07:45 AM, just before the irrigation of the bottom layers with ^{18}O labelled water, the SWC% of the pots' top and bottom layers were assessed to validate the assessment of further soil-related results. The triple interaction between plant Spp, WR and soil level profile (3 × 2 × 2) was significant for the soil volumetric water content ($p \le 0.001$). There was no significant difference amongst the top layers' SWC% of all pots just before the moment of the labelled irrigation, with all the measured values under PWP (≤0.0935 SWC%). Therefore, ^{18}O labelled water applied in the bottom layers was redistributed via hydraulic lift into homogenous recipients (top layers).

The hydraulic lift was observed and captured from SWC% fluctuations provided by the soil moisture sensors within the pots' top layers (indirect measurements), often occurring the day after the treatments' irrigation was applied. The hydraulic lift via SWC% was identified and measured via SWC% on five occasions (days 9, 16, 19, 22, 27) during the experimental period and was indirectly quantified as the differences between the highest and the lowest SWC% of each day (Table 2 and Fig. 4). The lowest SWC% values were registered around 7 to 8 AM, while SWC% peaks occurred consistently from 12 PM to 3 PM. The hydraulic lift registered as SWC% was significantly related to the Spp on the five occurrence dates ($p \le 0.05$) and was exhibited to a greater degree by Bv and Dg than Lp (Fig. 4).

Table 2

Effect of three plant species (Spp = Bv, Dg, Lp), two water level restrictions (WR = LR and HR) and interactions between treatments on hydraulic lift indirectly captured as the increase in soil volumetric water content presented by the top layer sensors at day 9, day 16, day 19, day 22 and day 27. Effect of three plant species (Spp = Bv, Dg, Lp), two water level restrictions (WR = LR and HR) and the interaction between treatments on hydraulic lift directly captured as $\delta^{18}O$ values by the isotopic analysis from the soil top layer sampled on day 33.

	Hydraulic Lift Soil volumetric water content (top layer)					$\delta^{18}O$
	Day 9 ($m^3\ m^{-3}$)	Day 16	Day 19	Day 22	Day 27	Day 33 (‰)
Spp						
Bromus valdivianus (Bv)	0.0102^a (±0.0008)	0.0058^a (±0.0007)	0.0046^a (±0.0006)	0.0055^a (±0.0005)	0.0052^a (±0.0005)	25.05^a (±4.36)
Dactylis glomerata (Dg)	0.0110^a (±0.0018)	0.0058^a (±0.0007)	0.0037^a (±0.0004)	0.0050^a (±0.0004)	0.0046^a (±0.0011)	13.70^b (±3.43)
Lolium perenne (Lp)	0.0050^b (±0.0023)	0.0031^b (±0.0014)	0.0019^b (±0.0009)	0.0027^b (±0.0012)	0.0025^b (±0.0004)	10.30^b (±3.82)
Significance	**	*	**	***	**	*
WR						
Low Restriction (LR)	0.0077 (±0.0020)	0.0039 (±0.0011)	0.0030 (±0.0009)	0.0039 (±0.0060)	0.0035 (±0.0009)	15.29 (±4.69)
High Restriction (HR)	0.0097 (±0.0007)	0.0059 (±0.0004)	0.0039 (±0.0003)	0.0049 (±0.0017)	0.0046 (±0.0004)	17.42 (±2.58)
Significance	NS	NS	NS	NS	NS	NS
Interaction Spp x WR						
BvLR	0.0108 (±0.0012)	0.0059 (±0.0014)	0.0058 (±0.0007)	0.0065 (±0.0003)	0.0057 (±0.0003)	30.23 (±5.86)
BvHR	0.0095 (±0.0010)	0.0057 (±0.0003)	0.0036 (±0.0005)	0.0046 (±0.0008)	0.0046 (±0.0007)	19.87 (±5.80)
DgLR	0.0124 (±0.0009)	0.0057 (±0.0009)	0.0034 (±0.0007)	0.0052 (±0.0007)	0.0049 (±0.0005)	12.22 (±5.66)
DgHR	0.0095 (±0.0020)	0.0059 (±0.0013)	0.0041 (±0.0005)	0.0049 (±0.0007)	0.0044 (±0.0009)	15.20 (±4.95)
LpLR	0.0000 (±0.0000)	0.0000 (±0.0000)	0.0000 (±0.0000)	0.0000 (±0.0000)	0.0000 (±0.0000)	3.40 (±3.17)
LpHR	0.0101 (±0.0008)	0.0062 (±0.0001)	0.0039 (±0.0004)	0.0052 (±0.0005)	0.0049 (±0.0003)	17.96 (±3.93)
Significance	NS	NS	NS	NS	NS	NS

Superscripted letters that differ within the same columns and lines for the same fixed effect and/or interaction of fixed effects indicate statistically significant values at * $p \leq 0.05$, ** $p \leq 0.01$, *** ≤ 0.001, **** $p = 0.0001$ and NS = non-significant. Following each least square mean is the (±) standard error of the mean.

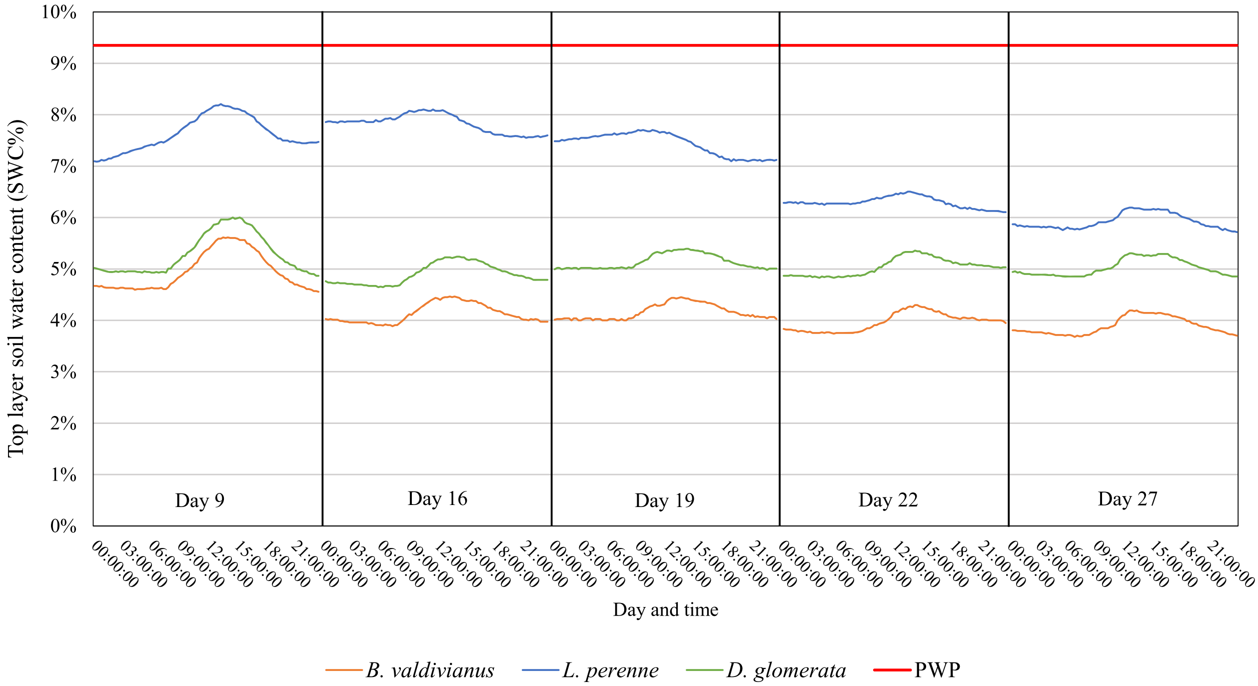

Fig. 4. Average daily fluctuation readings of volumetric water content captured by SWC% sensors located on the top-layer soil on the five days the hydraulic lift was captured. The SWC% values presented refer to the data by Spp (significantly different as per Table 2; B. valdivianus in orange, L. perenne in blue and D. glomerata in green) on day 9, day 16, day 19, day 22 and day 27. The line in red depicts the soil substrate's permanent wilting point (PWP).

In addition to the SWC% indirect measurements, the hydraulic lift was directly quantified using ^{18}O labelled water (day 33). The hydraulic lift registered using ^{18}O labelled water significantly differed between the Spp ($p \leq 0.05$). The Bv exhibited the phenomena to a greater degree, approximately double the $\delta^{18}O$ values found in the soil top layer of Dg and Lp pots (Table 2). For both indirect and direct methodologies of hydraulic lift assessment, no significant differences due to different WR and no interaction between the fixed effect (Spp x WR) were found ($p > 0.05$). The three pots without the barrier presented a $\delta^{18}O$ of 0.26‰, −0.50‰, 2.99‰ (μ = 0.92‰), while the three pots with the barrier presented a $\delta^{18}O$ of 1.10‰, 1.69‰, −0.73‰ (μ = 1.17‰).

3.3. *Physiological responses: species' metabolic responses regulated by soil water stress*

There were no significant differences in the plant physiological responses evaluated (leaf water potential, plant osmotic potential and MDA) amongst the three Spp ($p > 0.05$). However, these three variables were significantly affected by the WR ($p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$, respectively). The plants submitted to HR treatment presented more negative values of leaf water potential and plant osmotic potential and higher values of MDA than the plants under LR. There were no significant interactions between the fixed effects (Spp x WR) for leaf water potential and MDA ($p > 0.05$). Significant interactions were found for plant osmotic potential ($p \leq 0.05$), in which the LpHR presented the most negative value (Table 3).

3.4. Morphological responses: structural changes in species' strategies determined by soil water

There were no significant interactions (Spp x WR) for lamina, sheath and dead material biomass accumulation and the proportional distribution of lamina weight in relation to the total weight of above-ground biomass (lamina:total biomass proportion) per pot and per tiller ($p > 0.05$). The different WR significantly affected the biomass accumulation of lamina and lamina:total biomass proportion per pot and tiller ($p = 0.0001$ and $p \leq 0.05$, respectively). The plants subjected to the LR treatment accumulated 64.5% and 50.9% more lamina biomass per pot and tiller, respectively, than in HR. Significant differences were found amongst Spp's dead material biomass accumulation per pot and tiller (both at $p \leq 0.05$), where Bv accumulated dead material was higher than that of Lp and Dg at pot and tiller levels. Meanwhile, significant differences were also found amongst Spp's sheath accumulation per pot and tiller ($p \leq 0.001$ and $p = 0.0001$, respectively), in which Dg had the greatest sheath mass and Bv the lowest sheath mass for both sampling scales. For the lamina biomass accumulation, significant differences amongst Spp were found only in the tiller's sampling scale ($p \leq 0.05$), in which the Lp presented the lowest accumulated lamina biomass per tiller. At a pot sampling scale, the three species accumulated similar lamina biomass (Table 4).

The lamina surface area per tiller was significantly different between the Spp ($p \leq 0.001$) and the WR ($p \leq 0.001$) and had a significant interaction (Spp x WR) ($p \leq 0.05$) (Table 5). The DgLR had the greatest area of lamina area, with an average increment of 215.0% over the lowest areas presented by LpLR, LpHR and BvHR. There was no significant interaction (Spp x WR) and no significant differences between WR for specific lamina area (lamina area/lamina mass) ($p > 0.05$). Significant differences were only shown by the Spp ($p \leq 0.05$), where Dg had an average SLA 31.0% higher than the other species.

The leaf regrowth stage (LS) significantly differed due to the WR and presented a significant interaction (Spp x WR). The three lowest LS values were found on the plants subjected to a higher WR during the experimental period; DgHR and LpHR presented the lowest values, followed by the BvHR. (Table 5). In the five different five dates of assessment, the number of tillers did not present significant interactions between treatments (Spp x WR) and was not significantly affected by WR ($p > 0.05$). Significant differences were found only for the Spp at all five dates of assessment ($p \leq 0.001$ for all). The same pattern of vegetative growth was observed for all species, with an overall increment in the number of tillers from day 0 (first date of assessment) up to day 29 (final date). Amongst species, the Lp consistently had the highest number of tillers per pot, generally double the number of tillers that Bv and Dg presented. The Bv and Dg had a similar number of tillers per pot (Table 5).

The daily lamina elongation rate (LER) presented significant triple interactions (Spp x WR x Time) on days 4, 16, 19 and 22 ($p \leq 0.05$, $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.05$, respectively) and also presented a trend of difference on day 13 and 26 ($p \leq 0.1$). On day 4, BvLR and BvHR presented the highest LER, accumulating ~ 40 mm day^{-1} in total lamina length per tiller. At the same date, LpLR and LpHR elongated slower, accumulating 50% less than BvLR and BvHR, with an average lamina elongation rate of 20 mm day^{-1}. The following significant dates (days 16, 19 and 22), two to three weeks after the first assessment date (day 4), depicted the effect of water restriction throughout the experimental period as it presented a trend of diminishment on the LER for all species. On days 16, 19 and 22, DgHR accumulated less lamina length per tiller at a rate lower than 5 mm day^{-1}.

It is possible to observe the negative impact of water stress on the lamina development per tiller by the end of the experimental period, as DgHR accumulated 65% less lamina length than the DgLR (Fig. 5). The Bv and Lp plants subject to HR were less affected than the DgHR, with a smaller accumulation decrease of 37% and 25% than their LR pair, respectively. Within the three species submitted to HR treatment, Bv and Dg had a similar response pattern to the water stress, drastically decelerating the lamina elongation rate per tiller within the first ten days. Meanwhile, the Lp had a less accentuated drop in its elongation for the same period and presented a more noticeable deceleration towards the end of the experimental period.

3.5. Canonical variate analysis – identifying the differences amongst groups of individuals

The canonical variate analysis was significant (Wilk's Lambda = 0.0001), showing that the variables included in this study explained

Table 3

Effect of three plant species (Spp = Bv, Dg, Lp), two water level restrictions (WR = LR and HR) and the interaction between treatments on leaf water potential at day 27, plant osmotic potential and malondialdehyde leaf concentration (MDA) at day 32.

	Leaf water potential Day 27 *(MPa)*	Plant osmotic potential Day 32 *(MPa)*	MDA Day 32 *(μmol.L^{-1})*
Spp			
Bromus valdivianus (Bv)	- 1.9 (±0.10)	- 2.0 (±0.05)	1.1 (±0.16)
Dactylis glomerata (Dg)	- 1.9 (±0.09)	- 1.8 (±0.14)	0.7 (±0.12)
Lolium perenne (Lp)	- 1.7 (±−0.11)	- 2.2 (±0.27)	0.9 (±0.06)
Significance	NS	NS	NS
WR			
Low Restriction (LR)	- 1.7^{a} (±0.07)	- 1.7^{a} (±0.08)	0.7^{b} (±0.10)
High Restriction (HR)	- 2.2^{b} (±0.04)	- 2.3^{b} (±0.16)	1.0^{a} (±0.09)
Significance	***	**	*
Interaction Spp x WR			
BvLR	- 1.7 (±0.08)	- 1.9^{a} (±0.04)	0.8 (±0.260)
BvHR	- 2.1 (±0.00)	- 2.1^{a} (±0.06)	1.3 (±0.003)
DgLR	- 1.7 (±0.08)	- 1.7^{a} (±0.17)	0.5 (±0.038)
DgHR	- 2.1 (±0.00)	- 1.9^{a} (±0.21)	1.0 (±0.131)
LpLR	- 1.6 (±0.12)	- 1.7^{a} (±0.12)	0.9 (±0.050)
LpHR	- 1.9 (±0.02)	- 2.8^{b} (±0.20)	0.8 (±0.122)
Significance	NS	*	NS

Superscripted letters that differ within the same columns and lines for the same fixed effect and/or interaction of fixed effects indicate statistically significant values at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p = 0.0001$ and NS = non-significant. Following each least square mean is the (±) standard error of the mean.

Table 4

Effect of three plant species (Spp = Bv, Dg, Lp), two water level restrictions (WR = LR and HR) and interactions between treatments on the lamina, sheath and dead material accumulation and its proportional distribution in relation to the total weight of above-ground biomass (lamina:total biomass) per pot and per tiller on Day 32.

	Lamina		Sheath		Dead Material		Lamina:total biomass	
	Pot *(g)*	Tiller *(mg)*	Pot *(g)*	Tiller *(mg)*	Pot *(g)*	Tiller *(mg)*	Pot %	Tiller
Spp								
Bromus valdivianus (Bv)	3.6 (±0.5)	92.2^{a} (±14.5)	0.8^{b} (±0.2)	20.2^{b} (±2.2)	1.5^{a} (±0.20)	32.9^{a} (±3.5)	60.3 (±3.7)	61.3 (±4.2)
Dactylis glomerata (Dg)	3.5 (±0.5)	104.9^{a} (±14.8)	1.6^{a} (±0.2)	66.6^{a} (±6.2)	0.6^{b} (±0.19)	21.2^{ab} (±5.8)	62.0 (±3.4)	54.5 (±4.7)
Lolium perenne (Lp)	3.7 (±0.3)	44.9^{b} (±6.4)	1.9^{a} (±0.1)	24.5^{b} (±3.1)	1.3^{a} (±0.07)	11.8^{b} (±1.7)	54.0 (±1.8)	55.2 (±1.9)
Significance	NS	**	***	****	*	*	NS	NS
WR								
Low Restriction (LR)	4.4^{a} (±0.2)	97.0^{a} (±14.2)	1.6 (±0.2)	40.4 (±8.8)	1.1 (±0.2)	18.1 (±3.2)	63.0^{a} (±2.1)	62.3^{a} (±1.9)
High Restriction (HR)	2.7^{b} (±0.2)	64.3^{b} (±9.7)	1.2 (±0.2)	33.8 (±7.2)	1.1 (±0.2)	25.7 (±5.0)	54.6^{b} (±2.5)	51.7^{b} (±3.1)
Significance	****	*	NS	NS	NS	NS	*	*
Interaction Spp x WR								
BvLR	4.7 (±0.3)	109.7 (±12.4)	1.1 (±0.14)	24.8 (±0.3)	1.5 (±0.31)	27.3 (±4.9)	64.7 (±3.8)	67.7 (±0.9)
BvHR	2.4 (±0.2)	74.7 (±24.3)	0.4 (±0.04)	15.5 (±1.8)	1.5 (±0.33)	38.4 (±2.1)	56.0 (±6.1)	55.0 (±6.8)
DgLR	4.5 (±0.5)	132.0 (±16.4)	1.8 (±0.48)	73.1 (±9.8)	0.5 (±0.25)	16.2 (±4.9)	66.7 (±4.1)	60.7 (±4.4)
DgHR	2.6 (±0.2)	77.8 (±9.3)	1.4 (±0.22)	60.2 (±7.6)	0.7 (±0.34)	26.2 (±11.0)	57.3 (±4.4)	48.3 (±7.3)
LpLR	4.3 (±0.2)	49.3 (±12.7)	1.9 (±0.26)	23.3 (±5.3)	1.3 (±0.04)	10.7 (±1.7)	57.7 (±0.9)	58.7 (±1.2)
LpHR	3.2 (±0.2)	40.4 (±5.1)	1.9 (±0.08)	25.7 (±4.3)	1.2 (±0.16)	12.9 (±3.2)	50.3 (±1.5)	51.7 (±2.0)
Significance	NS	NS	NS	NS	NS	NS	NS	NS

Superscripted letters that differ within the same columns and lines for the same fixed effect and/or interaction of fixed effects indicate statistically significant values at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p = 0.0001$ and NS = non-significant. Following each least square mean is the (±) standard error of the mean.

Table 5

Effect of three plant species (Spp = Bv, Dg, Lp), two water level restrictions (WR = LR and HR) and interactions between treatments on lamina surface area, specific lamina area, leaf regrowth stage per tiller on Day 32, and the number of tillers on day 0, day 8, day 15, day 22, and day 29.

	Lamina surface area D32 *(cm^2)*	Specific lamina area D32 *(cm^2g^{-1})*	Leaf regrowth stage D32	Number of Tillers D0	D8	D15	D22	D29
Spp								
Bromus valdivianus (Bv)	16.9^{b} (±6.2)	47.9^{b} (±9.3)	2.7 (±0.5)	72.8^{b} (±19.9)	75.5^{b} (±22.3)	74.2^{b} (±18.6)	74.3^{b} (±19.4)	76.7^{b} (±22.5)
Dactylis glomerata (Dg)	24.0^{a} (±11.4)	62.3^{a} (±6.3)	2.6 (±0.9)	59.7^{b} (±22.6)	60.0^{b} (±24.2)	60.8^{b} (±23.5)	64.5^{b} (±23.3)	66.2^{b} (±24.0)
Lolium perenne (Lp)	9.5^{c} (±4.2)	47.2^{b} (±3.2)	2.5 (±0.4)	129.3^{a} (±21.1)	132.5^{a} (±21.6)	134.5^{a} (±23.8)	133.0^{a} (±23.6)	138.5^{a} (±25.1)
Significance	***	*	NS	***	***	***	***	***
WR								
Low Restriction (LR)	21.9^{a} (±10.8)	53.9 (±10.5)	3.0^{a} (±0.4)	90.1 (±38.9)	90.9 (±37.6)	90.7 (±37.3)	93.9 (±35.4)	100.6 (±39.9)
High Restriction (HR)	11.7^{b} (±4.6)	51.0 (±8.9)	2.1^{b} (±0.3)	84.4 (±37.0)	87.8 (±41.6)	89.0 (±42.8)	87.3 (±41.3)	87.0 (±40.8)
Significance	***	NS	****	NS	NS	NS	NS	NS
Interaction Spp x WR								
BvLR	21.3^{b} (±2.8)	49.3 (±9.7)	3.0^{ab} (±0.1)	81.3 (±22.9)	83.3 (±25.8)	81.7 (±20.8)	84.0 (±20.7)	87.7 (±25.9)
BvHR	12.6^{c} (±5.5)	46.5 (±10.7)	2.3^{bc} (±0.4)	64.3 (±15.9)	67.7 (±19.9)	66.7 (±16.2)	64.7 (±15.3)	65.7 (±15.4)
DgLR	33.4^{a} (±6.3)	65.9 (±2.0)	3.4^{a} (±0.3)	59.3 (±32.8)	62.3 (±35.9)	63.3 (±34.4)	69.7 (±33.7)	73.3 (±32.6)
DgHR	14.6^{bc} (±4.3)	58.6 (±7.5)	1.8^{d} (±0.2)	60.0 (±14.4)	57.7 (±12.5)	58.3 (±13.1)	59.3 (±12.1)	59.0 (±14.5)
LpLR	10.9^{c} (±5.9)	46.5 (±3.3)	2.8^{b} (±0.4)	129.7 (±23.7)	127.0 (±21.0)	127.0 (±27.1)	128.0 (±26.2)	140.7 (±29.3)
LpHR	8.1^{c} (±1.4)	47.8 (±3.7)	2.2^{cd} (±0.1)	129.0 (±23.4)	138.0 (±25.1)	142.0 (±22.6)	138.0 (±25.1)	136.3 (±26.6)
Significance	*	NS	*	NS	NS	NS	NS	NS

Superscripted letters that differ within the same columns and lines for the same fixed effect and/or interaction of fixed effects indicate statistically significant values at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p = 0.0001$ and NS = non-significant. Following each least square mean is the (±) standard error of the mean.

95.6% of the results (CAN 1 = 88.4%, $p = 0.0001$, CAN 2 = 7.2%, $p = 0.0031$; Fig. 6). Within the CAN 1 axe, the number of tillers (NT) was located in its negative direction, showing a contrast with below-ground bottom-layer biomass (BG_bott), hydraulic lift via SWC% sensor readings (HL_swc), and $\delta^{18}O$ values (HL_O18), dead material of the above-ground biomass (AG_demat) and MDA concentration (MDA), which were located in the positive direction of CAN1. In the positive direction of CAN 2, there were located the lamina area (LA), lamina and sheath of the above-ground biomass (AG_lam and AG_shth), below-ground top-layer (BG_top), BG_bott, HL_swc, and in the negative CAN 2 direction there were NT and MDA (Fig. 7).

4. Discussion

The three species displayed some degree of hydraulic lift assessed using both direct (isotopic labelling) and indirect (SWC% sensors) techniques, validating that they can exhibit the phenomena at their own capacity. Results from the current study showed that *B. valdivianus* (to a higher degree), *D. glomerata* (to a medium degree) and L. *perenne* (to a lower degree) redistributed water from the deep to shallower soil layer via the hydraulic lift phenomenon. Complementary information on morpho-physiological responses of each species subjected to different levels of water restriction allowed us to (a) infer how they perform under soil water restriction, (b) correlate their functional strategies with the hydraulic lift phenomenon, (c) hypothesise on future practical applications of the species in a pastoral ecosystem, and (d) suggest new methodologies that can be helpful to the development of this research topic. The role of the capillary barrier utilised in the experimental pots contributed to the robustness of the study data generated.

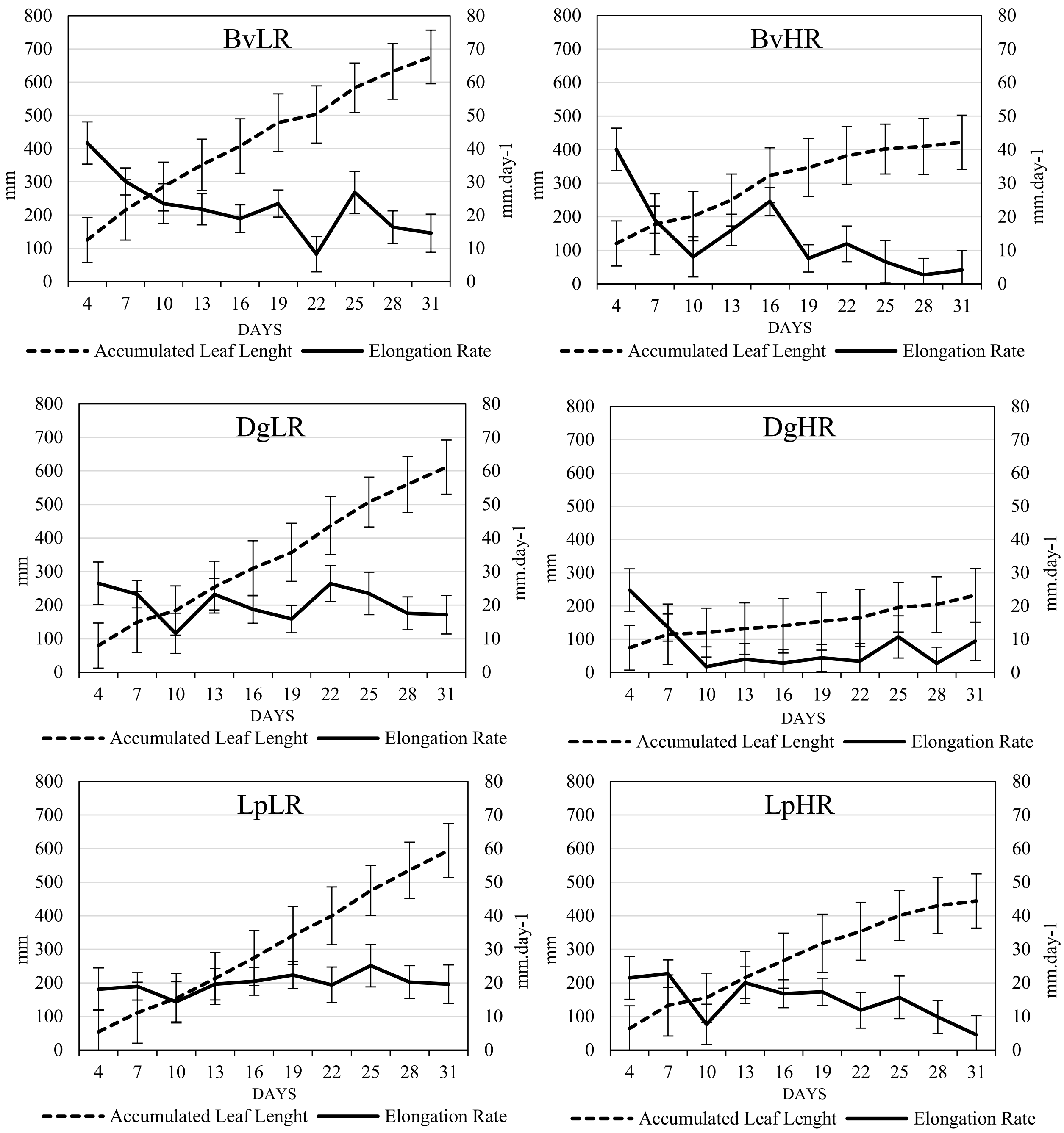

Fig. 5. The accumulated lamina length (mm - in dashed black) and daily lamina elongation rate (mm.day^{-1} - in solid black) per tiller of the interaction between species, levels of water restriction and time (Spp x WR x Time). Standard errors of the mean are presented as vertical bars and are significantly different across time between species and levels of water restriction for the same response variable.

4.1. Can the hydraulic lift be associated with grasses under water stress conditions? If so, what are the drivers that trigger the phenomenon?

Meunier et al. (2017) carried out a study to model the hydraulic lift of L. *multiflorum* in rhizo-boxes, and a day of the application of ^{18}O labelled water found an enrichment of 2.0 and 1.3‰ in comparison to control at the shallower layers (20–25 cm depth). In the present study, the $\delta^{18}O$ values found in the top layers evince that the three species exhibited the hydraulic lift at their capacity during daytime and peaking around midday. *Bromus valdivianus* can redistribute water to a greater degree than *D. glomerata* and L. *perenne* under water stress, showing approximately double the $\delta^{18}O$ values in the soil top layer (Table 2).

Recent studies have shown that the type of root plays an essential role in the plant's ability to uptake water under drought or water restriction conditions, such that species with shorter root hairs (i.e., *O. sativa* and *Z. mays*) have no contribution to the water uptake (Carminati et al., 2016; Ahmed et al., 2018; Cai and Ahmed 2022). Meanwhile, species with longer root hairs (i.e., *Hordeum vulgare*) had an explicit influence on the water uptake levels (Burak et al., 2021; Cai and Ahmed 2022). The root morphology and allocation in depth may partially explain the hydraulic lift occurrence. *Bromus valdivianus* and *D. glomerata* were the species that presented greater root mass in the bottom layer (deep-rooted if compared to L. *perenne*) and are known to present root growth in depth as a functional trait of both species (García-Favre et al. 2022; Crush et al., 2005). Meanwhile, L. *perenne* allocates fewer roots in depth (shallow-rooted) (Wedderburn et al., 2010;

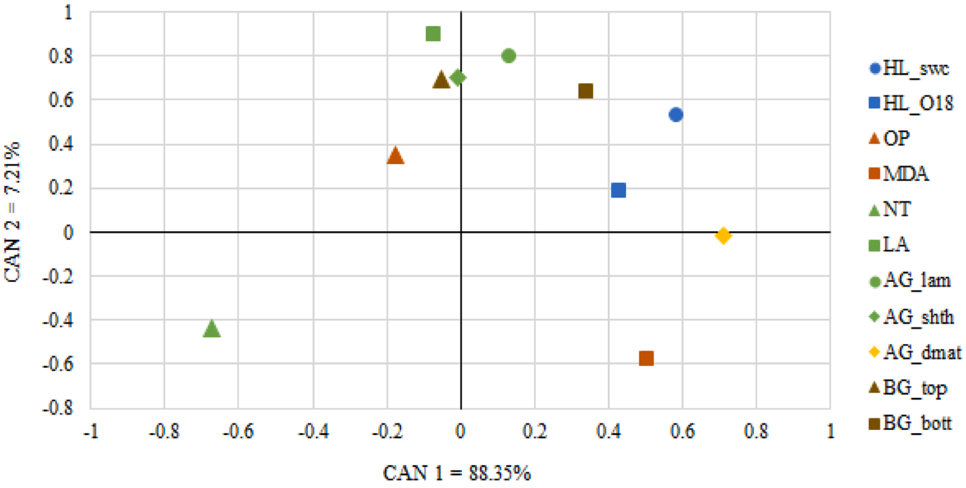

Fig. 6. Canonical variates of the original variables and their relationships for Bromus valdivianus, Dactylis glomerata and Lolium perenne due to high and low levels of water restriction: hydraulic lift via SWC% sensor readings (HL_swc), hydraulic lift via δ^{18}O values (HL_18O), plant osmotic potential (OP), MDA concentration (MDA), number of tillers (NT), lamina area (LA), lamina of the above-ground biomass (AG_lam), sheath of the above-ground biomass (AG_shth), dead material of the above-ground biomass (AG_demat), below-ground top-layer biomass (BG_top) and below-ground bottom-layer biomass (BG_bott).

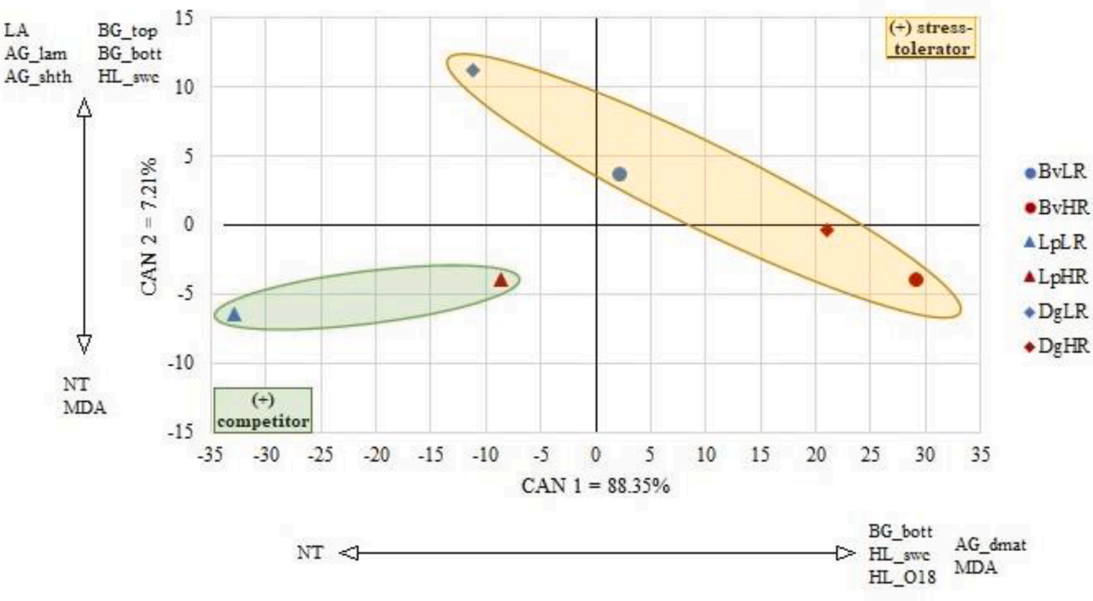

Fig. 7. Canonical scores for the interaction of fixed effects: species (Spp = Bromus valdivianus - Bv, Dactylis glomerata – Dg and Lolium perenne - Lp) and levels of water restriction (WR = high restriction – HR and low restriction - LR).

García-Favre et al. 2022), a functional trait likely to constrain the species' hydraulic lift occurrence. In the present study, a more even root system distribution was found in *B. valdivianus*, which had a dry matter ratio of approximately 4:1 (0–20cm:20–40 cm), while *D. glomerata* and L. *perenne* had approximately 6:1 and 5:1, respectively. In the present study, L. *perenne* under low water restriction did not exhibit hydraulic lift, as evidenced by δ^{18}O value [3.40‰ (±3.17)]. This leads us to conclude that probably shallow-rooted plants are less capable of exhibiting hydraulic lift under low levels of soil water restriction because of this morphological restrictive attribute.

The fluctuations of SWC% displayed on five different days, with an increase in the soil water availability during the hours of highest temperature (towards midday), demonstrated that *B. valdivianus* under low and high restriction, *D. glomerata* under low and high restriction, and L. *perenne* under high restriction can exhibit hydraulic lift depending on the levels of soil water stress (Fig. 4). On day 9, the highest fluctuations in SWC% were registered, interpreted as the day in which the highest hydraulic lift was exhibited. On this day, the vapour pressure deficit rose to 41.34 hPa, leading to a linear transpiration rate increase (Rawson et al., 1977). In addition, on these five dates, similar environmental conditions were observed, in which the daily light integral was at or above 80 mol m^{-2} s^{-2}, or double the value in relation to the previous day. The energy required to evaporate water from leaves and plants comes mostly from direct solar radiation, which also leads to an increment in the transpiration rate and, consequently, an increased movement of water and solutes by mass flow through the xylem to transpiring surfaces of shoots (Kramer and Boyer 1995). The peaking values of SWC % observed around midday, interpreted as the hydraulic lift, can be associated with two complementary factors: (a) increased transpiration rates that result in a higher sap rising flow within the xylem to subsidise water for leaves and shoots and (b) within a higher rate of water movement, some water was released (or lost) to the soil, driven by the extremely low top layer SWC% (below PWP). The most negative plant osmotic potential was found in the L. *perenne* under high restriction, which can be related to a higher physiological stress level due to the high level of water restriction applied (Table 3). In the present study, even though the water potentials were not specifically measured in all soil and plant compartments, it is possible to infer through the soil water retention curve and plant water potential measurements (plant osmotic potential and leaf water potential) that the hydraulic lift was driven by the water potential soil gradient. When the top layer was permanently under PWP (−15,000hPa), and the plants had a plant osmotic potential and leaf water potential of ~ 2.0 MPa, part of the water flowing more intensively during the transpiration peak is transferred passively to the soil due to differences in water potential.

Beyond that, differences in the root membrane permeability of each species could be hypothesised as an additional morpho-physiological factor that can facilitate water transit and, therefore, redistribution inter mediums. However, there is not enough evidence to support this argument in the present study. Specific functional traits of roots (i.e., maximum rooting depth and proportional distribution, water acquisition and utilisation, and association with mycorrhizal fungi) focusing on the potential hydraulic lift occurrence that a species can exhibit remain gaps to be fulfilled in this research topic (Prieto et al., 2012; Freschet et al., 2021). A major barrier to overcome in such studies includes methodologies to infer, non-destructively and accurately, the hydraulic lift's occurrence, magnitude and potential agricultural applicability (i.e., bioirrigation). Hence, the necessity to apply interdisciplinary approaches, such as the combination of high-frequency laser spectrophotometry to analyse the eddy covariance of isotopes during soil-plant-atmosphere processes (isotopic flux partitioning) (Good et al., 2014) or highly sensitive and real-time and non-invasive imaging techniques (e.g., δ^2H neutron imaging) (Cai et al., 2022).

4.2. Is the hydraulic lift a characteristic of competitors and/or stress-tolerators? How does it relate to morpho-physiological responses on grasses under soil water restriction?

The phenotypic plasticity of the three species under soil water restriction was evinced by the morphological responses of the plant components in contrast to the above-ground biomass accumulation (leaf growth x tillering). A compensatory effect in response to the environment alters resource utilisation, often seen as changes in the development rate amongst organs due to abiotic stress (Huber et al., 1999). In the present study, L. *perenne* presented a higher number of tillers per pot and a lower lamina area and lamina dry matter accumulation per tiller; *B. valdivianus* and *D. glomerata* presented an opposite pattern, with a lower number of tillers but a higher lamina surface and lamina dry matter accumulation per tiller (Tables 4 and 5). However, the final lamina dry matter mass accumulated and the ratio of 'lamina mass:total biomass' were similar between the three species. This is an important compensatory effect, previously assessed for grasses (López et al., 2013), in which the species present different growth strategies whilst accumulating similar lamina mass and total above-ground mass. These growth strategies can be taken to a field scale when trying to optimise the species selection for the establishment of diverse pastures (Nelson 2000) and can also be used to optimise the species' performance for sites of variable environmental conditions (i.e., dry season, topography, flood periods, etc.).

Regarding water restriction levels, the MDA concentration showed that when submitted to HR treatments, the three species were more stressed (1.04 μmol L^{-1}), negatively impacting total biomass accumulation (Tables 1 and 3). The LR pots' above-ground biomass was 41.2% higher than the HR pots for all three species, with increment over time driven mostly by the lamina accumulation, as this was the only component with significant differences in above-ground biomass accumulation due to water stress (Tables 1 and 4). Changes in soil water availability initially affect the lamina elongation rate rather than the tiller density (Bahmani et al., 2003; García-Favre et al., 2021a). During the three initial dates of lamina elongation assessment, the BvLR, BvHR, DgLR and DgHR treatments had a clear downward trend in their LER, and that can be related to the levels of water stress that the plants were subjected to. After the third assessment date, DgHR had a LER close to zero, corroborating previous studies on *D. glomerata* under water stress (Volaire et al., 1998).

Meanwhile, *L perenne* under both water restriction regimes were not substantially affected by the top-layer drought imposed and the two respective levels of water restriction on the bottom layers (Fig. 5). It is well established that soil water restriction results in an initial plateau in the herbage accumulation, followed by overall diminishment and, after a prolonged time, negatively affects plant persistence and survival (Thomas and James 1999; Arredondo and Schnyder 2003; Skinner et al., 2006; Nie and Norton 2009; Turner et al., 2012; Rogers et al., 2019). López et al. (2013) conducted a study showing that *B. valdivianus*, under water stress, tends to accentuate its below-ground accumulation rather than above-ground. In a drought resistance study with L. *perenne* and *D. glomerata* under full irrigation and prolonged summer drought, the higher survival rate after the drought was closely related to the species' ability to maintain roots at deeper depths and readjust their water potential on the surviving leaves (Volaire et al., 1998). Conversely, in another study with L. *perenne* and *B. valdivianus* as mixtures and monocultures, the deeper-rooted species (*B. valdivianus*) was responsible for a 15% increment in herbage mass production during the low water availability season, in comparison to the monoculture of the shallower-rooted species (L. *perenne*) (García-Favre et al. 2022).

The different responses of grasses to abiotic stress can determine pasture succession in the long term. Hence, it is possible to divide the three species studied here according to their short-term morpho-physiological responses to the drought conditions imposed, whereby L. *perenne* could be classified as a drought-resistant species that continues growing under moderate drought conditions (e.g., up to a month in the present study). In comparison, *B. valdivianus* and *D. glomerata* could be classified as drought survivors that almost or entirely cease their growth and can, therefore, survive periods of more prolonged drought (Volaire 2018). Conversely, the Competitors (C), Stress-tolerators (S), and Ruderals (R) theoretical model proposed by Grime (1974) categorises species due to their functional roles in the ecosystem, intra- and inter-specific competition ability under abiotic and biotic stress and succession mechanisms. According to the C-S-R model, competitors can monopolise resource capture faster by the spatially dynamic foraging of roots and shoots, stress-tolerators have long-lived tissues and mechanisms to cope with environmental stress where growth is severely restricted, and ruderals are short-lived with the tendency to invest in resources caption to produce offsprings rapidly. The present study analyses the three grass species according to the C-S-R model and, under current experimental conditions, Bv and Dg fit in the stress-tolerators class and L. *perenne* as a competitor. The CVA analysis highlighted the differences in morpho-physiological strategies adopted by the three species (*B. valdivianus, D. glomerata,* and L. *perenne*) under levels of water restriction (high and low water restriction), enforcing both Grime's and Volaire's propositions around abiotic stress effects on individuals (Figs. 6 and 7). It is important to reinforce that in this current study, the baseline for all species was a drought condition with the top layer continuously under PWP, and the corresponding amounts of water were irrigated only into the bottom layer. In that respect, the responsiveness of Lp under different levels of drought was very subtle. At the same time, *B. valdivianus* and *D. glomerata* exhibited varied morpho-physiological responses due to the imposed moderate (low restriction treatments) or severe (high restriction treatment) drought conditions.

The positive positioning of variables in CAN 1 and CAN 2 was closely related to the species' ability as a stress-tolerator, whereas the negative positioning was mostly explained by the species' ability as a competitor (Grime 1974). The L. *perenne* under both water restriction regimes was closely located in the left bottom zone of the CVA, towards the negative direction of CAN 1 and CAN 2 (Fig. 7). The close positioning and small variation within the CAN 2 axis showed that Lp strategies did not change under different levels of water stress, whereas the graph depicts a considerable differentiation of Bv and Dg strategies under the two water stress conditions. The *B. valdivianus* and *D. glomerata* under low water restriction were located towards the positive direction of CAN 2, exhibiting a high LA, lamina and sheath weight per tiller. Meanwhile, the *B. valdivianus* and *D. glomerata*, under high water restriction, were located around the central area of the CAN 2 axis, which shows a low mobilisation of resources to grow. This short-term strategic response is probably associated with the higher level of water stress applied. In addition, the CAN 1 location of *B. valdivianus* and *D. glomerata* under high water restriction in the far positive direction indicated how they were affected by the drought imposed, with clear responsiveness in the form of MDA and dead material per tiller. Although the *B. valdivianus* and *D. glomerata* plants had different responses in herbage growth due to water stress, they had root allocation as a common characteristic. Along the CAN 2 axis, *B. valdivianus* and *D. glomerata,* under low water restriction*,* were placed in the positive direction, following an increase in the top and bottom layer root accumulation. Concomitantly, along the CAN 1 axis, the *B. valdivianus* and *D. glomerata,* under high water restriction*,* were placed in the far positive direction, where the hydraulic lift and bottom-layer root allocation increased. Hence, *B. valdivianus* and *D. glomerata* plants can be characterised as drought survivors and stress-tolerators due to the root allocation and hydraulic lift (functional traits) but can also present competitor characteristics when under more favourable conditions of soil water availability, as seen by the greater herbage accumulation of the *B. valdivianus* and *D. glomerata* under low water restriction.

Grime's theory and Volaire's propositions facilitate the selection process of suitable species to compose a pastoral ecosystem given the chemical and physical attributes of the soil, rainfall conditions, latitude, and altitude. Moreover, potential species with the desirable characteristics to comprise a diverse pasture can be selected because of their complementary functionality, with different roles that fulfil agroecological spaces in a pastoral ecosystem. Such a combination of species can be a key process to strategically overcome pasture persistence threats related to more regular extreme climatic events.

5. Conclusion

The isotopic labelling methodology and soil water content monitoring methodologies adopted in the study enabled the validation and assessment of the hydraulic lift in grass species. These methodologies indicated that all three species exhibited hydraulic lift, *Bromus valdivianus* Phil. to a greater, *Dactylis glomerata* L. to a medium and *Lolium perenne* to a lesser degree. The phenomenon occurred between 10 AM and 3 PM daily, with the top layer SWC% peaking when the temperatures were the highest in the day, at around 12 PM. The root morphology and allocation at depth may explain the phenomenon occurrence, as a more even root system distribution was found in Bv, which had a dry matter ratio of approximately 4:1. In contrast, L. *perenne* and *D. glomerata* had approximately 6:1 and 5:1, respectively.

The negative impact of water stress affected the three species differently, with *B. valdivianus* and *D. gloemerata*, drastically decelerating the lamina elongation rate per tiller within the first ten days, while L. *perenne* did not completely slowed its growth, presenting a postponed response with a more noticeable deceleration towards the end of the experiment period. In addition to that, the multivariate analysis highlighted the three grass species' morpho-physiological strategies to cope with soil water stress according to the C-S-R model. *Lolium perenne* strategies did not change under different levels of water content, whereas the graph depicts a considerable differentiation of both *B. valdivianus* and *D. glomerata* strategies. Therefore, the study indicates that *B. valdivianus* and *D. glomerata* presented attributes of stress-tolerators, and under lower levels of water restriction can also present attributes of competitors, while L. *perenne* only presented attributes of competitors, highlighted by its lack of adaptative responsiveness under water stress.

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CRediT authorship contribution statement

Bia Anchão Oliveira: Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ignacio Fernando López:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Lydia Margaret Cranston:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis. **Peter David Kemp:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Daniel Joseph Donaghy:** Writing – review & editing, Funding acquisition. **José Dörner:** Writing – review & editing, Validation, Formal analysis, Conceptualization. **Nicolas López-Villalobos:** Writing – review & editing, Validation, Formal analysis. **Javier García-Favre:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Iván Pablo Ordóñez:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Robert Van Hale:** Data curation, Formal analysis, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw data were generated at the Plant Growth Unit, Massey University, New Zealand. Derived data supporting the findings of this study are available from the first author, Oliveira B A, upon reasonable request.

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