Chilean J. Agric. Anim. Sci., ex Agro-Ciencia (2025) 41(1): 44-58.
https://doi.org/10.29393/CHJAAS41-6CDPJ50006

ISSN 0719-3890 online

COIRÓN DULCE (*Festuca gracillima* Hook, f.) GROWTH PHYSIOLOGY UNDER DIFFERENT DEFOLIATION INTENSITY LEVELS AND WATER RESTRICTION REGIMES

Paula Oyaneder[2a, 1b], Iván Ordóñez[1a*], Sergio Radic-Schilling[2b], Ignacio López[3], and Jorge Ivelic-Sáez[4, 1c]

[1a] Agricultural Research Institute (INIA, Kampenaike), Punta Arenas (6200000), Chile. https://orcid.org/0000-0003-3397-0217
[1b] Agricultural Research Institute (INIA, Kampenaike), Punta Arenas (6200000), Chile.
[1c] Agricultural Research Institute (INIA, Kampenaike), Punta Arenas (6200000), Chile. https://orcid.org/0000-0001-6613-7382
[2a] University of Magallanes, Faculty of Sciences, Department of Agricultural and Aquaculture Sciences, Punta Arenas (6200000), Chile.
[2b] University of Magallanes, Faculty of Sciences, Department of Agricultural and Aquaculture Sciences, Punta Arenas (6200000), Chile. https://orcid.org/0000-0003-1812-9235
[3] School of Agriculture and Environment, College of Sciences, Massey University, Palmerston North (4442), New Zealand. https://orcid.org/0000-0001-8119-1037
[4] Doctoral Program in Agricultural Sciences, Austral University of Chile, Valdivia (5090000), Chile. https://orcid.org/0000-0001-6613-7382
* Corresponding author: ivan.ordonez@inia.cl

ABSTRACT

Festuca gracillima **Hook, f. is a tussock grass species that grows and dominates the rangelands of the Magellan Region in Chile. It may exhibit tolerance to water restriction, while low defoliation intensity could encourage *F. gracillima* growth regardless of the severity of water deficit. This study aimed to evaluate the effects of defoliation intensity under two water restriction regimes. Two factors were evaluated, defoliation intensity and water restriction regimes. The first factor was defoliation intensity, with four defoliation heights: 3, 5, 8 and 10 cm. The second factor was two levels of water restriction regimes: one emulating typical field conditions of the rangeland, and the other simulating severe water restriction. The experimental design was a randomised complete block design (four defoliation heights × two water restriction regimes × five blocks). The experiment was conducted in a glasshouse, and the data were interpreted using accumulated growing degree days (GDD; base temperature of 0 °C). Defoliation at a height of 10 cm promoted lamina elongation and plant growth under both water restriction regimes, compared to treatments with a greater defoliation intensity. The phyllochron for the species was estimated to range between 262 and 286 GDD across all treatments, increasing up to 504 GDD under severe water restriction at the end of the experimental period. Soil water restriction delayed plant development (i.e., increased the phyllochron) and extended leaf lifespan. *Festuca gracillima* exhibits a conservative growth strategy (slow traits), which enables it to tolerate high levels of water restriction.**

Keywords: Water restriction, defoliation heights, phyllochron, lifespan, turnover.

Received: December 16, 2024 Accepted: April 11, 2025

INTRODUCTION

Festuca gracillima Hook, f. is a grass species configured as tussocks that dominates and structures the sub-Antarctic rangelands of Patagonia (Radic-Schilling et al., 2021; 2022). These ecosystems contribute to 56% of the sheep livestock in Chile (INE, 2022) and serve as the primary food source for sheep during the late autumn, winter, and spring periods in Patagonia (Radic-Schilling et al., 2021; 2022). The presence of *F. gracillima* is associated with climatic and edaphic factors. It thrives in shallow soils characterized by slow organic matter decomposition (Pisano, 1977) and typically grows in xeric environments, enduring constant soil water restriction near the permanent wilting point (PWP = 1,500 kPa) throughout the growing season, particularly during summer when soil water potential often drops below the PWP (Ivelic-Sáez et al., 2021; Ordóñez et al., 2023).

Festuca gracillima has been reported to exhibit 'slow traits' (Ordóñez et al., 2022), a resource-conserving strategy that enhances its capacity to withstand severe environmental constraints according to Reich (2014). The vegetative stage of *F. gracillima* occurs in autumn and resumes after winter, typically starting in August, with regrowth initiating until the reproductive stage begins, which spans from late November to mid-February (Oliva, 1996). During the vegetative growth phase, particularly in spring and early summer, declining rainfall and frequent strong wind (wind gusts exceeding 100 km h^{-1}) contribute to persistent soil water restriction (Ivelic-Sáez et al., 2021; Ordóñez et al., 2023).

In the Magellan Region, the period of water restriction in rangeland ecosystems can extend from October to March and is characterized by high evapotranspiration rates and low rainfall (González-Reyes et al., 2017; Ivelic-Sáez et al., 2021). Climate change projections for the region suggest an increased atmospheric water demand, driven by rising temperatures and wind speed (Soto-Rogel et al., 2020), along with the higher likelihood of severe drought events (González-Reyes et al., 2017). These conditions create a scenario of severe water limitation, with uncertain consequences for the survival and growth of *F. gracillima*.

Defoliation targets, which determine both the frequency and intensity of defoliation, remain unidentified for native species in Patagonia, including *F. gracillima*. These parameters are crucial for: i) establishing defoliation criteria to promote regrowth (Fulkerson and Donaghy, 2001; Ordóñez et al., 2021); ii) enhancing growth under water-restricted conditions (García-Favre et al., 2021b); and iii) ensuring adequate accumulation of water-soluble carbohydrates to support the ability of the plant to survive climatic stress (Zwicke et al., 2015). Defoliation frequency refers to the stage of plant development at which energy reserves have accumulated sufficiently to secure plant growth and stimulate regrowth (García-Favre et al., 2021a; Ordóñez et al., 2021), while defoliation intensity refers to the appropriate residual height (defoliation height) that prevents damage to energy reserves and protects the apical meristems of grasses (Donaghy and Fulkerson, 1998; Fulkerson and Donaghy, 2001; Turner et al., 2007). Grasses exhibit a hierarchical allocation of photoassimilates, prioritizing: i) restoration of photosynthetic area, ii) replenishment of energy reserves, iii) root growth, and/or iv) tiller initiation (García-Favre et al., 2021a,b; Ordóñez et al., 2021). Consequently, defoliation impacts the growth of each organ, with root development and tiller initiation being the most negatively affected functions (Fulkerson and Donaghy. 2001; Ordóñez et al., 2021).

Accordingly, defoliation frequency and intensity are key parameters for implementing controlled grazing practices, as they influence plant growth and survival under stress conditions, ultimately affecting the persistence and productivity of desired species within forage ecosystems (Turner et al.. 2007; Ordóñez et al., 2021). However, these agronomic parameters have not been defined for *F. gracillima*, prompting the following research questions: i) what is the impact of defoliation height on plant regrowth? ii) can low defoliation intensity enhance the plant's tolerance to typical local and severe water restrictions?

We hypothesize that *F. gracillima* exhibits a high tolerance to water-limited conditions, reflected on its leaf growth dynamics, and that low defoliation intensity promotes growth regardless of the severity of water stress. The aim of the study was to evaluate the effects of defoliation intensity under two soil water restriction regimes: one simulating the conditions typically observed in the sub-Antarctic rangelands of the Magellan Region during spring, and the other representing a severe water restriction scenario.

MATERIALS AND METHODS

Experimental conditions and experimental design

The study was conducted in a glasshouse at the Institute of Patagonia, University of Magallanes, Punta Arenas, Chile (53° 7′ 51.21″

S and 70° 53′ 8.68″ W). The experiment was established on June 1, 2021. The study period began on September 3, 2021, with the application of defoliation intensity treatments and water restriction regimes, and concluded on December 15, 2021, with the harvest of the plants. Temperature inside the glasshouse was recorded hourly at pot height. The ventilation control system of the glasshouse prevented extreme temperatures exceeding 35°C (Sage and Kubien, 2007). A total of 40 pots, each with an of 18 L volume capacity, were used. The pot substrate, which was soil from INIA's experimental stations (52°42′17.23″S and 70°55′20.48″W), contained 25.5 mg kg^{-1} Olsen-P; 813.2 mg kg^{-1} exchangeable K; 84.18 mg kg^{-1} S-SO_4^{2-}; 0.01 ($cmol_{(+)}$ kg^{-1}) exchangeable aluminium; and 53.2 ($cmol_{(+)}$ kg^{-1}) total exchangeable bases (Agronomy Soil Laboratory, Universidad Austral de Chile). As nitrogen is typically a limiting nutrient in agroecosystems (Vitousek et al., 1991), an equivalent dose of 100 kg N ha^{-1}in the form of urea fertilizer, was applied to each pot to prevent nitrogen deficiency. The nitrogen supplementation ensured proper plant establishment and growth during the study period (García-Favre et al., 2021a, b).

The soil had a silty loam texture with 8.3% clay, 79.5% silt and 12.2% sand (Agronomy Soil Laboratory, Universidad Austral de Chile). In order to determine the water restriction treatments, the field capacity (FC) and PWP were assessed. Therefore, soil water retention curve was determined on soil samples (n=5) randomly collected from the pots (one sample per block) using metallic cylinders of 220 cm^3. The samples were saturated from below for 48 h to reach 0 kPa, and then equilibrated at the following matric potentials in pressure chambers: -6, −33 and -1,500 kPa (Agronomy Soil Laboratory, Universidad Austral de Chile) (Dörner et al., 2015; Ordóñez et al., 2018).

A total of 40 plants were randomly collected from the field, within an area of 10 × 10 meters, at INIA's experimental station (52°42′17.23″S and 70°55′20.48″W). From each plant, a total of five individual tillers with roots were obtained and transplanted into the same pot, positioned at five equidistant positions: one in the centre of the pot and the remaining 4 near the edges of the pot, to minimize bias due to edge effects. All evaluations at plant and tiller levels were performed on the plant positioned at the centre of the pot.

The study consisted of four defoliation intensity levels: i) defoliation at a height of 3 cm, ii) defoliation at a height of 5 cm h, iii) defoliation at a height of 8 cm, and iv) defoliation at a height of 10 cm; as well as two soil water restriction regimes: i) a control treatment, in which the first 10 cm of soil were maintained at soil water content (% SWC) around the PWP (±3.6% SWC around PWP = 21.3% SWC), and ii) a severe water restriction treatment, in which the first 10 cm soil depth was maintained at values below the PWP, ranging from 5.5% to 7.5% SWC.

The severe water restriction period was applied for about 1,000 accumulated growing degree days (GDD), as defined later. Following this, a four-day recovery period was implemented, during which water was added to achieve 21,7% SWC. After the recovery period, a second severe water restriction period was applied for another 1,000 GDD similar to the treatments applied by Zwicke et al. (2015). The SWC was monitored at a soil depth of 10 cm every 15 min using five soil moisture sensors, using the factory calibration for mineral soils (Teros 10, Meter Group, USA) connected to a datalogger (ZL6, Meter Group, USA) for each water condition treatment. To control experimental errors resulting from environmental variation within the glasshouse (e.g., light, temperature, and humidity), the study was arranged according to a randomised complete block design with a factorial treatment distribution (Steel et al., 1997; Gutiérrez and de la Vara. 2008). The design included four defoliation intensity levels, two soil water restriction regimes, and five blocks (eq. 1). Blocking was performed as recommended by Fernandez (2007). To minimize environmental differences within each block due to spatial variation in the glasshouse (e.g., incident light, humidity, and temperature), and reduce the edge effect, pots within each block were randomly moved every two weeks (Fernandez, 2007; Hardy and Blumental, 2008; Ordóñez et al., 2021).

$$Yijk = \mu + \alpha i + \tau j + \beta k + (\alpha\tau)ij + \varepsilon ijk \qquad (eq\ 1)$$

Where Yijk= the response for (i) defoliation intensity levels, (j) soil water restriction regimes, and (k) blocks); μ= overall response; αi= effect of the "i" defoliation intensity levels; τj= effect of the "j" water restriction regimes; βk= effect of the "k" blocks; ($\alpha\tau$)ij= interaction between defoliation intensity and water restriction regimes (ij). εijk = random error.

Leaf expansion and all defoliation frequency cycles were recorded, calculated, and described based on GDD, with a base temperature of 0°C and no upper-temperature limit (McMaster and Wilhelm 1997; Ordóñez et al., 2021). To isolate defoliation intensity as the sole defoliation-related variable (independent of timing or frequency), all treatments and pots underwent synchronized defoliation (Turner et al., 2007). Three defoliations events were carried out during the study: i) the first defoliation occurred at the beginning of the study period, coinciding with the application of defoliation intensity levels and soil water restriction regimes; the herbage mass obtained from this defoliation event was discarded, ii) the second defoliation was performed when 1,000 GDD were completed, and iii) the third defoliation took place at the end of the experiment, following an additional 1,000 GDD accumulated after the recovery period.

Evaluated variables

Shoot growth

At the beginning of the experimental period (September 3, 2021), three tillers from the plant located in the centre of each pot were selected and marked by placing a coloured wire at their base. To determine growth as dry matter, the central plant in each pot was cut at the height corresponding to its assigned defoliation intensity treatment during each defoliation event. The harvested herbage mass was collected and then dried in an oven at 70 °C for 72 h (Ordóñez et al., 2021). Foliage from plants located close to the edges of the pots was also harvested but subsequently discarded. All calculations related to plant and tiller herbage mass were based on combined data from the second and third defoliations. To evaluate tiller population dynamics, the number of tillers on the central plant in each pot was recorded every 20 days. To evaluate the effects of defoliation intensity and water restriction regimes on plant growth, leaf appearance, leaf number, and lamina length were recorded for each tiller every 2 days. Lamina length was measured as the distance between the lamina tip and collar, considering only the green portion of the leaf. A leaf was considered fully expanded when its growth had ceased, it had reached its maximum length, the ligule was visualized, and the sheath was fully developed (Ordóñez et al., 2021).

To determine the phyllochron of *F. gracillima*, the appearance of a new laminae was recorded. A new lamina was considered to have 'appeared' when its tip became visible within the sheath of the preceding leaf (Wilhelm and McMaster, 1995). The average time between successive leaf appearances was determined and the phyllochron was calculated.

In order to determine leaf lifespan and the onset of senescence, quadratic equations were calculated from the leaf elongation data (Ordóñez et al., 2021). From the quadratic equations, the slope at 50 GDD was determined, as this is when energy reserves exert a greatest influence on leaf regrowth (Donaghy and Fulkerson, 1997; Fulkerson and Donaghy, 2001). The point at which the slope of each quadratic equation reached zero slope was also identified to estimate the number of GDD required to reach the plateau of lamina growth, which represents the onset of leaf senescence.

Root growth

At the final harvest, soil was carefully washed from the pots to evaluate root mass. The central plant in each pot, along with its entire root system, was separated from the surrounding four plants to allow for individual assessment and avoid edge effects. Live and dead roots were classified based on colour, with blackened roots indicating dead tissue and white or light- coloured indicating active, living tissue (Freschet et al., 2021). Root mass was dried at 70 °C for 72 h and weighed.

Statistical analysis

The normality of the data was evaluated using the Shapiro–Wilk test ($P<0.05$), and residual values and homogeneity of variance were assessed by Levene's test ($P<0.05$). Natural logarithm was used for data normalization for total plant mass, shoot mass, root mass, tiller weight, lamina length, and sheath length. The root square was used for data normalization for tiller number. ANOVA was performed on normally distributed data and the statistical significance was set at $P<0.05$. Treatment effects were analysed using Fisher's Least Significance Difference (LSD) test. Descriptive and non-parametric statistics were applied to data that could not be normalised (active root mass), using the Kruskal–Wallis test with a statistical significance set at $P<0.05$. Each pot was considered as an experimental unit. Data presented in graphs are shown as means with standard error of the mean (SEM). Regressions were employed to examine the relationships between phyllochron and GDD, phyllochron and soil water content, and lamina growth and GDD. Quadratic equations were used to explain lamina elongation (Ordóñez et al., 2021).

RESULTS

Significant differences were observed in plant mass, shoot mass, root mass, tiller weight, lamina length, and sheath length across defoliation intensities ($P<0.01$) and water restriction regimes ($P<0.001$). However, no interaction between these factors was detected ($P\geq0.05$). Consequently, each factor (defoliation intensity and water restriction regimes) was analysed separately for the parameters described above.

Soil water content dynamic and air temperature

Fig. 1A indicates that PWP was reached at 21.3% SWC (grey solid line), and FC at 33.2% SWC (dashed grey line). The SWC dynamics indicate that the water control treatment varied between 17.0% and 26.0%, with an average of 20.4% (SD±1.56%). In the severe water restriction treatment, the average SWC was 11.0% (SD±3.84%). During the first period, the

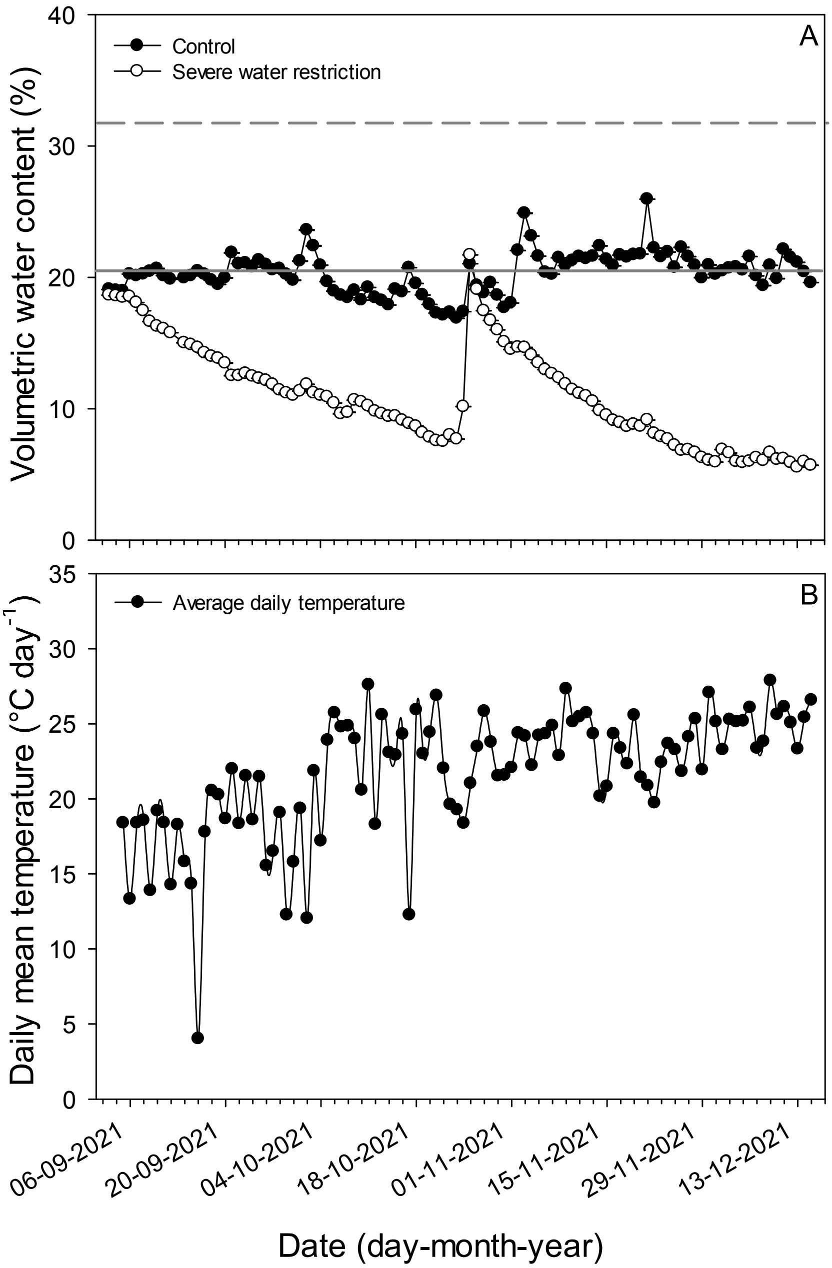

Fig. 1. Soil water content for the control and severe water restriction treatments. The dashed grey horizontal line indicates the volumetric water content at field capacity (FC; -33 kPa), and the solid grey horizontal line indicates the volumetric water content at permanent wilting point (PWP; -1.500 kPa) (A). Average daily temperature under glasshouse conditions during the experimental period (B). Bars represent the standard error of the mean (n= 5).

minimum SWC was 7.5% on October 24, 2021. In the second period of severe water restriction, the minimum SWC was 5.6% on December 14, 2021. During the recovery period for the severe water restriction, the SWC reached 21.7% on October 26, 2021 (Fig. 1A).

Average temperatures in the glasshouse ranged from 4,0 to 27 °C, with a mean of 21.7 °C (SD±4.2 °C) throughout the experimental period. Temperature steadily increased from the start of the experiment (September 3, 2021) until its completion (December 15, 2021) (Fig. 1B).

Effect of defoliation intensity on plant growth parameters

Significant differences were found in total plant dry matter ($P<0.01$; Fig. 2A). Defoliation at a height of 10 cm showed greater growth in terms of plant dry matter, with 2.94 g plant^{-1} (SEM±0.63), and shoot mass ($P<0.05$; Fig. 2B), with 2.38 g plant^{-1} (SEM±0.52), compared to the other defoliation treatments. For shoot mass, however, the same treatment was statistically similar to defoliation at a height of 5 cm.

Significant differences were found in total root mass ($P<0.01$; Fig. 2C). Total root mass for defoliation at heights of 10, 8 and 5 cm were statistically similar, with values of 0.56 g plant^{-1} (SEM±0.13), 0.33 g plant^{-1} (SEM±0.10) and 0.27 g plant^{-1} (SEM±0.06), respectively.

Significant differences were also observed in tiller weight ($P\leq0.01$; Fig. 2D). Defoliations at heights of 10 and 8 cm showed greater tiller weight, with values of 0.040 g plant^{-1} (SEM±0.006) and 0.029 g plant^{-1} (SEM±0.005), respectively.

Effect of water restriction regimes on plant growth parameters

Differences were found for the active mains roots (g plant^{-1}) for the two water treatments evaluated ($P<0.001$; Fig. 3B). The median for the water control treatment was 0.147 g root plant^{-1}, while the median for the severe water restriction treatment was 0.000 g root plant^{-1}. The third quartile for the control treatment was 0.223 g root plant^{-1}, compared to 0.027 g root

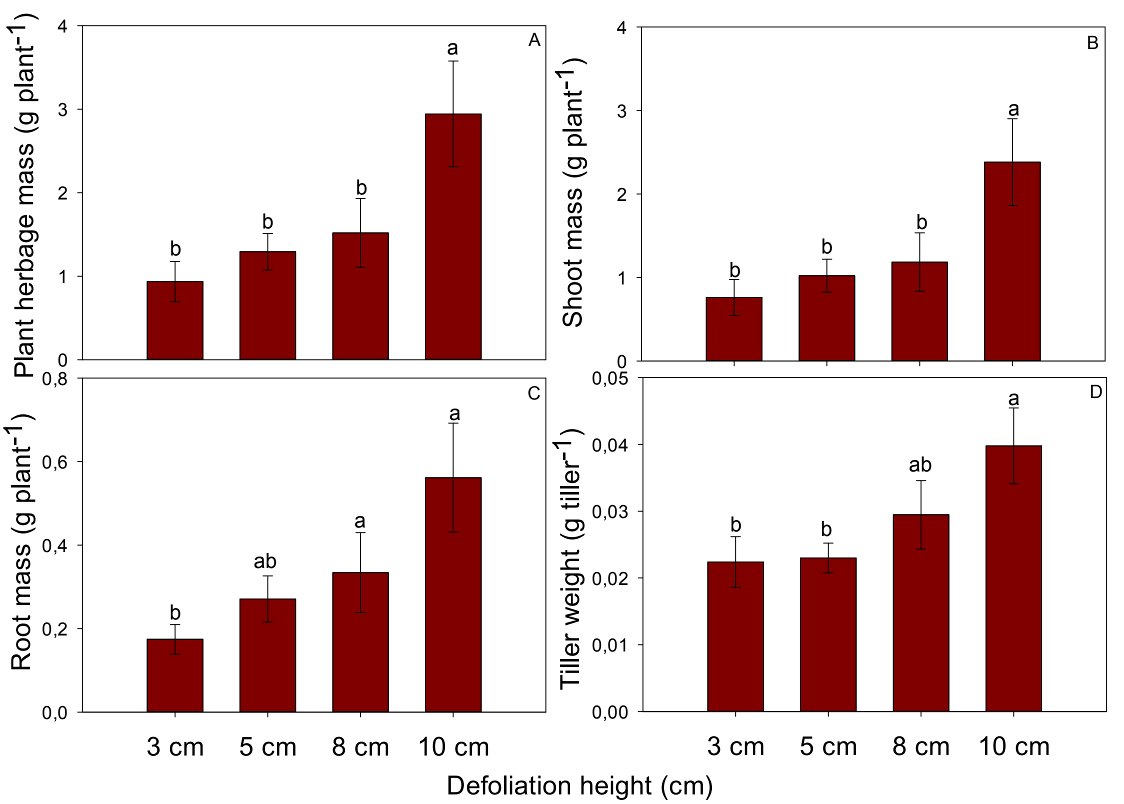

Fig. 2. Plant herbage mass (A), shoot herbage mass (B), root mass (C), and tiller weight (D) for all defoliation treatments. Bars represent the standard error of the mean (n= 10). Lowercase letters indicate statistical differences between treatments.

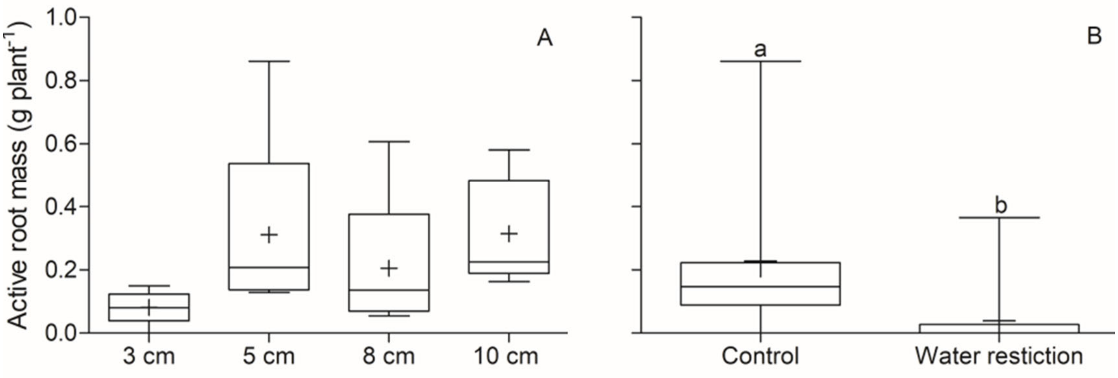

Fig. 3. Box plot for the active root for all defoliation treatments (A) and water restriction regimes (B). (+) indicates the mean. Lowercase letters indicate differences between treatments, for defoliation treatments (n=10) and for water restriction regimes (n=20).

plant^{-1} for severe water restriction. The fourth quartile for the control was 0.861 g root plant^{-1}, whereas it was 0.366 g root plant^{-1}under severe water restriction. These results indicate that 50% of the plants submitted to severe water restriction did not exhibit active main roots.

Table 1 shows higher values for the water control treatment compared to severe water restriction for the following parameters: total plant mass ($P<0.001$), shoot mass ($P<0.001$), root mass ($P<0.001$), tiller number ($P<0.01$), lamina length ($P<0.05$), and sheath length ($P<0.001$). Tiller weight was higher under the severe water restriction treatment compared to the water control treatment ($P<0.01$).

Tiller dynamics, lamina elongation, and phyllochron

Fig. 4 shows that the maximum lamina elongation of new leaves achieved with the defoliation treatment of 10 cm height (Fig 4A, 4B, 4C, and 4D). For the water control treatment, lamina length was 9.70 cm (Fig. 4A) and 11.2 cm (Fig. 4C). For the severe water restriction treatment, the lamina length was 9.4 cm (Fig. 4B) and 7.7 cm (Fig. 4D). Fig. 4E and F show the tiller dynamics for all defoliation treatments under the control (Fig. 4E) and severe water restriction treatments (Fig. 4F). No statistical differences were found between defoliation treatments in terms of tiller population for each water restriction regime ($P\geq0.05$).

Significant differences were found in average lamina length ($P<0.001$) and sheath length ($P<0.01$) (Table 2). Defoliation at a height of 10 cm achieved a lamina length of 6.92 cm plant^{-1} (SEM±0.25) and a sheath length of 2.60 cm plant^{-1} (SEM±0.59) (Table 2). Defoliations at a height of 3 cm resulted in the lowest lamina elongation, reaching a value of 2.87 cm plant^{-1} (SEM±0.31) and sheath elongation of 0.90 cm plant^{-1} (SEM±0.31) (Table 2).

Table 3 presents all quadratic equations, slope at 50 GDD, and the GDD required to reach zero slope for the lamina elongations showed in Fig. 4A, 4B, 4C, and 4D. All fitted equations exhibited an R^2 value greater than 0.81. Table 4 shows the slope at 50 GDD for the defoliation intensity treatment ($P\geq0.05$) and for the water restriction regimes ($P<0.01$). Additionally, Table 4 presents the GDD required to reach a zero slope for the water restriction regimes ($P<0.001$) and defoliation intensity ($P\geq0.05$). The zero slope for the quadratic equation in the water control treatment occurred at 468.7 GDD (±29.6), whereas it occurred at 896.3 GDD in the severe water restriction treatment (±84.6).

Fig. 5 shows the evolution of phyllochron values during the experimental period, expressed in GDD, for both the water control treatment (5A; $P<0.05$) and the severe water restriction treatment (5B; $P<0.001$). Additionally, it presents the linear regression between phyllochron and SWC (5C; $P<0.001$). At the beginning of the study, the phyllochron was estimated at 262 GDD for the water control treatment and 286 GDD for the severe water restriction treatment. The mean phyllochron over the experimental period was 247.6 GDD (SEM±4.5) under the water control treatment and

Table 1. Average values of growth parameters for the water control and severe water restriction treatments (n=20).

Water treatment	Total plant mass (g plant^{-1})	Shoot mass (g plant^{-1})	Root mass (g plant^{-1})	Tiller number (n° plant^{-1})	Tiller weight (g tiller^{-1})	lamina elongation (cm leaf^{-1})	Sheath elongation (cm leaf^{-1})
Control	2.48 a (±0.38)	2.00 a (±0.32)	0.48 a (±0.08)	34.5 a (±3.6)	0.024 b (±0.002)	5.02 a (±0.41)	2.46 a (±0.34)
SWC	0.93 b (±0.14)	0.72 b (±0.12)	0.20 b (±0.04)	15.0 b (±2.2)	0.035 a (±0.004)	4.29 b (±0.36)	0.92 b (±0.09)
P-value	<0.001	<0.001	<0.001	<0.001	<0.01	<0.05	<0.001

Data normalization with natural logarithm for total plant mass, shoot mass, root mass, tiller weight, and sheath length. Data normalization with root square for tiller number.

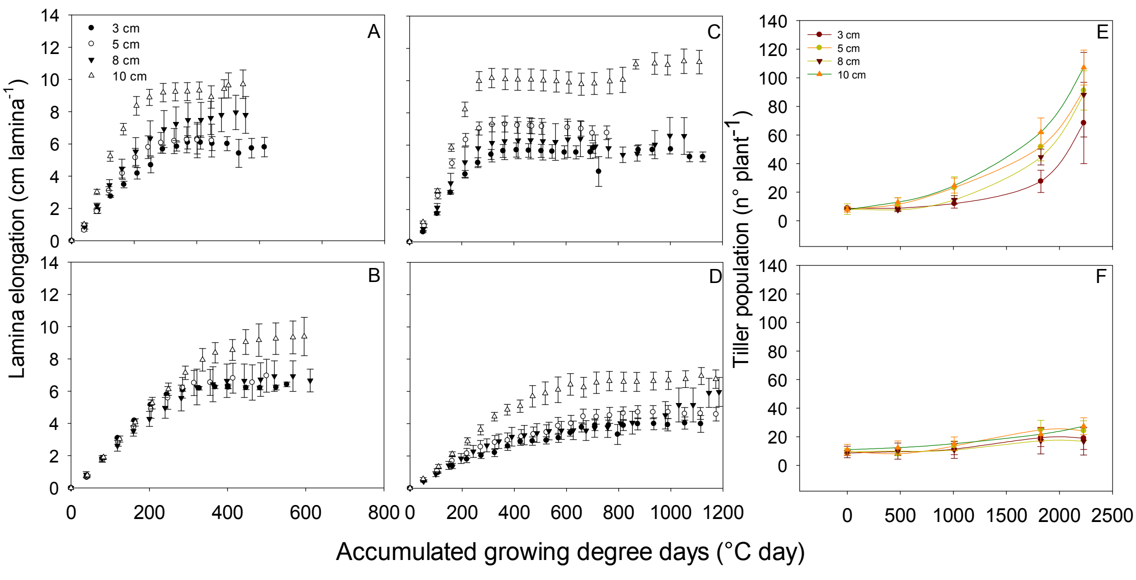

Fig. 4. Lamina elongation for all new leaves appeared during the evaluation period. Lamina elongation for the water control treatment for the first growing period (A). Lamina elongation for the water control treatment for the second growing period (B). Lamina elongation for the severe water restriction treatment for the first growing period (C). Lamina elongation for the severe water restriction treatment for the second growing period (D). Tiller dynamics for the control water treatment and defoliation treatments (E). Tiller dynamics for severe water restriction treatment and defoliation treatments (F). Bars represent standard error of the mean (n=5).

Table 2. Average lamina and sheath elongation for the different defoliation height treatments (n=10).

Defoliation height	Lamina length (cm plant^{-1})	Sheath length (cm plant^{-1})
3 cm	2,87 c (±0.31)	0,90 c (±0.11)
5 cm	4,07 b (±0.22)	1.67 ab (±0.28)
8 cm	4,75 b (±0.35)	1.58 bc (±0.42)
10 cm	6,92 a (±0.25)	2.60 a (±0.59)
P-value	<0.001	<0.01

Data normalization with natural logarithm lamina length and sheath length.

Table 3. *Festuca gracillima* quadratic equations for leaf elongation. Slope for the first 50 GDD and the GDD required to reach the plateau (zero slope) for all leaves and treatments shown in Fig. 5.

Figure	Defoliation height	Water treatments	Quadratic equations	R^2	Slope at 50 GDD	GDD with zero slope
5A	3	Control	y = -0.00003x2 + 0.0271x - 0.1256	0.98	0.0241	451.7
5A	5	Control	y = -0.00005x2 + 0.0361x - 0.4224	0.99	0.0311	361.0
5A	8	Control	y = -0.00004x2 + 0.0344x - 0.1831	0.99	0.0304	430.0
5A	10	Control	y = -0.00005x2 + 0.0431x + 0.2577	0.95	0.0381	431.0
5B	3	SWR	y = -0.00003x2 + 0.0289x + 0.0111	0.97	0.0135	725.0
5B	5	SWR	y = -0.00004x2 + 0.0329x - 0.3946	0.99	0.0270	500.0
5B	8	SWR	y = -0.00002x2 + 0.0244x + 0.1135	0.98	0.0156	830.0
5B	10	SWR	y = -0.00003x2 + 0.0344x - 0.4376	0.99	0.0225	956.3
5C	3	Control	y = -0.00001x2 + 0.0145x + 0.7957	0.84	0.0259	481.6
5C	5	Control	y = -0.00003x2 + 0.03x + 0.2186	0.94	0.0289	411.2
5C	8	Control	y = -0.00001x2 + 0.0166x + 0.9693	0.81	0.0224	610
5C	10	Control	y = -0.00002x2 + 0.0245x + 1.792	0.82	0.0314	573.3
5D	3	SWR	y = -0.000004x2 + 0.0081x + 0.0966	0.99	0.0077	1012.5
5D	5	SWR	y = -0.000005x2 + 0.0094x + 0.1333	0.99	0.0089	940.0
5D	8	SWR	y = -0.000003x2 + 0.0075x + 0.3522	0.95	0.0072	1250.0
5D	10	SWR	y = -0.000008x2 + 0.0153x + 0.0049	0.99	0.0145	956.3

Table 4. Average values for each water restriction regime and defoliation height treatments. Slope for the first 50 GDD and the GDD required to reach the plateau for all treatments. ± indicates the standard error of the mean (n = 8 for water restriction regime treatment; n=4 for defoliation treatment).

Treatments	Slope at 50 GDD	GDD to zero slope
Control	0.0290 a (±0.0017)	468.7 b (±29.6)
SWR	0.0146 b (±0.0025)	896.3 a (±77.7)
P-value	<0.01	<0.001
Defoliation at 3 cm	0.0178 (±0.0043)	667.7 (±130.2)
Defoliation at 5 cm	0.0240 (±0.0051)	553.3 (±132.1)
Defoliation at 8 cm	0.0189(±0.0049)	985.0 (±159.5)
Defoliation at 10 cm	0.0267 (±0.0052)	729.2 (±134.3)
P-value	≥0.05	≥0.05

405.8 GDD (SEM±16.9) under the severe water restriction treatment. A significant positive linear trend in phyllochron was observed under the severe water restriction condition over the experimental period, reaching a phyllochron of 504.8 GDD over 1,400 GDD, with an R^2 of 0.73. Moreover, a negative linear regression was observed between phyllochron and SWC, indicating that phyllochron increased as SWC decreased, with an R^2 of 0.70 and a slope of -15.189.

DISCUSSION

The present experiment showed no interaction between the factors of defoliation intensity and water condition. Nevertheless, the absence of interaction suggests that plant growth parameters responded similarly to the different levels of defoliation height and water restriction regimes evaluated in this experiment. Therefore, plants defoliated at a height of 10 cm exhibited a greater growth response under both

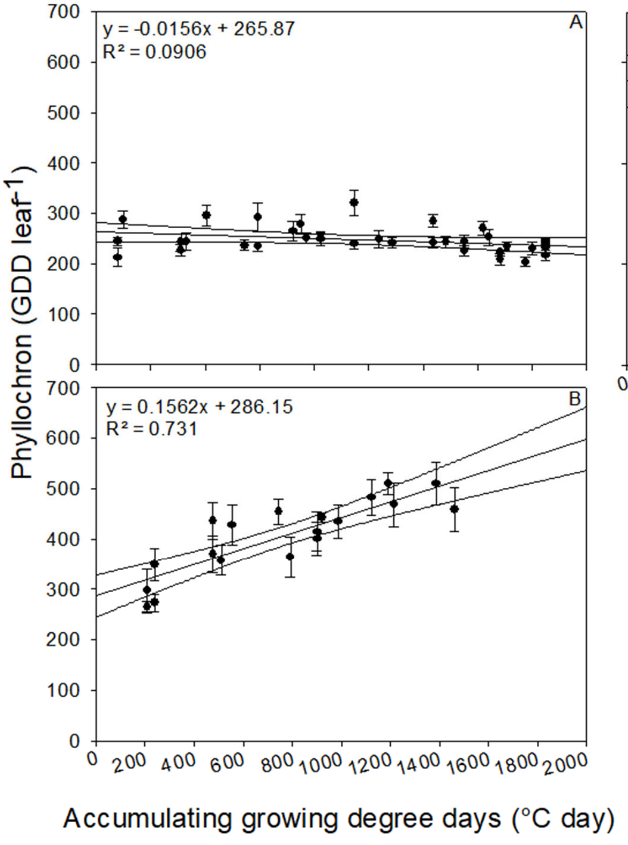

Fig. 5. Phyllochron for newly emerged leaves during the evaluation period. Phyllochron under the water control treatment in relation with the experimental period in terms of accumulated growing degree days (A). Phyllochron under the severe water restriction treatment in relation with the experimental period in terms of accumulated growing degree days (B). Linear regression between phyllochron and soil water content (SWC) over the entire experimental period (C). Bars represent the standard error of the mean (n=5). Interval of confidence of 95% for A, B and C.

water restriction regimes. When soil moisture reached 7.5% and 5.5% SWC, which constituted the minimum SWC values reached in the first 10 cm over the experiment al period [well below the PMP (21.3% SWC)], plants were capable to growth across all defoliation treatments. These results show that *F. gracillima* is capable of growing even under severe water restriction at 10 cm soil depth, suggesting that it may follow a conservative growth strategy (i.e., slow traits) as defined by Reich (2014). Therefore, under a hypothetical severe drought event as indicated by González-Reyes et al. (2017) and Aryal and Zhu (2020), *F. gracillima* may be able to maintain its physiological functioning throughout the period. Phyllochron showed to slow down its development in response to decreasing SWC. During this process, leaf elongation rate declined, requiring nearly twice the amount of GDD to reach maximum growth (i.e., a zero slope), which could offset the observed increase in phyllochron values. These physiological responses to soil water restriction can be agronomically relevant because defoliation management for grass forage species uses phyllochron (or leaf regrowth stage) as the parameter to define defoliation frequency (Fulkerson and Donaghy, 2001; Ordóñez et al., 2021). Consequently, the rate change in phyllochron in relation to SWC should be determined to order to incorporate this variable as a parameter when evaluating forage ecosystems under water-limited conditions.

Defoliation height

Several studies have demonstrated that defoliation at a height of 5 cm height allows certain species to retain sufficient water-soluble carbohydrates (WSC) reserves to support subsequent regrowth cycles (Donaghy and Fulkerson, 1998; Fulkerson and Donaghy, 2001; Turner et al., 2007). In grass species, the highest concentrations of WSC are located within the first 5 cm of the stubble, approximately twice the concentration found within the first 2 cm (Donaghy and Fulkerson 1998). In the case of *F. gracillima*, the effect of defoliation height on energy reserves has been poorly studied. However, growth strategy and defoliation management are critical factors that need to be evaluated to ensure the sustainable use of the sub-Antarctic rangelands, which are frequently subjected to water-limited conditions (Ivelic-Sáez et al., 2021; Ordóñez et al., 2023).

The results of the present study indicate that defoliation below 10 cm is detrimental for plant growth when soil water availability is restricted near and below the PWP. A more intense defoliation height (i.e., below 10 cm) resulted in a reduced lamina elongation, sensitive parameter to both water restriction (García-Favre et al., 2021b) and defoliation intensity (Ordóñez et al., 2021). In the present study, defoliation at a height of 10 cm promoted lamina elongation, with each new leaf being 2-3 cm longer than those under the other treatments. At the plant level, this response translates into a greater leaf area, which has the potential to enhance plant photosynthesis for growth and energy reserve storage, both of which are critical for improving plant survival during periods of water stress (Zwicke et al., 2015; Bristiel et al., 2019).

Water stress conditions

Severe water restriction negatively affected every measured parameter related to *F. gracillima* foliage and root mass production, except for tiller weight. Root quantity and structure are key determinants of soil exploration capacity, which in turn influences plant survival and growth during drought events (Zwicke et al., 2015; Bristiel et al., 2019). Furthermore, soil exploration determines water uptake (Neal et al., 2012), affecting leaf water status (Ordóñez et al., 2024). In the present study, root and shoot mass were greater under less intense defoliation for both water treatments, with no significant interaction observed between defoliation intensity and water regime. These results suggest that under water-limited conditions, defoliation intensity management may play a critical role in enhancing *F. gracillima* growth and soil exploration. In addition, the results indicate that root turnover was negatively influenced by severe water restriction, as evidenced by the lower mass of active roots compared to the water control treatment. A potential management strategy for sub-Antarctic rangelands could involve promoting plant growth during periods of greater water availability through the stimulation of new root formation. The greater availability of water resources occurs during early spring (Ivelic-Sáez et al., 2021; Ordóñez et al., 2023). During that time, promoting *F. gracillima* growth and development implies the closure of affected areas, which means that no grazing should occur during that period. Accordingly, when a severe drought event occurs as projected by González-Reyes et al. (2017), plants will exhibit greater growth and have a greater number of tillers and new roots, being capable of exploring a greater soil volume. In practical terms, this strategy can be implemented through a Deferred-Rotational Grazing system (Borrelli, 2001) and/or by adjusting the stocking rate (Radic-Schilling et al., 2022).

***Festuca gracillima* growth strategy**

In the present study, the growth rates exhibited by *F. gracillima* were lower compared to those reported for other grass species. For example, the lamina elongation rate of *Bromus valdivianus* Phill. under water-limited conditions was 0.22 mm °Cd^{-1} (García-Favre et al., 2021b). On the other hand, the phyllochron of *B. valdivianus* has been calculated to range between 92 and 101 GDD (base temperature of 0°C; Ordóñez et al., 2021) and 74.4 GDD (base temperature of 5°C; Calvache et al. 2020). For *L. perenne*, the reported phyllochron has been 87.9 GDD (base temperature of 5°C; Calvache et al., 2020). In the present study, the phyllochron of *F. gracillima* under the control treatment is 2 to 3 times slower than that of other grasses, such as *L. perenne* (Calvache et al., 2020) and *B. valdivianus* (Calvache et al., 2020; Ordóñez et al., 2021). The phyllochron determined for *F. gracillima* ranged between 265 and 286 GDD in soil moisture values below (i.e., severe water restriction) and around the PWP (i.e., water control), which is the water supply determined in the sub-Antarctic rangelands during spring and summer (Ivelic-Sáez et al., 2021; Ordoñez et al., 2023). Van Loo (1992) and Fulkerson and Donaghy (2001) indicated that phyllochron, or leaf appearance interval, is a generic "clock" primarily regulated by temperature. Although phyllochron is certainly dominated by temperature, the results of the present study indicate that decreasing soil water content under severe water restriction treatment significantly increased phyllochron values, thereby slowing the rate of leaf development. These results align with those of Bartholomew and Williams (2006) for *L. multiflorum* Lam., who reported a steady increase of 87% in the temperatures required for the appearance of one new leaf under water-limited conditions. Likewise, Calvache et al. (2020) showed that the phyllochron can vary across different seasons within a year.

This indicates that climatic variables (e.g., light and water) influence plant development in grasses. Additionally, the quadratic equations showed that plants required a significantly longer period to achieve the lamina´s maximum length (zero slope), with 468.7 GDD under the water control treatment and 896.3 under severe water restriction. The negative slope following the plateau in the quadratic equations can be interpreted as the onset of lamina senescence. This suggests that increasing leaf lifespan, delaying senescence, and extending the phyllochron may be adaptative responses of *F. gracillima* to reduced soil water content. From an agronomic perspective, if the soil water content can significantly affect the phyllochron, it should be considered a key variable in the development of pasture/grassland/rangeland management strategies.

CONCLUSIONS

Defoliation at a height of 10 cm enhanced plant tolerance under water-limited conditions (near and below PWP), promoting growth and leaf elongation across varying water restriction regimes-including one simulating sub-Antarctic rangeland soil moisture and another with severe water deficit.

Reduced soil water supply delayed the phyllochron, increasing thermal requirements for new leaf emergence. However, this was offset by prolonged leaf lifespan, demonstrating physiological adaptations to soil moisture levels below the PWP, as observed in this study.

These findings suggest that *Festuca gracillima* exhibits traits characteristic of a conservative growth strategy (slow-trait species), enabling it to survive, tolerate, and grow under severe water restriction conditions in the topsoil.

ACKNOWLEDGEMENTS

The authors thank INIA for funding this study (research project number 503083-70), Universidad de Magallanes (Chile) and Fondecyt 11231013. The present work reflects only the authors' views, and the funding agency cannot be held responsible for any use that may be made of the information it contains.

Funding statement

This work was funded by Instituto de Investigaciones Agropecuarias (INIA, Chile) (project No. 503083-70) and Universidad de Magallanes. FONDECYT Initiation grant 11231013 supported the preparation of the manuscript as part of a previous work.

Competing interests

The authors declare there are no conflicts of interest.

Author Contributions

The authors declare active participation in the bibliographic review by Paula Oyaneder, Iván Ordóñez and Ignacio López; in the development of the methodology: Iván Ordóñez, Sergio Radic-Schilling, Jorge Ivelic-Sáez and Paula Oyaneder; in the discussion of the results: Paula Oyaneder, Iván Ordóñez, Ignacio López, Sergio Radic-Schilling and Jorge Ivelic-Sáez; in review and approval of the final version of the article Iván Ordóñez, Ignacio López, Sergio Radic-Schilling and Jorge Ivelic-Sáez.

LITERATURE CITED

Aryal, Y., and J. Zhu. 2020. Multimodel ensemble projection of meteorological drought scenarios and connection with climate based on spectral analysis. Int. J. Climatol. 40(7): 3360–3379. doi: 10.1002/joc.6402

Bartholomew, P.W., and R.D. Williams. 2006. Effects of exposure to below-freezing temperatures, soil moisture content and nitrogen application on phyllochron in cool-season grasses. Grass Forage Sci. 61(2): 146–153. doi: 10.1111/j.1365-2494.2006.00518.x

Borrelli, 2001. Producción animal sobre pastizales naturales. p. 129-160. In P. Borrelli and G. Oliva. Ganadería sustentable en la Patagonia Austral. INTA Estación Experimental Santa Cruz. Argentina. https://opac.siunpa.unpa.edu.ar/cgi-bin/koha/opac-detail.pl?biblionumber=12786&query_desc=su%3A%22INTA%22%20and%20ccode%3ACG#!

Bristiel, P., C. Roumet, C. Violle, and F. Volaire. 2019. Coping with drought: root trait variability within the perennial grass *Dactylis glomerata* captures a trade-off between dehydration avoidance and dehydration tolerance. Plant Soil 434 (1–2): 327–342. doi: 10.1007/s11104-018-3854-8

Calvache, I., O. Balocchi, M. Alonso, J.P. Keim, and I.F. López. 2020. Thermal time as a parameter to determine optimal defoliation frequency of perennial ryegrass (*Lolium perenne* L.) and pasture brome (*Bromus valdivianus* Phil.). Agronomy 10(5): 1–13. doi: 10.3390/agronomy10050620

Donaghy, D.J., and W.J. Fulkerson. 1998. Priority for allocation of water-soluble carbohydrate reserves during regrowth of *Lolium perenne*. Grass Forage Sci. 53(3): 211–218. doi: 10.1046/j.1365-2494.1998.00129.x

Dörner, J., J. Huertas, J.G. Cuevas, C. Leiva, L. Paulino, and J.L. Arumí. 2015. Water content dynamics in a volcanic ash soil slope in southern Chile. J. Plant Nutr. Soil Sci., 178: 693-702. https://doi.org/10.1002/jpln.201500112

Fernandez, G. C. 2007. Design and Analysis of Commonly Used Comparative Horticultural experiments. HortScience. 42: 1052-1069. https://doi.org/10.21273/HORTSCI.42.5.1052

Freschet, G.T., L. Pagès, C.M. Iversen, L.H. Comas, B. Rewald, C. Roumet, J. Klimešová, M. Zadworny, H. Poorter, J.A. Postma, T.S. Adams, A. Bagniewska-Zadworna, A.G. Bengough, E.B Blancaflor, I. Brunner, J.H.C Cornelissen, E. Garnier, A. Gessler, S.E Hobbie, I.C Meier, L. Mommer, C. Picon-Cochard, L. Rose, P. Ryser, M. Scherer-Lorenzen, N.A Soudzilovskaia, A. Stokes, T. Sun, O.J. Valverde-Barrantes, M. Weemstra, A. Weigelt, M. Wurzburger, L.M. York, S.A. Batterman, M. Gomes de Moraes, Š. Janeček, H. Lambers, V. Salmon, N. Tharayil, and M.L. McCormack. 2021. A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. New Phytol. 232(3): 973–1122. doi: 10.1111/nph.17572

Fulkerson, W.J., and D.J. Donaghy. 2001. Plant-soluble carbohydrate reserves and senescence - Key criteria for developing an effective grazing management system for ryegrass-based pastures: A review. Aust. J. Exp. Agric. 41(2): 261–275. doi: 10.1071/EA00062

García-Favre, J., I.F. López, L.M. Cranston, D.J. Donaghy, and P.D. Kemp. 2021a. The growth response of pasture brome (*Bromus valdivianus* Phil.) to defoliation frequency under two soil-water restriction levels. Agronomy 11(2). doi: 10.3390/agronomy11020300

García-Favre, J., Y. Zhang, I.F. López, D.J. Donaghy, L.M. Cranston and P.D. Kemp. 2021b. Decreasing defoliation frequency enhances *Bromus valdivianus* Phil. Growth under low soil water levels and interspecific competition. Agronomy 11(7). doi: 10.3390/agronomy11071333

González-Reyes, Á., J.C. Aravena, A.A. Muñoz, P. Soto-Rogel, I. Aguilera-Betti, and I. Toledo-Guerrero. 2017. Variabilidad de la precipitación en la ciudad de Punta Arenas, Chile, desde principios del siglo XX. An. del Inst. la Patagon. 45(1): 31–44. doi: 10.4067/s0718-686x2017000100031

Gutiérrez, H., and R. de la Vara. 2008. Análisis y diseño de experimentos. McGraw-Hill/Interamericana editores. Mexico D.F. Mexico.

Hardy, E.M., and D.M Blumenthal. 2008. An efficient and inexpensive system for greenhouse pot rotation. hortscience. 43: 965-966. doi: 10.21273/HORTSCI.43.3.965

INE. 2022. Censo Agropecuario y Forestal. Instituto Nacional de estadísticas (INE). Santiago, Chile. https://www.ine.gob.cl/censoagropecuario

Ivelic-Sáez, J., J. Dörner, J.L. Arumí, P. Cisternas, J. Valenzuela, E. Muñoz, R. Clasing, S. Valle, S. Radic, H. Alonso, R. López, H. Uribe, R. Muñoz, I. Ordóñez, and J. Carrasco. 2021. Balance hídrico de humedales de uso agropecuario: El primer paso para el mejoramiento en la gestión hídrica a nivel predial en Magallanes. Una investigación multidisciplinaria. Boletín INIA, N° 435. Investigaciones Agropecuarias, Centro Regional de Investigación Kampenaike, Punta Arenas, Chile. https://hdl.handle.net/20.500.14001/67466

Van Loo, E.N. 1992. Tillering, leaf expansion and growth of plants of two cultivars of perennial ryegrass grown using hydroponics at two water potentials. Ann. Bot. 70(6): 511–518. doi: 10.1093/oxfordjournals.aob.a088511

McMaster, G.S., and W.W. Wilhelm. 1997. Growing degree-days: one equation, two interpretations. Agric. For. Meteorol. 87(1): 291–300. doi: 10.1016/S0168-1923(97)00027-0

Neal, J.S., S.R. Murphy, S. Harden, and W.J. Fulkerson. 2012. Differences in soil water content between perennial and annual forages and crops grown under deficit irrigation and used by the dairy industry. F. Crop. Res. 137: 148–162. doi: 10.1016/j.fcr.2012.07.013

Oliva, G. 1996. Biología de poblaciones de *Festuca gracillima*. Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Doctoral thesis in Biology. Buenos Aires, Argentina.

Ordóñez, I., I.F. López, P.D. Kemp, C.A. Descalzi, R. Horn, F. Zúñiga, D. Dorota and J. Dörner 2018. Effect of pasture improvement managements on physical properties and water content dynamics of a volcanic ash soil in southern Chile. Soil Tillage Res. 178(May 2017): 55–64. doi: 10.1016/j.still.2017.11.013

Ordóñez, I.., I. López, P.. Kemp, D. Donaghy, J. Dörner, J. García-Favre, and Y. Zhang, 2024. A short-term effect of multi-species pastures and the plant's physiological response on pasture growth during water stress periods. Eurpre-print: 42. doi: http://dx.doi.org/10.2139/ssrn.4615828

Ordóñez, I.P., I.F. López, P.D. Kemp, D.J. Donaghy, Y. Zhang and P. Herrmann. 2021. Response of *Bromus valdivianus* (Pasture Brome) growth and physiology to defoliation frequency based on leaf stage development. Agronomy 11(10): 2058. doi: 10.3390/agronomy11102058

Ordóñez, I., P. Oyaneder, S. Radic, J. Valenzuela, J. Ivelic and J. Yagello (Eds.). 2022. Respuesta fisiológica de *Festuca gracillima* a la altura de defoliación y estrés hídrico severo. Informativo INIA, N° 119. Investigaciones Agropecuarias, Centro Regional de Investigación Kampenaike , Punta Arenas, Chile. https://hdl.handle.net/20.500.14001/68573

Ordóñez, I.P., S. Radic-Schilling and R. Lira (Eds).2023. Descripción de la vegetación, suelo y tasas de crecimiento de vegas y coironales en la región de Magallanes. Boletín INIA, N° 486. nvestigaciones Agropecuarias, Centro Regional de Investigación Kampenaike , Punta Arenas, Chile. https://hdl.handle.net/20.500.14001/69085

Pisano, E. 1977. Fitogeografía de Fuego-Patagonia chilema. An. Inst. La Patagon. 8: 121–250. http://www.bibliotecadigital.umag.cl/handle/20.500.11893/628?show=full

Radic-Schilling, S., P. Corti, R. Muñoz-Arriagada, N. Butorovic, and L. Sánchez-Jardón. 2021. Ecosistemas de estepa en la Patagonia chilena: distribución, clima, biodiversidad y amenazas para su manejo sostenible. p. 223–256. In M. Martinez-Harms, J. Castilla and J. Armesto (Eds.). Conservación en la Patagonia chilena: evaluación del conocimiento, oportunidades y desafíos. Publisher: Ediciones-UC, Santiago, Chile.

Radic-Schilling, S., F. Sales, R. Lira, R. Muñoz-Arriagada, P. Corti, N. Covacevich, J. Ivelic-Sáez, I. Ordóñez, O. Vidal, R. Eccheverría and C. Sandoval. 2022. Magallanes Sheep Farming. In M. González and Palacios, R (Eds.).Sheep Farming - Herds Husbandry, Management System, Reproduction and Improvement of Animal Health. IntechOpen. doi: 10.5772/intechopen.95705

Reich, P.B. 2014. The world-wide "fast-slow" plant economics spectrum: A traits manifesto. J. Ecol. 102(2): 275–301. doi: 10.1111/1365-2745.12211

Sage, R.F., and D.S. Kubien. 2007. The temperature response of C_3 and C_4 photosynthesis. Plant, Cell and Environment 30: 1086-1106. doi: j.1365-3040.2007.01682.x

Soto-Rogel, P., J.C. Aravena, W.J.H. Meier, P. Gross, C. Pérez, R. Gonzáles Reyes, and J. Griessinger. 2020. Impact of extreme weather events on aboveground net primary productivity and sheep production in the Magellan region, southernmost chilean patagonia. Geosci. 10(8): 1–17. doi: 10.3390/geosciences10080318

Steel, R.G.D., J.H. Torrie, and D.A. Dicky. 1997. Principles and procedures of statistics, a biometrical approach. 3rd Edition, McGraw Hill series in probability and statisticos. Inc. Book Co., New York. USA.

Turner, L.R., D.J. Donaghy, P.A. Lane, and R.P. Rawnsley. 2007. Distribution of water-soluble carbohydrate reserves in the stubble of prairie grass and orchardgrass plants. Agron. J. 99(2): 591–594. doi: 10.2134/agronj2006.0223

Vitousek, P.M., and R.W. Howarth. Nitrogen limitation on land and in the sea: How can it occur? 1991. Biogeochemistry 13: 87–115. https://doi.org/10.1007/BF00002772

Wilhelm, W.W., and G.S. McMaster. 1995. Importance of the phyllochron in studying development and growth in grasses. Crop Sci. 35(1): 1–3. doi: 10.2135/cropsci1995.0011183x003500010001x.

Zwicke, M., C. Picon-Cochard, A. Morvan-Bertrand, M.P. Prud'homme, and F. Volaire. 2015. What functional strategies drive drought survival and recovery of perennial species from upland grassland? Ann. Bot. 116(6): 1001–1015. doi: 10.1093/aob/mcv037