






## Article

# Effect of Water Restriction and Supplementary Nitrogen on the Growth Dynamics of *Bromus valdivianus* Phil.

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## Abstract

Decreasing summer precipitation is negatively affecting global productivity of grassland plant species. This study evaluated the effect of three levels of soil plant available water [80–90% PAW-H (high), 50–60% PAW-M (medium), and 20–30% PAW-L (low), which were soil water restriction (SWR) equivalent to ( $v/v\%$ ) 10–20%, 40–50%, and 70–80%, respectively] and nitrogen (N 0 and 110 kg ha<sup>-1</sup>) on growth and nutritional quality of *Bromus valdivianus* Phil. (Bv) mini-swards (MS; 125 L containers), arranged in three blocks. Total lamina length (TLL), leaf expansion rate (LER; cm d<sup>-1</sup>), phyllochron (Phy) expressed as “days” and “°C day”, tiller mass (TM, g tiller<sup>-1</sup>), number of live leaves (NLL), number of dead leaves (NDL), and accumulated herbage mass [AHM, g DM (dry mass) m<sup>2</sup>] were measured. Defoliation events, leaving 5 cm residual height, were carried out every 320 GDD (using a base growth temperature of 5 °C), and foliage samples for nutritive quality [DM, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), water-soluble carbohydrates (WSC), and metabolic energy (ME)] were collected. Reducing PAW to 20–30% decreased the AHM by 60.7%, TLL by 52.7%, LER by 50%, and TM by 50%, with significant interaction between the main effects for AHM, TLL, and LER. The addition of N increased the AHM by 31.6%, LER by 21.6%, and TLL by 19.6%. The Phy remained undisturbed by decreasing PAW and increasing the N rate. Nutritive quality was generally not statistically different for the interaction or between N levels. However, low PAW levels resulted in statistically ( $p < 0.05$ ) lower ME and higher concentrations of NDF. In general, growth, AHM, and nutritional quality of Bv during the summer period were driven by PAW levels and by the availability of N. Plant available water levels of 50% to 60% at 20 cm soil depth, with the addition of N, allowed Bv to reach its highest production.

**Keywords:** abiotic stress; drought; pasture brome; soil fertility



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## 1. Introduction

Increasing temperature and variability in the intensity and frequency of seasonal rainfall events in the Southern Pacific (Southern Chile and New Zealand) [1,2] constrain pasture species growth and productivity. Regional climate data indicate that, since 1979, climate variability has been evident; however, from 2014, the intensity and rate of climate change have become more pronounced [1].

Regional climatic changes in these predominately temperate humid zones have increased pasture growth seasonality, resulting in higher growth rates in spring, lower growth rates in summer, and a general decrease in the persistence of fast-growing perennial pastures dominated by *Lolium perenne* L. (perennial ryegrass) and *Trifolium repens* L. (white clover) [3]. *Lolium perenne* and *T. repens* persistence and survival are negatively affected by decreasing soil water availability, commonly associated with periods of drought [4]. In the South of Chile, during drought events, pasture soil has been reported to reach permanent wilting point (PWP) at 60 cm depth [3], with soil increasingly experiencing a negative soil water deficit between November and April (summer through autumn; Southern Hemisphere) [5].

Plants have an array of morphophysiological mechanisms to cope with soil water restriction. Reduced soil water availability may trigger changes in plant morphophysiological and metabolic functions, which may alter photosynthesis, growth, and tissue formation [6]. Soil water restriction (SWR) is one of the main environmental factors that constrain pasture growth during the summer [7]. In temperate species, stomata closure is a short-term mechanism that prevents the loss of water through the laminae to maintain the plant's turgor [8,9]. While stomata closure prevents water loss from the stomata cavity, CO<sub>2</sub> and O<sub>2</sub> exchange between the air and the plant is reduced/stopped. In C3 species, this results in reduced growth because of decreased photosynthesis, net C assimilation, and herbage mass accumulation [6].

The plant response to water stress can also be physiological, biochemical, and/or molecular, reflecting a reduction in net photosynthetic rate, decreased Rubisco efficiency, and/or increased expression of ABA biosynthetic genes, respectively [6,10]. Some plant species may adjust and modify their phenotypic expression in response to environmental fluctuations/stresses (i.e., water restriction) [5]. In addition, the intensity and duration of SWR may influence the plant response, with the effects becoming more evident once soil moisture has returned to 'pre-stress' levels [8,11].

In general, plant tolerance responses within a population drive plant survival and persistence. Tolerant characteristics, including movement and allocation of resources to shoot and root tissues [10,12]; deep-root development [13]; number, size, and distribution of stomata and hairs [9]; and osmotic adjustment [14], drive plant selection and genetic segregation. In early stages of plant development, soil water restriction reduces lamina elongation and final lamina length. In more advanced morphophysiological stages, plants may increase leaf senescence and foliage loss in response to SWR [15,16]. Under this condition, plants decrease their turgor and reduce their growth as a result of decreased photosynthesis and C translocation. Furthermore, as leaf water loss is usually greater than the volume of water entering the roots, the plant may enter into a negative water balance, depending on conditions. As water restriction progresses, leaves may start to sag to the point where plant tissue weight cannot be maintained, and the plant wilts [17].

Nitrogen (N) is an important nutrient for plant growth and development. Fluctuations and availability of soil N [18] limit plant growth and development in a variety of ecosystems [19]. Nitrogen deficiency in pasture systems is frequently addressed and managed at key points in the year (e.g., during spring) when the soil water availability and temperatures favour plant growth [20] through application of fertiliser N and/or incorporation of N-fixing legumes in the sward.

Nitrogen absorbed by plants from the soil solution is used in a variety of metabolic processes, including protein and cofactor formation and nucleic acid production [21]. Nitrogen is a structural component of chlorophyll and is fundamental for photosynthetic processes and for the formation of signalling and storage molecules [22]. Application of N stimulates and sustains lamina elongation [23] and tiller appearance [24] and increases pas-

ture dry matter (DM) production [25] and N content in harvested plant material. However, increased plant N content has a limited effect on forage digestibility [26].

Naturalised pastures in temperate humid climates frequently include plant species with root and leaf morphophysiological attributes that allow them to tolerate annual periods of soil water restriction. These adapted/ecotypic pasture species have important roles in the development of permanent pastures in areas of increasing SWR and climate variability as a result of their morphological plasticity [12,27], ability to use water from deep soil layers [3,7], and morphological adjustments in leaf and root structure [9,13].

*Bromus valdivianus* Phil. (Bv) is a dominant grass species native to the southern temperate humid regions of South America, commonly forming naturalised pastures on deep, well-drained, medium-textured, fertile, allophanic volcanic ash soils. Similar environmental conditions favour *L. perenne* and *T. repens* growth [27,28]. *Bromus valdivianus* has a deep root system [13], which can capture available soil water located at depth in the soil profile [3]. *Bromus valdivianus* has larger tillers than *L. perenne* (~2.3 times) but with a lower tiller number per plant (~2.2 times [5,29]). Furthermore, Bv has a high growth rate during the spring and summer [30]. Despite the importance of Bv, there is limited research outlining a clear N fertility response.

Initial field fertilisation evaluations have demonstrated that Bv is responsive to fertiliser application, producing high amounts of annual accumulated herbage mass [13,14], and is even capable of colonising a degraded soil–pasture complex when soil fertility growth constraints are released [7]. However, in general, there is limited information on N response of Bv to fertilisation. The hypothesis was that the response of Bv to fertiliser is not just related to soil nutrient status but also to soil water availability. Therefore, the objectives of this research were to evaluate the effect of the interaction of three levels of soil water availability and the addition of N on Bv growth parameters, including leaf extension rate (LER), total leaf length (TLL), tiller mass (TM), number of live leaves (NLL), and accumulated herbage mass (AHM), in volcanic soils.

## 2. Materials and Methods

### 2.1. Growth Conditions and Soil Nitrogen Treatment

This study was carried out in a rainout shelter (6.5 m width × 8.5 m length × 2 m high) at the Institute of Animal Production, Universidad Austral de Chile (39°45'30" South, 73°14'55" West), Valdivia, Chile. Temperature and relative humidity were measured and recorded hourly for the study duration by a weather station (model MetPRO Weather Station, Campbell Scientific Inc., Logan, UT, USA) adjacent to the study site. Average, maximum, and minimum day/night temperature and relative humidity were 15.85 ± 3.67, 37.2 ± 4.42, 1.0 ± 2.92, and 69.7%, respectively. Daily maximum and minimum air temperatures were used to calculate growing degree days (GDD) [31–33] and accumulated growing degree days (AGDD) [16]. Average solar radiation for the study period was 21.75 Mj/m<sup>2</sup>.

Eighteen mini-swards (MS; 125 L volume; 48 cm diameter × 80 cm depth drums) of *Bromus valdivianus* Phil. cv. "Bronco" (Bv) were arranged under the rainout shelter with a 2 m wide perimeter strip (from the edge of the rainout shelter to the external line of the containers) to avoid wind-borne moisture/edge effects. Containers were arranged in randomised complete blocks with a 0.5 m corridor between each block and a 0.2 m separation between each container to allow for ease of access/data collection during the study.

Each container was filled with 0.1 m<sup>3</sup> of well-drained, silty loam, disturbed allophanic volcanic ash soil (Valdivia soil series), classified as Andisol, Duric Hapludand [34], collected from the upper horizon (0–20 cm). Container soil had a high OM content (12.6%) and a soil fertility analysis of 16.1 mg kg<sup>-1</sup> mineral N (N-NO<sub>3</sub>+NH<sub>4</sub>), 17.4 mg kg<sup>-1</sup> Olsen-

phosphorus test (Olsen-P), 130 mg kg<sup>-1</sup> exchangeable potassium (K<sup>+</sup>), 0.04 cmol<sup>+</sup> kg<sup>-1</sup> exchangeable sodium (Na<sup>+</sup>), 3.93 cmol<sup>+</sup> kg<sup>-1</sup> exchangeable calcium (Ca<sup>2+</sup>), 0.39 cmol<sup>+</sup> kg<sup>-1</sup> exchangeable magnesium (Mg<sup>2+</sup>), 4.7 cmol<sup>+</sup> kg<sup>-1</sup> total exchangeable bases, 0.08 cmol<sup>+</sup> kg<sup>-1</sup> exchangeable aluminium (Al<sup>3+</sup>), 4.78 cmol<sup>+</sup> kg<sup>-1</sup> cationic exchangeable capacity, and 1.8% Al saturation (Laboratory of Soil and Water Analysis, Institute of Agricultural Engineering and Soil Science, Facultad de Ciencias Agrarias y Alimentarias, Universidad Austral de Chile, Valdivia, Chile). All containers were fertilised (18 g of CaCO<sub>3</sub> and 10.8 g of 5N-30P<sub>2</sub>O<sub>5</sub>-20K<sub>2</sub>O-1Mg-1S-7Ca) before sowing, as determined by soil chemical analysis. Two N treatments were also imposed at this time. Briefly, half of the MS received 5 g m<sup>-2</sup> of N fertiliser (50 kg N ha<sup>-1</sup> year<sup>-1</sup>, equivalent) at the beginning of the measuring period. The study involved 3 cutting events; after each cut/defoliation event, these MS received 2 g m<sup>-2</sup> of N fertiliser, equivalent to 20 kg N ha<sup>-1</sup>, which made up to a total of 110 kg N ha<sup>-1</sup> applied during the study. The remaining containers received no N (0 kg N ha<sup>-1</sup> treatment).

Soil samples were then collected from six containers using a 250 cm<sup>3</sup> metallic cylinder, and a soil water retention curve was determined [35]. Field capacity (FC) was determined at 60 hPa [36] and a soil moisture ( $v/v\%$ ) of 42%. Plant available water (PAW) was considered as the water remaining in the soil between 60 hPa and 15,430 hPa (permanent wilting point; PWP), which corresponded to 27.2% ( $v/v\%$ ) of the total soil volumetric water content (VWC), with 35% of the macropores being >50 µm. Soil temperature and moisture sensors (EM50 data logger, METER Group, Pullman, WA, USA) were installed in each container at 5, 20, and 60 cm soil depths and calibrated as described by Dec et al. [37].

Four hundred Bv seeds were sown (winter) in each container to a depth of ~1 cm. A cardboard template (square grid; holes 1 × 1 cm) was used to manually space the seeds at sowing. After seed emergence (~12 days), the soil was maintained at FC (60 hPa) until the irrigation/water restriction treatments were imposed at 320 AGDD, equivalent to 4 phyllochrons of Bv [one phyllochron for Bv is equivalent to 80 AGDD [38]].

## 2.2. Soil Water Restriction

Three SWR [ $v/v\%$ : 10–20%, 40–50%, and 70–80%, which were equivalent to 80–90% PAW-H (high), 50–60% PAW-M (medium), and 20–30% PAW-L (low), respectively], based on the PAW calculated from the soil water retention curve, were imposed at 320 AGDD (Time 0) and maintained throughout the study period according to the soil moisture content at 20 cm soil depth, with irrigation events determined according to the soil moisture content of each container. The 320 AGDD were used as targets to apply the treatments and to indicate a defoliation event, as explained and justified in the following section.

## 2.3. Defoliation/Cutting Events and Nitrogen Treatment

At Time 0 (Summer; 320 AGDD from seedling emergence: Cut 0), all the MS were cut (defoliated), leaving a 5 cm residual height; cut material (forage) from each container was collected and oven dried (68 °C 48 h<sup>-1</sup>), and the dry mass (DM) was determined. Thereafter, each time 320 AGDD was reached, all 18 MP were cut to 5 cm above soil level, plant material was collected, and DM was determined. This process was repeated for 3 additional cuts/defoliation events (Cut 1, 2, and 3, respectively). Cut/defoliation event and frequency were based on Bv post-defoliation recovery of the water-soluble carbohydrate (WSC) reserves. Post-defoliation recovery was also confirmed through visual observation of Bv morphophysiological stage, i.e., appearance of the 4th leaf on an individual tiller, based on one Bv phyllochron corresponding to 80 AGDD [39], which is within the optimal range for Bv defoliation [40]. The study ended (day 270) with the final defoliation event (Autumn) at Cut 3, where all above-ground plant material was harvested.

#### 2.4. Assessment of Plant Growth and Morphophysiological Variables

Morphophysiological variables of the tillers were measured over time. In each mini-sward, three individual tillers were marked by placing a small coloured clip at their base. Every three days, the lamina length, defined as the distance from the collar section to the lamina tip, was measured [40], and the total lamina length per tiller (TLL) was calculated. Lamina appearance was recorded, and the lamina elongation process ended with the appearance of the ligule, with the lamina length being the tip–ligule distance (cm). This information allowed the lamina extension rate to be calculated (LER;  $\text{cm d}^{-1}$ ). The AGDD between the appearance of two consecutive lamina was used to calculate the phyllochron (Phy), expressed in thermal time (AGDD) and as the number of days. At each defoliation event, the number of live leaves (NLL) and dead leaves (NDL) was recorded.

After each defoliation event, the herbage mass harvested per mini-sward was oven dried ( $60\text{ }^{\circ}\text{C}$  for 72 h), and DM was determined, along with the weight of individual tillers (tiller mass; TM), and the accumulated herbage mass (AHM) was calculated per mini-sward as the addition of the herbage grown between cutting events. For the last cut, the residual herbage was cut to soil level, harvested, dried, weighed, and added to the AHM calculation.

At each defoliation event, a 200 g sub-sample of fresh pasture was collected from the harvested plant material from each mini-sward and oven-dried ( $60\text{ }^{\circ}\text{C}$  per 72 h) and DM, crude protein (CP), water-soluble carbohydrates (WSC), neutral detergent fibre (NDF), acid detergent fibre (ADF), digestibility (DV), and metabolisable energy (ME) were determined through NIRS (MODEL 6500, Near Infrared Spectrophotometry System, FOSS NIRSystem Inc., Silver Spring, MD, USA). Prediction equations used were developed at the Animal Nutrition Laboratory, Universidad Austral de Chile, and calibrated on wet chemistry results.

#### 2.5. Experimental Design and Statistical Analysis

This study was arranged as a randomised complete block design (3 blocks, random effects), with a factorial distribution of the treatments (3 PAW  $\times$  2 N, fixed effects), for a total of 18 MS. All the data collected was analysed using SAS V9.0 (SAS Institute, Cary, NC, USA), and the PROC GLMMIX adjusted model was applied. Tukey was used to separate treatment means with significant ( $p < 0.05$ ) differences, and PDIFF was applied when there was significant interaction.

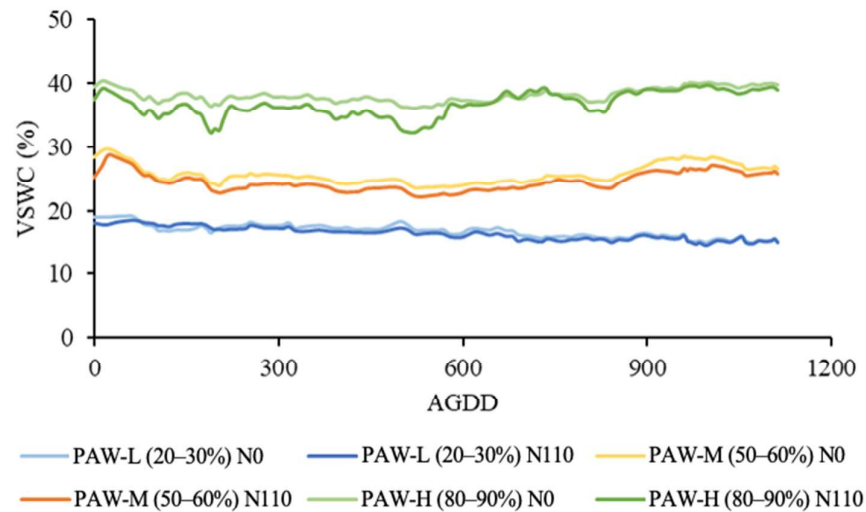
### 3. Results

#### 3.1. Soil Water Content According to Water Restriction and Nitrogen Addition

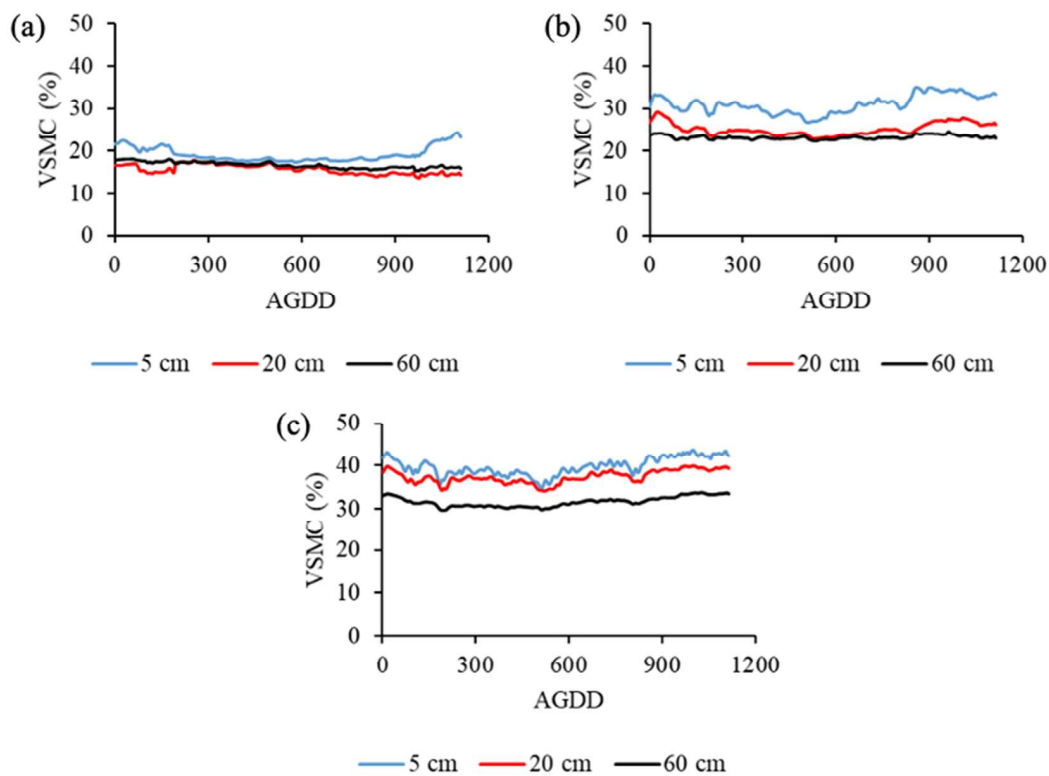
During the study period, the soil moisture sensors (located at 20 cm soil depth) provided soil moisture content values used to calculate the MS irrigation needs. Thus, PAW ( $v/v\%$ ) levels were successfully maintained within each treatment (Figure 1), and the corresponding PAW for each mini-sward was different when compared across treatments. This was particularly evident when the two extreme PAW levels were contrasted (80–90% PAW-H vs. 20–30% PAW-L). The 80–90% PAW-H displayed 37.2% average volumetric soil water content (VSWC) for the entire study period, with a 40.1% maximum and 33.7% minimum. The average VSWC for 50–60% PAW-M was 27.07%, with 29.2% maximum and 23.2% minimum, while the average VSWC for 20–30% PAW-L was 16.3%, with 18.2% maximum and 14.2% minimum, values that were close to PWP (Figure 1).

As a general trend, the PAW treatments over the study time reached the targets set by the objectives (Figure 2), such that the pastures with 20–30% PAW-L created a stressful situation for the pastures to grow and perform, as reflected in the AHM (Table 1). The 50–60% PAW-M and 80–90% PAW-H water restriction treatments also generated a contrasting situation for the pastures, being mirrored in the statistical differences shown by AHM (Table 2). The AHM

resulted from the water accumulated stress during the whole study period, which meant that it was not the result of a single water restriction stress event. Figure 2 shows a higher VSMC at the shallower depth for all treatments, reflecting the irrigation being applied to the soil surface to mimic precipitation events.



**Figure 1.** Volumetric soil water content (VSWC %) of *Bromus valdivianus* Phil cv. “Bronco” (Bv) mini-swards (MS) at 20 cm soil depth, according to the plant available water (PAW) ranges applied [PAW: 80–90% (high), 50–60% (medium), and 20–30% (low)] with increasing accumulated growing degree days (AGDD). Mini-swards were fertilised with either 0 or 110 kg N ha<sup>-1</sup>, equivalent.



**Figure 2.** Volumetric soil moisture (VSMC %) of *Bromus valdivianus* Phil. cv. “Bronco” (Bv) mini-swards (MS) at 5, 20, and 60 cm soil subjected to three plant available water (PAW) levels, (a) PAW-L 20–30%, (b) PAW-M 50–60%, and (c) 80–90% PAW-H, over accumulated growing degree days.

**Table 1.** Morphophysiological growth parameters of *Bromus valdivianus* Phil. adjustments as a consequence of the soil water restriction and the nitrogen (N) availability.

PAW <sup>1</sup> (%)	LER <sup>4</sup> (cm day <sup>-1</sup> tiller <sup>-1</sup> )	TLL <sup>5</sup> (cm tiller <sup>-1</sup> )	NLL <sup>6</sup> (No tiller <sup>-1</sup> )	NDL <sup>7</sup> (No tiller <sup>-1</sup> )	TM <sup>8</sup> (g tiller <sup>-1</sup> )	Phy <sup>9</sup> (N° days)	Phy <sup>9</sup> (°C day)	AHM <sup>10</sup> (g DM m <sup>-2</sup> )
20–30	0.61 b	18.76 c	4.22 b	0.47	0.04 b	12.53	136.61	284.41 c
50–60	1.15 a	29.92 b	4.56 ab	0.75	0.06 b	11.99	129.50	607.09 b
80–90	1.22 a	35.50 a	4.80 a	0.69	0.08 a	11.61	124.41	723.49 a
Sem <sup>2</sup>	0.061	1.525	0.129	0.089	0.008	0.586	6.892	28.267
<i>p</i> -Value	0.0001	0.0001	0.006	0.073	0.030	0.164	0.061	0.0001
<b>N<sup>3</sup> (kg ha<sup>-1</sup>)</b>								
0	0.87 b	24.72 b	4.38	0.63	0.05	12.24	132.61	437.31 b
110	1.11 a	30.73 a	4.65	0.64	0.07	11.88	128.22	639.08 a
sem	0.050	1.243	0.101	0.073	0.007	0.481	5.619	23.082
<i>p</i> -Value	0.0008	0.012	0.196	0.965	0.130	0.553	0.508	0.0001
<b>Interaction</b>					<b><i>p</i>-Value</b>			
PAW × N	0.042	0.047	0.170	0.931	0.914	0.339	0.259	0.0094

<sup>1</sup> PAW: plant available water (%); <sup>2</sup> sem: standard error of the mean; <sup>3</sup> N: nitrogen addition; <sup>4</sup> LER: leaf extension rate (cm tiller<sup>-1</sup>); <sup>5</sup> TLL: total lamina length (cm tiller<sup>-1</sup>); <sup>6</sup> NLL: number of live leaves (N° tiller<sup>-1</sup>); <sup>7</sup> NDL: number of dead leaves (N° tiller<sup>-1</sup>); <sup>8</sup> TM: tiller mass (g tiller<sup>-1</sup>); <sup>9</sup> Phy: phyllochron (expressed as “days” and “°C day”); and <sup>10</sup> AHM: accumulated herbage mass. Different letters in the same column indicate significant differences according to 5% Tukey test.

**Table 2.** Nutritive value of *Bromus valdivianus* Phil. cv. “Bronco” (Bv) under increasing soil water restriction (SWR) and nitrogen (N) fertiliser.

PAW <sup>1</sup> (%)	DMC <sup>4</sup> (%)	CP <sup>5</sup> (%)	DV <sup>6</sup> (%)	ME <sup>7</sup> (Mcal kg <sup>-1</sup> DM)	ADF <sup>8</sup> (%)	NDF <sup>9</sup> (%)	WSC <sup>10</sup> (g kg <sup>-1</sup> DM)
20–30	26.86 b	22.17 a	70.07 b	2.56 b	27.76 a	56.76 a	87.05
50–60	23.95 b	19.25 b	71.73 a	2.61 a	27.03 ab	54.81 b	87.27
80–90	22.64 a	19.83 ab	72.29 a	2.63 a	26.75 b	53.20 b	87.06
Sem <sup>2</sup>	0.051	0.596	0.232	0.009	0.254	0.281	3.467
<i>p</i> -Value	0.0002	0.016	0.0001	0.0003	0.037	0.0001	0.998
<b>N<sup>3</sup> (kg ha<sup>-1</sup>)</b>							
0	25.18	19.92	71.01	2.59	26.92	54.90	88.48
110	23.79	20.9	71.72	2.60	27.44	54.89	88.77
sem	0.720	0.486	0.190	0.007	0.207	0.229	2.831
<i>p</i> -Value	0.1924	0.204	0.023	0.561	0.095	0.840	0.451
<b>Interaction</b>				<b><i>p</i>-Value</b>			
PAW × N	0.684	0.830	0.477	0.769	0.931	0.294	0.758

<sup>1</sup> PAW: plant available water; <sup>2</sup> sem: standard error of the mean; <sup>3</sup> N: nitrogen addition; <sup>4</sup> DMC: dry matter content; <sup>5</sup> CP: crude protein; <sup>6</sup> DV: digestibility value; <sup>7</sup> ME: metabolisable energy; <sup>8</sup> ADF: acid detergent fibre; <sup>9</sup> NDF: neutral detergent fibre; and <sup>10</sup> WSC: soluble water carbohydrates. Different letters in the same column indicate significant differences according to 5% Tukey test.

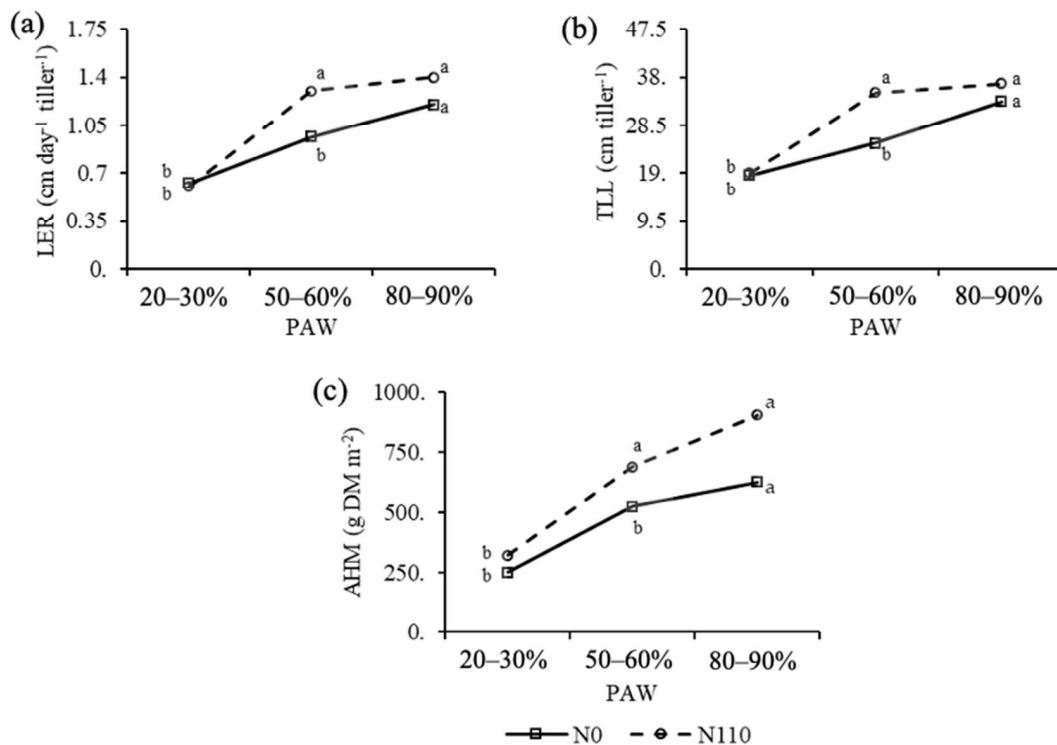
### 3.2. Growth Dynamics of the Mini-Swards

The effects of the soil PAW diminishment and N addition on Bv MS growth dynamics and herbage mass accumulation (g DM m<sup>-2</sup>) are shown in Table 1. The NLL and TM significantly decreased with decreasing PAW (NLL<sub>80–90%</sub> 4.8 > NLL<sub>20–30%</sub> 4.2 cm,  $p < 0.01$ ; and TM<sub>80–90%</sub> 0.04 > TM<sub>20–30%</sub> 0.08 g,  $p < 0.05$ ) but not the NDL ( $p > 0.05$ ). These three tiller attributes were not statistically modified by N addition. The Phy, expressed as “number of

days" ( $p > 0.05$ ; days) and as "thermal time" ( $p > 0.05$ ; TT), was not statistically altered by decreasing PAW or by N addition (Table 1).

The pastures reduced (%) their growth with decreasing PAW (Table 2). The 80–90% PAW-H constituted the referential pasture grown without water restriction and was used to quantify the negative effect of the restricted PAW reflected on Bv growth. In 50–60% PAW-M, there was a 5% and 25% reduction in the NLL and TM, respectively, while in 20–30% PAW-L, the reduction was 12.1% and 50% for NLL and TW, respectively (Table 1).

There was a significant interaction between PAW levels and N addition (Table 1) for LER ( $p < 0.001$ ), LL ( $p < 0.05$ ), and AHM ( $p < 0.001$ ) (Figure 3). Plant growth response to N addition, expressed as LER, LL, and AHM, was strongly dependent on the PAW restriction, in that MS that received N were more tolerant to decreasing PAW (increasing SWR) than MS that did not receive N. This response was consistent for LER, LL, and AHM and was observed at 50–60% PAW-M. However, at the lowest (20–30% PAW-L; highest SWR) and highest (80–90% PAW-H; lowest SWR) PAW levels, plant growth response to N addition was suppressed (Figure 3).



**Figure 3.** *Bromus valdivianus* Phil. cv. "Bronco" (Bv) growth response to increasing plant available water (PAW) and soil nitrogen (N) addition expressed as (a) leaf elongation rate (LER); (b) total lamina length (TLL); and (c) accumulated herbage mass (AHM). Symbols followed by different letters indicate significant differences according to LSD test ( $p < 0.05$ ).

### 3.3. Mini-Sward Nutritive Value

Nutritive value variables did not have statistically significant interactions between PAW and N addition. All the measured nutritive value variables had statistical differences when the PAW decreased (Table 2), except for the WSC content, which did not change with decreasing PAW or when N was added.

Decreased PAW-M (50–60%) induced an increase in the dry matter content (DM) of the MS. Crude protein content decreased with 50–60% PAW-M, but as PAW increased to 10–20%, the CP content increased to similar levels to MS at 80–90% PAW-H (Table 2). Pasture metabolis-

able energy (ME) and digestibility value (DV) significantly decreased ( $p < 0.001$ ) when the PAW decreased from 50–60% (PAW-M) to 10–20% (PAW-L), and NDF and ADF significantly increased ( $p < 0.05$  and  $p < 0.001$ , respectively) when the PAW decreased. Interestingly, DV was the only variable related to the nutritive value that increased with N addition (0-N = 71.01 vs. 110-N = 71.72;  $p < 0.023$ ).

## 4. Discussion

### 4.1. Pasture Growth Dynamics

This study demonstrates that PAW and N addition modified Bv growth, yield components, and nutritive value, which may modify plant phenology [6,41,42]. The differences measured for LER and TLL among the PAW levels were associated with reduced soil water availability (Table 1, Figure 3). As soil water stress increased, plant growth decreased because PAW became a major factor that constrained plant growth [43]. Where PAW was higher (80–90%), Bv LER and TLL increased (Figure 3). These LER and TLL reduction responses, as a consequence of the increased PAW restriction (20–30%), have been previously reported by [5], where *L. perenne* LER and TLL decreased when PAW restriction corresponded to a prolonged summer drought [29].

Cellular expansion is one of the more sensitive plant physiological responses to the PAW restriction [44] due to the reduction in turgor pressure in the growing cells within the leaves [45]. It has also been reported that perennial grasses may initiate early flowering to avoid drought effects, while a reduction in LER and TLL provides plant protection and promotes plant development and survival for the following year [4]; however, this is not always achieved [46] and may reflect the variability in timing, severity, and duration of drought events.

Reduction in Bv LER and TLL when N was not added (Table 1, Figure 3) follows the results reported by [47]; however, when PAW restriction was not imposed at its highest level, Bv was able to utilise added N and increase LER and TLL (Figure 3). It has been suggested that N fertilisation increases TLL as a result of increased LER [48]. Nitrogen fertilisation has been reported to increase *Bromus auleticus* Trin. ex Nees LER when compared to the non-fertilised treatments [49].

The overall response of Bv LER and TLL demonstrates how a PAW, near FC levels, stimulates plant growth, while low PAW restricts plant growth to the point that N fertilisation was not utilised, and plant growth (herbage mass) did not increase. Increased LER is normally linked to longer and wider laminae, and tillering stimulation, when added N is utilised, which we suggest, explains the positive AHM response to N addition [47,50]. When Bv leaf growth near FC and PWP were compared, there was an evident increase in plant stress (reduced plant growth; AHM diminishment) due to the higher water restriction (Figure 3). We suggest that a soil water deficit limits plant nutrient uptake from the soil solution, coupled with a decrease in plant growth with decreasing water availability [51]. When a soil has enough nutrients to satisfy pasture growth demand, it must also have sufficient soil water content to enable nutrient uptake [52]. In general, higher soil moisture and N application have been shown to increase yield in shallow-rooted species, but there was no significant interaction [53]. However, there was an intrinsic link between water availability and N uptake in deep-rooted grass species [54], where PAW governed plant response to N.

Tiller mass significantly ( $p < 0.05$ ) decreased with decreasing PAW. Similar results were reported by [5,29], where decreased LER, TLL, and NLL per tiller reflected TM diminishment and added to the other pseudostem components (e.g., leaf sheath), which resulted in a lesser AHM per unit area but also per tiller (Table 1, Figure 3). It has been reported that, under SWR conditions (PAW restriction), TM decreases due to a reduction

in the LER and leaf area ratio (LAR) and the accelerated leaf senescence rate (LSR) [55], suggesting that plant growth has an inverse relationship between the tiller number and TM, where an increased tiller size accompanies a decreased tiller number. However, in the present study, PAW and/or N addition levels did not significantly modify either LAR, Phy expressed as “days” or “TT”, or NDL (Table 1, Figure 3). When Bv competes with perennial ryegrass, Bv produces fewer but heavier tillers, probably reflecting the greater reserves necessary to maintain foliage and root growth during the PAW restriction period [5]. Similar results have been found in *Dactylis glomerata* L. (cocksfoot) [42] and *Phalaris aquatica* L. (Phalaris) [56].

The growth patterns of Bv in response to PAW diminishment at the tiller level are similar to those reported by [16], where Bv aerial phytomass decreased with decreasing soil water availability and greater phytomass was accumulated when irrigation was applied. Similar results have also been reported by [5,29]. Studies with *Bromus inermis* Leyss. (smooth brome), cocksfoot, perennial ryegrass, and *Phleum pratense* L. (Timothy) have shown decreased herbage and root mass with severe SWR [41]. Plant response and tolerance to SWR are attributes that are dependent on plant species, ecotype, and cultivar features, which are also linked to soil water use efficiency [57,58]. In the current study, Bv herbage mass accumulation (Figure 3) was similar when subjected to PAW-M (50–60%) and PAW-L (20–30%).

In our current study, the PAW and N addition results strongly suggest that Bv survival-colonisation strategy combines stress tolerance and competitive attributes [59], which are capable of down-regulating plant growth when PAW is restricted (LER and TLL, Figure 3) [16,60]. It is important to note that, in our study, when water stress was decreased and allowed growth, the water restriction was only partly released, as shown by 50–60% PAW, and Bv took the opportunity to utilise available nutrients, as was the case with the N added treatment (Figure 3).

The lack of difference for the AHM when N was added with 80–90% PAW-H indicated that the N supplied by the soil was high enough to support a statistically similar AHM as Bv MS that received N (Figure 3). However, Bv reached significantly greater AHM levels when PAW was at 50–60% (PAW-M). We suggest that these results are related to the soil microorganism N mineralisation activity, where N released from the soil OM to the exchangeable soil complex in the rhizosphere and where there is ‘sufficient’ soil moisture to allow N uptake by roots [61]. The capacity of the soil to store water and nutrients is an important driver of crop yield, as it regulates irrigation frequency and the efficiency of water and nutrient management [37]. When soil moisture is close to FC, N mineralisation activity can effectively fulfil a high AHM for Bv. However, at 80–90% PAW-H, the N addition treatment generated 34.4% higher AHM when compared to the control. Total N application rate and the frequency of N split application exhibited a positive N responsiveness of increased growth and mass of *Bromus willdenowii* Kunth (prairie grass), smooth brome, and Bv [62–64].

It has been suggested that there is lower soil N availability for plants when the soil is dry (PAW-L) [65], in that N addition will not generate a pasture growth response until soil water availability is increased, underlining the close relationship between soil water availability and N utilisation by plants (as discussed by [66]). It was reported that N addition can increase soil water utilisation in some pasture species [52], which may explain the observed responses in the current study, where PAW–N addition had significant interactions for Bv LER, TLL, and AHM (Figure 3).

The restriction of PAW diminished the NLL per tiller, with the most pronounced effect at low PAW-L (20–30%), which agreed with the decrease in Bv fully expanded leaves reported when available soil water was reduced from PAW-H (80–85%) to PAW-L

(20–25%) of FC [16]. We suggest that the reduction in the TLL and NLL in response to decreased PAW reflects a reduction in cell size and division in the basal region of the growing leaves, which will also decrease the LAR [67]. In the current study, NLL did not differ significantly between the N fertiliser treatments, suggesting that N addition does not increase the NLL per tiller [68] and that NLL corresponds more to a genotypic constant [69]. Furthermore, N addition has been suggested to have little effect on the rate of leaf appearance per tiller, both as individual species (grown as isolated plants) and/or as part of a plant community [70]. This response agrees with [71], who reported similar results with species of *Bromus* and *Stipa*.

Drought conditions reduce leaf expansion and length and increase leaf senescence and death in perennial grasses [72,73] and reduce growth rate, leaf initiation, and photosynthetic rates [43,74]. Under these conditions, leaf senescence and death are considered to represent survival strategies for grass species [4]. Therefore, in the current study, we expected that Bv would have increased NDL and decreased AHM as the PAW decreased; however, Bv NDL remained stable (not statistically different) with decreasing PAW. Nitrogen addition did not modify the NDL, even though studies have reported an increase in the leaf senescence with medium to high N addition, with moderately frequent defoliations [75,76]. The lack of response to N addition by NDL has also been reported by [49], where *Bromus* daily LSR, expressed as either accumulated or per unit of area, did not differ between the 0 kg ha<sup>-1</sup> and the 100 kg N ha<sup>-1</sup> N fertilised treatments, with AGDD accounting for more than 90% of the daily LSR. In a Bv tiller, the oldest fully mature leaf starts senescing when the seventh leaf appears [3]; at this point, there are three completely expanded leaves and three actively expanding leaves. Therefore, it is possible that the defoliation frequency used in the current study may not allow the NDL to be fully expressed as a consequence of decreased PAW because the MS were defoliated when Bv had four live leaves per tiller. To minimise leaf losses due to senescence, the optimal frequency of defoliation for Bv has been set at between 3.5 and 4.0 leaf regrowth stage [3,16]. Leaf regrowth stage has been defined and explained in detail by [3,13].

The phyllochron of Bv, expressed as days leaf<sup>-1</sup> or as AGDD leaf<sup>-1</sup> (°C day), did not statistically change with decreasing PAW or with N addition. It has been reported that the phyllochron has low dependency on soil fertility factors [18,77] and that temperature is more closely related to phyllochron length, with temperature as the main environmental variable that controls the leaf appearance rate [78,79]. Similar responses have been observed with *Bromus* spp., where N addition has shown a limited effect on modifying the phyllochron [7]. However, N addition may speed up structural changes at the tiller level, for example, increasing leaf length [50]. Phyllochron has been reported to be unresponsive to N addition by [80,81], which suggests that soil N level modifies other variables related to plant growth dynamics but not LAR.

Increased PAW restriction did not modify Bv phyllochron (Table 1). PAW restriction reduced plant growth/mass, accelerated plant morphological development, and decreased survival. Cell expansion is usually affected first when plants are grown under restricted PAW due to the turgor pressure reduction in the leaf cells. Under a moderate water deficit, the leaf development continues at the same rate per unit of thermal time; thus, the phyllochron is not modified [82]. However, it has been reported that the phyllochron decreases with high PAW restriction due to the reduction in both cell size and cell division rates [16]. In the current study, while LAR slightly increased with decreasing PAW ( $p = 0.06$ ; Table 1), we suggest that these values do not allow us to conclude that Bv phyllochron was modified by decreasing PAW.

#### 4.2. Nutritive Value

There were no significant interactions between the main factors, demonstrating that the PAW restriction and N addition on Bv nutritive value are not codependent. Increased PAW restriction stimulated changes in the nutritive value, for example, Bv nutritive value decreased as FDN and FDA increased and ME and DV decreased (Table 2). Decreasing PAW (increasing SWR) may trigger plant phenological processes related to reproduction, for example, early progression into the reproductive stage, stopping tillering, and/or favouring stem elongation and early flowering [54], which are related to plant nutritional decrease [39,83]. It is important to note that these aforementioned plant phenological processes are also stimulated by day length [84]. Increasing PAW encourages tillering for a longer period, maintains pasture's nutritional value, and slows the start of the reproductive period [85,86]. Thus, high PAW retards the reproductive stage of a pasture and maintains a higher nutritive value (until commencement of the reproductive stage) [85]. In the current study, Bv subjected to the medium PAW-M (50–60%) had greater nutritive values when compared to Bv at the low PAW-L (20–30%) (Table 2).

A negative effect of severe restriction of PAW during the summer period on the nutritive value of pasture grass species has been reported by [29,87]. A decline in nutritive value may follow structural changes within the plant, for example, decreased leaf–stem ratio and accumulation of dead plant material [88] and increased thickness of the pseudostem bundle sheath wall and the upper and lower epidermis cuticle [9]. Plant cells also undergo structural changes due to the thickening of their primary and secondary walls and the reduction in the cell contents as the plants move towards heading [89,90]. These types of structural changes to the cell wall, in response to decreased PAW, were probably associated with increased cellulose, hemicellulose, and lignin, which are associated with decreased PAW (Table 2).

The physiological factors that are involved in the process of production and storage of WSC (water-soluble carbohydrates) are likely species (and cultivar and variety) dependent and governed by the relationships between the soil type, water uptake, transpiration, and water requirements [91]. However, the WSC concentration in the tissues did not differ between the PAW treatments; therefore, the WSC concentration was not sensitive to the changes in the PAW in this study.

It has been reported that Bv increases the WSC ( $\text{mg tiller}^{-1}$ ) in the base of the tillers with high PAW restriction [16]. After defoliation, Bv recovers 80% of its WSC when a thermal time of 195 AGDD has been reached [16,38,40] and when Bv completes 3.5–4.0 leaf regrowth stage and is ready to be defoliated again [16,40], meaning that a tiller at that regrowth stage will have five live leaves: three fully expanded leaves and two elongating leaves, one at 0.75% of its final length and a new one at 0.25% of its final length [40].

The CP content in Bv increased with higher PAW restriction, a response associated with the amount of herbage mass accumulated, where the PC content decreases with the increase in accumulated herbage mass (dilution effect). This has been demonstrated for pastures growing under low, adequate, and excessive N fertilisation [92]. The PC dilution effect of the N content commonly happens in pasture species and responds to eco-physiological plant development stages [93].

The addition of N did not modify the nutritive quality of Bv. Studies have reported that increasing N application rates modifies the nutritive quality and productive performance of both Bv and perennial ryegrass [62,64,94], for example, increased herbage mass and tissue CP and decreased NDF and WSC [62,64,94]. In the current study, N addition did not modify the nutritive quality, which may suggest that there was already enough N available in the soil; thus, N fertilisation did not have a significant effect on Bv nutritive quality adjustments and changes.

The nutritive values obtained in the Bv MS when subjected to the PAW treatments represent pastures of high nutritional quality, which suggests that these pasture species contain adequate nutrient concentration and high digestibility and can produce a good productive response on grazing livestock [39]. According to [95], pastures of high nutritive value are those that offer 18 to 24% DM, 18 to 25% CP, 2.5 to 2.9 Mcal kg<sup>-1</sup> DM EM, and 40 to 50% NDF, values that are similar to those obtained in the present study (Table 2). This shows that Bv subjected to different PAW levels had good productive performance and maintained a high nutritive value.

## 5. Conclusions

The soil water availability and N fertilisation generated changes in the growth dynamics of Bv during the summer period. A high or moderate PAW produces a greater herbage mass accumulation, longer laminae, greater leaf elongation rate, and heavier tillers than Bv under a low PAW. The high PAW in this study produced a greater number of live leaves per tiller, while moderate PAW and N addition to the soil resulted in Bv growth responses similar to those under high PAW (or a low SWR). However, the PAW and N addition did not modify the phyllochron, expressed as days and thermal time.

The nutritional value of Bv did not vary with the N addition. However, greater PAW restriction decreased pasture ME and digestibility and increased the DM content and NDF and ADF values. In general, even though some nutritive variables were reduced, they remained within ranges considered as high-quality forage. *Bromus valdivianus* has morphophysiological growth attributes that allow it to adjust growth according to changing environmental conditions, as demonstrated by the PAW restriction and the N supply. This capacity for morphophysiological adjustment and/or growth modification, ranging from competitive to stress tolerance, is a desirable attribute that provides persistence and resilience for grazing/pasture systems under increasing climate uncertainty.

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