



# Root traits contributing to water stress tolerance in two perennial grasses in semiarid rangelands of central Argentina

Características de las raíces que contribuyen a la tolerancia al estrés hídrico en dos gramíneas perennes de pastizales semiáridos del centro de Argentina

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## ABSTRACT

Limited water availability poses a significant challenge for plants in arid and semiarid environments, which cover two-thirds of Argentina's territory. The objective of this study was to quantify the effects of different water regimes (natural precipitation versus irrigation) on aboveground and belowground growth and production parameters of two native perennial, cool-season, grass species: *Nassella longiglumis* and *Amelichloa ambigua*. Seedlings were obtained from seeds and grown in bags containing sieved soil (n=6). Over two growing seasons, plants with supplemental irrigation (200 mm) were compared to control plants (natural precipitation). Plants were harvested in three key phenological phases: vegetative, reproductive, and dormant. For each plant, basal area, tiller production, height, aboveground and belowground biomass, root length density, and detailed characteristics of a complete adventitious root (length, width, and number of lateral roots) were determined. Scanned images were processed using specialized software

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for root image analysis. The observed differences between species reflect contrasting survival strategies. Control plants of *N. longiglumis* exhibited longer, finer adventitious roots and a higher length density than irrigated and *A. ambigua* plants. This latter was less sensitive to irrigation. Belowground biomass varied with irrigation during the cycle, with no differences between species. Unlike *N. longiglumis*, *A. ambigua* showed greater vegetative development, with more basal area and tillers, resulting in greater aerial biomass. In contrast, *N. longiglumis* prioritized its reproductive cycle. Both species had greater aerial biomass under irrigation. In conclusion, *N. longiglumis*, the most competitive species, exhibited adaptations in its root system for water acquisition, maintaining its aerial growth and reproductive effort even under water restriction. These traits possibly contribute to the dominance of *N. longiglumis* in these rangelands.

**Keywords:** Adventitious roots; aerial and belowground productivity; digital images; root length density.

## RESUMEN

La disponibilidad limitada de agua impone un gran desafío para las plantas en ambientes áridos y semiáridos, que cubren dos tercios del territorio argentino. El objetivo de este estudio fue cuantificar los efectos de distintos regímenes hídricos (precipitación natural versus riego) sobre parámetros de crecimiento y producción aéreas y subterráneas de dos especies de gramíneas perennes nativas, de ciclo invernal: *Nassella longiglumis* y *Amelichloa ambigua*. Se obtuvieron plántulas desde semilla que crecieron en bolsas conteniendo suelo tamizado (n=6). Durante dos temporadas de crecimiento, se compararon plantas con riego suplementario (200 mm) y plantas control (precipitación natural). Las plantas se cosecharon en tres fases fenológicas clave: vegetativa, reproductiva y dormante. En cada planta se determinaron: área basal, producción de macollas, altura, biomasa aérea y subterránea, densidad de longitud de raíces y características detalladas de una raíz adventicia completa (longitud, ancho y número de raíces laterales). Se emplearon imágenes escaneadas y procesadas mediante un software especializado en el análisis de imágenes de raíces. Las diferencias observadas entre las especies reflejan estrategias de supervivencia contrastantes. Las plantas control de *N. longiglumis* presentaron raíces adventicias más largas y finas y mayor densidad de longitud que las irrigadas y que plantas de *A. ambigua*. Esta última fue menos sensible al riego. La biomasa subterránea varió con la irrigación durante el ciclo, sin diferencias entre especies. A diferencia de *N. longiglumis*, *A. ambigua* mostró mayor desarrollo vegetativo, con más área basal y macollas, resultando en mayor biomasa aérea. En cambio *N. longiglumis* priorizó su ciclo reproductivo. Ambas especies tuvieron mayor biomasa aérea bajo riego. En conclusión, *N. longiglumis*, la especie más competitiva, exhibió adaptaciones en su sistema radical para la adqui-

sición de agua, manteniendo su crecimiento aéreo y esfuerzo reproductivo incluso bajo restricción hídrica. Estos rasgos posiblemente contribuyan a la dominancia de *N. longiglumis* en estos pastizales.

**Keywords:** Densidad de longitud radical; imágenes digitales; productividad aérea y subterránea; raíces adventicias.

## INTRODUCTION

Water availability stands out as the primary environmental factor shaping plant distribution, growth, and survival worldwide (Brown, 1995). In most of the world's rangelands, including Argentina, vast areas experience climatic conditions where water becomes a limiting resource for plant development, at least during specific parts of their growth cycle (Busso & Fernández, 2018). Water stress in these semiarid regions limits plant productivity by negatively affecting various growth parameters. Root-mediated soil exploration, crucial for resource acquisition, represents a significant energy investment for mature plants. Research indicates that this activity can consume over half of the available photoassimilates (Fogel, 1985). Furthermore, drought-tolerant species often allocate considerably more biomass belowground than aboveground, emphasizing the critical role of an optimal root system for their survival and productivity (Fogel, 1985).

In semiarid and arid environments, competition for resources occurs mainly underground. Success in this belowground competition depends on the plant's ability to occupy available soil space, especially through root length and root biomass (Casper & Jackson, 1997). Thus, differences in root proliferation capacity could explain why some species are more competitive or drought-tolerant than others. To understand plant productivity under drought conditions, research often focuses on root traits such as total length and diameter, root length density, belowground biomass, and the ability to reach deeper soil layers with more available water, along with the degree of lateral root development (Hartnett *et al.*, 2013). The combined analysis of belowground and aboveground traits, particularly those that influence plant productivity, allows a more complete evaluation of the differences between species with different forage values for livestock purposes. Information about maximum rooting depth and lateral spread can better predict functional variations among plant species under current and future climate change scenarios (Comas *et al.*, 2013). Although studies in Argentina that simultaneously examine aboveground and belowground components have increased over the past three decades, this type of research remains scarce (López-Mársico *et al.*, 2020). The use of appropriately sized containers offers a valuable and complementary approach to field investigations in elucidating the belowground characteristics of vegetation and for understanding the root system's dynamics (Poorter *et al.*, 2012).

Rangelands of central Argentina, traditionally dominated by mid-to-late successional perennial forage species, have been significantly impacted by land-use practices and climatic factors (Peláez *et al.*, 2017). Extensive livestock production, especially bovine, which relies heavily on grazing native vegetation, is the dominant activity in these regions. However, poor land management practices, including overgrazing, excessive clearing, and tillage, coupled with adverse climatic factors like severe droughts, strong winds, and irregular rainfalls, have resulted in the loss and degradation of these valuable grasslands. In many areas, the original vegetation has been replaced by species of lower forage quality and earlier successional stages, compromising local livestock production (Loydi & Distel, 2010). Perennial grasses of late successional stages have been found to have greater competitive ability and drought and defoliation tolerance than early successional species. This increased competitiveness has been observed in different perennial grass species of central Argentina. This is attributed to several morphological traits like denser and more extensive root systems, a higher prevalence of beneficial mycorrhizal fungi in their roots, greater biomass allocation to belowground biomass and faster growth rates of their aerial tissues even after defoliation events (Busso *et al.*, 2008; Cardillo *et al.*, 2018; Distel & Fernández, 1988; Moretto & Distel, 1997; Torres *et al.*, 2020).

Cool-season perennial grasses, the most abundant vegetation type in these rangelands, have adapted to semiarid environments by developing shallow root systems, with a high proportion distributed in the upper soil layers (Peláez *et al.*, 1994). Shallow root allocation allows rapid water uptake from small rain events, critical during dry periods. Even rainfall events of small magnitude can rapidly stimulate physiological processes in these grasses, such as new root production (Sala & Lauenroth, 1982). Low rainfall events are the most prevalent in this semiarid region, highlighting the importance of this adaptation for drought tolerance (Fresnillo Fedorenko *et al.*, 1992).

Considering this background, two native, cool-season perennial grasses, of different successional stages, were selected for the study. The relative abundance of these species in the plant community depends on historical and current land uses (Giorgetti *et al.*, 1997). *Nassella longiglumis* (Phil.) Barkworth (ex *Stipa clarazii*, “flechilla grande”) is a late-seral, palatable, dominant, and highly competitive species under exclosure or light grazing conditions. Selective heavy grazing results in their replacement by *Amelichloa ambigua* (Speg.) Arriaga & Barkworth (ex *Stipa ambigua*, “paja vizcachera”), a less-competitive, unpalatable, early-seral grass (Cano, 1988; Saint Pierre *et al.*, 2004a, 2004b). These C<sub>3</sub> grasses have adapted to the semiarid climate by concentrating their growth during the cooler and wetter seasons (autumn and spring), completing their life cycle before the hot, dry summer. As a result, their green leaf production ceases during the summer months, increasing only in response to significant rainfall events (Distel & Fernández, 1988). Another crucial adaptation is the presence of well-devel-

oped, highly branched root systems exhibiting continuous activity throughout their growing season (Busso & Bolletta, 2007; Saint Pierre *et al.*, 2003). However, root growth and decomposition rates vary seasonally, with higher values observed in spring and early fall compared to late fall and winter (Distel & Fernández, 1988). Climatic factors significantly influence root growth. Wet periods and low soil temperatures can lead to reduced root activity. The plant's phenological phase also plays an important role, with root growth potentially decreasing during the spring reproductive period (Becker *et al.*, 1997). Studies consistently show enhanced root development (proliferation, biomass, and density) is crucial for *N. longiglumis*'s competitive ability across varying water availability (Becker *et al.*, 1997; Flemmer *et al.*, 2002; Saint Pierre *et al.*, 2004a, 2004b). Their robust root system likely confers additional benefits beyond competition, such as improved drought tolerance and enhanced nutrient acquisition. However, a limitation of these studies is their reliance on soil and root samples taken from mature, field-grown plants of different ages and developmental stages. This approach does not simultaneously account for aboveground and belowground components nor does it analyze whole-plant functioning under natural conditions.

This study aimed to quantify the effects of contrasting water regimes (natural precipitation versus irrigation) on aboveground and belowground growth and production parameters of two perennial grass species differing in palatability and successional stage. These parameters were investigated simultaneously, on different plant's phenological stages. The results of this work are expected to contribute to a better interpretation of the adaptive mechanisms that confer these species a competitive advantage in environments with limited water availability. This information, in turn, could then be used to identify key morphological characteristics for improving the productivity and resilience of perennial forage grasses for restoration purposes.

## MATERIALS AND METHODS

### Study site

The study was carried out over two years at the Chacra Experimental Patagones, in the southwest of Buenos Aires Province (40° 39' S, 62° 54' W; 40 m asl), within the Monte Phytogeographic Province (Cabrera, 1976; Fig. 1). This province exhibits a more pronounced water deficit in its southernmost region compared to other semiarid areas like the Caldenal. The climate is temperate with concentrated rainfall in winter and spring (Giorgetti *et al.*, 2000). Long-term mean annual precipitation (40 years) is 430.4 mm with a maximum of 877 mm in 1984 and a minimum of 196 mm in 2009; mean annual temperature is 14.1 °C. The soil type is a typical haplocalcid. The community is characterized by an open shrub layer including herbaceous species of different quality for livestock production (Giorgetti *et al.*, 1997).



**Fig. 1.** Geographical location of the Chacra Experimental Patagones, within the Buenos Aires Province (in black) and the Monte Phytogeographic Province, Argentina (in green).

**Fig. 1.** Ubicación geográfica de la Chacra Experimental Patagones, dentro de la Provincia de Buenos Aires (en negro) y la Provincia Fitogeográfica del Monte, Argentina (en verde).

Dominant woody species are *Condalia microphylla*, *Chuquiraga erinacea*, *Larrea divaricata*, *Schinus johnstonii*, *Geoffroea decorticans*, *Neltuma alpataco* and *Prosopidastrum angusticarpum*. The herbaceous layer is dominated by perennial grasses as *Nassella longiglumis*, *N. tenuis*, *Piptochaetium napostaense* and *Poa ligularis*. Other common perennial grasses in the region include *Pappophorum vaginatum*, *Pappostipa speciosa*, *Jarava plumosa*, *Amelichloa ambigua* and *A. brachychaeta* (Torres *et al.*, 2024). Precipitation data during the study were collected by a meteorological station located at the site.

## Experimental design and treatments

In late spring 2011 (before the study), seeds of *N. longiglumis* and *A. ambigua* were collected from the study site. Seeds were germinated on moistened filter paper in Petri dishes at 24°C under natural photoperiod conditions with constant humidity. The following year, 36 seedlings of each species were obtained and transferred to the field in April. Individual plants were transplanted into black polyethylene bags (with basal perforations) of increasing volumes (small: 0.03 m<sup>3</sup>, intermediate: 0.045 m<sup>3</sup>, large: 0.06 m<sup>3</sup>) filled with previously sieved soil (0.04 cm<sup>2</sup> mesh screen) from the study site. The bag volumes were selected based on a literature review to provide adequate space for root growth throughout the study (Distel & Fernández, 1988; Poorter *et al.*, 2012). All plants were kept in an enclosure to domestic livestock, under natural conditions, from April to February of the following year. To assess the impact of water availability on plant growth, two treatments were applied: natural precipitation (control) and natural precipitation supplemented with weekly irrigation (irrigated) to achieve an additional 200 mm of precipitation during the entire 11-month growth cycle. Weekly irrigation volumes were calculated based on the surface area of each pot and were applied manually. To assess plant response throughout the year, six plants per species per treatment (n=6) were sampled at three key phenological stages defined by Giorgetti *et al.* (2000): vegetative (mid-fall-winter; plants from small bags), reproductive (spring-early summer; plants from intermediate bags), and dormant (late summer; plants from large bags). To assess potential year-to-year variation, the entire procedure was repeated over a second year using new plants grown from seeds. Figure 2 shows the monthly water balance components for the two-year period, spanning 11 months per year, and the experimental design used. The accumulated natural precipitation up to the time of plant removal in the dormancy phenological stage was 342 mm and 307.3 mm, for the first and second year, respectively.

## Sampling procedures

**1.1. Aboveground analysis.**— At each phenological stage, plant components contributing to aboveground production, including basal area, number of vegetative and reproductive tillers, and height to the tallest tiller were measured (Fig. 3). Subsequently, the aerial biomass was harvested at 5 cm and oven-dried at 70°C for 72 hours to determine aboveground biomass production.

**1.2. Belowground analysis.**— To minimize damage, the entire root system of each plant with some soil attached was extracted from the bags and transported to the laboratory for further processing. The longest adventitious root of each plant was then carefully removed. For enhanced visualiza-

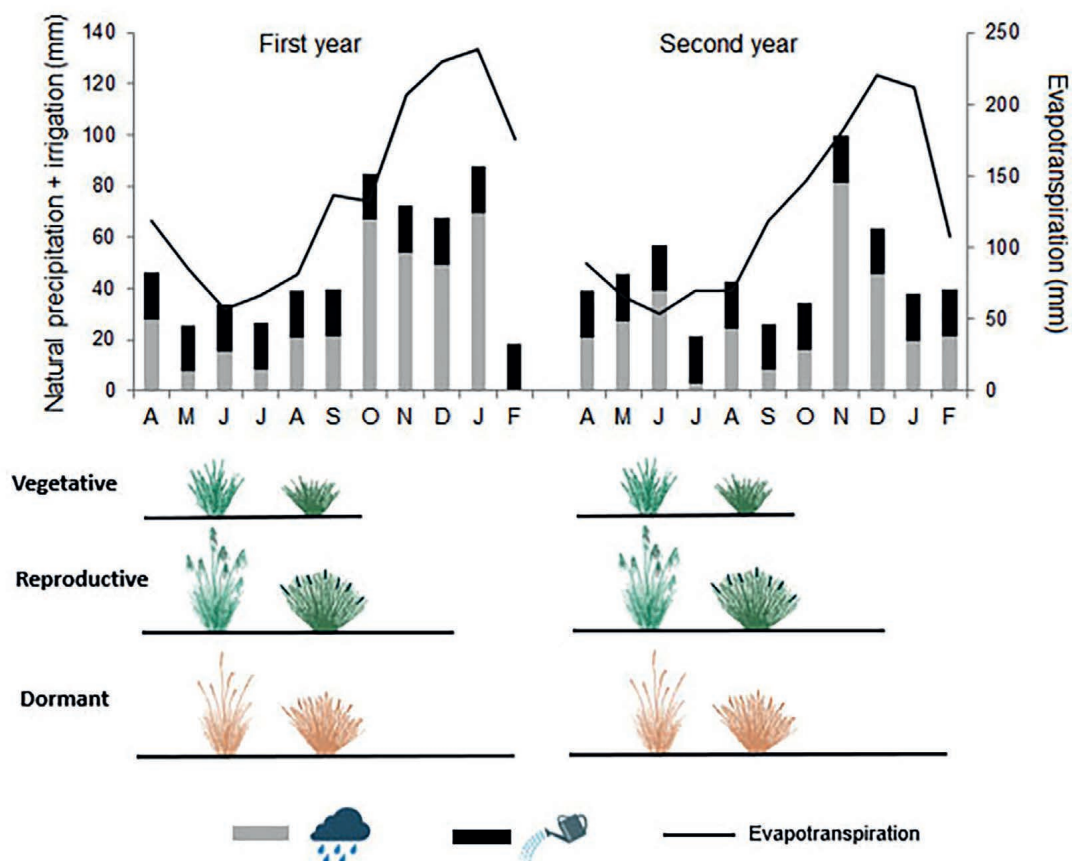


Fig. 2. Monthly natural precipitation, irrigation and evapotranspiration for the phenological phases evaluated (vegetative, reproductive and dormant), in *Nassella longiglumis* (left) and *Amelichloa ambigua* (right) plants, during the study period (11 months, two years).

Fig. 2. Precipitación natural mensual, riego y evapotranspiración para las fases fenológicas evaluadas (vegetativa, reproductiva y dormante), en plantas de *Nassella longiglumis* (izquierda) y *Amelichloa ambigua* (derecha), durante el periodo de estudio (11 meses, dos años).

tion, it was stained with a 0.05% w/v Trypan Blue solution and placed on transparent adhesive paper for scanning. The remaining root system was thoroughly sieved through a 0.05 cm<sup>2</sup> mesh sieve to recover roots from the soil, which were also stained and scanned in sections between two large 20 x 35 cm glasses to capture its entirety. Digital images of both the adventitious roots and the whole root systems were analyzed using the free software Rootedge 2.3b (Kaspar & Ewing, 1997) and ImageJ 1.54g (Abramoff *et al.*, 2004). Adventitious root traits, including total length, average width, number of lateral roots, and total lateral root length, were quantified from the images (Fig. 3). Additionally, root length density was determined from the images of the complete root system and expressed as cm of root length per cm<sup>3</sup> of soil volume. After scanning, roots were dried at 70 °C for 72 hours and then placed in a muffle furnace at 550 °C for 6 hours (McNaughton *et al.*, 1998). The belowground biomass production of the ash-free organic matter was obtained by subtracting the dry weight at 550 °C from that at 70 °C (Fig. 3).

## Statistical analysis

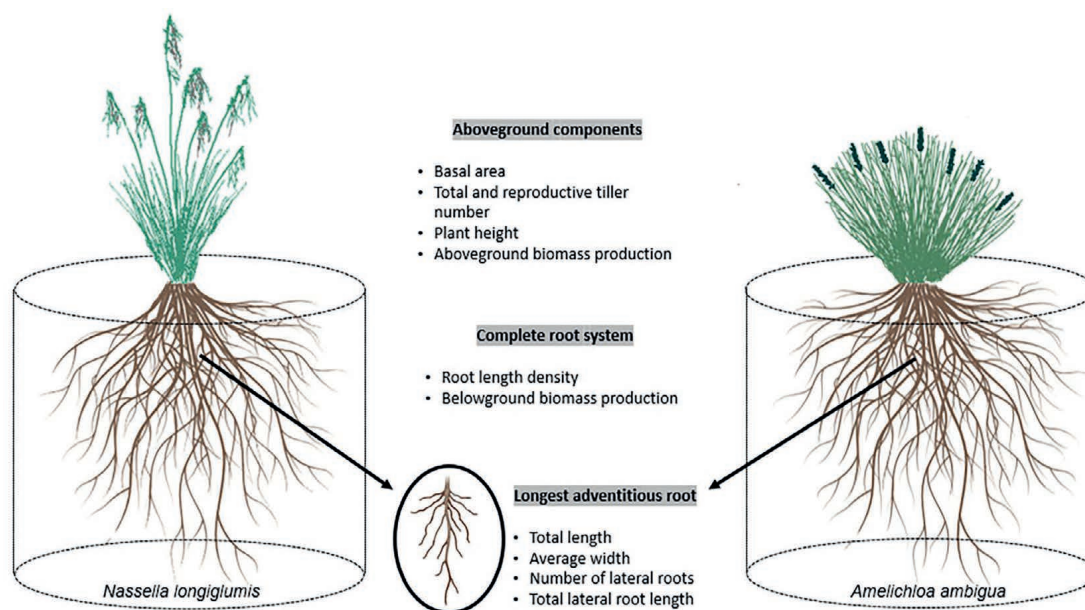
A four-way ANOVA was used to analyze the data, considering year (two levels), species (*N. longiglumis* and *A. ambigua*), treatment (control and irrigated), and phenological phase (vegetative, reproductive, and dormancy) as independent factors. When interactions between factors were significant ( $p \leq 0.05$ ), each interaction was analyzed separately. Tukey's test ( $\alpha = 0.05$ ) was used for mean comparison. To meet the assumptions of normality and homoscedasticity, the number of lateral roots, tillers, and root length density data were transformed using  $\sqrt{x}$ , while aboveground and belowground dry weight data were transformed with  $\ln(x+1)$  (Sokal & Rohlf, 1984). Untransformed values are shown in the figures. All statistical analyses were performed with Infostat version 2020 (Di Rienzo *et al.*, 2020).

## RESULTS

As statistical analysis revealed no significant year-to-year differences the results presented are mean values for the entire two-year study period.

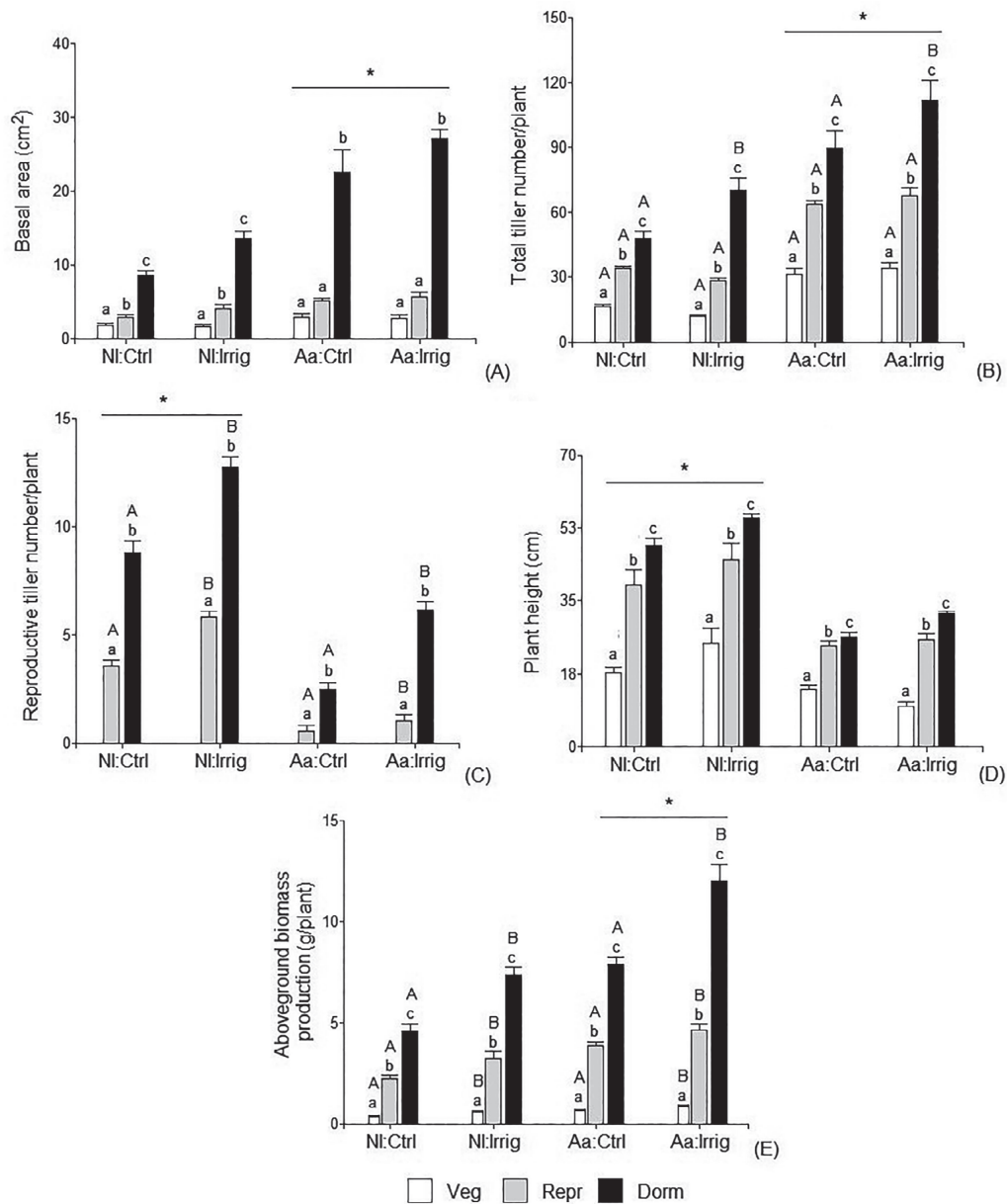
### Aboveground analysis

*Amelichloa ambigua* plants consistently exhibited a greater basal area compared to *N. longiglumis* plants throughout the entire growth cycle, in both treatments (Fig. 4A). A larger basal area was recorded during the dormant



**Fig. 3.** Parameters evaluated in aboveground and belowground portions of *Nasella longiglumis* and *Amelichloa ambigua* plants, cultivated in pots under field conditions.

**Fig. 3.** Parámetros evaluados en porciones aéreas y subterráneas de plantas de *Nasella longiglumis* y *Amelichloa ambigua*, cultivadas en macetas en condiciones de campo.



**Fig. 4.** (A) basal area; (B) total tiller number; (C) total reproductive tiller number; (D) height and (E) aboveground biomass production (mean  $\pm$  1 S.E.,  $n=12$ ) in *Nassella longiglumis* (NI) and *Amelichloa ambigua* (Aa) plants exposed to natural precipitation (Ctrl) or natural precipitation plus irrigation (Irrig) during the vegetative (Veg), reproductive (Repr), and dormant (Dorm) phenological phases. Within each phenological phase and species different uppercase letters indicate significant differences ( $p \leq 0.05$ ) between treatments; within each species and treatment, different lowercase letters indicate significant differences ( $p \leq 0.05$ ) between phenological phases; \* indicate significant differences ( $p \leq 0.05$ ) between species within treatments.

**Fig. 4.** (A) área basal; (B) número total de macollas; (C) número total de macollas reproductivas; (D) altura y (E) producción de biomasa aérea (media  $\pm$  1 E.S.,  $n=12$ ) en plantas de *Nassella longiglumis* (NI) y *Amelichloa ambigua* (Aa) expuestas a precipitación natural (Ctrl) o precipitación natural más riego (Irrig) durante las fases fenológicas: vegetativa (Veg), reproductiva (Repr) y dormante (Dorm). Dentro de cada fase fenológica y especie, letras mayúsculas distintas indican diferencias significativas ( $p \leq 0,05$ ) entre tratamientos; dentro de cada especie y tratamiento, letras minúsculas distintas indican diferencias significativas ( $p \leq 0,05$ ) entre fases fenológicas; \* indican diferencias significativas ( $p \leq 0,05$ ) entre especies dentro de los tratamientos.

phase, for both species, but this increase was more pronounced in the unpalatable species. The irrigation treatment did not significantly affect this parameter.

The total number of tillers (green + dry) per plant also increased throughout the growth cycle, with *A. ambigua* plants having a significantly higher number of tillers compared to *N. longiglumis* at all sampling dates (Fig. 4B). The irrigation treatment only led to a significant increase in tiller production compared to the control group in the final phenological stage (Fig. 4B). The evaluation of reproductive tillers per plant was carried out during the last two phenological stages. Irrigation also increased the production of reproductive tillers per plant, with higher values for *N. longiglumis* (Fig. 4C). The production of reproductive tillers continued to increase until the plants were removed in the dormant phase.

Plant height increased naturally throughout the growth cycle, unaffected by irrigation in either *N. longiglumis* or *A. ambigua* (Fig. 4D). The palatable species consistently achieved greater height in both treatments.

Aboveground biomass production per plant increased steadily throughout the growth cycle, with *A. ambigua* consistently exceeding *N. longiglumis* (Fig. 4E). Irrigation further enhanced biomass accumulation in both species, and this pattern was maintained throughout the study.

### Belowground analysis

Regardless of irrigation treatment, adventitious root length increased in both species throughout the study (Fig. 5A). However, the treatment had different effects depending on the species considered. In *N. longiglumis*, control plants exhibited longer roots compared to irrigated ones. Conversely, *A. ambigua* root length remained unaffected by the treatment. Additionally, under control conditions, the preferred species had longer adventitious roots compared to the non-preferred one.

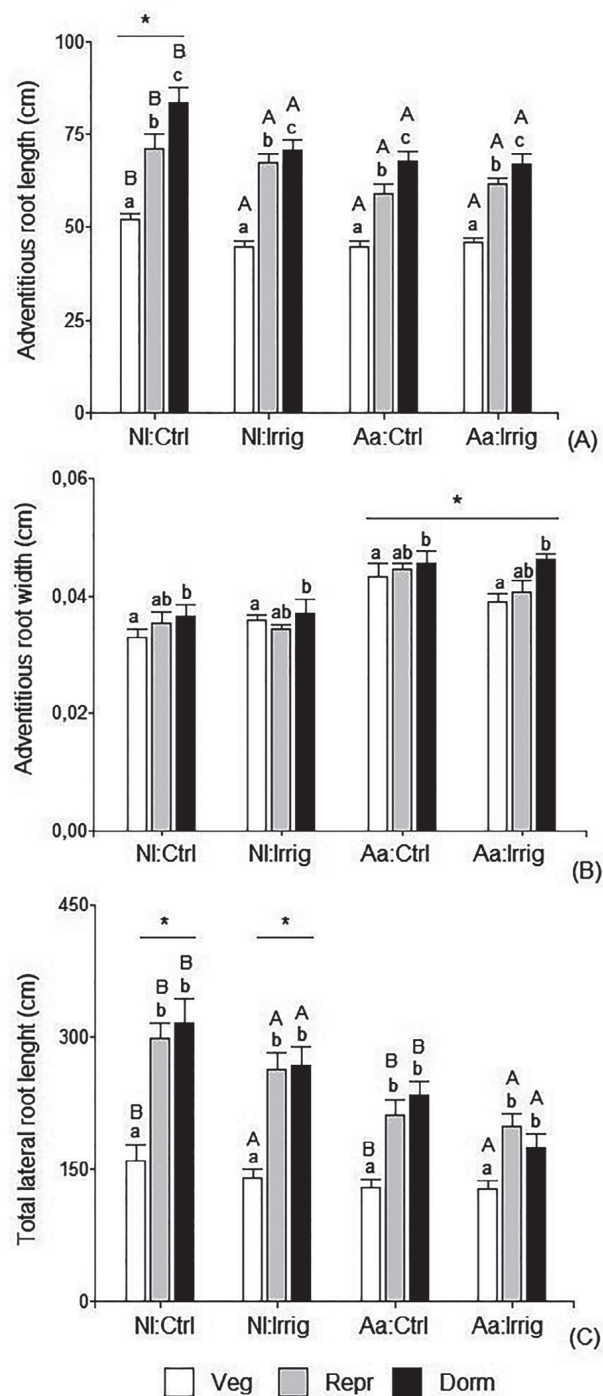
Adventitious root width varied between both species and growth stages. *Amelichloa ambigua* showed a greater mean root width compared to *N. longiglumis* (Fig. 5B). In addition, root width only increased between the early vegetative and late dormant stages, despite a general trend of increasing width throughout the growth cycle. The treatment produced no effect.

The number of lateral roots per adventitious root remained consistent across species and treatments, exhibiting only a steady increase throughout the growth cycle. Across the three phenological phases (vegetative, reproductive, and dormant) average values (n=48) with standard error were  $133.46 \pm 4.55$ ,  $150.69 \pm 6.15$ , and  $182.08 \pm 7.06$ , respectively.

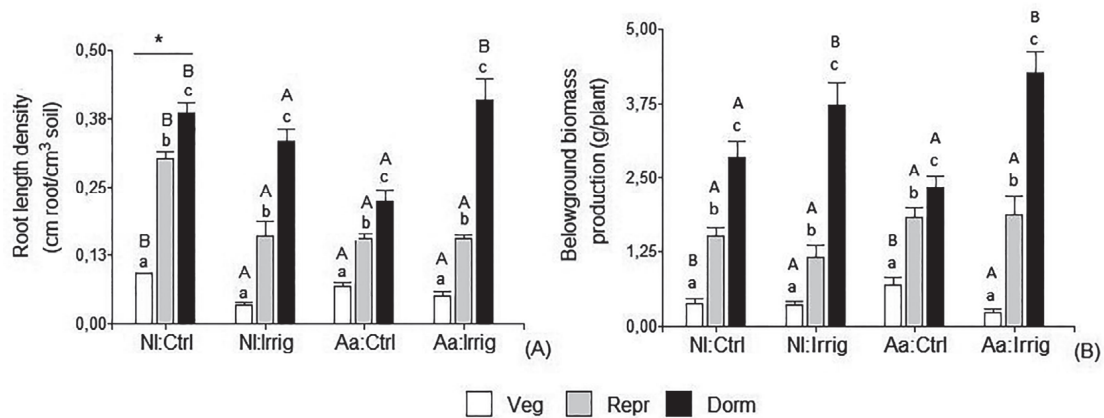
For both species, the control treatment resulted in longer total adventitious lateral roots compared to irrigation (Fig. 5C). Total length increased in the last two phenological phases (reproductive and dormant) compared to the vegetative one. In addition, *N. longiglumis* surpassed *A. ambigua* during these final two stages, in both treatments.

**Fig. 5.** (A) adventitious root length, (B) adventitious root width and (C) total adventitious lateral roots length (mean  $\pm$  1 S.E.,  $n=12$ ) in *Nassella longiglumis* (NI) and *Amelichloa ambigua* (Aa) plants exposed to natural precipitation (Ctrl) or natural precipitation plus irrigation (Irrig), during the vegetative (Veg), reproductive (Repr), and dormant (Dorm) phenological phases. Within each phenological phase and species different uppercase letters indicate significant differences ( $p \leq 0.05$ ) between treatments; within each species and treatment, different lowercase letters indicate significant differences ( $p \leq 0.05$ ) between phenological phases; \* indicate significant differences ( $p \leq 0.05$ ) between species within treatments.

**Fig. 5.** (A) longitud de la raíz adventicia, (B) ancho de la raíz adventicia y (C) longitud total de raíces laterales de la raíz adventicia (media  $\pm$  1 E.S.,  $n=12$ ) en plantas de *Nassella longiglumis* (NI) y *Amelichloa ambigua* (Aa) expuestas a precipitación natural (Ctrl) o precipitación natural más riego (Irrig), durante las fases fenológicas vegetativa (Veg), reproductiva (Repr) y dormante (Dorm). Dentro de cada fase fenológica y especie, letras mayúsculas distintas indican diferencias significativas ( $p \leq 0,05$ ) entre tratamientos; dentro de cada especie y tratamiento, letras minúsculas distintas indican diferencias significativas ( $p \leq 0,05$ ) entre fases fenológicas; \* indican diferencias significativas ( $p \leq 0,05$ ) entre especies dentro de los tratamientos.



Considering the complete root system, root length density consistently increased throughout the year for both species, regardless of irrigation treatment. Species differences were only found in the control group, where *N. longiglumis* exhibited greater values than *A. ambigua* (Fig. 6A). Besides, for *N. longiglumis*, the control group showed a higher length density compared to irrigation throughout the year, while for *A. ambigua* no treatment effect was observed, except in the final phase, where irrigation surpassed the control.



**Fig. 6.** (A) root length density and (B) belowground biomass production in *Nassella longiglumis* (NI) and *Amelichloa ambigua* (Aa) plants exposed to natural precipitation (Ctrl) or natural precipitation plus irrigation (Irrig), during the vegetative (Veg), reproductive (Repr), and dormant (Dorm) phenological phases. Within each phenological phase and species different uppercase letters indicate significant differences ( $p \leq 0.05$ ) between treatments; within each species and treatment, different lowercase letters indicate significant differences ( $p \leq 0.05$ ) between phenological phases; \* indicate significant differences ( $p \leq 0.05$ ) between species within treatments.

**Fig. 6.** (A) densidad de longitud de raíces y (B) producción de biomasa subterránea en plantas de *Nassella longiglumis* (NI) y *Amelichloa ambigua* (Aa) expuestas a precipitación natural (Ctrl) o precipitación natural más riego (Irrig), durante las fases fenológicas vegetativa (Veg), reproductiva (Repr) y dormante (Dorm). Dentro de cada fase fenológica y especie, letras mayúsculas distintas indican diferencias significativas ( $p \leq 0,05$ ) entre tratamientos; dentro de cada especie y tratamiento, letras minúsculas distintas indican diferencias significativas ( $p \leq 0,05$ ) entre fases fenológicas; \* indican diferencias significativas ( $p \leq 0,05$ ) entre especies dentro de los tratamientos.

Belowground biomass production increased throughout the growth cycle in both species, but the effect of irrigation treatment shifted over time (Fig. 6B). During the vegetative phase, control plants showed higher biomass. However, this difference disappeared in the reproductive period and then reversed in the dormant phase, where irrigated plants accumulated more biomass. There were no differences between species.

## DISCUSSION

This region receives precipitation levels that ranges between arid and humid regimes. However, it experiences significant year-to-year variation, with a trend towards more frequent and intense droughts (Stritzler *et al.*, 2007). This increasing aridity makes the region highly dependent on short-term climatic events (Ferrelli *et al.*, 2019). Therefore, evaluating how perennial plant species cope with this evolving scenario becomes increasingly crucial. Throughout the two-year study period, recorded precipitation remained below historical averages, but total water availability exceeded historical values when combined with irrigation. Such patterns are characteristic of this site, where precipitation levels fell below the 20-year average

approximately 50% of the time. Despite a 10.2% difference in cumulative rainfall between the two years, the parameters studied showed no significant interannual differences. This suggests that the amplitude of precipitation experienced during the study may not have been a determining factor for these parameters. On the other hand, while this design allowed for the evaluation of plant responses to contrasting precipitation conditions (water stress vs. improved soil water levels), high evapotranspiration throughout most of the study period resulted in a water deficit for the plants, as shown in Figure 2.

All measured parameters, both aboveground and belowground, exhibited continuous growth throughout the year. However, responses to increased water availability from irrigation treatment varied. The non-forage species consistently produced the greatest biomass throughout the study due to its larger basal area and abundant vegetative tillers. This aligns with findings by Ithurrart (2015), who attributed similar results to a greater tiller size in the studied species (as measured by total leaf length of blades, sheaths, and both dry and green leaves per tiller). In *N. longiglumis*, taller tillers contributed to aerial biomass accumulation, but this wasn't enough to match *A. ambigua*'s aerial production. Previous field studies reported higher aerial biomass for *N. longiglumis* than *A. ambigua* under various disturbances and water availability scenarios, mainly due to higher relative height growth and dry matter production rates (Busso *et al.*, 2016a; Saint Pierre *et al.*, 2004a). Two limitations likely contributed to this discrepancy with our results. First, our trial period of 11 months might not have been long enough for *N. longiglumis* to reach its full growth potential. Second, there is a lack of data on litter production, a relevant component of total biomass. While Ambrosino *et al.* (2019) reported higher litter production in the late-seral forage species *Poa ligularis* compared to *A. ambigua* under field conditions, our study on *N. longiglumis* lacked litter production data, potentially underestimating total aerial biomass due to material loss. Furthermore, non-palatable species exhibit slower leaf turnover rates, partially attributed to their tissue chemistry, which enhances leaf retention (Ambrosino *et al.*, 2019). Leaf emergence is another key driver of biomass production in grasses. During the vegetative stage, new leaves emerge faster than old leaves die off (senescence). In contrast, this pattern shifts during the reproductive phase when the rate of new leaf emergence slows down (Langer, 1972). *Nassella longiglumis* exhibits earlier spring flowering compared to the later cycle of *A. ambigua* (late spring flowering) (Armando *et al.*, 2024; Ithurrart, 2015). This earlier shift to reproduction in *N. longiglumis* likely translates into a shorter period of leaf production, potentially resulting in lower biomass accumulation than *A. ambigua*. The extended foliage retention and longer vegetative growth cycle of *A. ambigua* may have contributed to its higher biomass production in this study.

Irrigation only promoted increased aerial plant growth compared to the control treatment towards the study's end, through a higher tiller per

plant production. This coincides with what was previously mentioned for cool-season species that increase their growth in summer (January and February) only if water supply is maintained (Distel & Fernández, 1988). However, when evaluating aerial biomass production, a positive effect of irrigation during the whole cycle was observed for both species. In line with this, Busso *et al.* (2004) reported precipitation as a key factor influencing biomass variation in these species at this specific site. Similarly, Ithurrart (2015) found a strong dependence between aerial growth and rainfall in *A. ambigua* and *P. ligularis*, in the same rangeland. In a pot study, Moretto and Distel (1997) found that limited water availability negatively impacted both aboveground and seed yield in *N. longiglumis* and *Jarava ichu* (unpalatable grass species) grown in greenhouse conditions. Limited soil moisture is well-documented to reduce plant biomass due to water stress impacting growth parameters such as leaf production and growth rates (Brown, 1995). Our findings further support this established link between water availability and biomass production.

Increased water availability also favored reproductive tiller production, especially in *N. longiglumis*. Consistent with the previously mentioned that *A. ambigua* exhibits delayed or even absent reproduction compared with other perennial grass species, our study found this species produced significantly fewer reproductive tillers than *N. longiglumis* under both water regimes. The number of reproductive tillers can indicate the species' reproductive effort, especially under stressful conditions. In this regard, these species can produce two types of reproductive structures: open chasmogamous flowers located on terminal panicles, which facilitate cross-pollination, and self-fertilizing cleistogamous flowers found at various nodes on axillary panicles, enclosed within leaf sheaths (Lerner *et al.*, 2008). Previous research has shown that *N. longiglumis* favors the chasmogamous flowers over cleistogamous, whereas the opposite occurs in *A. ambigua* (Armando *et al.*, 2024). In this way, *N. longiglumis* may expend more energy when resources are plentiful to ensure successful cross-pollination, while *A. ambigua* prioritizes self-assured reproduction even under stress. Although the production of cleistogamous flowers was not evaluated in this study, it is possible the species that produced fewer reproductive tillers (*A. ambigua*) favored this method instead. The unseen flower production might account for *A. ambigua*'s lower reproductive tiller count.

While root architecture (length, density, branching) and morphological characteristics (dry biomass) are determined by plant genetics, they can also be significantly affected by environmental factors (biotic and abiotic) and by the plant's physiological and phenological state (Bellini *et al.*, 2014). Changes in root architecture can be caused by adjustments in the number, growth, and distribution of lateral roots (Malamy, 2005). Particularly in grass species, the characteristics of both components, adventitious roots and their lateral branches, collectively define their fibrous root system's overall complexity and density (Fitter, 1994). In our study, belowground

parameters showed variable results between species, water availability, and phenological stages. *Nassella longiglumis* showed root system traits potentially beneficial for water stress tolerance. This preferred species displayed enhanced adventitious root growth when subjected to water stress, exceeding both the irrigated treatment and the control plants of the other species. In *A. ambigua* no changes in root length were observed under differential water availability conditions. No differences were found in the number of lateral roots per adventitious root between the two species and treatments. However, an increase in this parameter was observed throughout the growing season. In semiarid environments, plants develop larger lateral root spreads to maximize water uptake from the shallowest layers of soil, where the majority of infiltrated water is accessible (Schenk & Jackson, 2002). This adaptation is particularly beneficial in areas experiencing shrub encroachment, where grasses compete intensely with woody species for scarce soil moisture. At this study site, a notable increase in woody plant density has been observed in recent years (Torres *et al.*, 2024). *Chuquiraga erinacea*, a shrub characterized by a dense canopy of persistent leaves, is the dominant species. This shrub's high rainwater retention capacity, due to its leaf structure, can reduce water infiltration into the subsoil thereby limiting water availability for other plant species (Álvarez Redondo *et al.*, 2018; Álvarez Redondo & Adema, 2018). Our results also align with previous research on the dynamics of root growth in drought-tolerant native perennial grasses (Distel & Fernández, 1998). This study revealed that lateral roots are continuously produced, and those produced in autumn can remain viable for up to 10 months, undergoing a suberization process towards summer. Root suberization is crucial for drought-stressed plants to survive in semiarid conditions, as it creates a physical barrier that minimizes water loss and maintains root structural integrity in harsh soil conditions (Barberon *et al.*, 2016). Furthermore, recent research has demonstrated that suberization can also enhance drought stress tolerance in plants by promoting lateral root development (Kim *et al.*, 2022). On the other hand, *Nassella longiglumis* showed smaller diameter roots and greater total lateral root length than *A. ambigua*. These differences were more important towards the end of the study. Ithurrart *et al.* (2018) also reported greater root diameter in *A. ambigua* compared to other perennial preferred grass species. Small-diameter roots, which have a higher specific root length, increase plant efficiency by maximizing surface area contact with soil water, effectively expanding the volume of soil accessible for water exploration (Comas *et al.*, 2013). This increased contact facilitates improved water acquisition and enhanced plant productivity. Reducing root diameter is a desirable trait for plants facing drought, as it enhances water acquisition and overall productivity (Wasson *et al.*, 2012). Additionally, thinner roots offer an advantage in terms of root growth rate, further contributing to plant resilience and efficiency in resource acquisition (Larreguy *et al.*, 2012).

When examining the entire root system differences between species were also found. *Nassella longiglumis* control plants consistently displayed a higher root length density compared with regular irrigation treatment and *A. ambigua* plants. This suggests a more extensive root network in *N. longiglumis*, allowing for greater access to water throughout the soil volume. Also, the presence of a higher root length density in *N. longiglumis* than *A. ambigua*, together with similar root biomass between both species, indicates the presence of finer roots in the first species, which is supported by the observation of the smaller diameter found in their adventitious roots. In *A. ambigua*, root length density was affected by irrigation only during the last phenological stage, suggesting that continuous water availability stimulated uninterrupted root growth in this species. On the other hand, during the growth cycle, a higher production of belowground biomass was observed in control plants of both species during the vegetative stage, coinciding with the period of highest plant activity, and then reversing this trend towards summer, when plants maintain their growth in response to water supply. This behavior aligns with observations in other studies and reflects the ability of perennial grasses to respond to pulses of water availability by strategically allocating resources during the drier months to fine-root production (Becker *et al.*, 1997; Larreguy *et al.*, 2012).

Plants tend to optimize resource acquisition in semiarid environments by balancing their aerial and belowground growth rates. Drought-stressed plants may adapt to water deficiency by stimulating or preserving root growth while reducing shoot growth (Bloom *et al.*, 1985). By favoring root growth over aerial growth, plants can enhance their hydraulic status under mild to moderate drought stress, thanks to an increased root-to-shoot ratio, continuous production of new root tips, and improved water acquisition to sustain existing shoots (Comas *et al.*, 2013). Our study observed that water stress led to reduced aerial growth in control plants compared to irrigated ones. Yet, all aerial and belowground production components still showed an increase throughout the growing season. These findings confirm previous research highlighting the stress tolerance of these species, as they maintain growth while adapting to water-limited conditions (Becker *et al.*, 1997; Saint Pierre *et al.*, 2003). Particularly in *N. longiglumis*, these traits likely contribute to its higher precipitation use efficiency, as previously reported, compared to *A. ambigua* (Busso *et al.*, 2016b).

In their natural habitat, these grasses experience not only water scarcity but also adverse thermal conditions, especially during the summer months. While the presence of woody species mitigates heat stress by providing shade, this concomitant condition decreases incident solar radiation, which can limit the aboveground biomass production of understory grasses compared to those inhabiting open areas, particularly in *N. longiglumis* plants (Blazquez *et al.*, 2020). Nevertheless, lower temperatures under the canopy typically increase soil moisture, generating conditions that favor the growth and production of most adapted herbaceous perennials. The

coexistence of woody and herbaceous species in the Monte region is driven by complementary resource use and synergistic interactions between species with different functional traits (Torres *et al.*, 2014).

The findings of this study hold significant implications for Monte's rangeland management in the context of future climate scenarios. With increasing precipitation variability and drier conditions projected for the region, plant adaptations for tolerance will be essential for their survival. The identification of functional traits, like those observed in the more competitive grass species *N. longiglumis*, provides a valuable tool for enhancing plant breeding programs and restoration efforts in degraded rangelands. This knowledge can be directly applied to develop resilient grass varieties suitable for implementing effective productive restoration strategies, ultimately ensuring the long-term health and productivity of these valuable rangelands of Argentina.

## CONCLUSIONS

The results obtained confirm that both grass species maintained continuous aboveground and belowground growth under different water-deficit levels. However, *N. longiglumis*, the more competitive species, exhibited distinct root system adaptations for water acquisition. It displayed increased growth of adventitious and lateral root length, a higher density of finer roots, and crucially, maintained its aerial growth and reproductive effort even under greater water limitations. These superior root traits likely contribute to *N. longiglumis*' dominance in these rangelands by enhancing its water stress tolerance.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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