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Do populations of *Festuca pallescens* from a rainfall gradient differ in the expression of morpho-physiological traits under drought stress?

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ABSTRACT

In arid environments, stochastic rainfall and high evapotranspiration force plants to optimize water resources. North Patagonia is characterized by a deep rainfall gradient that gives rise to environments with very contrasting water availability. *Festuca pallescens* is a key native forage species, growing widely in those environments. To explore morphological and physiological traits involved in the response to drought, we exposed plants from populations sampled along the rainfall gradient to different water availability conditions (Well-watered, Water-pulse, Water-drought). We evaluated morphological traits in all populations to assess inter-population variability and physiological traits between selected populations from sub-humid and arid environments to explore possible macro-environmental responses. Populations showed variation in survival after 45 days of drought conditions and differences in the expression of morphological traits. Also, populations from arid environments were less affected than those from humid environments, showing a longer recovery when they received water pulses. Although a population survival pattern related to the rainfall gradient was not evident, populations from arid environments exhibited local adaptation to their home environments, taking better advantage of water pulses. These results provide information about the response of a non-model species to environments with contrasting water availability and possibly, to changing rain patterns in arid environments under climate change.

Keywords:

perennial grasses; arid environments; abiotic stress; tillering; Water use efficiency

1. INTRODUCTION

Drylands are highly vulnerable to altered climatic conditions such as changing rain events and increasing temperature, like those predicted by climate change (IPCC, 2019). Scarcity of water and a great temporal variability in water availability are main constraints in arid environments (Snyder & Fartowski, 2005). In addition, rainfall is stochastic and scarce while evapotranspiration is high (Heinrich, 1979). To cope with these constraints, plants display morphological and physiological responses at whole-organism level (Farooq et al. 2009; Kørup et al. 2018; Manslin et al. 2019; Rosová et al. 2022) and economize resources to minimize losses by reducing growth rate or resource absorption (Chapin, 1991; Couso, 2011; Grime, 1979; Lambers & Oliveira, 2019). This creates a trade-off between the ability to quickly use resources when they are available (rapid resource absorption to growth) and tolerate shortage (lower growth rates) (Couso et al. 2010). Accordingly, dryland rangeland species usually show adaptations to water loss (Munns, 2001; Oyiga et al. 2020; Wang et al. 2007), as well as phenotypic (Jump & Peñuelas, 2000; Moreno, 2011; Pigliucci, 2001) and adaptive plasticity (Nicotra et al. 2010). Either of the mentioned, solely or in combination, might provide the capacity of the species to overcome the predicted climatic alterations.

In arid environments, plant survival is closely related to soil water availability (Bertiller et al., 1995; Snyder & Fartowski, 2006). Unlike other environmental stresses, drought stress occurs gradually due to progressive soil drying and a high rate of evapotranspiration. Therefore, it is considered one of the main causes of death in plants (Luna Flores et al. 2012). First, plants close stomata reducing the exchange of carbon dioxide (CO₂); these has a negative impact on plant growth (Engelbrecht & Schulz, 2001; Garreaud et al. 2009; Archer, 2003) and instantaneous water use efficiency (WUE) could either show a decline or an increase in C3 species (Taylor et al. 2011). On the other hand, the water deficit also can produce morphological changes on plants, such as a decrease in leaves and stems growth, smaller leaf area and a reduction of the specific leaf area (SLA, cm² gr⁻¹) (Engelbrecht & Schulz, 2001). These changes are related to a decrease in transpiration rate, water potential (Ψ) and relative water content. In general, there is also a reduction of the aboveground biomass (Khurana & Singh, 2004; Luna Flores et al. 2012; Singh & Singh, 2006) and an increment of below ground biomass (Oyiga et al. 2020; Wang et al. 2007).

Grasses constitute one of the most prominent functional groups in North Patagonian meadows and steppes (Fernández et al. 1991). Many of these species share functional traits relevant to arid and semiarid environments, for example, phenology is coupled with rainfall regimes and the root system is deeper than shrubs (Golluscio et al. 2009). Likewise, many

morphological and physiological aspects of some grasses are closely related to the Patagonian environment's heterogeneity, such as root anatomy (Leva et al., 2013), the ability to recover after a drought event (Yahdjian & Sala, 2006) and adjustments in the above-ground and underground biomass ratio (R/S^{-1} ; Austin & Sala, 2002). Many Patagonian grass steppes located in the Subandino District of Patagonia phytogeography province (Golluscio et al., 1988; Soriano, 1956) are dominated by *Festuca pallescens* (Gaitan et al., 2019; León et al., 1998). This species is a dominant native species with intense forage use and wide distribution. It inhabits different environments in Patagonia where hydric balance is favorable for their establishment (i.e. low evapotranspiration rate and deep soils) (León et al., 1998), from forests (with more than 1000 mm of rain) to steppes (with less than 240 mm of rain) (Bertiller et al., 1990; López et al., 2019; 2020). Therefore, it can be a dominant species in both wet-meadows (humid environments) and highland steppes (semiarid environments) (Bran et al., 2000). In addition, this species opportunistically takes advantage of water and nutrient pulses (Coronato & Bertiller, 1990; Moreno, 2012), presents phenological differences associated with air temperature variation (Bertiller et al., 1990) and shows local adaptations to its original environments (López et al., 2020).

North Patagonia is characterized by climatic gradients (rainfall, temperature and altitude), a highly complex geomorphology (Gaitan et al., 2019), with low organic matter (OM) and poorly developed soils that favors soil compaction and reduces water retention (Godagnone & Bran, 2009). The rain-shadow effect of the Andes Mountains creates different environmental settings from very humid to arid. This west-east decreasing rainfall gradient defines the floristic physiognomic types, aridity index and seasonal variations of three ecological regions (Leon et al., 1998; Aruelo et al., 1998; Godagnone & Bran, 2009). Towards the west, more humid environments dominate the Patagonian region: the Cordillera and Pre-cordillera ecological regions. These environments are dominated by temperate forest and higher rainfall regimes with sandy loam soils (Bianchi et al., 2016; Esterheld et al., 1998). However, towards the east predominates the Hills and Plateaus ecological region, dominated by shrubs and grass steppes (Oliva et al., 2016) with less developed soils (very low OM), sporadic rains and abrupt daily changing temperatures (Bran et al., 2000). Therefore, populations of the same species that grow along the rainfall gradient between the Cordillera and the Hills and Plateaus ecological regions, could present large interspecific variation in morphological and physiological traits.

Global precipitation patterns are expected to change in the next decades, altering the rainfall regime of North Patagonia, further deepening the region's water deficit (Crego et al., 2014; Muñoz et al., 2009). Recent studies have shown a denoted decline of *F. pallescens* in Patagonian rangelands due to increasing aridity and overgrazing (Gaitan et al., 2019). Drought affects the expression of morphological and physiological traits in plants (Zhen et al., 2011; Luong & Hoik, 2022) and drought avoidance is common in perennials species, relying on high

water use efficiency (WUE), limited vegetative growth, and high biomass allocation to roots (Kooyers, 2011; Chandregowda et al., 2022). However, perennial grasses have the ability to overcome long periods of moderate and severe drought by different strategies that involve both morphological and physiological traits (Volaire & Thomas, 1999; Poirier et al., 2012; Benzano et al., 2013; Malachowski & Volaire, 2018; Norton et al., 2016). Plants are increasingly exposed to changing environmental conditions on their physiological limits (Shaw and Eppers, 2012). Thus, ecophysiological traits could play an important role in adaptation to changing climate (Kosova et al., 2022). In previous works, we found that the populations inhabiting the easternmost environments of Hills and Plateaus ecological region experienced stronger thermal and hydric restrictions for germination while populations from the central area of the gradient (Pre-cordillera ecological region) have fewer germination restrictions (López et al., 2019; 2021). Under this scenario, we wonder whether populations of this keystone species show differences in the capacity to cope with long periods of water deficit. We hypothesized that, macro-environmental conditions imposed strong selection pressure on populations that inhabit the different environments located along the rainfall regime in North Patagonia. Therefore, the populations growing in more restrictive environments (eastern environments) will be less affected by drought at morphological and physiological levels than populations from more humid environments (western environments), denoting local adaptation to their home environments. We tested this hypothesis by subjecting plants from populations from the whole rainfall gradient to different irrigation regimens, searching for a) differences in the expression of morphological traits in all populations and b) differences in physiological traits between two groups of populations from sub-humid environments (Pre-cordillera) and arid environments (Hills and Plateaus). This information will increase the scarce knowledge about how non-model species are likely to cope with current and predicted increase of droughts, providing information to propose natural resource management strategies that reinforce the resilience of ecosystems to climate change.

2.METHODOLOGY

2.1 Sampling sites

The sampling sites covered three ecological regions in North Patagonia that include diverse communities of vegetation: Cordillera, Pre-cordillera and the Hills and Plateaus (Bran et al., 2000; Gaitan et al., 2019). A total of eight source populations of *F. pallescens* were selected at four sampling sites: towards the west, Peninsula Huemul (PH) in Cordillera ecological region (humid: 1000 mm) and San Ramon (SR) in Pre-Cordillera ecological region (semi-humid: 600 mm), and towards the east, Pilcaniyeu (P) and Ing. Jacobacci (J) in Hills and Plateaus ecological region (arid: 250-150 mm). At each site, we sampled one source population at a highland steppe

(A) and another at a meadow (B) (Fig. 1). Our sampling sites were located in relatively similar latitudes, so photoperiodic characteristics were similar. Therefore, to reach possible differences in temperature, we sampled at two altitudes in each sampling site. Nonetheless, longitude changes notoriously between sampling sites and followed the sharp rainfall gradient, decreasing from 3000 mm in the west (near the Andes Mountains), to less than 150 mm towards the east (the Patagonian steppe) in only 235 km. In consequence, there are many differences in evapotranspiration rate, aridity index, and seasonal precipitation variation between sampling sites, but these variables do not change abruptly between the two sampled altitudes at each sampling point (Bianchi et al. 2016). We gathered seeds from 30–50 plants separated 25 m from each other in each population for greenhouse experiments. All sites were distributed along the west-east decreasing rainfall gradient of about 500 km in North Patagonia (Argentina) (rainfall gradient: 1000 to less than 150 mm) (Table 1).

2.2 Plant material

Plants were obtained from seeds germinated at 15.5°C and saturated moisture, in 200 ml cavity plugs with inert substrate. In order to favor the initial development, the seedlings were fertilized once with Hakaphos NARANJA (1 gr l⁻¹; 15% N, 5%-P₂O₅, 30%-K₂O and 2% MgO, 4% S, 0.01% B, 0.02% Cu, 0.005 Fe, 0.05 Mn, 0.001 Mo, 0.02 Zc). When the seedlings developed three leaves, they were transplanted into 3 L plastic pots with a substrate containing a 1:1:2 ratio of volcanic ash to black earth soil to turf (0.15 mS electrical conductance (EC) and pH = 6.97). Before starting the experiments, plants of *F. pallescens* were grown for three months in a common environment (greenhouse). The plants were maintained at pot capacity (volumetric water content in the pot close to 15%) measuring the pot volumetric water content (% v v⁻¹) with a TDR (“Time domain reflectometer”, Trime FM, Eijelkamp) every four days along the entire essay.

2.3 Drought stress experiment

Five plants from each of the eight populations were randomly assigned to one of the four experimental blocks and one of the three watering regimes in a greenhouse (n=480). The experiment followed a split-plot experimental design. The main plot was the effect of water availability with three levels and the sub-plot was population factor with eight levels. Levels for the “water availability” factor were: i) water content in the pot capacity of 15% (WW, “well-watered”), ii) water pulses of 350 cm³ simulating an erratic steppe precipitation event of 5 mm in two moments along the experiment (WP, “water pulse”) and iii) a single irrigation at the beginning up to pot capacity, and no additional water till the end of the experiment (WD, “water deficit”) (Fig. 2). The levels for population factor were the eight source populations (Table 1)

The experiment was done from January to March (summer season) and water pulses were held on February 4th and 27th when pot moisture was below 3% in more than 50% of the plants in WP treatment. Each pulse consisted of 5 mm of water based on the daily inter-annual average of rainfall in the southeast and northeast Patagonian steppes (Coronato Bertiller, 1996; López, 2011).

In order to monitor the progress of the drought stress, water pot volumetric content was measured with a TDR buried 10 cm deep in each pot every four days. The stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) was monitored three times a week with a SC-1 porometer (Decagon Devices Inc.) in four fully expanded leaves in each plant of all populations per treatment. The pre-dawn leaf water potential (Ψ_H , MPa) was measured in a fully expanded leaf every 15 days in all treatment levels using a pressure chamber (PMS Instruments Co., Corvallis, USA). For this, the leaves of three plants for each population were obtained by cutting a tiller at ground level that was then placed in a paper envelope and the measurement was carried out in a period of no more than 5 min. The sampled plants were different on each measurement date, chosen at random within each treatment. Plant survival was evaluated 45 days after the initial date of the trial, when more than 50% of the plants in the water deficit (WD) treatment presented a pre-dawn leaf water potential lower than -2 MPa, indicating a moderate to severe water stress (Caballé et al., 2011).

2.4 Measured variables and statistical analysis

We evaluated the following morpho-physiological variables:

2.4.1 Morphological variables:

- a. **Tillering:** Four tillers per plant were marked with metal hoops at the beginning of the experiment in ten plants per population per treatment to calculate the average difference in number of tillers produced by a marked tiller per plant at the end of the experiment (n=240).
- b. **Biomass production:** the difference between the total biomass at the beginning and at the end of the trial was estimated by destroying three random selected plants per population and treatment sample. These samples were used to determine the dry weight (total biomass) (n=72).
- c. **Biomass distribution:** the difference between the root/shoot ratio at the beginning and at the end of the trial in the same plants selected at random and used to calculate biomass production (n=72).

We analyzed tiller production, biomass production and distribution with a two-way ANOVA with two fixed factors (WT, water treatment and P, population) and their interaction in GraphPad Prism demo version 5.00 for Windows, GraphPad Software, San Diego California

USA (www.graphpad.com). Heterogeneous groups were separated by Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$) to discriminate differences between the two factors and their interaction.

2.4.2 Physiological variables:

All physiological variables were measured using a Li-Cor 6400 infrared gas analyzer (Lincoln, NE, USA). The measurements were made at the beginning of the experiment, one day before and two consecutive days after the application of the water pulses, between 10 a.m. and 4 p.m., on days coinciding with the pre-dawn water potential and volumetric potted water content measurements. Physiological measurements had to be carried out in a narrow window of time (8.30 am to 11.30 am) and we could not measure more than 40 plants per day, each Amax (maximum net photosynthesis rate) took at least 4 minutes. Therefore, due to time-limitations we measured five plants per population per treatment of two western (SRA and SRB) and two eastern (JA and JB) populations ($n=40$). These populations were selected because they are located in the most characteristic environments for the species (by the abundance of populations in these ecoregions): meadows and grass steppes of the Subandino District in the phytogeography province of Patagonia (Golluscio et al., 1982; Soriano, 1956). Besides, they represent two eco-regions: Pre-cordillera (SRA and SRB) and Hills and Plateaus (JA and JB). A LED lamp (6400-02 LED Light Source, Lincoln, NE, USA) was used to control the light intensity within the measurement chamber.

- a. **Maximum net photosynthesis rate (Amax, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)**. The measurements before and after the application of the water pulses were carried out only with saturating photosynthetically active radiation (PAR, $1800 \mu\text{mol m}^{-2} \text{ s}^{-1}$), considering a minimum pulse of 180 s and 3% coefficient of variation. The chamber temperature was the ambient air temperature and the partial pressure of CO_2 was set at $400 \mu\text{mol mol}^{-1}$. The air flow varied between 50 and $500 \mu\text{mol s}^{-1}$ to keep the ΔCO_2 above $5 \mu\text{mol mol}^{-1}$, the minimum sensitivity value of Li-Cor 6400.
- b. **Leaf-level water-use efficiency (WUE, $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$)** calculated as the relationship between CO_2 assimilation rate and stomatal conductance (Amax gs^{-1}).

In order to analyze the effect of the water pulse, we tested differences in both Amax and WUE between treatments (WW vs WP) in the different stages of the water pulse (Initial irrigation (0); Pre-pulse (1); 24 h after the water pulse application (2) and 48 h after the water pulse application (3)) for each water pulse (4th and 27th February) in populations from the west (SRA and SRB) and the east (JA and JB) sampling sites (see table 1). Populations situated in San Ramon and Ing. Jacobacci are typical rangelands from Pre-Cordillera ecological region and Hills and Plateaus ecological region respectively. Populations from each ecological region were

jointly evaluated because we search for differences in the physiological responses based on possible adaptations at a macro-environmental scale. Differences were tested using a general linear model with two fixed factors and two levels for each factor: water treatment (WW and WP) and sample site (San Ramon and Ing. Jacobacci) in InfoStat 2020 (Di Rienzo et al. 2020) applying a posteriori LSD Fisher test if differences were significant at $\alpha=0.05$.

3. RESULTS:

Water availability was effectively reduced under water pulse (WP) and water deficit (WD) treatments (Fig.2). At the end of the experiment, the mean pre-dawn leaf water potential in plants from WW treatment was -0.75 ± 0.14 MPa, while plants from WP and WD treatments showed values close to -1.5 MPa (moderate stress) and -2.28 MPa (severe stress) respectively. The mean volumetric water content was above pot capacity ($19.73 \pm 5.32\%$) in plants from WW treatment through the entire trial. The mean volumetric water content did not increase after receiving the water pulses in WP treatment, dropping to less than 5% volumetric water content at the end of the trial. Finally, the volumetric water content dropped continuously in WD treatment, reaching 1% at the end of the experiment.

3.1 Responses to drought stress

Different survival percentages were observed between the treatments WP and WD, with a positive effect of the water pulse. Overall, the application of water pulses improved survival between 45 and 60%. Under the lowest water level (WD) populations from the east (e.g. JB) showed a higher percentage of survival than populations from the west (e.g. PHA), however the highest survival percentage was registered in populations from the west (SRB) while the lowest was found in a population from the east (PB) (Fig. 3).

3.2 Responses to water availability expressed in morphological variables measured in all populations

Tiller production was significantly different between water treatments and population source, as well as their interaction ($F=25.10$; $p<0.001$; $F=7.39$; $p<0.001$ and $F=2.27$; $p<0.01$ respectively). Biomass production differed significantly between water treatments ($F=44.89$; $p<0.001$) and between population source ($F=6.20$; $p=0.001$). However, changes in biomass distribution ($R\ S^{-1}$) were not significant between water treatments, population source or their interaction.

Overall, populations from the east (PA, PB, JA and JB) produced more tillers than populations from the west (PHA, PHB, SRA, SRB) in WW treatment. However, a population from the west (SRA) and two populations from the east (PA and JB) showed the highest tiller

production in WP treatment. Hence, populations PA, and JA produced more tillers than PHA, PHB, SRA and SRB in WW treatment. In WD treatment all populations diminished their tiller production but populations from the east (with the exception of PB) showed more tiller production than populations from the west (Fig. 4a).

Differences in biomass production among populations were significant in the WP and WD, but not WW treatments ($F=9.73$; $p<0.001$, $F=9.55$; $p<0.001$, $F=1.06$; $p=0.43$ respectively). Two populations from the east (PA, PB) and one from the west (PHA) produced less biomass than the rest under both WP and WD treatments. Biomass distribution ($R\ S^{-1}$) did not show significant differences under either treatment. However, there is a trend of a higher proportion of root biomass in western populations under both WP and WD treatments than populations from the east (Fig. 4b).

3.3 Responses to water pulse expressed in physiological variables measured in populations from Pre-cordillera and Hills and Plateaus Ecoregions.

Overall, well-watered plants showed higher net photosynthesis rate values (A_{max} , $7.54\ \mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$ vs $3.14\ \mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$; $F=20.88$; $p<0.001$) and slightly lower values of leaf-level water-use efficiency values (WUE, $52.04\ \mu\text{mol CO}_2\ \text{mol H}_2\text{O}^{-1}$ vs $60.46\ \mu\text{mol CO}_2\ \text{mol H}_2\text{O}^{-1}$; $F=0.16$; $p=0.69$) than water-limited plants. At the beginning of the experiment (stage 0), well-watered plants and water-limited plants showed no significant differences in net photosynthesis rate (A_{max}) and leaf-level water-use efficiency (WUE) ($F=3.32$, $p=0.07$; $F=1.05$, $p=0.31$; respectively). Before the application of the first water pulse (stage 1), all populations showed lower values in A_{max} in WP than in WW treatment ($F=23.05$, $p<0.001$). However, populations did not show significant differences in WUE values at this stage ($F=3.78$, $p=0.06$). At stage 2 (24 h after the water pulse application), there were not significant differences between treatments ($F=3.21$, $p=0.08$), populations ($F=0.02$, $p=0.88$) or their interaction ($F=0.28$, $p=0.61$) in A_{max} or WUE. Then, at stage 3 (48 h after the water pulse application) mean values of A_{max} were significantly different between treatments ($F=8.27$, $p=0.01$) and populations ($F=11.11$, $p=0.003$). Values of A_{max} decreased in all population in WP treatment and populations from sub-humid environments showed higher values of A_{max} than populations from arid environments. However, values of WUE did not differ between treatments ($F=0.64$, $p=0.43$), populations ($F=1.93$, $p=0.18$) or their interaction ($F=0.84$, $p=0.37$). Therefore, this physiological response only lasted 24 h in all populations. On the other hand, populations from both environments did not show significant differences in either physiological variable between treatments, populations or their interactions at any stage of the 2nd water pulse (Fig. 5).

4. DISCUSSION

Species from arid and semiarid environments face large periods of water shortage; therefore, leveraging stochastic rain events in summer constitute an advantageous strategy. Our results reinforce the opportunistic behavior of *Festuca pallescens* to profit by stochastic rain events (Defossé et al., 1997). Populations of *F. pallescens* showed differences in morphological traits and low variation in physiological traits related to drought stress. However, our hypothesis was only partially supported. Populations from arid and semiarid conditions (except for one) showed higher tiller production than those from humid environments in well-watered and water drought treatments, but biomass production and distribution and physiological responses were similar under greenhouse conditions. In addition, there was not a population survival pattern related to the rainfall regime established in North Patagonia, instead populations showed variation in survival percentages after 45 days of drought conditions, expressing the inter-population variability. Noteworthy, the root/shoot ratio did not change substantially.

Overall, the application of water pulses in the WP treatment allowed a small increase in water content (Ψ_H ; % v v⁻¹; Fig. 2) in *F. pallescens* populations, although insufficient to reach the same moisture content levels of well-watered plants. Nonetheless, there were differences in plant survival percentages after 45 days of drought conditions between populations in WP and WD treatments, expressing the species sensibility to drought. Surprisingly, there was not a survival pattern related to the original environments of each population. Towards the west, populations PHA and PHB are situated in extreme humid environments with sandy-stony soils but different edaphic characteristics. For instance, PHA is a high-altitude rangeland, that might be exposed to freezing/desiccation due to cold winters. Then, populations SRA and SRB are located in meadows or steppes with fairly deep sandy loam soils. Therefore, soils from these humid environments, Cordillera and Pre-Cordillera ecoregions, accumulate more organic matter (OM) and volcanic sediments, which increases the capacity to retain water. On the other hand, towards the east of the decreasing rainfall gradient, in Hills and Plateaus ecological region, precipitation is sporadic and soils are shallower with less OM, therefore water retention capacity is lower for PA, PB, JA and JB populations (Godagnone & Aran, 2009). Therefore, populations have different strategies to overcome drought stress and it is not entirely related to macro-environmental conditions (e.g. rains).

Rainfall is asymmetrically distributed in North Patagonia, with strong winter precipitations (Puelo et al., 2008) and discrete “pulses” during the summer growing season (Snyder & Tartowski, 2006). Rainfall pulses in conjunction with temperature, wind, infiltration, surface flow, and evapotranspiration, outcome the length of time that the soil is wet or dry (Snyder & Tartowski, 2006), leaving an ecological legacy that influences subsequent responses of plant species to rain and drought. In this sense, our results show two main outcomes. First, some populations of *F. pallescens* from Hills and Plateaus ecological region produced more

tillers than populations from western humid environments whether water was a limiting condition or not. Moreover, tiller morphology differed between populations; those from Hills and Plateaus ecological region presented smaller leaves than those from Cordillera and Pre-Cordillera ecological regions. This tiller morphology might be related to a trade-off between reducing the exposure to drying agents without standing down foliage production and potential photosynthetic structure (i.e. without reducing the number of leaves). On the other hand, in Cordillera and Pre-Cordillera ecological regions trees and shrubs are dominant surrounding vegetation, so long leaves might be an advantage vegetative feature to intercept more radiation in environments with a higher incidence of shade (e.g. in undergrowth) (Fernández et al. 2010, López et al. 2020).




Overall, plants from all populations diminished tiller production under drought. However, plants from the easternmost populations (Hills and Plateaus ecological region) produced more tillers than the rest of the populations in WP treatment. This suggests that these populations could better leverage the sporadic steppe rains in terms of biomass production. In other conspicuous perennial grasses from the Patagonian steppe (*Bromus pictus*, *Poa ligularis*, *Pappostipa speciosa*) tiller production also decreased under drought (Couso & Fernández 2012). *P. ligularis* showed a water-saving strategy, similar to the results that we obtained for *F. pallescens*. On the contrary, *B. setifolius* was more affected by drought than *P. ligularis* and *P. speciosa*, but had the highest potential growth (i.e. the largest potential to capitalize on water pulses for improving performance traits), fitting into a water-spending, or Grime's competitive strategy. Drought stress also reduced the number of tillers and biomass in *Hordeum vulgare* (Farooq et al. 2009) and *F. arundinacea* (Kørup et al. 2018). On the other hand, ecotypes of *Trichloris crinita* growing in arid and semiarid Argentinian rangelands with salt and drought stress, showed local adaptations to their arid environments, displaying high shoot and root production and a low percentage of dead shoot biomass under drought conditions (Quiroga et al. 2011, Marinoni et al. 2020) like *F. pallescens* (i.e. Ing. Jacobacci populations Hills and Plateaus Ecological region, the driest ecological region). As tiller production is directly related to aboveground net primary productivity (ANPP) which determines forage availability, and it is also closely linked to energy flow and nutrient and carbon cycles (Gaitan et al. 2014), these populations stand out in terms of forage and ecosystems attributes.









Secondly, biomass allocation did not significantly change between treatments, showing that most of the *F. pallescens* populations did not modify their R/S^{-1} ratio in the face of the hydric deficit (Fernández et al. 2006). Biomass allocation is a trait with a low phenotypic divergence for this species (López et al. 2020). The lack of an increased allocation to root biomass was also found in other forage perennial grasses (i.e. *Festuca ovina*, *Poa alpina*), indicating that the capacity of seedlings to adjust root biomass under drought conditions might be influenced by their ontogeny, available resources and characteristics of the drought event

(Haslin et al. 2019). Although we did not deepen on root architecture, we observed that most plants increased rooting depth, and produced a very low proportion of lateral roots. This is an important trait that should be further explored.

Physiological responses to water pulses under greenhouse conditions were similar among populations in spite of coming from environments with contrasting macro-environmental conditions. Populations from semi-humid and arid environments (Pre-Cordillera and Hills and Plateaus, respectively) showed a photosynthesis rate recovery after the application of the 1st water pulse, but this response only lasted 24 h. A similar response was shown by adult plants of *P. speciosa* (Golluscio et al. 2009) from a shrub-grass steppe. This shallow-rooted plant exhibited an increase in leaf water potential (MPa) after two days of watering, which is a short-term response (1 - 10 days) to the summer water pulse. Hence, when re-watered, *P. speciosa* also showed significant photosynthetic responses, increased leaf conductance and transpiration rate suggesting an opportunistic behavior which could translate in the ability to produce new leaves under favorable conditions (Golluscio et al. 2005). On the contrary, we worked with young plants grown in pots under the same greenhouse conditions, and we observed some results in days (24 – 48 h), at least in terms of water potential. So it is possible that in our experiment we only observed the beginning of the physiological processes occurring in young shallowed rooted plants after receiving a water pulse that demand low costs of carbon and energy, but not what might occur afterwards.

After the application of the 2nd water pulse, populations could not recover. This pulse was given at the end of February, after a longer period of drought conditions than the 1st pulse, and both well-watered and water-limited plants showed lower values of photosynthesis rate and leaf-level water-use efficiency (Fig. 5). It is possible that, during this period (late summer) plants close their stomata in their natural environments to avoid water loss (Caballé et al. 2011). Stomata closure should last some hours but not days, at least in most plants (Flexas et al. 2008), then there are other specific changes (i.e. structural) related not only to stomatal but also mesophyll conductance that occur before stomatal closure. These authors suggest that mesophyll conductance differs between species, changes faster than stomatal conductance and is influenced by leaf structure and certain proteins (i.e. aquaporins) (Flexas et al. 2008). Aquaporins are directly involved in plant response to arid environments in perennial grasses (Chen et al. 2018). So, it is possible that *F. pallescens* might decrease mesophyll conductance by chloroplast movements (Flexas et al. 2008) or by the regulation of aquaporin activity during the periods of water limitation. In agreement with this suggestion, variations in the proportion of girders of sclerenchyma, increasing in leaves of populations from most xeric environments (i.e. Ing. Jacobacchi) were observed (Guidalevich et al. in press). This leaf structure might enlarge mesophyll cell wall thickness and therefore limit CO₂ diffusion through the mesophyll tissues, decreasing mesophyll diffusion conductance g_m (g_m ; Xiong et al. 2018). In addition, water

transpiration in *F. palleescens* could be prevented by anatomical leaf features, such as thicker leaves due to large proportions of girders of sclerenchyma (as we mentioned before) or a greater degree of leaf rolling and folding (Greco  Cavagnaro, 2003), which might be a protective strategy against water deficit (Valladeres  Niinemets, 2007). In addition to these two structural features, stomata are located only on the adaxial leaf surface (Caballe et al.  2011), reducing gas exchange exposure.

On the other hand, water deficit promotes the reduction of photosynthesis intensity, the loss of photosynthetic pigments, impairs the activity of enzymes and of the photosystem II (Hura et al.  2007  Johnson et al.  2018). After the application of the water pulse, all populations showed a photosynthesis rate recovery, therefore photosynthetic machinery was probably negatively affected, but damage was overcome allowing photosynthetic recovery. Nonetheless, dehydration and rehydration is very likely to cause mechanical damage to the photosynthetic mesophyll cells (Johnson et al.  2018). In addition, in the short term response, plants can regulate WUE by modifying stomatal conductance involving the regulation of stomatal opening and closing or stomata density (Meng, 2018). In arid environments, grasses (mostly C3) exhibit an adaptive strategy in water use consisting of a slow extraction of soil water under drought stress associated with a low transpiration rate (Quiroga et al.  2013). Our results showed that under water deficit (WP treatment), plants had slightly higher values of leaf-level water-use efficiency than well-watered plants, exhibiting higher values of WUE than those generally found for herbaceous species ($43 \mu\text{mol mol}^{-1}$) (Webster et al.  2016). Also, tall fescue E+ plants under water stress (Swarthout et al.  2009) are more similar to values of WUE found for evergreen shrubs and deciduous trees (64 and $66 \mu\text{mol mol}^{-1}$, respectively) (Webster et al.  2016). So far, we observed that populations of *F. palleescens* could increase A_{max} and WUE after rewatering. However further studies focusing on both g_s (stomatal conductance) and g_m (mesophyll diffusion conductance) could give more information about mechanisms involved in these recoveries.

5. CONCLUSION

As many perennial grasses, our results indicated that *F. palleescens* is a drought evasive species with large intraspecific variation to drought stress and potentially interesting traits to cope with this stress. It is important that species could continue growing and producing under limiting water availability, so searching only for tolerant genotypes is not appropriate if we look for genotypes that could reinforce the resilience of ecosystems. Some populations, particularly those from arid environments (Hills and Plateaus ecological regions), could produce more tillers regardless of the water deficit and maintain high levels of WUE, though mechanisms that are

still unknown. These populations are interesting genotypes for environments such as poor meadows or poor productive steppes, because with low water availability, plants could still manage to produce above ground biomass. As global precipitation patterns are expected to change in the next decades (Crego et al. 2014), species such as *F. pallescens*, constitute important ecological pieces to face possible alterations in the rainfall regimes that might deepen water deficit in arid and semi-arid environments. The results obtained in this research provide relevant knowledge to current programs of conservation and genetic improvement of grassland species.

Author contributions

A.S.L., P.M. and D.R.L. conceptualized the study, performed supervision and acquired funding. P.M. and G.C. contributed to the design and implementation of the research. G.C. and D.R.L. specified the physiological measurements to be done, A.S.L. carried out the experiment. P.E. collected part of the data. A.S.L and G.C. analyzed the data. All authors contributed to the writing of the manuscript.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure captions

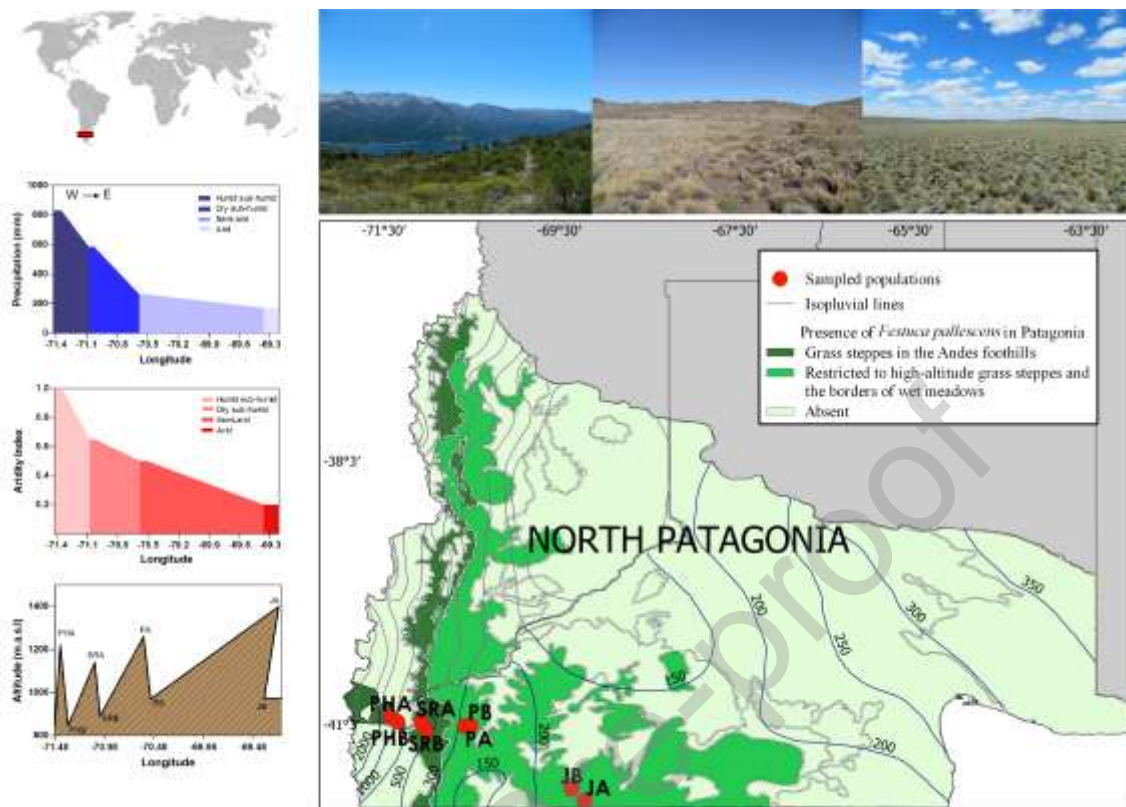


Figure 1: Map of the sampling sites distribution of populations of *Festuca pallescens* along the rainfall gradient. Above: pictures of the most representative rangelands of *F. pallescens* rangelands along the rainfall gradient. On the right: the map of the sampling sites with the whole distribution of the species, isopleth lines and the sampling sites in North Patagonia. Population PHA and PHB belong to Cordillera ecological region, SRA and SRB belong to Pre-cordillera ecological region and PA, PB, JA and JB belong to Hills and Plateaus ecological region. On the left: worldwide location of the sampling zone. Precipitation (mm), Aridity Index and Altitude (m a.s.l.) along the decreasing rainfall gradient. AI, defined as the ratio of potential evaporation to precipitation, was based on Gaitan et al. (2019).

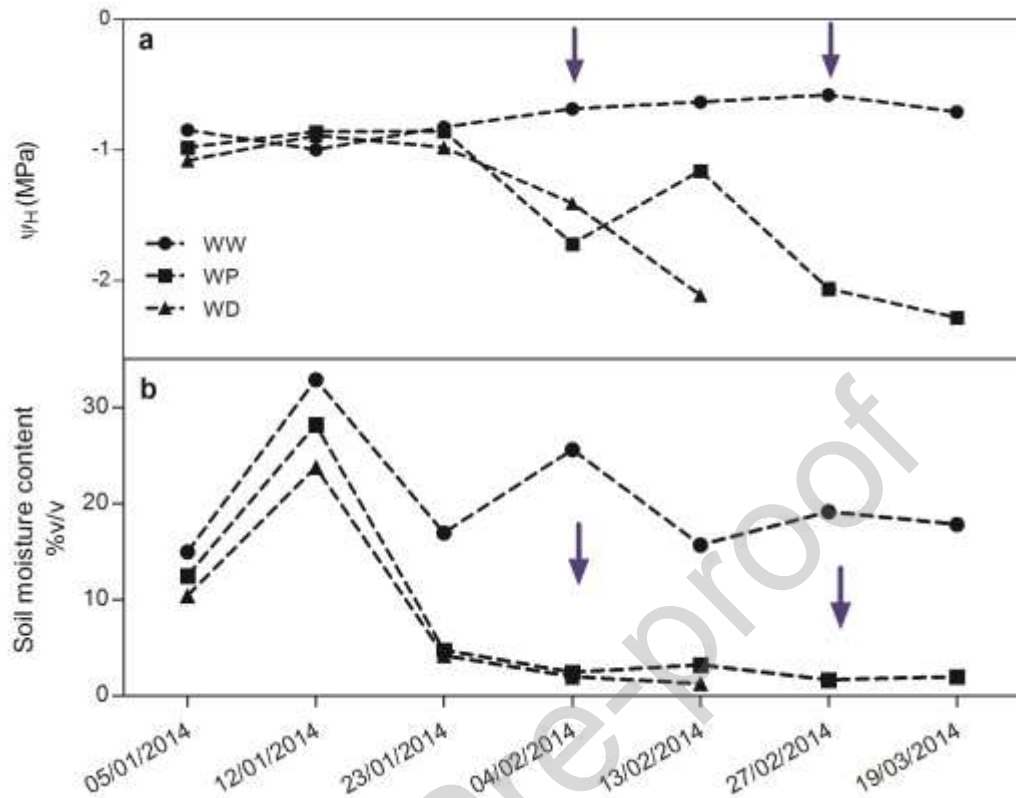


Figure 2: Physiological conditions in the greenhouse trial. a) Pre-dawn water potential (ψ_H , MPa), b) Mean percentage volumetric water content (%v/v). Levels of water availability: high (WW: Well-watered), intermediate (WP: Water pulses) and low (WD: Water deficit). The experiment started on January 1st and ended on March 19th (“x” axis). Arrows show the application of the water-pulse.

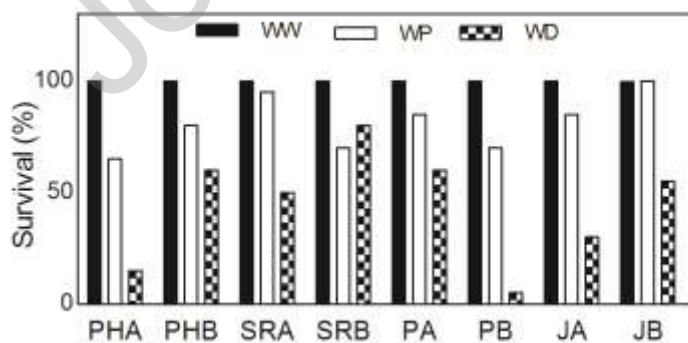


Figure 3: Survival percentage after 45 days. The colored bars indicate the treatments with different levels of water availability: WW (Well-watered), WP (Water pulses) and WD (Water deficit). Populations are shown in the “x” axis.

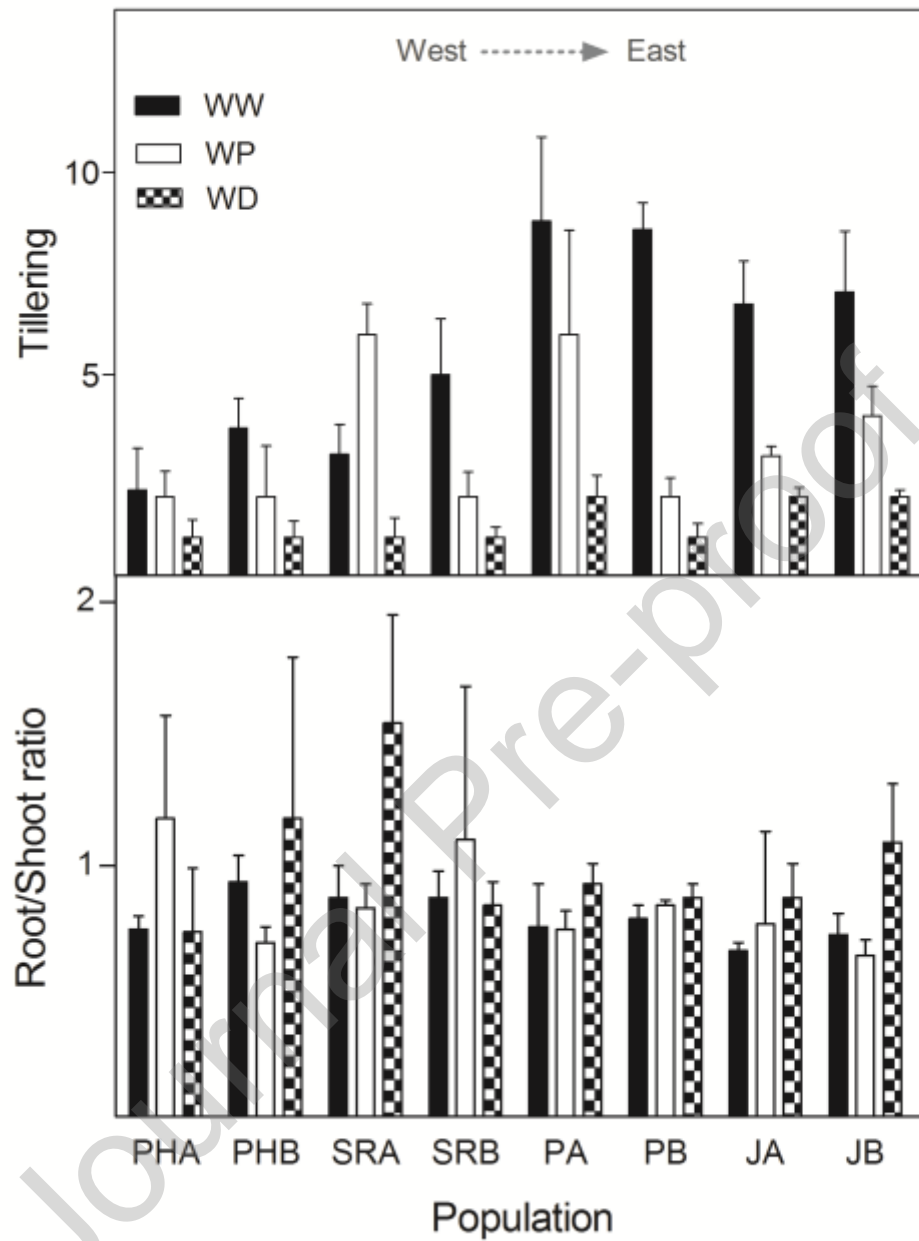


Figure 4: Differences in morphological variables between treatments and populations. Water availability levels for each treatment: WW (Well-watered), WP (Water pulses) and WD (Water deficit). Bars indicate the standard deviation. Populations are shown in the “x” axis.

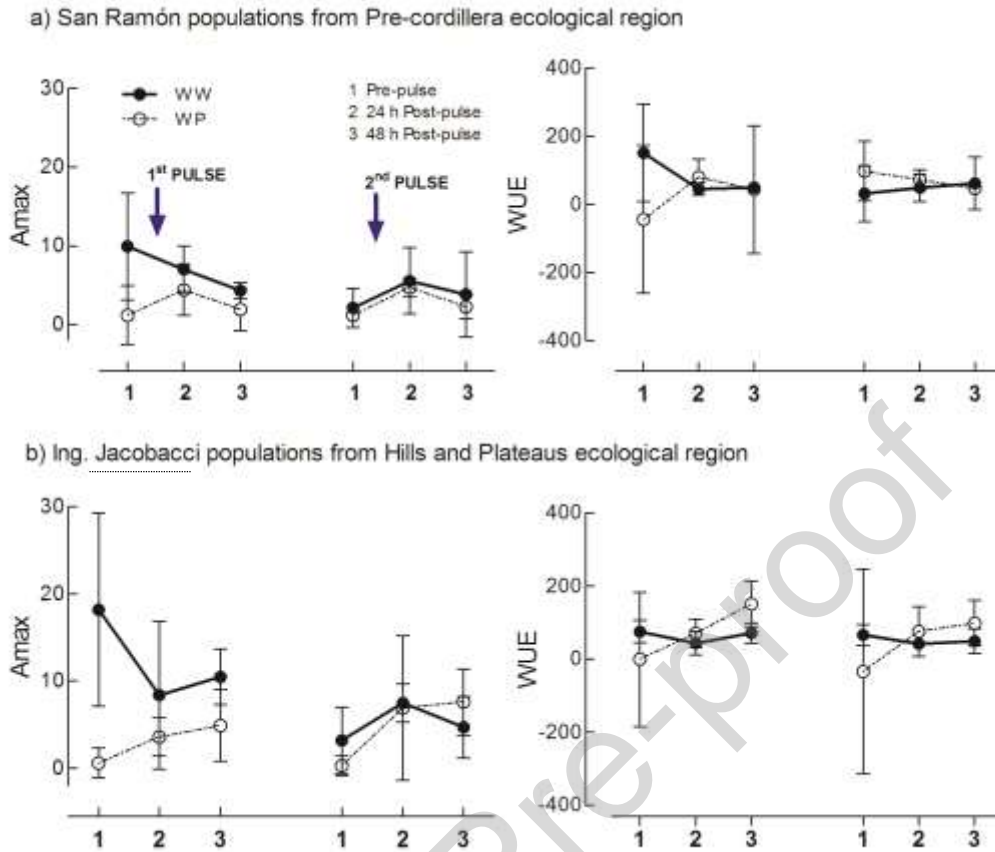


Figure 5: Physiological variables (A_{max} and WUE) measured in San Ramón populations from Pre-Cordillera ecological region (a) and Ing. Jacobacci populations from Hills and Plateaus ecological region (b) before and after receiving the water pulse. The left panel shows the maximum net photosynthesis rate (A_{max} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and right panel shows the leaf-level water-use efficiency (WUE, $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$). Arrows indicate the water-pulse application. The “x” axis shows the different moments of the water pulse application: pre-pulse (1), 24 h post-pulse (2), and 48 h post-pulse (3).

Table 1: Environmental and genetic characterization of sampled populations of *Festuca pallescens*. Floristic physiognomic type (FPT) was characterized following Bran et al. (2000). *Pop*: population; *Pp*: Precipitation (mm); *Lat*: Latitude; *Long*: Longitude; *Alt*: Altitude (m). The populations are listed from west to east. Acronyms: *NQN*: Neuquén, Argentina. *RN*: Río Negro, Argentina.

	Ecological region	Sampling site	<i>Pp</i>	<i>Pop</i>	<i>Lat</i>	<i>Long</i>	<i>Alt</i>	<i>FPT</i>	Soil type and main edaphic characteristics
WES	Pre-cordillera	Península Huemul (NQN)	831	PHA	40°57′	71°25′	1220	Shrub-grass steppe	Fairly deep volcanic soil. Cold, with snow in winter. Water deficit in summer.

			<i>PHB</i>	41°1′	71°20′	845	Shrub-grass steppe	Deep sandy soil. Water deficit in the summer.
			<i>SRA</i>	41°7′	71°4′	1139	Grass steppe	Fairly deep sandy loam soil. Without water deficit in the summer.
<i>Pre-cordillera</i>	San Ramon Ranch (RN)	584	<i>SRB</i>	41°1′	71°1′	902	Meadow	Deep sandy loam soil with high proportion of Organic Soil Matter (OSM). Cold. Moderate water deficit in summer.
<i>EAST</i>	<i>Hills and Plateaus</i>	264	<i>PA</i>	41°4′	70°34′	1260	Shrub-grass steppe	Fairly deep, loamy texture and deep clayey Cold, with snow in winter, exposed to the West wind, water deficit in summer.
			<i>PB</i>	41°3′	70°30′	970	Meadow	Deep loamy sand, alkaline with high proportion of OSM. Cold in winter, water deficit in late summer
	<i>Hills and Plateaus</i>	170	<i>JA</i>	41°55′	69°12′	1400	Grass steppe	Fairly deep sandy loam soil. Cold, water deficit in summer.
			<i>JB</i>	41°46′	69°21′	970	Salty Meadow	Deep silty loam, saline-alkaline soil with high proportion of OSM. Water deficit in late summer

CRediT authorship contribution statement

A.S.L., P.M. and D.R.L. conceptualized the study, performed supervision and acquired funding. P.M. and G.C. contributed to the design and implementation of the research. G.C. and D.R.L. specified the physiological measurements to be done, A.S.L. carried out the experiment. P.E. collected part of the data. A.S.L. and G.C. analyzed the data. All authors contributed to the writing of the manuscript.

Declaration of interests

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Populations of *F. pallescens* showed variation in survival after 45 days of drought.
- A population survival pattern related to the rainfall gradient was not evident.
- Morphological traits were less affected in populations from arid environments.
- Populations from arid environments took better advantage of water pulses.

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