

# Functional Ecology

## Loss of fungal symbionts at the arid limit of the distribution range in a native Patagonian grass – resource ecophysiological relations

### AUTHORS

Cecilia Casas<sup>1,2,3\*</sup>, Pedro E. Gundel<sup>2,4,5</sup>, Eluney Deliens<sup>1</sup>, Leopoldo J. Iannone<sup>6,7</sup>, Guillermo García Martínez<sup>8</sup>, María V. Vignale<sup>6,7,9</sup>, Hans Schnyder<sup>3</sup>

### AFFILIATIONS

<sup>1</sup> Universidad de Buenos Aires, Facultad de Agronomía, Departamento de Recursos Naturales y Ambiente, Cátedra de Edafología, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentina

<sup>2</sup> IFEVA, Universidad de Buenos Aires, CONICET, Facultad de Agronomía, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentina

<sup>3</sup> Technische Universität München, Lehrstuhl für Grünlandlehre, Alte Akademie 12, 85354 Freising-Weihenstephan, Germany

<sup>4</sup> Universidad de Buenos Aires, Facultad de Agronomía, Departamento de Recursos Naturales y Ambiente, Cátedra de Ecología, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentina

<sup>5</sup> Instituto de Ciencias Biológicas, Universidad de Talca, Av. Lircay s/n, Talca, Chile

<sup>6</sup> Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales. Departamento de Biodiversidad y Biología Experimental. Laboratorio de Micología, Fitopatología y Liquenología, Av. Intendente Güiraldes 2160. Pab. II, 4° piso (C1428EHA), Buenos Aires, Argentina

<sup>7</sup> CONICET- Universidad de Buenos Aires, Instituto de Micología y Botánica (INMIBO), Av. Intendente Güiraldes 2160, Pab. II, 4° piso (C1428EHA), Buenos Aires, Argentina

<sup>8</sup> INTA EEA Esquel-Regional Patagonia Sur, Chacabuco 513, Esquel Chubut, 9200, Argentina

<sup>9</sup> Instituto de Biotecnología Misiones (InBioMis), Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones e Instituto Misionero de Biodiversidad (IMiBio), Ruta 12 km 7.5 (3300), Posadas, Argentina.

### CORRESPONDING AUTHOR

Cecilia Casas

Universidad de Buenos Aires, Facultad de Agronomía, Departamento de Recursos Naturales y Ambiente, Cátedra de Edafología, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentina

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/1365-2435.13974](https://doi.org/10.1111/1365-2435.13974)

This article is protected by copyright. All rights reserved

IFEVA, Universidad de Buenos Aires, CONICET, Facultad de Agronomía, Av. San Martín 4453  
(C1417DSE), Buenos Aires, Argentina  
Tel: +54 11 5287 0739  
Email: ccasas@agro.uba.ar

## **ACKNOWLEDGEMENTS**

We thank Nicolas Nagahama for helping us with botanical survey during field sampling. We also thank Carolina del Toro, M. Paula Casabella, Anja Schmidt, Monika Michler and Rudi Schäufole for sample processing and analyses, Markus Moser for climatic data processing, Regina Hirl for contributing to the discussion and interpretation of C and O isotope data, Francisco González Pinto for discussions about data analyses, and Victoria Novas for her support. Jennifer A. Rudgers and Amy Austin are thanked for valuable comments on an early version of the manuscript. This research was supported by a DAAD postdoctoral scholarship to CC (German Academic Exchange Service; ALE-ARG program; 2015-2017) and the Deutsche Forschungsgemeinschaft (DFG SCHN 557/9-1). CC, MVV, PEG and LI are supported by the University of Buenos Aires and the Argentinian National Scientific and Technical Research Council (CONICET).

## **CONFLICT OF INTEREST**

The authors have no conflicts of interest to declare that are relevant to the content of this article.

## **AUTHOR CONTRIBUTIONS**

CC designed the study and theoretical framework with the assistance from PEG and HS. CC, ED, MVV, LI and GGM performed the research. CC performed the statistical analyses and designed the figures and tables. CC, HS and PEG wrote the manuscript. All authors contributed to the discussion of the final version.

## **DATA AVAILABILITY STATEMENT**

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.c59zw3r8k> (Casas et al., 2021). The R code is available upon request to the corresponding author.

DR CECILIA CASAS (Orcid ID : 0000-0003-3538-6081)

Article type : Research Article

Editor : Adam Frew

Section : Ecophysiology. Functional ecology. Microbial ecology

## Abstract

1. Crucial to our understanding of plant ecology is the consideration of the eco-physiological responses and constraints of plant-fungal symbioses throughout the native distribution range of their host.
2. We examined key eco-physiological roles of two co-occurring fungal symbionts [*Epichloë* endophytes and arbuscular mycorrhizal fungi (AMF)] in the endemic grass *Hordeum comosum* across a wide bioclimatic gradient and contrasting grazing severity. We sampled *H. comosum* plants along four humid-to-arid transects in Patagonia, Argentina, covering its entire distribution range and determined *Epichloë* presence, AMF root colonization, nitrogen and phosphorus concentration, intrinsic water use-efficiency (iWUE, the ratio of photosynthesis to stomatal conductance) and  $^{18}\text{O}$ -enrichment of cellulose in shoots.
3. Root colonization by AMF increased with *Epichloë*-presence. All plants hosted *Epichloë* in the humid range of the gradient, but symbioses occurrence decreased towards arid sites which also displayed severe grazing symptoms at site level.
4. Symbiosis with *Epichloë* correlated positively with shoot nitrogen concentration in the centre of the distribution range, and with shoot phosphorus concentration across the entire distribution range.
5. The site-level relationship of AMF colonization with  $^{18}\text{O}$ -enrichment and iWUE suggested that mycorrhiza boosted stomatal conductance in humid environments but curbed it in arid environments.
6. While the interpretation of interactions and potential causalities from observational studies should be done with caution, this study demonstrates distinct correlations

between plant-fungal symbiont associations and key resource parameters (phosphorus, nitrogen, and iWUE vs  $^{18}\text{O}$ -enrichment). Such correlations may suggest particular functional roles for these symbionts in the ecology of their host plant.

### **Keywords**

Arid-to-humid gradients,

Carbon and oxygen isotopic composition,

*Epichloë* and arbuscular mycorrhizal fungi,

Grazing severity,

Intrinsic water-use-efficiency (iWUE),

Non-pathogenic symbioses,

Nutrient relations,

Stomatal

conductance.

## 1. INTRODUCTION

Symbiotic microorganisms are thought to assist plants to cope with biotic and abiotic stress factors of global change (Compant et al., 2010). However, stressful environments may also constrain the ability of host plants to sustain symbiotic heterotrophic microorganisms (Delgado-Baquerizo et al., 2018). Yet, knowledge about the symbionts' relationship with the eco-physiology of the host plants throughout their distribution range is scarce. Natural climatic gradients in combination with biotic factors such as grazing severity, offer opportunities for addressing general eco-physiological questions regarding patterns of symbiotic interactions in plant adaptation to environmental conditions (Pellissier et al., 2013; Smit et al., 2009).

Many grass species of the *Pooideae* subfamily establish persistent, symptomless, and specific symbioses with asexual fungal endophytes of the genus *Epichloë* (Family *Clavicipitaceae*; Clay & Schardl, 2002). The fungus grows inside the intercellular spaces of aboveground tissues and is transmitted to progeny *via* seed (Gundel et al., 2011). The incidence of *Epichloë* in wild grass populations increased with mean annual temperature but not with mean annual precipitation world-wide (Semmartin et al., 2015) and with evapotranspiration and water deficit at regional scales (Afkhani et al., 2014; Lewis et al., 1997). In addition to *Epichloë*, most grasses are colonized by arbuscular mycorrhizal fungi (AMF; phylum Glomeromycota), establishing one of the most ubiquitous root symbiotic associations of vascular plants (Smith & Read, 2008). Generally, the abundance of AMF hyphae is positively associated with mean annual precipitation (Gao et al., 2016) whilst negatively associated with nitrogen addition (Kim et al., 2015) regardless of increase in temperature in steppe ecosystems. Therefore, optimal environmental conditions for symbiosis prevalence may differ between *Epichloë* and AMF plant-symbiont associations.

*Epichloë* and AMF symbionts depend on plants for the supply of photo-assimilates, while in return hosts may obtain a suite of nutritional and/or non-nutritional benefits (Clay & Schardl, 2002; Delavaux et al., 2017). *Epichloë* endophytes increase plant resistance to herbivores through bioactive alkaloids such as ergots, peramine, lolines and indole diterpenes (Clay & Schardl, 2002; Schardl et al., 2013). The effect of different alkaloids produced by *Epichloë* have been well studied in forage grasses [e.g., tall fescue (*Festuca arundinacea*) toxicosis and ryegrass (*Lolium perenne*) staggers] (Schardl et al., 2013). The widespread occurrence of *Epichloë* endophytes in wild grasses suggests that alkaloids may have important ecological roles in defensive mutualisms (Schardl et al., 2013). However, the

relation between grazing severity and endophyte frequency in wild grasses has received far less attention (Hernández-Agramonte & Semmartin, 2016; Rudgers et al., 2016). Particularly, in arid environments, severe grazing decreased endophyte frequency and tiller biomass of the grasses that were preferred by grazers (*Hordeum comosum* and *Poa lanuginosa*) indicating that symbiosis frequency may depend on overall plant performance (Hernández-Agramonte & Semmartin, 2016). While AMF also provide their host plants with protection against (insect)-herbivory under certain conditions (Gehring & Whitham, 1994), severe grazing reduced root colonization by AMF possibly related with photo-assimilates limitation (Gehring & Whitham, 1994; van der Heyde et al., 2017). In arid environments, severe grazing decreased AMF colonization of preferred (*Bromus pictus* and *Poa ligularis*) and non-preferred grass species (*Pappostipa speciosa* and *Pappostipa humilis*) grazed by sheep (Cavagnaro et al., 2018).

In addition to plant resistance to herbivory, *Epichloë* may also increase plant tolerance to abiotic stress factors (Clay & Schardl, 2002). Thus, increased drought tolerance in *Leymus chinensis* in symbiosis with *E. bromicola* was related to antioxidant compounds accumulation (Liu et al., 2017) and to a higher photosynthetic nitrogen use efficiency (Ren et al., 2014). Under severe drought, *Lolium arundinaceum* displayed higher water use efficiency (WUE, the ratio of carbon gain to water loss) when in symbiosis with *E. coenophiala*, an effect mainly attributed to higher photosynthetic rates (Swarthout et al., 2009). Conversely, *E. festucae* did not increase the drought tolerance of *Festuca rubra*, but enhanced plant nutrition (nitrogen and phosphorus), irrespective of water availability (Vázquez-de-Aldana et al., 2013). AMF are known to improve host access to phosphorus, nitrogen and water (Augé, 2001; Smith & Read, 2008); however, they are also implied in plant resistance to abiotic stress factors (Delavaux et al., 2017). Similar to *Epichloë*, the mycorrhizal associations increased plant tolerance to drought by inducing several mechanisms such as improving host nitrogen and phosphorus nutrition, increasing water uptake, stomatal conductance and photosynthesis (Augé, 2001; Ruiz-Lozano et al., 2012; Wu & Xia, 2006).

Besides the stress caused by the almost-permanent water deficit in arid and semiarid lands, plant species that are preferred by herbivores are particularly threatened (Golluscio et al., 1998). In these conditions, symbioses with both *Epichloë* and AMF may improve access to water and nutrients, and increase resistance to herbivores (e.g., Clay & Schardl, 2002; Delavaux et al., 2017). Field surveys and controlled-environment experiments found that root colonization by AMF is higher in plants with *Epichloë* compared to endophyte-free plants

(e.g., Vignale et al., 2016; Zhong et al., 2019). Nonetheless, a meta-analysis showed that inhibitory effects of *Epichloë* on root colonization by AMF are also frequent (Omacini et al., 2012). In a greenhouse experiment, Liu *et al.* (2017) found that *Epichloë* presence had a stronger effect than – and even nulled – AMF inoculation effects on drought tolerance to *Leymus chinensis* plants. Additionally, the simultaneous presence of *Epichloë* and AMF boosted phosphorus uptake, and improved drought tolerance in *Lolium perenne* (Li et al., 2019), indicating that the presence of both symbionts had complementary effects on host plant adaptation to stressful conditions.

Effects of fungal symbionts on host adaptation capacity under stressful conditions may be connected with physiological adjustments of intrinsic water used efficiency (iWUE), the ratio of net photosynthesis and stomatal conductance to water vapor. The simultaneous measurement of  $^{13}\text{C}$ -discrimination ( $\Delta^{13}\text{C}$ ) and  $^{18}\text{O}$ -enrichment of cellulose ( $\Delta^{18}\text{O}$ ; details in Materials and Methods) in  $\text{C}_3$  plants, can shed light on the interplay of plant carbon and water relations in the field (Flanagan & Farquhar, 2014). While iWUE can be estimated from  $\Delta^{13}\text{C}$  (Farquhar & Richards, 1984; Ma et al., 2020),  $\Delta^{18}\text{O}$  has been interpreted as an indicator of stomatal conductance (Barbour, 2007; Moreno-Gutiérrez et al., 2012; Scheidegger et al., 2000), particularly in comparative studies at site level (Baca Cabrera et al., 2021), and thus, may help separating the (growing season-integrated, assimilation-weighted) effects of both net photosynthesis and stomatal conductance on iWUE (Baca Cabrera et al., 2021; Barbour, 2007; Scheidegger et al., 2000). Whether the dual association with *Epichloë* and AMF affects iWUE or  $\Delta^{18}\text{O}_p$  at site-level or across natural aridity ranges of host plants has not been studied so far.

Here, we used a natural gradient of aridity combined with contrasting grazing pressures to explore the relationship between the prevalence of *Epichloë* and AMF colonization, with eco-physiological traits in a native Patagonian grass (see Figure S1 in Supporting Information). The species *Hordeum comosum* (*Pooideae*) is a  $\text{C}_3$  perennial grass, highly preferred by endemic [Guanaco (*Lama guanicoe*)] and domestic [sheep (*Ovis aries*)] large grazers. Thus, overgrazing can reduce its abundance in vegetation communities (Golluscio et al., 1998)□. *H. comosum* associates with *E. tembladera*, which produces alkaloids that are usually considered toxic for herbivorous insects and mammals (Iannone et al., 2015; Yi et al., 2018). However, this effect has not been experimentally validated for the *H. comosum*–*E. tembladera* association. Although it is known that *H. comosum* establishes symbioses with AMF (Casanova-Katny et al., 2011), environmental controls on mycorrhization are unknown.

Therefore, we asked specifically: (i) Is host plant symbiotic status with *Epichloë* and AMF related with environmental aridity or grazing severity? and (ii) What is the relationship between host symbiotic status and plant (eco)physiological parameters such as nitrogen and phosphorus concentration, iWUE, and  $^{18}\text{O}$ -enrichment of cellulose? To answer these questions, we collected *H. comosum* plants throughout the entire natural arid-to-humid distribution range of the species in the northwest Patagonia steppe (Argentina).

## 2. MATERIAL AND METHODS

### 2.1. *H. comosum*, sites and survey area

Individual plants of *H. comosum* were collected in January 2015. We selected 35 sites along four west-east, humid-arid transects, covering an area of ~5500 km<sup>2</sup> (41°3' S to 44°14' S and 69°38' W to 71°33' W). Transects were designed to cover the natural west-east gradient of mean annual precipitation (MAP, from ~900 to 150 mm). *H. comosum* was not found in three sites in the most humid and in two sites in the most arid extremes of the survey range (Figure S1).

We recorded the geographical coordinates, elevation, and plant vegetation cover of each site. Geographical coordinates were used to characterize each site according to mean annual precipitation (MAP) and mean annual temperature (MAT) at a latitude/longitude resolution of 30 seconds as obtained from 50-year climatic means (1950-2000) of the WorldClim database (Hijmans et al., 2005). Mean annual temperature (MAT) ranged between 6.3 and 9.7 °C, was mainly determined by site elevation and longitude, and not influenced by latitude (Pearson correlation = 0.64, 0.48 and -0.01, respectively). Environments inside the survey area were defined according to MAP (mm) as: arid (<200 mm), semi-arid (200-500 mm), dry subhumid (500-650 mm) and humid (>650 mm) (Method S1). Geographical coordinates were also used to estimate the mean oxygen isotopic composition of local meteoric waters ( $\delta^{18}\text{O}_{\text{rain}}$ ) with the ECHAM5-wiso global circulation model (Werner, 2019). Vegetation cover was described by total plant species (including grasses and shrubs) or bare soil cover in rectangular plots (50 m<sup>2</sup>) at each site (Winkworth et al., 1962).

Atmospheric relative humidity (RH) was calculated as the ratio between actual vapor pressure ( $e_a$ , kPa) and saturation vapor pressure ( $e_{\text{sat}}$ , kPa), with  $e_{\text{sat}}$  estimated by using the minimum and maximum annual temperatures from the WorldClim database (1950-2000) according to Allen et al. (1998). Actual vapor pressure was obtained from the WorldClim database (1970 – 2000). The Pearson correlation between MAP and RH was 0.84. Therefore,



RH was not included in the statistical models ( $RH = 50.13 + 0.017 \text{ MAP}$ ;  $R^2 = 0.7$ ;  $p < 0.0001$ ; Figure S2A). We considered additional environmental variables (i.e., minimum, and maximum annual temperature, potential evapotranspiration, aridity index, soil pH, nitrogen concentration in the rhizosphere; described in Methods S1) which, however, were also excluded from the analyses because of strong collinearity with parameters retained in the model.

Grazing severity was classified at site-level as either severely or mildly grazed vegetation, independently of grazing symptoms (yes or no) on the local *H. comosum* population. The two categories were discerned based on regional guidelines that assess the natural vegetation status as affected by grazing and with assistance of two regional experienced rangeland specialists (GGM and PhD Nicolas Nagahama). Grazing severity assessment considered ground cover and floristic composition of vegetation (total cover of valuable forage species, as indicators of grazing severity; Table S1), and vigor of forage species (symptoms of herbivory, height and anchoring of plants to the soil) (e.g. Siffredi et al., 2011)□.

## 2.2. Material collection

At each site, we collected eight *H. comosum* plants at haphazard locations, but with at least 10 m spacing among each other. All sites were sampled within 12 days, ensuring that differences in plant species phenology did not bias the analysis. Sites were not located in protected areas and a corresponding agreement for material transfer was celebrated between the Faculty of Agronomy of the University of Buenos Aires (Argentina) and the Technical University of Munich (Germany). The transferred material was only used for analysis purpose which implies the destruction during the process. Rhizosphere soil was collected by shaking generously the roots of each plant, dried and conserved for further analyses (Methods S1). Root samples were washed with tap water to remove adhering soil, then roots were conserved in FAA solution (10% formalin, 5% acetic acid, 50% ethanol, 35% distilled water, v/v) until AMF colonization assessment at individual plant level. All measurements were performed at plant level for each plant.

## 2.3. Plant traits measurements

Shoot biomass produced during the sampling-year growing season (including leaves, stems, and inflorescences) was identified based on greenness or the presence of spikes and weighed after drying. This material was used for elemental analyses of nitrogen and phosphorus and isotopic analyses of carbon and oxygen in  $\alpha$ -cellulose ( $\delta^{13}\text{C}_p$  and,  $\delta^{18}\text{O}_p$ ,

respectively). The concentration of nitrogen in the shoot and in rhizosphere soil of each plant was determined with an elemental analyzer (NA 1110; Carlo Erba Instruments). Shoot phosphorus was quantified by phosphovanado-molybdate colorimetry (Hanson, 1950). Nitrogen and phosphorus were expressed as dry wt fractions (w/w).

## 2.4. Carbon and oxygen isotopic composition of cellulose

$\alpha$ -cellulose was extracted from 50 mg of dry shoot material according to the protocol of Brendel et al. (2000) as modified by Gaudinski et al. (2005).  $\delta^{13}\text{C}_p$  and  $\delta^{18}\text{O}_p$  of  $\alpha$ -cellulose were determined by isotope ratio mass spectrometry using established protocols and instrumentation as detailed by Köhler et al. (2012) for  $\delta^{13}\text{C}_p$  and Hirl et al. (2021) for  $\delta^{18}\text{O}_p$ .  $\delta^{13}\text{C}_p$  was used to calculate  $\Delta^{13}\text{C}$  from which iWUE was estimated according to Ma et al. (2020) as detailed in Methods S1.  $^{18}\text{O}$ -enrichment of cellulose above source water, taken as rain water ( $\delta^{18}\text{O}_{\text{rain}}$ ), was calculated as  $\Delta^{18}\text{O}_p = (\delta^{18}\text{O}_p - \delta^{18}\text{O}_{\text{rain}})/(1 + \delta^{18}\text{O}_{\text{rain}})$ , with  $\delta^{18}\text{O}_{\text{rain}}$  estimated with the ECHAM5 global circulation model (Methods S1) (Werner, 2019).  $\delta^{18}\text{O}_{\text{rain}}$  was estimated as the average of June–December (growing season) from 1958 to 2013, as no seasonal precipitation pattern was evident.

## 2.5. Symbiotic microorganisms

*Epichloë* sp. presence was determined in stem tissues and seeds (10 to 15) of each plant by staining and microscopic observation (Clark et al., 1983)□. *Epichloë* presence was recorded as a logical value (positive or negative) for each plant. *Epichloë* identification was not possible in 17 out of 238 plants because there were not filled seeds or the aerial tissues were not well preserved enough to enable a reliable diagnosis. *Epichloë* was isolated on potato dextrose agar (PDA in darkness at 24°C) from superficially sterilized stem tissues and seeds. One isolate from one plant chosen at random from each site was morphologically and genetically characterized as described in Iannone et al. (2009) and phylogenetically characterized following the methodology in Mc Cargo et al. (2014)□. This identified *E. tembladera* as the endophyte species colonizing *H. comosun* plants, in agreement with previous studies (Iannone et al., 2015; Yi et al., 2018).

Root samples were stained with Trypan Blue (Phillips & Hayman, 1970) for AMF colonization analyses at individual plant level. The percentage of AMF colonization was determined according to McGonigle et al. (1990)□ and adjusted for observer bias prior to statistical analyses (Methods S1). Briefly, whole roots fixed in FAA were heated at 90°C for about 1 h in 10% KOH (w/v), rinsed in water acidified with dilute HCl, stained by simmering

for 5 min in 0.05% (w/v) trypan blue in lactophenol, and the excess stain removed with clear lactophenol. For each plant, thirty 1 cm-long segments were randomly selected from each sample, mounted on slides in lactophenol and observed at a magnification of 40× (Phillips & Hayman, 1970)□.

## 2.6. Statistical analysis

We first modelled site-level traits to describe the influence of environmental variables (i.e., MAP and MAT) or grazing severity on vegetation cover and *Hordeum comosum* cover. The vegetation cover model was a normal error distribution generalized least squared model and included MAP, MAT, grazing severity and the interaction between MAP and grazing severity as predictors. The *H. comosum* cover model was a generalized linear model with beta error distribution and included MAP, MAT, grazing severity, vegetation cover and the interaction between MAP and grazing severity as predictors. In both models including transects as random effect or not gave a very similar likelihood (LRT: 2.4e-08; p-value = 0.99 and  $X^2 = 4e-04$ ; p-value = 0.98, respectively).

Then, we modelled plant-level traits to study the influence of environmental variables or grazing severity on *Epichloë* presence, AMF colonization, nitrogen and phosphorus concentration in shoot biomass,  $\Delta^{18}O_p$  and iWUE. *Epichloë* presence was analyzed with a generalized linear mixed model which accounted for individual plants nested in sites and sites nested in transects (Pinheiro & Bates, 2000)□ with binomial error distribution (Bates et al., 2015). This model also included MAP, MAT, grazing severity and the interaction between MAP and grazing severity as fixed effects.

The other models at plant-level were analyzed by using linear mixed-effects models with the same nesting hierarchies but with normal distribution of errors. Specifically, all models (AMF colonization, shoot nitrogen and phosphorus,  $\Delta^{18}O_p$  and iWUE) included MAT, MAP, grazing severity, *Epichloë* presence and the double interaction between the last three factor as fixed effects. Then, shoot nitrogen and phosphorus concentration,  $\Delta^{18}O_p$  and iWUE models also included AMF colonization and its double interactions with MAP, grazing severity and *Epichloë* presence.  $\Delta^{18}O_p$  and iWUE models included shoot nitrogen concentration and its double interactions with MAP, grazing severity and *Epichloë* presence. And iWUE models included  $\Delta^{18}O_p$  and its double interactions with MAP, grazing severity and *Epichloë* presence as fixed effects (Table 1).

Before defining the initial model, we analyzed collinearity between given climatic and environmental variables with a Pearson correlation of 0.7 as threshold (Dormann et al., 2013)

(Methods S1). Main predictors were centered by subtracting the mean (Schielzeth, 2010). We selected the most parsimonious random intercepts structure (transect/site or site) by comparing the Akaike information criterion of the complete models estimated by REML (Zuur et al., 2009). We evaluated multicollinearity on the initial and final models by means of the variance inflation factor (VIF; Table S2) (Dormann et al., 2013). We performed a graphical inspection of the models with normal error distribution to evaluate their adequacy (Pinheiro & Bates, 2000; Zuur et al., 2009). When necessary, the variances were modelled by using specific variance functions (Table S3). We did not find spatial correlation among residuals. In the case of the *Epichloë* presence model the dispersion parameter indicated no overdispersion ( $\phi = 0.37$ ). The initial models estimated by ML were reduced by removing non-significant terms in a stepwise fashion (Likelihood ratio tests; Table 1 and Table S4-S5) (Zuur et al., 2009). When *Epichloë*, grazing severity or an interaction involving them were significant, least squares means adjusted by “sidak” and p-value  $< 0.05$  were used to compare means between levels of factors or slopes (emmeans and emtrends functions, respectively with emmeans package; Lenth, 2017) (Table S6). To validate the strength of fixed effects, we calculated the difference between the AIC of the null (i.e., minimum model excluding all fixed effects) and the minimum model ( $\Delta AIC$ ). The conditional and marginal coefficients of determination ( $R^2_c$  and  $R^2_m$ , respectively from r.squaredGLMM function in MuMIn package; Barton, 2016) were also calculated. See Table S3 for traits of the models.

Being aware of the effects of atmospheric humidity (RH) and temperature on  $\Delta^{18}O_p$  (e.g., Hirl et al., 2021), we graphically evaluated the relationship between  $\Delta^{18}O_p$  and MAP for both iso-RH and isothermal gradients. The iso-RH transect was defined as the range of  $\Delta^{18}O_p$  and MAP within an RH of  $58.6 \pm 2\%$ . This included 12 sites situated between  $\sim 300$  and  $700$  mm MAP (Figure S2). The isothermal transect was defined as  $8.4 \pm 0.25^\circ\text{C}$  and included 10 sites distributed along the entire MAP gradient (Figure S3). A detailed description of data processing and statistical analyses are given in Methods S1. All analyses and figures were made in the R-cran environment; version 3.6.1 (R Development Core Team, 2019) and with RStudio; version 1.2.1335 (RStudio, 2019). Data available from: <https://doi.org/10.5061/dryad.c59zw3r8k> (Casas et al., 2021). R codes are available from the authors upon request.

### 3. RESULTS

#### 3.1. Distribution and abundance of *Hordeum comosum* and its symbionts

Total vegetation cover increased at a rate of 5% for every 100 mm increase in MAP (Figure 1A; Table 1). The cover of *H. comosum* was related to MAP, with a maximum ( $\approx 4\%$ ) near the center of the gradient ( $\sim 490$  mm; Figure 1A; Table 1). From that point, *H. comosum* cover decreased towards sites with greater and lesser MAP. *H. comosum* was not found beyond 950 mm and below 150 mm of MAP. Severely and mildly grazed sites were found over a wide MAP range, but the most humid sites were mildly grazed, while the most arid sites were classified as severely grazed.

The symbiotic status of *H. comosum* with *Epichloë* was related with environmental aridity, although there was some overlap in the MAP-range among sites with either all, none or some (mixed) of the *H. comosum* plants being symbiotic with *Epichloë*. However, grazing severity had no significant effect on the probability of *H. comosum* hosting *Epichloë*. The probability of finding a *H. comosum* plant hosting *Epichloë* was half at a MAP of  $\sim 300$  mm, increased toward more humid and decreased toward more arid sites (Figure 1B). Although, sites with a population incidence (i.e., proportion of plants with *Epichloë*, Figure S4) of 100% were found over a MAP-range of 330 to 860 mm (57% of all sites), mixed sites with *Epichloë*-symbiotic and -non-symbiotic plants over a range of 150 to 730 mm (33%), and populations with all *H. comosum* individuals free of *Epichloë* only in sites with  $< 370$  mm MAP (10%).

Except for three out of a total of 220 individuals, all the *H. comosum* plants presented roots colonized by AMF. Root colonization by AMF was highly variable (0-100%), but on average, it increased by 6% for every 100 mm increase of MAP (Figure 1C; Table 1) and was 8% higher in *Epichloë*-symbiotic than in *Epichloë*-free plants (Figure 1D), independently of site aridity.

#### 3.2. Plant nitrogen and phosphorus concentration

Nitrogen concentration in shoots varied along the MAP gradient and depended on the *Epichloë*-symbiotic status of the plants (Table 1). In *Epichloë*-free plants, shoot nitrogen concentration presented a quadratic relationship with MAP, reaching a minimum at  $\sim 400$  mm, whereas in *Epichloë*-symbiotic plants, nitrogen concentration in shoots increased by 0.05% with each 100 mm increase in MAP (Figure 2A; Table S6).

Phosphorous concentration in shoots was, on average, 0.03% higher in *Epichloë*-symbiotic plants than in endophyte-free plants (i.e., 0.19% and 0.16%, respectively; Table 1; Table S6)

and also varied with MAP depending on grazing severity (Figure 2B). It increased by 0.01% and decreased by 0.02% with every 100 mm increase in MAP in sites with severe and mild grazing, respectively.

### 3.3. $^{18}\text{O}$ -enrichment of cellulose and intrinsic WUE

$\Delta^{18}\text{O}_p$  showed a hyperbolically decreasing relationship with MAP under severe grazing (Figure 3A; Table 1), with  $\Delta^{18}\text{O}_p$  increasing on average by 8.5‰ between the subhumid (35.8‰ at 550 mm) and arid limit (44.3‰ at 150 mm) of the MAP range. However, we observed no relation between  $\Delta^{18}\text{O}_p$  and MAP in mildly grazed sites which did not extend into the arid (the confidence intervals for the linear and quadratic terms included zero; Table S6). These relationships between  $\Delta^{18}\text{O}_p$  and MAP under different grazing severities were not influenced by temperature or RH effects on  $\Delta^{18}\text{O}_p$  (Figure S2-3).

The variation of  $\Delta^{18}\text{O}_p$  along the MAP gradient also depended on the level of AMF colonization of plants (Table 1). In particular, the hyperbolic relationship between  $\Delta^{18}\text{O}_p$  and MAP was steeper for highly AMF colonized plants in comparison to plants with low levels of AMF colonization under severe grazing (Figure 3A). Thus, under severe grazing,  $\Delta^{18}\text{O}_p$  of highly AMF colonized plants was greater than that of plants with low AMF colonization in the arid environments, while the opposite was the case in the semiarid to humid environments. In a similar way, in mildly grazed sites the highly AMF colonized plants had generally lower values of  $\Delta^{18}\text{O}_p$  in comparison to plants with low AMF colonization in semiarid to humid environments (Figure 3A). There were no mildly grazed sites in arid environments.

iWUE did not vary significantly with MAP under severe grazing but, remarkably, increased with MAP under mild grazing (Figure 3B; Table 1; Table S6). Again, changes of iWUE with MAP depended on the level of AMF colonization of plants. Thus, in the arid environments, iWUE of highly AMF colonized plants was greater than that of plants with low AMF colonization under severe grazing. Meanwhile, in the semiarid to humid environments iWUE was similar for plants with high and low AMF colonization under severe grazing. In a similar way, in mildly grazed sites highly AMF colonized plants had generally lower values of iWUE in comparison to plants with low AMF colonization in humid environments while they presented similar iWUE values in semiarid environments (Figure 3B). Accordingly, the interaction between MAP and AMF colonization for both,  $\Delta^{18}\text{O}_p$  and iWUE determined that at site level, the relationships of AMF root colonization with  $\Delta^{18}\text{O}_p$  and iWUE were negative (or neutral) in subhumid and humid environments, neutral in the semiarid environment, and

tended to positive in the arid environment (Figure S5-S6). Overall, variation in iWUE tended to be positively associated with  $\Delta^{18}\text{O}_p$  (Table 1).

## 4. DISCUSSION

### 4.1. The interaction of *H. comosum* plants with *Epichloë* and mycorrhizal fungi changed along the aridity gradient

The *H. comosum* symbiotic status with *Epichloë* and AMF was favored toward humid sites and also coincided with mildly grazed sites in the most humid extreme of our transects. That pattern agrees with previous regional observations that studied the symbionts separately [i.e., *Epichloë* sp. (Afkhani et al., 2014; Lewis et al., 1997) and AMF (Gao et al., 2016)]. Along the same line, the abundance of AMF spores in the soil decreased with grazing severity in arid sites in Patagonia (Dudinszky et al., 2019). Beyond the regional pattern, the positive association between *Epichloë* and AMF colonization was also evident in *Bromus setifolius* – another perennial grass endemic to Patagonia (Novas et al., 2005) – although the relation between these symbioses and eco-physiological parameters of the host has not been explored in that study.

The symbiotic pattern that we observed in *H. comosum*, integrated with plant nutritional and eco-physiological characteristics (i.e.,  $\Delta^{18}\text{O}_p$  and iWUE), suggests that symbioses were associated with improved fitness of the host particularly in productive and mildly grazed sites. We assume that *H. comosum* plants would face more intense inter-specific competition in productive vegetation communities in the humid environment of our transects. As has been shown in other species (Zhou et al., 2018), it is very feasible that the co-symbiosis with *Epichloë* endophytes and AMF increases the competitive performance of *H. comosum* plants. Additionally, *Epichloë* in *H. comosum* plants could confer resistance to herbivores by production of N-rich alkaloids (e.g., peramine and terpenoles; Yi et al., 2018). This could be particularly vital in semiarid to humid sites, where the *Epichloë*-AMF consortium was associated with an improved nutritional quality of *H. comosum*. However, the role of herbivores in determining differential fitness of *Epichloë*-symbiotic and *Epichloë*-free *H. comosum* plants along the entire arid-humid gradient remains to be verified experimentally. Conversely, the two symbioses were much more limited in less productive, permanently stressful arid and severely grazed sites.

Interestingly, the maximum *H. comosum* cover occurred in the center of the MAP range

(~500 mm) along with maximum symbiotic status variability. At this point of the gradient, (i) *H. comosum* populations were either entirely symbiotic with *Epichloë* or presented a mixture of symbiotic and non-symbiotic plants, (ii) *Epichloë*-symbiotic plants showed higher nitrogen and phosphorus concentrations than *Epichloë*-free plants and (iii) plants with greater AMF colonization associated with decreased  $\Delta^{18}\text{O}_p$ , which is indicative of enhanced stomatal conductance for comparisons at site level (e.g., Baca Cabrera et al., 2021). Understanding to what extent these eco-physiological characteristics among *H. comosum* plants are driven by the symbiotic associations and how they may contribute to niche differentiation (Valladares et al., 2015) could unveil the symbioses-driven mechanisms explaining the host species abundance (either high or low) along the natural distribution range. The alignment of the response variables at plant and site level in this field study, certainly provides directions for manipulative experiments that are required to explore these mechanisms.

#### **4.2. AMF-colonization enhanced the aridity-response of cellulose- $^{18}\text{O}$ -enrichment**

This work detected strong variation of  $\Delta^{18}\text{O}_p$  with MAP that was indicative of ecohydrological effects related to variation of stomatal conductance (Barbour, 2007; Hirl et al., 2019; Ramírez et al., 2009) or effects of soil water dynamics (Hirl et al., 2019) and symbionts on the  $^{18}\text{O}$  of (source) water taken up by the root system. The exact identity of the physiological mechanism underlying these patterns remains to be ascertained, particularly at the between-site or MAP gradient level. Consistent with empirical evidence,  $\Delta^{18}\text{O}_p$  increased strongly with decreasing MAP (positively correlated with RH) (e.g. Barbour, 2007; Hirl et al., 2021). Interestingly, however,  $\Delta^{18}\text{O}_p$  also increased towards low MAP along isothermal and iso-RH gradients (Figure S2-3), demonstrating that changes of  $\Delta^{18}\text{O}_p$  with MAP were not driven by RH or temperature alone (Hirl et al., 2021). Variation of stomatal conductance, independent of RH and temperature, is a likely explanatory factor (Baca Cabrera et al., 2021). In addition, absence of significant variation in iWUE towards arid and severely grazed sites would indicate that eventual variations of stomatal conductance were matched by proportional variations in photosynthesis (Scheidegger et al., 2000) in that range. Meanwhile, increasing iWUE towards more humid and mildly grazed environments was likely due to an enhancement of photosynthetic capacity relative to stomatal conductance (Scheidegger et al., 2000), possibly linked to greater phosphorus and nitrogen concentration in *Epichloë*-symbiotic plants with greater AMF colonization in humid sites.

In particular, the relation of  $^{18}\text{O}$ -enrichment and iWUE with the degree of AMF



colonization at within-site level may be taken to suggest an effect of the degree of AMF colonization on stomatal conductance. Although an enhancement of stomatal conductance by AMF has been reported previously (Augé, 2001; Querejeta et al., 2006; Ruiz-Lozano et al., 2012), the site-level AMF colonization vs  $\Delta^{18}\text{O}_p$  (and iWUE) relationships is consistent with the idea that increasing AMF colonization may boost host plant stomatal conductance in the (semi)humid range, but curb it in the arid range. While such an interpretation aligns with findings in woody species in semiarid environments (Querejeta et al., 2003, 2006), further research is needed to elucidate the differential effects of *Epichloë* and AMF colonization on host nutritional and physiological status under stress conditions as given by either drought or severe grazing.

## 5. CONCLUSION

This field study demonstrated that *Epichloë* and AMF prevail especially under (semi)humid and mildly grazed conditions within the distribution range of their host *H. comosum*. Also, AMF colonization appeared to be related with greater stomatal sensitivity towards aridity. Importantly, our findings support the idea that both symbionts do indeed affect host fitness through resource-economic variables such as nutrients, water and carbon. The alignment of the response variables at plant and site level opens interesting questions to be studied in future experimental approaches: *i*) what are the physiological mechanisms underlying the interaction between AMF and associated *Epichloë* and the components of iWUE in the different locations in the MAP range of *H. comosum*?; *ii*) what are the ecological mechanisms explaining the loss of the symbionts (i.e., *Epichloë* absence and lower levels of AMF colonization) under arid conditions?; *iii*) Is it possible to determine a cost:benefit threshold which ultimately explains the range of conditions for a mutualistic relation? Such knowledge would be key to achieve a real understanding of the functional roles of fungal symbionts in the ecology of host plants.

## 6. REFERENCES

- Afkhami, M. E., McIntyre, P. J., & Strauss, S. Y. (2014). Mutualist-mediated effects on species' range limits across large geographic scales. *Ecology Letters*, *17*(10), 1265–1273. <https://doi.org/10.1111/ele.12332>
- Allen, R. G., Pereira, L. S., Raes, D., & Smith, M. (1998). Crop evapotranspiration - Guidelines for computing crop water requirements. In *Irrigation and Drainage Paper No. 56, FAO* (p. 300). FAO. <https://doi.org/10.1016/j.eja.2010.12.001>
- Augé, R. M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, *11*(1), 3–42. <https://doi.org/10.1007/s005720100097>
- Baca Cabrera, J. C., Hirl, R. T., Schäufele, R., Macdonald, A., & Schnyder, H. (2021). Stomatal conductance limited the CO<sub>2</sub> response of grassland in the last century. *BMC Biology*, *19*(50), 50. <https://doi.org/https://doi.org/10.1186/s12915-021-00988-4>
- Barbour, M. M. (2007). Stable oxygen isotope composition of plant tissue: a review. *Functional Plant Biology*, *34*(2), 83–94. <https://doi.org/10.1071/FP06228>
- Barton, K. (2016). *MuMIn: Multi-Model Inference. R package version 1.15.6*. <https://cran.r-project.org/package=MuMIn>
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). *lme4: Linear mixed-effects models using eigen and S4. R package version 1.1–7. 2014*. <https://doi.org/10.1016/j.jorganchem.2015.04.040>
- Brendel, O., Iannetta, P. P. M., & Stewart, D. (2000). A rapid and simple method to isolate pure alpha-cellulose. *Phytochemical Analysis*, *11*(1), 7–10. [https://doi.org/10.1002/\(SICI\)1099-1565\(200001/02\)11:1<7::AID-PCA488>3.0.CO;2-U](https://doi.org/10.1002/(SICI)1099-1565(200001/02)11:1<7::AID-PCA488>3.0.CO;2-U)
- Casanova-Katny, M. A., Torres-Mellado, G. A., Palfner, G., & Cavieres, L. A. (2011). The best for the guest: high Andean nurse cushions of *Azorella madreporica* enhance arbuscular mycorrhizal status in associated plant species. *Mycorrhiza*, *21*(7), 613–622. <https://doi.org/10.1007/s00572-011-0367-1>
- Casas, C., Gundel, P. E., Deliens, E., Iannone, L. J., García Martínez, G., Vignale, M. V., & Schnyder, H. (2021). *Data from: Loss of fungal symbionts at the arid limit of the distribution range in a native Patagonian grass – resource ecophysiological relations*. <https://doi.org/https://doi.org/10.5061/dryad.c59zw3r8k>
- Cavagnaro, R. A., Pero, E., Dudinszky, N., Golluscio, R. A., & Grimoldi, A. A. (2018). Under pressure from above: Overgrazing decreases mycorrhizal colonization of both preferred and unpreferred grasses in the Patagonian steppe. *Fungal Ecology*.

<https://doi.org/10.1016/j.funeco.2018.09.003>

- Clark, E. M., White, J. F., & Patterson, R. M. (1983). Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of in vitro culture of the fungus. *Journal of Microbiological Methods*, *1*(3), 149–155. [https://doi.org/10.1016/0167-7012\(83\)90033-7](https://doi.org/10.1016/0167-7012(83)90033-7)
- Clay, K., & Schardl, C. L. (2002). Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *The American Naturalist*, *160*, 99–127. <https://doi.org/10.1086/342161>
- Compan, S., Van Der Heijden, M. G. A., & Sessitsch, A. (2010). Climate change effects on beneficial plant-microorganism interactions. *FEMS Microbiology Ecology*, *73*(2), 197–214. <https://doi.org/10.1111/j.1574-6941.2010.00900.x>
- Delavaux, C. S., Smith-Ramesh, L. M., & Kuebbing, S. E. (2017). Beyond nutrients: A meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. *Ecology*, *98*, 2111–2119. <https://doi.org/10.1002/ecy.1892>
- Delgado-Baquerizo, M., Eldridge, D. J., Maestre, F. T., Ochoa, V., Gozalo, B., Reich, P. B., & Singh, B. K. (2018). Aridity decouples C:N:P stoichiometry across multiple trophic levels in terrestrial ecosystems. *Ecosystems*, *21*(3), 459–468. <https://doi.org/10.1007/s10021-017-0161-9>
- Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J. R. G., Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean, C., Osborne, P. E., Reineking, B., Schröder, B., Skidmore, A. K., Zurell, D., & Lautenbach, S. (2013). Collinearity: A review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, *36*(1), 027–046. <https://doi.org/10.1111/j.1600-0587.2012.07348.x>
- Dudinszky, N., Cabello, M. N., Grimoldi, A. A., Schalamuk, S., & Golluscio, R. A. (2019). *Role of Grazing Intensity on Shaping Arbuscular Mycorrhizal Fungi Communities in Patagonian Semiarid Steppes* ☆. <https://doi.org/10.1016/j.rama.2019.02.007>
- Farquhar, G. D., & Richards, R. A. (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Functional Plant Biology*, *11*(6), 539. <https://doi.org/10.1071/PP9840539>
- Flanagan, L. B., & Farquhar, G. D. (2014). Variation in the carbon and oxygen isotope composition of plant biomass and its relationship to water-use efficiency at the leaf- and ecosystem-scales in a northern Great Plains grassland. *Plant, Cell and Environment*,

37(2), 425–438. <https://doi.org/10.1111/pce.12165>

- Gao, C., Zheng, Y., Yang, W., & Chen, L. (2016). Increased precipitation, rather than warming, exerts a strong influence on arbuscular mycorrhizal fungal community in a semiarid steppe ecosystem. *Botany*, *94*(6), 459–469. <https://doi.org/10.1139/cjb-2015-0210>
- Gaudinski, J., Dawson, T., Quideau, S., Schuur, E., Roden, J., Trumbore, S., Sandquist, D., Oh, S.-W., & Wasylishen, R. (2005). Comparative analysis of cellulose preparation techniques for use with  $^{13}\text{C}$ ,  $^{14}\text{C}$ , and  $^{18}\text{O}$  isotopic measurements. *Analytical Chemistry*, *77*, 7212–7224. <https://doi.org/10.1021/ac050548u>
- Gehring, C. A., & Whitham, T. G. (1994). Interactions between aboveground herbivores and the mycorrhizal mutualists of plants. *Trends in Ecology & Evolution*, *9*(7), 251–255.
- Golluscio, R. A., Deregibus, A. V., & Paruelo, J. M. (1998). Sustainability and range management in the Patagonian steppes. *Ecologia Austral*, *8*(2), 265–284. [https://doi.org/http://hdl.handle.net/20.500.12110/ecologiaaustral\\_v008\\_n02\\_p265](https://doi.org/http://hdl.handle.net/20.500.12110/ecologiaaustral_v008_n02_p265)
- Gundel, P. E., Garibaldi, L. A., Martínez-Ghersa, M. A., & Ghersa, C. M. (2011). *Neotyphodium* endophyte transmission to *Lolium multiflorum* seeds depends on the host plant fitness. *Environmental and Experimental Botany*, *71*, 359–366. <https://doi.org/10.1016/j.envexpbot.2011.02.002>
- Hanson, W. C. (1950). The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex. *Journal of the Science of Food and Agriculture*, *1*(6), 172–173. <https://doi.org/10.1002/jsfa.2740010604>
- Hernández-Agramonte, I. M., & Semmartin, M. (2016). The role of grazing intensity and preference on grass-fungal endophyte symbiosis in a Patagonian steppe. *Journal of Arid Environments*, *134*, 122–124. <https://doi.org/10.1016/j.jaridenv.2016.07.006>
- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*(15), 1965–1978. <https://doi.org/10.1002/joc.1276>
- Hirl, R. T., Ogée, J., Ostler, U., Schäufole, R., Baca Cabrera, J. C., Zhu, J., Schleip, I., Wingate, L., & Schnyder, H. (2021). Temperature-sensitive biochemical  $^{18}\text{O}$ -fractionation and humidity-dependent attenuation factor are needed to predict  $\delta^{18}\text{O}$  of cellulose from leaf water in a grassland ecosystem. *New Phytologist*, *229*(6), 3156–3171. <https://doi.org/10.1111/nph.17111>
- Hirl, R. T., Schnyder, H., Ostler, U., Schäufole, R., Schleip, I., Vetter, S. H., Auerswald, K., Cabrera, J. C. B., Wingate, L., Barbour, M. M., & Ogée, J. (2019). The  $^{18}\text{O}$

eco-hydrology of a grassland ecosystem—predictions and observations. *Hydrol. Earth Syst. Sci.*, 23, 2581–2600. <https://doi.org/10.5194/hess-23-2581-2019>

Iannone, L. J., Cabral, D., Schardl, C. L., & Rossi, M. S. (2009). Phylogenetic divergence, morphological and physiological differences distinguish a new *Neotyphodium* endophyte species in the grass *Bromus auleticus* from South America. *Mycologia*, 101(3), 340–351. <https://doi.org/10.3852/08-156>

Iannone, L. J., Irisarri, J. G. N., Mc Cargo, P. D., Pérez, L. I., & Gundel, P. E. (2015). Occurrence of *Epichloë* fungal endophytes in the sheep-preferred grass *Hordeum comosum* from Patagonia. *Journal of Arid Environments*, 115, 19–25. <https://doi.org/10.1016/j.jaridenv.2014.12.008>

Kim, Y.-C., Gao, C., Zheng, Y., He, X.-H., Yang, W., Liang, C., Wan, S.-Q., Liang, & Guo, D. (2015). Arbuscular mycorrhizal fungal community response to warming and nitrogen addition in a semiarid steppe ecosystem. *Mycorrhiza*, 25, 267–276. <https://doi.org/10.1007/s00572-014-0608-1>

Köhler, I. H., Macdonald, A., & Schnyder, H. (2012). Nutrient supply enhanced the increase in intrinsic water-use efficiency of a temperate seminatural grassland in the last century. *Global Change Biology*, 18(11), 3367–3376. <https://doi.org/10.1111/J.1365-2486.2012.02781.X>

Lenth, R. V. (2017). *emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.0*. <https://cran.r-project.org/package=emmeans>

Lewis, G. C., Ravel, C., Naffaa, W., Astier, C., & Charmet, G. (1997). Occurrence of *Acremonium* endophytes in wild populations of *Lolium* spp. in European countries and a relationship between level of infection and climate in France. *Annals of Applied Biology*, 130, 227–238. <https://doi.org/10.1111/j.1744-7348.1997.tb06828.x>

Li, F., Deng, J., Nzabanita, C., Li, Y., & Duan, T. (2019). Growth and physiological responses of perennial ryegrass to an AMF and an *Epichloë* endophyte under different soil water contents. *Symbiosis*, 79(2), 151–161. <https://doi.org/10.1007/s13199-019-00633-3>

Liu, H., Chen, W., Wu, M., Wu, R., Zhou, Y., Gao, Y., & Ren, A. (2017). Arbuscular mycorrhizal fungus inoculation reduces the drought-resistance advantage of endophyte-infected versus endophyte-free *Leymus chinensis*. *Mycorrhiza*, 28(8), 791–799. <https://doi.org/10.1007/s00572-017-0794-8>

Ma, W. T., Tcherkez, G., Wang, X. M., Schäufele, R., Schnyder, H., Yang, Y., & Gong, X. Y. (2020). Accounting for mesophyll conductance substantially improves  $^{13}\text{C}$ -based estimates of intrinsic water-use efficiency. *New Phytologist*, 0.

<https://doi.org/10.1111/nph.16958>

- Mc Cargo, P. D., Iannone, L. J., Vignale, M. V., Schardl, C. L., & Rossi, M. S. (2014). Species diversity of *Epichloë* symbiotic with two grasses from southern Argentinean Patagonia. *Mycologia*, *106*(2), 339–352. <https://doi.org/10.3852/106.2.339>
- McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., & Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, *115*(3), 495–501. <https://doi.org/10.1111/j.1469-8137.1990.tb00476.x>
- Moreno-Gutiérrez, C., Dawson, T. E., Nicolás, E., & Querejeta, J. I. (2012). Isotopes reveal contrasting water use strategies among coexisting plant species in a mediterranean ecosystem. *New Phytologist*, *196*(2), 489–496. <https://doi.org/10.1111/j.1469-8137.2012.04276.x>
- Novas, M. V., Cabral, D., & Godeas, A. M. (2005). Interaction between grass endophytes and mycorrhizas in *Bromus setifolius* from Patagonia, Argentina. *Symbiosis*, *40*(1), 23–30. <https://doi.org/ISSN:0334-5114>
- Omacini, M., Semmartin, M., Pérez, L. I., & Gundel, P. E. (2012). Grass–endophyte symbiosis: A neglected aboveground interaction with multiple belowground consequences. *Applied Soil Ecology*, *61*(0), 273–279. <https://doi.org/http://dx.doi.org/10.1016/j.apsoil.2011.10.012>
- Pellissier, L., Pinto-Figueroa, E., Niculita-Hirzel, H., Moora, M., Villard, L., Goudet, J., Guex, N., Pagni, M., Xenarios, I., Sanders, I., & Guisan, A. (2013). Plant species distributions along environmental gradients: do belowground interactions with fungi matter? *Frontiers in Plant Science*, *4*(DECEMBER 2013). <https://doi.org/10.3389/fpls.2013.00500>
- Phillips, J. M., & Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, *55*(1), 158–161. [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3)
- Pinheiro, J., & Bates, D. (2000). *Mixed-Effects Models in S and S-PLUS* (J. Chambers, W. Eddy, W. Härdle, S. Sheather, & L. Tierney (eds.)). Springer.
- Querejeta, J. I., Allen, M. F., Caravaca, F., & Roldán, A. (2006). Differential modulation of host plant  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  by native and nonnative arbuscular mycorrhizal fungi in a semiarid environment. *New Phytologist*, *169*(2), 379–387. <https://doi.org/10.1111/j.1469-8137.2005.01599.x>

- Querejeta, J. I., Barea, J. M., Allen, M. F., Caravaca, F., & Roldán, A. (2003). Differential response of  $\delta^{13}\text{C}$  and water use efficiency to arbuscular mycorrhizal infection in two aridland woody plant species. *Oecologia*, *135*(4), 510–515. <https://doi.org/10.1007/s00442-003-1209-4>
- R Development Core Team. (2019). *R: a language and environment for statistical computing* (3.6.1; p. Vienna, Austria). R Foundation for Statistical Computing. <https://www.r-project.org/>.
- Ramírez, D. A., Querejeta, J. I., & Bellot, J. (2009). Bulk leaf  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  reflect the intensity of intraspecific competition for water in a semi-arid tussock grassland. *Plant, Cell and Environment*, *32*(10), 1346–1356. <https://doi.org/10.1111/j.1365-3040.2009.02002.x>
- Ren, A., Wei, M., Yin, L., Wu, L., Zhou, Y., Li, X., & Gao, Y. (2014). Benefits of a fungal endophyte in *Leymus chinensis* depend more on water than on nutrient availability. *Environmental and Experimental Botany*, *108*, 71–78. <https://doi.org/10.1016/j.envexpbot.2013.11.019>
- RStudio, T. (2019). *RStudio: Integrated Development for R* (1.2.1335). RStudio, Inc. <http://www.rstudio.com/>.
- Rudgers, J. A., Fletcher, R. A., Olivas, E., Young, C. A., Charlton, N. D., Pearson, D. E., & Maron, J. L. (2016). Long-term ungulate exclusion reduces fungal symbiont prevalence in native grasslands. *Oecologia*, *181*(4), 1151–1161.
- Ruiz-Lozano, J. M., Porcel, R., Bárzana, G., Azcón, R., & Aroca, R. (2012). Contribution of arbuscular mycorrhizal symbiosis to plant drought tolerance: state of the art. In *Plant responses to drought stress* (pp. 335–362). Springer Berlin Heidelberg. [https://doi.org/10.1007/978-3-642-32653-0\\_13](https://doi.org/10.1007/978-3-642-32653-0_13)
- Schardl, C. L., Florea, S., Pan, J., Nagabhyru, P., Bec, S., & Calie, P. J. (2013). The epichloae: alkaloid diversity and roles in symbiosis with grasses. *Current Opinion in Plant Biology*, *16*(4), 480–488. <https://doi.org/https://doi.org/10.1016/j.pbi.2013.06.012>
- Scheidegger, Y., Saurer, M., Bahn, M., & Siegwolf, R. (2000). Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia*, *125*(3), 350–357. <https://doi.org/10.1007/s004420000466>
- Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, *1*(2), 103–113. <https://doi.org/10.1111/j.2041-210x.2010.00012.x>
- Semmartin, M., Omacini, M., Gundel, P. E., & Hernández-Agramonte, I. M. (2015). Broad-

scale variation of fungal-endophyte incidence in temperate grasses. *Journal of Ecology*, 103(1), 184–190. <https://doi.org/10.1111/1365-2745.12343>

Siffredi, G. L., Boggio, F., Giorgetti, H., Ayesa, J. A., Kropfl, A., & Alvarez, J. M. (2011). *Guía para la Evaluación de Pastizales para las Áreas Ecológicas de Sierras y Mesetas Occidentales y de Monte de Patagonia Norte*. Ediciones Instituto Nacional de Tecnología Agropecuaria.

Smit, C., Rietkerk, M., & Wassen, M. J. (2009). Inclusion of biotic stress (consumer pressure) alters predictions from the stress gradient hypothesis. *Journal of Ecology*, 97, 1215–1219. <https://doi.org/10.1111/j.1365-2745.2009.01555.x>

Smith, S. E., & Read, D. J. (2008). Mycorrhizal Symbiosis. In *Soil Science Society of America Journal* (3rd ed.). Academic Press. <https://doi.org/10.1097/00010694-198403000-00011>

Swarthout, D., Harper, E., Judd, S., Gonthier, D., Shyne, R., Stowe, T., & Bultman, T. (2009). Measures of leaf-level water-use efficiency in drought stressed endophyte infected and non-infected tall fescue grasses. *Environmental and Experimental Botany*, 66(1), 88–93. <https://doi.org/10.1016/j.envexpbot.2008.12.002>

Valladares, F., Bastias, C. C., Godoy, O., Granda, E., & Escudero, A. (2015). Species coexistence in a changing world. In *Frontiers in Plant Science* (Vol. 6, p. 866). Frontiers Research Foundation. <https://doi.org/10.3389/fpls.2015.00866>

van der Heyde, M., Bennett, J. A., Pither, J., & Hart, M. (2017). Longterm effects of grazing on arbuscular mycorrhizal fungi. *Agriculture, Ecosystems & Environment*, 243, 27–33. <https://doi.org/10.1016/J.AGEE.2017.04.003>

Vázquez-de-Aldana, B. R., García-Ciudad, A., García-Criado, B., Vicente-Tavera, S., & Zabalgoageazcoa, I. (2013). Fungal endophyte (*Epichloë festucae*) alters the nutrient content of *Festuca rubra* regardless of water availability. *PLoS ONE*, 8(12), 1–14. <https://doi.org/10.1371/journal.pone.0084539>

Vignale, M. V., Iannone, L. J., Pinget, A. D., de Battista, J. P., & Novas, M. V. (2016). Effect of epichloid endophytes and soil fertilization on arbuscular mycorrhizal colonization of a wild grass. *Plant and Soil*, 405(1–2), 279–287. <https://doi.org/10.1007/s11104-015-2522-5>

Werner, M. (2019). *ECHAM5-wiso simulation data - present-day, mid-Holocene, and Last Glacial Maximum*. PANGAEA. <https://doi.org/10.1594/PANGAEA.902347>

Winkworth, R. E., Perry, R. a., & Rossetti, C. O. (1962). A comparison of methods of estimating plant cover in an arid grassland community. *Journal of Range Management*,



15(4), 194–196. <https://doi.org/10.2307/3895247>

- Wu, Q. S., & Xia, R. X. (2006). Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology*, 163(4), 417–425. <https://doi.org/10.1016/j.jplph.2005.04.024>
- Yi, M., Hendricks, W. Q., Kaste, J., Charlton, N. D., Nagabhyru, P., Panaccione, D. G., & Young, C. A. (2018). Molecular identification and characterization of endophytes from uncultivated barley. *Mycologia*, 5514, 1–20. <https://doi.org/10.1080/00275514.2018.1464818>
- Zhong, R., Xia, C., Ju, Y., Zhang, X., Duan, T., Nan, Z., & Li, C. (2019). A foliar *Epichloë* endophyte and soil moisture modified belowground arbuscular mycorrhizal fungal biodiversity associated with *Achnatherum inebrians*. *Plant and Soil*, 1–18. <https://doi.org/10.1007/s11104-019-04365-7>
- Zhou, Y., Li, X., Gao, Y., Liu, H., Gao, Y.-B., van der Heijden, M. G. A., & Ren, A.-Z. (2018). Plant endophytes and arbuscular mycorrhizal fungi alter plant competition. *Functional Ecology*, 32(5), 1168–1179. <https://doi.org/10.1111/1365-2435.13084>
- Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R* (M. Gail, K. Krickeberg, J. M. Samet, A. Tsiatis, & W. Wong (eds.)). Springer.

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

**Appendix S1.** Supporting methods include detailed description of (1) climate, (2) soil and, (3) rhizosphere data collection as well as (4) water use efficiency calculus, (5) considerations about oxygen isotope composition and stomatal conductance; (6) data processing and (7) statistical analyses.

**Appendix S2.** Supporting tables, includes:

**Table S1.** Criteria for the classification of sites in terms of the grazing conditions (mild and severe) according to vegetation cover and floristic composition.

**Table S2.** VIF values of the initial and final regression models for *Hordeum comosum* analyses.

**Table S3.** Best-fitting regression models for *Hordeum comosum* analyses.

**Table S4.** Likelihood ratio test of regression models for *Hordeum comosum* analyses.

**Table S5.** Likelihood ratio test of regression models for shoot nitrogen and phosphorus concentration in *Hordeum comosum*.

**Table S6.** Post-hoc comparisons of regression models for *Hordeum comosum* analyses.

**Appendix S3.** Supporting figures, includes:

**Figure S1.** Conceptual analytical framework of the study.

**Figure S2.** Relations between mean annual precipitation, relative humidity, and  $^{18}\text{O}$ -enrichment of cellulose in shoot biomass of *H. comosum* plants ( $\Delta^{18}\text{O}_\text{P}$ ).

**Figure S3.** Relations between oxygen isotope composition of meteoric waters ( $\delta^{18}\text{O}_\text{rain}$ ), oxygen isotope composition of *H. comosum* shoot cellulose ( $\delta^{18}\text{O}_\text{P}$ ),  $^{18}\text{O}$ -enrichment of cellulose ( $\Delta^{18}\text{O}_\text{P}$ ) and mean annual precipitation.

**Figure S4.** Proportion of *Epichloë* symbiotic plants per site in relation to mean annual precipitation (MAP, mm)

**Figure S5.**  $^{18}\text{O}$ -enrichment of cellulose ( $\Delta^{18}\text{O}_\text{P}$ ) in shoot biomass of *H. comosum* plants in relation with the degree of arbuscular mycorrhizal colonization (AMF, %).

**Figure S6.** iWUE of cellulose in shoot biomass of *H. comosum* plants in relation with the degree of arbuscular mycorrhizal colonization (AMF, %).

## TABLES

**TABLE 1** Parameter estimates of minimum models. Columns show the models for: *H. comosum* cover, vegetation cover, *Epichloë* presence and AMF colonization, nitrogen (N) and phosphorus (P) concentration,  $\Delta^{18}\text{O}_p$  and iWUE. Rows show the fixed factors, the  $\Delta\text{AIC}$  (AIC null - minimum model), the marginal and conditional coefficient of determination ( $R^2_m$  and  $R^2_c$ , respectively). The intercept shows the value for *Epichloë*-free plants under severe grazing when all the other predictors are averaged (centered). Cells show the estimated parameter for significant predictors, predictor evaluated but non-significant (n.s.) and non-included in the model (shadow). See p-values in Table S4.

	<i>H. comosum</i> cover (%) <sup>†</sup>	Vegetation cover (%)	<i>Epichloë</i> presence <sup>†</sup>	AMF colo- nization (%)	Shoot (%)	N	Shoot P (%)	$\Delta^{18}\text{O}_p$ (‰)	iWUE
<b>Intercept</b>	-29.04	50.23	2.1	39.18	0.83	0.15	38.078	50.92	
<b>Vegetation cover (%)</b>	n.s.								
<b>MAP* (mm)</b>	<b>Lin. -11.9</b> <b>Quad. -122.45</b>	<b>0.05</b>	<b>0.01</b>	<b>0.06</b>	<b>Lin. 2.52</b> <b>Quad. 3.55</b>	<b>0.0001</b>	<b>Lin. -20.2</b> <b>Quad. 31.4</b>	0.0005	
<b>MAT (°C)</b>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
<b>Grazing (Mild)</b>	n.s.	n.s.	n.s.	n.s.	n.s.	<b>0.017</b>	-1.45	-2.52	
Grazing x MAP	n.s.	n.s.	n.s.	n.s.	n.s.	<b>-0.0003</b>	<b>Lin. 46.7</b> <b>Quad. -55.5</b>	<b>0.02</b>	
<b><i>Epichloë</i> (present)</b>				<b>8.1</b>	<b>0.04</b>	<b>0.036</b>	n.s.	n.s.	
<i>Epichloë</i> x Grazing				n.s.	n.s.	n.s.	n.s.	n.s.	
<i>Epichloë</i> x MAP				n.s.	<b>Lin. -2.01</b> <b>Quad. -2.92</b>	n.s.	n.s.	n.s.	
<b>AMF (%)</b>					n.s.	n.s.	<b>-0.009</b>	0.01	
<i>Epichloë</i> x AMF					n.s.	n.s.	n.s.	n.s.	

	<i>H. comocum</i> cover (%) <sup>†</sup>	Vegetation cover (%)	<i>Epichloë</i> presence <sup>†</sup>	AMF colo- nization (%)	Shoot (%)	N	Shoot P (%)	$\Delta^{18}\text{O}_p$ (‰)	iWUE
Grazing x AMF					n.s.	n.s.	n.s.	n.s.	n.s.
MAP x AMF					n.s.	n.s.	<b>Lin. -0.26</b> <b>Quad. 0.21</b>		<b>-0.0002</b>

TABLE 1: Cont.

	<i>H. comocum</i> (%)	cover	Vegetation cover (%)	<i>Epichloë</i> presence	AMF colo- nization (%)	Shoot (%)	N	Shoot (%)	P	$\Delta^{18}\text{O}_p$ (‰)	iWUE
<b>Shoot N (%)</b>										n.s.	2.63
Shoot N x <i>Epichloë</i>										n.s.	n.s.
Shoot N x Grazing										n.s.	<b>-9.23</b>
Shoot N x MAP										n.s.	n.s.
<b><math>\Delta^{18}\text{O}_p</math> (‰)</b>											<b>0.60</b>
$\Delta^{18}\text{O}_p$ x <i>Epichloë</i>											n.s.
$\Delta^{18}\text{O}_p$ x Grazing											n.s.
$\Delta^{18}\text{O}_p$ x MAP											n.s.
$\Delta\text{AIC}$	23.7		11.8	12.35	27.66	15.48		21.42		21.11	19.8
$R^2_m$	0.32		0.31	0.28	0.32	0.25		0.28		0.50	0.46
$R^2_c$	-		-	0.81	0.57	0.53		0.64		0.82	0.798

\*poly(MAP, 2) in *H. comosum* cover, shoot nitrogen concentration and  $\Delta^{18}\text{Op}$  model. †Link functions are cauchit and logit in the case of *H. comosum* cover and *Epichloë* presence models, respectively; and identity in the other models. As AMF contains NAs, when AMF was not a significant predictor (shoot nitrogen and phosphorus concentration), we present models considering the whole dataset (see Table S5 for p-values).

## FIGURE LEGENDS

**FIGURE 1** (A) Cover of *Hordeum comosum* (left axis; black symbols) and total vegetation cover (right axis; open dots) per site; (B) *Epichloë* presence (1; E+) or absence (0; E-) for each plant; (C) root AMF colonization (%) in relation to mean annual precipitation (MAP, mm) and (D) root AMF colonization (%) of *Epichloë*-symbiotic (E+) and non-symbiotic (E-) *H. comosum* plants. (A - C) show *H. comosum* plants from sites with severe (squares) and mild (triangles) level of grazing; black and grey colors indicate *Epichloë*-symbiotic (E+) and non-symbiotic (E-) *H. comosum* plants, respectively. (A - C) lines and shadows show model fit and 95% prediction interval, respectively. In (C), black and grey lines show model fit for E+ and E- plants, respectively. In (B), symbols were jittered to avoid overlapping, the proportion of *Epichloë* symbiotic plants per site is presented in Fig. S4. Vertical dotted lines in (A-C) delimit the environments determined by MAP range: humid (>650 mm), dry subhumid (650 to 500 mm), semiarid (500 to 200 mm), and arid (<200 mm). In (D) the bars show the mean  $\pm$  standard error and grey points show raw data. Different letters show significant differences with  $p$ -value < 0.05.

**FIGURE 2** Relationship between nitrogen (A) and phosphorus (B) concentration (both in %) in shoots of *Hordeum comosum* plants with mean annual precipitation (mm). Symbols show *Epichloë*-symbiotic (E+; black symbols) and non-symbiotic (E-; grey symbols) *H. comosum* plants from sites with severe (squares) and mild (triangles) level of grazing. In (A) and (B) black and grey lines show model fit for E+ and E-, respectively. In (B) full and dashed lines show model fits for severe and mild grazing, respectively. In all cases, shadows show 95% prediction intervals. Vertical dotted lines delimit the environments determined by MAP as in Figure 1.

**FIGURE 3** (A)  $^{18}\text{O}$ -enrichment of cellulose ( $\Delta^{18}\text{O}_p$ ; ‰) and (B) iWUE in shoot biomass of *H. comosum* plants in relation to mean annual precipitation (mm). Symbols indicate *H. comosum* plants from sites with severe (squares) and mild (triangles) level of grazing with less than 40% (grey) or more than 60% (black) AMF colonization. Full and dashed lines show model fit for mild and severe grazing, respectively while, grey and black lines indicate model fit for 20 and 80% AMF colonization, respectively; shadows show 95% prediction intervals. Vertical dotted lines delimit the environments determined by the MAP as in Figure 1.







