Effect of soil nitrogen and water content on the establishment of a *Lolium perenne* L. and *Trifolium repens* L. pasture

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Abstract

Climate change has decreased rainfall in Southern Chile affecting the productivity of the grasslands that sustain the dairy and beef cattle industries. These grasslands are mainly mixtures of *Lolium perenne* L. and *Trifolium repens* L. Thus, we study the response on the establishment of a mixture of these two species to three levels of soil water content and two levels of nitrogen (N) fertilization. The trial was carried out in containers sowed in a complete randomized block´s design. Soil moisture measurements were taken daily. N fertilizations was applied at the establishment and every 60 days. Leaf appearance rate was measured every 3 days. Intercepted radiation, plant water potential, botanical composition, dry matter content and nutritional forage quality were measured at each harvest. Soil samples were taken at the beginning, peak and end of the establishment period to study the microbial communities. Results showed that the rate of leaf appearance of *L. perenne* and *T. repens* were not influenced by soil water content, nor by nitrogen fertilization level. *L. perenne* yield was influenced by soil water content and N fertilization level. *T. repens* yield was affected only by the soil water content. All the variables measured were affected by the harvest date. Soil water content and abundance of *T. repens* had a significant effect on the nutritional quality of the pasture. Soil water content and nitrogen fertilization level modified the microbial communities in the soil.

Introduction

Livestock systems in southern Chile are based on grasslands. Climate change poses a risk to grassland development and production due to increased temperatures and decreased precipitations. Therefore, the objective of this work was to study the impact of a water restriction on yield, nutritive value, competitive ability and the rhizobacterial community in the establishment of a mixture of *Lolium perenne* L. and *Trifolium repens* L., the most widely sown species in southern Chile.

Methods

Eighteen mini-meadows of a mixture of *L. perenne* and *T. repens* were established in 125 L containers at Universidad Austral de Chile, Valdivia, Chile (elevation 12 m.a.s.l., latitude 39º48'S, longitude 73º15'W, annual rainfall 2500 mm). These containers were filled with an disturbed Hapludand soil from the Valdivia Series. The mini-meadows were established on September 8, 2016, using a template with the area of the container (0.18 m2) in which 180 holes were made where *L. perenne* and *T. repens* seeds were placed alternately, i.e. a mixture of 50% *L. perenne* and 50% *T. repens* seeds. The *T. repens* seeds were inoculated before sowing with Rizofix Gel®, an inoculant based on Rizhobium spp. according to the manufacturer's specifications. A soil analys indicated no chemical limitations for pasture growth and a pH: 6.3; Organic Matter: 4,1%; Mineral N: 23.1 mg kg^{-1}; Olsen-P: 16.4 mg kg^{-1}; K: 175 mg kg^{-1}; CICE: 10.23 cmol kg^{-1}; Al saturation: 0.05%. The mini-meadows were subjected to three levels of water restriction, 20-30%, 40-45% and 85-90% of soil available water for plants (AWP) and two levels of N, 0 and 9 gr m^{-2} (90 kg ha^{-1}), applied as urea in three partializations of 3 gr m^{-2} (30 kg ha^{-1}) at the establishment and 3 gr m^{-2} every 60 days, for a total of 6 treatments and 3 replicates in a complete randomized block´s design. To determine the daily irrigation water supply, Decagon® 5TM temperature and humidity sensors located at 5 and 20 cm depth and the Decagon® EM50 ProCheck logger were used. A daily moisture log was kept in order to maintain the desired water levels. The system was calibrated with the water retention curve characteristic for the soil used (Pf curve) to determine the water content of the soil at any given barometric tension.

Biomass yield and nutritive value

During the trial period, the mini-meadows of the different treatments were harvested each time *L. perenne* of the intermediate irrigation treatment reached 3 leaves completely elongated (leaf stage 3). Harvesting was done with hand shears leaving a 5 cm high residue to favor regrowth (Fulkerson and Donaghy, 2001). Four harvests were performed, on January 10, 2017, February 21, 2017, April 6, 2017 and May 25, 2017. Collected biomass was oven dried at 60ºC for 48 hours before determining the herbage dry mass (HM). Accumulated herbage mass (AHM) was calculated by adding together the HM obtained from each mini-sward at the 4 harvests. The HM collected from each mini-sward at each trimming event was evaluated for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), digestible organic matter on dry matter basis (DOMD) and water-soluble carbohydrates (WSC). Metabolizable energy (ME) was estimated from DOMD: ME (Mcal kg^{-1} DM) = 0.279 + 0.0325 × DOMD. Analyses were performed at the Animal Nutrition Laboratory, Universidad Austral de Chile.

Competitive ability

It was determined through the Relative Efficiency Index (REI) proposed by Connolly (1987) as an unbiased indicator of the competitive dynamics of a mixture.

$$REI = (\ln Wia - \ln Woa) - (\ln Wib - \ln Wob) \quad \text{[Equation 1]}$$

Where for two competing species, *Wia* refers to the biomass of species *a* in the mixture at time *i* and *Woa* refers to the biomass of species *a* in the mixture at time zero. Therefore, REI is the difference between the relative growth rates of competing species *a* and *b*. In this study species *a* was *T. repens*, while species *b* was *L. perenne*. Although REI is not a measure of competition per se, it is used to describe the trajectories of species in the mixture over time (Grace, 1995).

Soil microbial diversity

Soil samples were taken at the beginning (control), during the period of maximum growth of the pasture and at the end of the trial. These samples were analyzed by PCR-DGGE (Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis) to determine the diversity of bacteria, nitrogen-fixing bacteria and arbuscular mycorrhizae, using different molecular markers. To study the change in the bacterial communities present in the soil of the treatments over time, the marker gene 16S rRNA, which is the universal bacterial marker gene, and the universal primers 358F-907R were used, according to the methodology of Gallardo et al. (2012). It was also observed how the communities of nitrogen-fixing bacteria change over time within the different treatments applied in this study. The nifH marker gene was used with the universal primers PolF/PolR and PolFI/AQER according to the methodology described by Wartiainen (2008). To assess the diversity of arbuscular mycorrhizae, nested PCR techniques were used, the first NS1/NS41 and the second AM1/NS31 described and used by Beauregard (2013). Using this technique, it is possible to evaluate how AMF communities are affected with nitrogen and water supply over time. Band pattern analysis of DGGE gels were analyzed with Phoretix one-dimensional gel analysis software (version 4.00; Phoretix International, Newcastle upon Tyne, United Kingdom). The diversity of microbial communities in the different treatments was analyzed with Shannon's diversity (H) and Simpson's richness (D) indices. The data matrix obtained from the image analysis of the gels was subjected to multivariate NMDS analysis with PAST software.

Results and Discussion

Biomass yield and nutritive value

The treatment with 80-85% AWP and a N supply equivalent to 90 kg ha^{-1} showed a significant higher yield in the four consecutive harvests ($p<0.05$). Over time, there were significant differences among harvests ($p<0.0001$). Harvest 4 presented the lowest yield in all treatments, being the treatment with 25-30% AWP the one that presented the lowest yield for both N levels (Figure 1).

There were significant differences in yield of *L. perenne* between treatments. Among both, soil moisture and nitrogen level, the differences were more significant for nitrogen ($p=0.0011$) than for soil moisture ($p=0.0405$).

There was a significant difference in the yield of *T. repens* among treatments due soil moisture ($p<0.0001$), but not for the N fertilization level ($p=0.1134$).

CP was affected by soil moisture ($p=0,0016$) and N fertilization ($p=0.0058$) only in the 4th cut, with 27.89% CP at N=0, and 26.15% CP at N=90 kg ha^{-1} and 25.29% CP at 25-30 % AWP and >27% CP at higher soil moisture levels.

Soil moisture ($p=0.05$) and nitrogen level ($p=0.0085$) affected ADF. The highest ADF was obtained with limited soil moisture (25-30% AWP) and 90 kg N ha^{-1} at the end of the summer (22.44% ADF).

NDF showed significant differences between treatments in both soil moisture ($p=0.0447$) and nitrogen level ($p=0.0034$). The level of 90 kg ha^{-1} presented the highest NDF in all cuts.

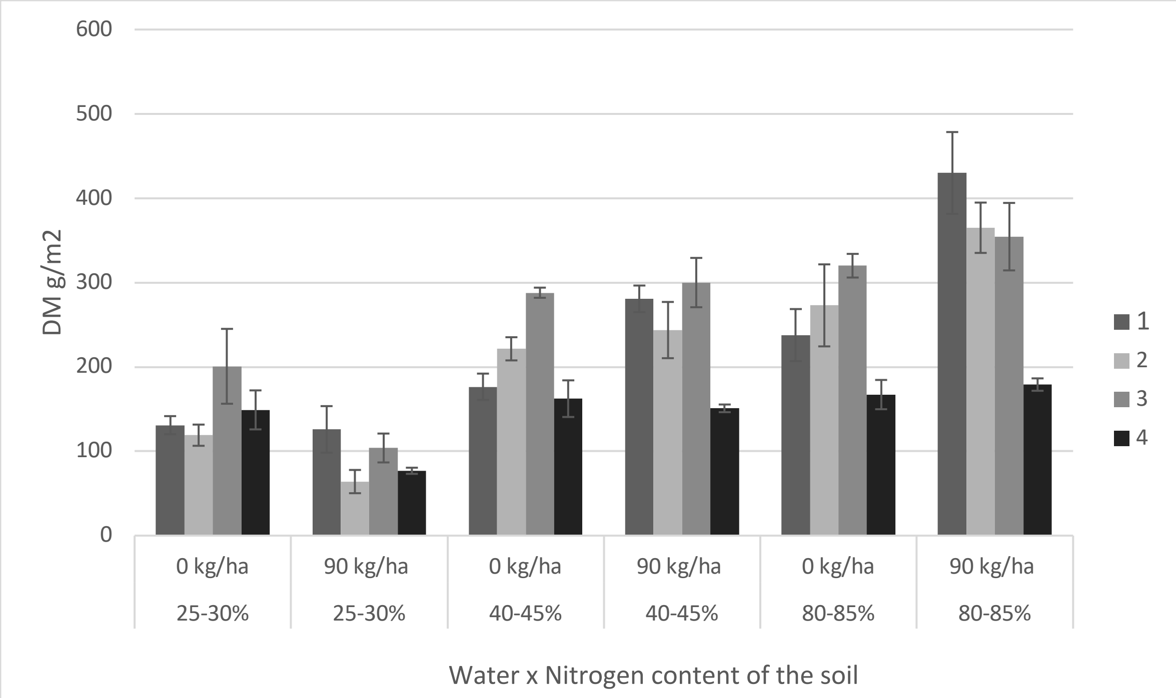

Figure 1. Total yield (gr m^{-2} DM) for the treatments combining two levels of nitrogen (0 and 90 kg ha^{-1}) and three levels of available water in the soil (25-30%; 40-45% and 80-85%) in four consecutive harvests (1-4). Bars represent the SEM.

There were no significant differences in WSC between treatments due to soil moisture (p=0.0561) or soil nitrogen level (p=0.4316). The highest WSC values were observed at the first cut (141.76 gr kg^{-1}), showing a clear trend to decrease through the growing season.

There was a significant effect of soil moisture on ME (p=0.0074) with higher ME values (3.08 Mcal kg^{-1} DM) at higher AWP. There was no effect of N fertilization on ME (p=0.1194). ME values varied slightly through the growing season.

Competitive ability

Soil moisture (p=0,4136) and nitrogen level (p=0,9071) did not affect the competitive ability of *L. perenne* and *T. repens* (Figure 2). However, there was a clear effect of the harvest date (p<0,0001) on the REI of the species. *T. repens* increased its competitive ability during the summer, and *L. perenne* became more competitive in fall.

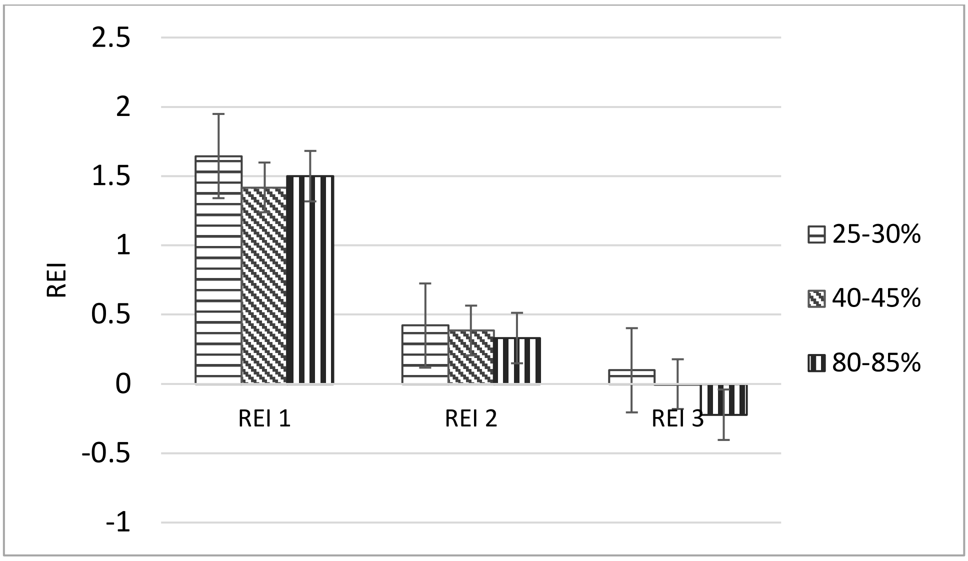

Figure 2. Relative Efficiency Index of *T. repens* - *L. perenne* under different soil water contents: 25-30%, 40-45% and 80-85% of soil available water for the plants (AWP)

Soil microbial diversity

In the soil samples taken in spring, at the maximum growth rate of the pasture, the NMDS showed a separation of the rhizobacterial community on the treatments at 25-30% AWP, with and without N fertilization. In the soil samples taken in fall, at the end of the trial, analyses showed that the rhizobacterial community growing at the 25-30% AWP without N fertilization was significantly different from the community growing in the rest of the treatments.

Conclusions and/or Implications

What most influenced yield and growth rate of *L. perenne* was nitrogen fertilization. *T. repens* performance was more influenced by soil moisture level, in both yield and growth rate. The interaction between soil moisture and nitrogen fertilization contributed to a higher cumulative yield and growth rate of the mixture.

Soil water content and percentage of *T. repens* in the mixture had a significant effect on the nutritive value of the mixture.

Both, nitrogen fertilization and water deficit caused changes in the diversity of the microbial communities present in the soil; however, the most influential variable was the soil water level.

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