

Biomass production, arbuscular mycorrhizae and soil plant-available P under water stress in native perennial grasses

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Abbreviations

AM Arbuscular Mycorrhizae

R Regrowth Biomass Production at the Initiation of the Growing Season

SMC Soil Moisture Contents

TAABP Total Annual Aboveground Biomass Production

Introduction

Precipitation determines vegetation type and species relative composition in plant communities [1]; in addition, it is a primary variable affecting soil water storage and availability. This availability is the most important factor which affects plant distribution, growth and survival in rangelands throughout the world [2]. Plant productivity can be reduced in these rangelands because of the negative effects of water stress on plant growth [2].

The generally adverse effect of high soil plant-available phosphorus levels on AM formation is well documented [3, 4], and is mainly caused by higher P concentrations in the roots [5]. In soils well supplied with phosphate, mycorrhizal plants typically grow worse than non-mycorrhizal ones, because mycorrhizae can behave as parasitic rather than mutualistic in the fungi-plant association [6]. Plant species with lower root length density are more dependent on mycorrhizal colonization for resource acquisition than species with higher root length density [7]. *Nassella clarazii* has greater root length density [8] and mycorrhizal colonization [9] than *N. tenuis* and *Amelichloa ambigua*. These species are abundant in rangelands of Central Argentina [10]. While *N. clarazii*, a late-seral, highly competitive species, is selectively consumed by domestic herbivores, *N. tenuis*, an earlier-seral and less competitive species, is an intermediate species to herbivory, and *A. ambigua* and *S. gynerioides*, both early-seral, low competitive species are only cut-off when a better forage is not available [10].

Nassella clarazii is a more competitive species than *N. tenuis* and *A. ambigua* [11, 12, 13]. Shoot growth, root proliferation, root length densities and nutrient uptake rates have been shown to be greater in *N. clarazii* than in *N. tenuis* and *A. ambigua* [11, 12, 13, 14]. Covacevich et al. (2005) [15] reported that AM colonization was not depressed in wheat at soil plant-available P concentrations of up to 15 ppm; beyond these soil plant-available P concentrations, root colonization of wheat by AM was highly depressed. Soils in our study site had between 24 to 55 ppm soil plant-available P concentrations. The shoot and root physiological traits in *N. clarazii*, and the high soil plant-available P concentrations at the study site, suggest that high AM colonization in *N. clarazii* could act in a parasitic rather than a mutualistic way. If so, aboveground plant biomass in the late-seral, highly competitive *N. clarazii* would be expected to decrease as percentage arbuscular mycorrhizal colonization increase. On the other hand, lower values of the above mentioned physiological traits in *N. tenuis* and the early seral, low competitive *S. gynerioides* suggest that aboveground biomass production would not diminish with increases in root percentage AM colonization.

Arbuscular mycorrhizal fungi are common mutualistic symbionts of plant roots in grasslands [16]. Arbuscular mycorrhizal associations have been found in areas that cover a wide range of soil moisture [17]. Under experimental conditions [18] and [19] reported that arbuscular mycorrhizal colonization lessened with decreasing soil moisture availability in the perennial and annual grasses *Schizachyrium scoparium* (Michx.) Nash and *Sorghum bicolor* (L.) Moench., respectively. Jupp and Newman (1987) [20] reported the effect of soil moisture on soil plant-available phosphorus availability. Plant phosphorus uptake and transport, and diffusion coefficients of ^{32}P , were significantly reduced as soil moisture content (SMC) decreased [21, 22, 23, 24, 25]. Root phosphorus is also added to the fresh organic phosphorus pool upon its death and/or incorporation into the soil. Decomposition of fresh (and stable) organic matter may result in net mineralization of organic phosphorus [26]. In addition, plant growth rate reductions under water stress [27] directly influence phosphorus uptake. As a result, phosphorus concentrations in the soil solution have increased when SMC decreased [28].

Various morphological and physiological characteristics have been associated with an effective nutrient acquisition and plant competition. Plants of low productive environments characterize by low nutrient acquisition rates, nutrient retention and low growth rates to maintain a balance between availability and demand [29]. In these less productive environments, a high soil resource competition is assumed. As a result, high infection frequencies by AM [30] are common root characteristics of semiarid rangeland species. However, linear relationships between soil versus root characteristics and plant growth should not necessarily be expected [31, 32], and these relationships are often difficult to identify in natural systems [33]. In addition, roots of various species may show a high morphological and physiological plasticity which allow them an increased exploration of heterogeneously-distributed soil resources [32].

The following general hypotheses will be tested in this field study: Total annual production of biomass is reduced under severe water stress (that imposed during the vegetative plus internode elongation developmental stages) in all study species. However, total annual aboveground plant biomass under all study water levels, and that produced during the initial regrowth period in the following year, when all plants are released from water stress, are greater in *N. clarazii* than in *N. tenuis*. Aboveground biomass production of *S. gynerioides* is highly stimulated by greater than lower SMC. High mycorrhizae

percentages in soils with high soil plant-available P concentrations decrease aboveground biomass production in the late-seral, high competitive species *N. clarazii*, but not in the earlier-seral, comparatively less competitive species *N. tenuis* and *S. gynerioides*. Root AM colonization decrease with decreasing SMC in all study species. Finally, soil plant-available P concentrations under the canopy of all study species increase meanwhile SMC decreases. Objectives of this work included to determine (1) the effects of different SMC (water stress, rainfed or irrigated conditions) on aboveground biomass production, colonization levels by AM on *N. clarazii* and *N. tenuis* in competition with plants of *S. gynerioides*, and available soil plant-available P concentrations, and (2) the relationships among AM colonization levels, plant biomass production and soil plant-available phosphorus concentrations. As emphasized by [34], these studies will contribute to increase our understanding of the interdependency of the responses between shoot and root tissues in the study species.

Materials and methods

Study site

This research was conducted during 1995, 1996 and early 1997 in proximities of the Departamento de Agronomía – Centro de Recursos Renovables de la Zona Semiárida (CERZOS) in Bahía Blanca city (38° 48' S, 62° 13' W). Plots were established in a typical Haplustol soil (L. Sánchez, Dept. Agronomía, UNS, personal communication). In this soil, 5 horizons are distinguished (A-AC-C-2Ck-3Ckm); a calcium carbonate horizon ($\text{CaCO}_3 > 300 \text{ gr kg}^{-1}$) is present at 1.80 m depth, and pH is 6.6-7 in the root zone [35].

Rainfall, temperature and potential evapotranspiration data during the study period were obtained in a meteorological station located 100 m apart from the experimental plots (Figure 1). During 1995, 1996 and early (January-March) 1997 rainfall was 447.2, 621.3 and 285 mm, respectively. Rainfall distribution was seasonal in 1995, with peaks in fall and spring. Rainfall distribution was more uniform during 1996. Absolute minimum and maximum, and mean monthly temperatures were similar in both years. Mean minimum temperature was 7°C during June and July, and mean maximum temperature was 22 to 24°C in January. Potential evapotranspiration was also similar in both years. In general, 1996 was a wetter year than 1995.

Plant material

Research was conducted on the late-seral, highly competitive *Nassella clarazii*, the comparatively earlier-seral, comparatively less competitive *Nassella tenuis* and the early-seral, poorly competitive *Stipa gynerioides* [36]. These are three C_3 perennial grass species native to the Distrito Fitogeográfico del Caldén [37]. Their growing cycle occurs during fall, winter and spring. The first two species have a high forage value (desirable, preferred, palatable) [37] while *S. gynerioides* is a non-preferred species. This species was included in the experimental plots because it is the most abundant, undesirable perennial grass at the south of such District [38].

Experimental design

Between December 1993 and April 1994, 28 experimental plots (1.8 x 1.8 m) were established in the field on unplowed, weeded soil. Plants were obtained from a 20 year-exclosure to domestic animals located southeast of La Pampa Province (38° 45' S, 63° 45' W). Within each plot, transplants were placed 30 cm apart from one another in seven horizontal and vertical rows such that each plant of *N. clarazii* or *N. tenuis* was surrounded by four plants of *S. gynerioides*. Disposition of plants within a uniform matrix contributes to reduce potentially confounding effects on plant responses as a result of plant competition. A total of 1372 transplants were used for the whole study. Crown-level plant diameters (n=56) were similar among species at time of transplanting: 13.47 ± 0.56 cm (mean \pm 1 SE) for *N. clarazii*, 10.02 ± 0.51 cm for *N. tenuis*, and 12.27 ± 0.61 cm for *S. gynerioides*. All tussocks of *N. clarazii* and *N. tenuis* were hand-clipped to a 5-cm stubble height in January 1995, during the plant quiescent period. From a total of 28 experimental plots, eight were randomly assigned to the irrigated and eight to rainfed treatments, and 4 plots to each of the water stress treatments (vegetative, internode elongation, and vegetative plus internode elongation).

Water levels

Plants were exposed to rainfed, irrigated or water stress conditions. Rainfed plots received rainfall all year round (Figure 2). A drip irrigation system watered the irrigated plots, which were additionally rainfed. Soil tensiometers installed in the irrigated plots allowed watering of these plots to saturation whenever they reach 60% of field capacity. Periods of irrigation and imposition of water stress during 1995 and 1996 are depicted in Figure 2. Transparent plastic sheets covered the water-stressed plots whenever rain fell at periods when these species are often exposed to water stress in their native environment [10]: vegetative or early internode elongation or both phenological periods (Figure 2). Water-stressed plots were surrounded with plastic sheets up to 1.8 m soil depth to prevent lateral movement of water into these plots. All 28 experimental plots received 313.7 mm from mid-October 1995 to late-April 1996, and 487.8 mm from late-October 1996 to March 1997. Water-stressed plots were thus alleviated from water stress during these periods by receiving natural rainfall.

Sampling procedures

Leaf water potentials

Leaf water potential was determined periodically at mid-day in all treatments to provide a measure of plant water status during the study period. Measurements were done using a pressure-chamber on sunny days only between 1200 to 1300 h. Youngest, fully expanded leaf blades were taken for these measurements using one tiller per species within each replicate plot and sampling date. From excision to end of each determination, leaves were cut one at a time and maintained in a plastic bag to reduce water loss [39].

Aboveground biomass production

In January (summer) 1996, the dormant season, all plants of the desirable species (*N. clarazii* and *N. tenuis*) were defoliated to 5-7 cm stubble under all water levels to measure new plant growth from that height. On 21 December of the same year, all plants were defoliated to that stubble height within each plot to quantify biomass production during 1996. With this purpose, 4 to 8 plants were sampled within each water level. Defoliation on 21 December 1996 also allowed to measure biomass production from this date to 28 February 1997. At this date, biomass production of all three species was evaluated, defoliating for the first time during the study the plants of *S. gynerioides*. *Stipa gynerioides* remained undefoliated during the study because this species is only cut off when there is not better forage [37]. Defoliated material was oven-dried to 60° C during 72 h and weighed.

Soil available phosphorus and AM

No P supplements were provided during the study. Soil plant-available P concentrations at the outset of the experiment (1995) were not determined because parallel, labor-intensive studies were conducted on these plots at that time. We recognize that this limits use of plant and soil plant-available P data as a measure of plant productivity and the influence of root characteristics including AM. However, during the study period (1996-early 1997), samplings for soil available phosphorus determinations were not only conducted under water stress and irrigated but also rainfed (untreated control) conditions.

A total of 460 soil plus root samples were obtained between 0-15 cm soil depth using a soil corer (8.4 cm diameter, 15 cm height: 831.3 cm³ volume) during April, June, September and October in 1996, and February in 1997. Samples were obtained diagonally from the plant periphery to the plant center to assure that sampled soil plus roots corresponded to the sampled plant. One plant of each species was used per replicate at each sampling date. Roots were obtained after washing soil samples through a 60 mesh screen [40], and they were maintained at 4°C in a solution of formaldehyde, glacial acetic acid and ethanol [41] for AM determinations. A parallel, similar soil sampling was conducted on each plant for available phosphorus determinations.

Soil available phosphorus was determined by the method of [42]. Soil was air-dried and then screened through a mesh of 0.5 mm, and 2.5 g soil was weighted. This sample was placed within a tube which contained 20 ml of Bray and Kurtz's extractant. The tube was agitated during 5 min. at 190 agitations per minute. Content of the tube was filtered, and P (ppm) was determined by colorimetry using a UV-Visible Recording Spectrophotometer, UV-2100 Shimadzu.

Washed roots were cut into 15 mm segments, cleared and stained for determination of mycorrhizae colonization at 100-400X magnification [43]. Three fields on each of thirty root segments were scored for presence or absence of hyphae, vesicles and arbuscules for each plant.

Statistical analysis Leaf water potentials

Leaf water potentials were analysed using a three-way ANOVA (5 water levels x 3 species x 4 sampling dates: April, June and September 1996, and February 1997) in split plot. Soil water levels were the main factor applied to randomly distributed plots, in an unbalanced but proportional manner; there were 4 replicates for the irrigated and rainfed treatments, and 2 replicates for each of the water stress treatments (vegetative, internode elongation and vegetative plus internode elongation). There were plants of the three species within each plot; one plant of each species was assigned to be sampled within each sampling date. Secondary factors were sampling dates and species. Interactions were open to evaluate the effects of water levels, dates and species. Since interactions involved water levels, species and sampling dates, comparisons were conducted (1) among water levels for each sampling date and (2) among sampling dates for each water level on each of the study species individually. Means were compared by LSD at 5 %, when the F tests indicated that the variables were different at 5 % [44].

Aboveground biomass production

Biomass production in 1996 and that produced between 21 December 1996 and 28 February 1997 were individually analyzed using a two-way ANOVA (5 water levels x 3 species) in split plot. Soil water levels were the main factor, applied to randomly distributed plots, in an unbalanced but proportional manner. There were plants of the three species within each plot, assigning one plant of each species for analysis within each sampling date. Secondary factors were the species.

Available soil plant-available P

At first, soil plant-available phosphorus concentration data were analyzed using a three-way split plot ANOVA (5 water levels x 3 species x 5 sampling dates: April, June, September and October 1996, and February 1997). Soil water levels acted as main factors, applied to randomly distributed plots, in a proportional but unbalanced manner. Eight replicates were used for the irrigation and rainfed treatments, and 4 replicates were utilized for each of the water stress treatments (vegetative, internode elongation, vegetative plus internode elongation). Plants of the three species were within each plot, assigning one plant of each species for analysis at each sampling date. Secondary factors were sampling dates and species. Within each plot, plants were assigned for sampling previous to the sampling dates. This allowed avoiding measurements of available soil plant-available phosphorus corresponding to nearby plants previously sampled. However, this rigid scheme did not allow replacement of lost plants (i.e., plants which died as a result of treatment application) during the study. Because of this, it was necessary to adapt the statistical analysis when the lost of sampling units resulted in an unbalance not proportional among the species within each plot. This phenomenon was mainly presented in June and September.

In this way, we have a 'Design 1' using a three-way ANOVA for those months where information was complete: April, October and February. Months with missing data (June and September) were analyzed with a 'Design 2': a split-plot two-way ANOVA with the same main factor (soil water levels) and a unique secondary factor (the species). Uncompleted plots, which lack information on the three species, were eliminated to apply this analysis, leaving an unbalanced, proportional design. Interactions were open to

evaluate the effects of water levels, dates and species. Means were compared with Fisher's protected LSD at 5% when the F test indicated that the variables differed at that significance level [44].

Relationships among study variables

According to the available variables on each sampling date and for each species [% AM: Percentage AM colonization; Soil plant-available P: available soil plant-available Phosphorus; Biomass: Annual aboveground biomass (1996) and that produced between 21 December 1996 and 28 February 1997; Water Pot: Leaf water potential] correlation coefficients (r) were calculated according to the scheme shown in Figure 3. Correlation coefficients obtained at the different dates were compared using statistics with Chi-square distribution based on the 'Z' transformation of Fisher, where r is the correlation coefficient, k is the number of correlation coefficients being compared, and n is the number of data pairs which are used to calculate each correlation coefficient. Used formulae were as follows:

$$X^2 = \sum_{i=1}^k (n_i - 3) \cdot Z_i^2 - \frac{[\sum_{i=1}^k (n_i - 3) \cdot Z_i]^2}{\sum_{i=1}^k (n_i - 3)} \quad Z = \frac{1}{2} \ln \left(\frac{1 + r}{1 - r} \right)$$

If correlation coefficients did not differ, a correlation coefficient combining all dates was calculated. If differences were detected, pair comparisons were effected using LSD at $p < 0.05$. According to the results, dates were grouped to find the corresponding combined correlation coefficients. Its significance ($Q=0$ versus $Q \neq 0$) was tested on each of these using a normal distribution test. If any correlation coefficient remained isolated (i.e., was not the result of combining several dates), its significance was tested with a t test [45]. Only statistically significant, biologically meaningful correlations will be reported ($p \leq 0.05$).

Results

Leaf water potentials

In April 1996 and February 1997, leaf water potentials of *N. clarazii*, *S. gynerioides* and *N. tenuis* did not differ ($p > 0.05$) among soil water levels (Figure 4). However, leaf water potentials were lower ($p < 0.05$) under water stress at the (1) vegetative and (2) vegetative plus internode elongation developmental stages than under rainfall or irrigated conditions in June 1996 for all three species (Figure 4). Also, leaf water potentials of *N. clarazii*, *N. tenuis* and *S. gynerioides* were lower ($p < 0.05$) under water stress in the internode elongation and vegetative plus internode elongation stages than under the other water levels in September of the same year. Lowest leaf water potentials ($p < 0.05$) were found in September 1996 and February 1997 under rainfed and irrigated conditions in *N. clarazii* and *S. gynerioides* (data not shown). In June 1996, leaf water potentials were lower ($p < 0.05$) than in September of the same year when water stress occurred at the vegetative stage in *N. clarazii* y *S. gynerioides*. In September 1996, leaf water potentials were lower

($p < 0.05$) than values in June in the internode elongation stage, and greater ($p < 0.05$) than values in June at the vegetative stage in all three species (Figure 4).

Aboveground biomass production

Plants of *N. clarazii* reached a greater ($p < 0.05$) annual aboveground biomass under irrigated, rainfed and water stress conditions at the vegetative or internode elongation developmental stage than under water stress in both phenological stages in 1996 (Figure 5). Plants of *N. tenuis*, however, have a similar ($p > 0.05$) biomass under all water levels. *Nassella clarazii* showed a greater ($p < 0.05$) annual biomass than *N. tenuis* under irrigated and rainfed conditions, and under water stress in the vegetative or internode elongation stage in 1996 (Figure 5). In February 1997, *N. clarazii* showed a greater ($p < 0.05$) biomass under water stress in the vegetative stage than under the remaining water levels (Figure 5). In *Nassella tenuis*, there were no differences ($p > 0.05$) among water levels (Figure 5). *Nassella clarazii* reached a greater ($p < 0.05$) biomass than *N. tenuis* under water stress conditions in the vegetative stage. *Stipa gynerioides* had a similar ($p > 0.05$) biomass production under all water levels in 1997 (Figure 6).

Available Soil Plant-Available P

When soil plant-available Phosphorus concentration was analyzed on average for the April, October and February sampling dates, there was an interaction ($p < 0.05$) between water levels, dates and species. This implies that the response in the various water levels was different ($p < 0.05$) during these months in all three species. Because of this, each species was analyzed individually comparing dates and water levels (Table 1). In *N. clarazii* and *N. tenuis*, there was a different response in the different water levels in April, October and February ($p < 0.05$) (Table 1). In April 1996, soil plant-available Phosphorus concentration was greater ($p < 0.05$) than in October of the same year and February 1997 where *N. clarazii* had been exposed to water stress during the vegetative developmental stage. On the other hand, soil plant-available Phosphorus concentrations were greater ($p < 0.05$) in October 1996 than in April 1996 and February 1997 when *N. clarazii* and *N. tenuis* were exposed to water stress in both developmental stages. In all sampling dates, each species had a similar ($p > 0.05$) soil plant-available Phosphorus concentration under all water levels (Table 1). Soil plant-available Phosphorus concentrations under *S. gynerioides* were similar ($p > 0.05$) among water levels and sampling dates (Table 1).

In June 1996, soil plant-available Phosphorus concentrations were similar among species ($p > 0.15$) and water levels ($p > 0.80$) (Table 2). However, in September, soil below *N. clarazii* plants had a greater ($p < 0.05$) phosphorus concentration when plants were exposed to water stress under the internode elongation developmental stage than under irrigation (Table 2). Under *S. gynerioides*, soil plant-available Phosphorus concentrations were lower ($p < 0.05$) under water stress in the vegetative stage than under irrigated, rainfed and water stress conditions in the vegetative plus internode elongation developmental stages (Table 2). There were no differences ($p > 0.05$) in soil plant-available Phosphorus concentrations among water levels in *N. tenuis*. Soil under the canopies of *N. clarazii* and *S. gynerioides* had a greater ($p < 0.05$) phosphorus concentration than that under the canopy of *N. tenuis* under rainfed conditions (Table 2). However, soil plant-available Phosphorus concentrations were greater ($p < 0.05$) under plants of *N. clarazii* than under

those of the other two species under water stress conditions in the vegetative or internode elongation developmental stage (Table 2).

Relationships between root and shoot variables

Total annual biomass production in 1996 and the subsequent R during 21 December 1996-28 February 1987 after water stress was released, were negatively correlated ($p < 0.05$) with percentage AM colonization (Figure 7) in *N. clarazii* under all water levels. This relationships were not significant ($p > 0.05$) for neither *N. tenuis* nor *S. gynerioides* under all water regimes. In *N. tenuis*, leaf water potential (X, -MPa) and AM colonization (Y, %) showed a negative correlation ($Y = 34.217 - 6.3323X$, $r = 0.699$, $p = 0.05$, $n = 8$) in June 1996. Percentage AM colonization (Y, %) of *N. clarazii* correlated negatively ($Y = 181 - 6.62X + 0.0677X^2$, $r = 0.51$, $p = 0.003$, $n = 42$) with soil plant-available phosphorus availability (X, ppm) in April and June 1996.

Discussion

The scope of data interpretation might be limited in this study because samplings were conducted for only one growing season (January 1996-February 1997). However, 1996 appeared to be a rather typical, average year at the study site. For example, rainfall during this year (621 mm) was similar to the long-term mean (633.9 ± 58.3 mm, $\text{mean} \pm 1 \text{e.e.}$) at the same research area during 1987-1996. No long-term evapotranspiration data were available. In general, we were successful in imposing water stress conditions at the field in all three species in comparison to irrigated plots. Lack of significant differences ($p > 0.05$) in leaf water potentials in April 1996 and February 1997 was due to the fact that (1) water stress was imposed at the end of April 1996, and (2) all plots were exposed to natural rainfall after 26 October 1996, respectively. Leaf water potentials found in June and September are similar to results reported in these and other perennial grasses by [46] and [47].

In arid and semiarid environments, soil water availability affects plant growth and survival in the plant community [2]. Water stress, or years with precipitations below the annual mean, decrease dry matter production in several perennial grass species [48]. Plants of *N. clarazii* and *N. tenuis* were not shaded by plants of *S. gynerioides* under water stress, even though these plants remained undefoliated until the end of the study. In agreement with the general hypothesis, biomass production of *N. clarazii* was lower under water stress in the vegetative plus internode elongation stage than under the remaining soil moisture regimes. Similar results were obtained by [2] and [48] in other perennial grass species. However, and despite using a strict experimental plot setup to obtain plant water stress under field conditions, annual biomass in *N. tenuis*, and that accumulated by *S. gynerioides*, were similar among soil water levels, which disagrees with the general hypothesis. This calls the attention of the difficulty of imposing water stress conditions at the field in an environment where annual precipitation may be higher than 600 mm. Similar results were obtained by [49, 50, 51]. *Nassella clarazii* showed a higher average degree of osmotic adjustment (-2.72 MPa) than *N. tenuis* (-1.94 MPa) and *S. gynerioides* (-2.34 MPa) under water stress in the internode elongation stage of development [36]. This drought tolerance mechanism will very likely helped these species to keep growing in the face of declining leaf water potentials [39], and would help explain, at least partially, why aboveground

biomass production of these species, except that on *N. clarazii*, was similar under all soil water regimes. The lower biomass production of *N. clarazii* under water stress in the vegetative plus internode elongation than in the other developmental stages suggests that this species may be more sensitive than *N. tenuis* and *S. gynerioides* to long-term water stress.

In 1996, annual biomass production was greater in *N. clarazii* than in *N. tenuis* under irrigated and rainfed conditions and under water stress in the vegetative or internode elongation developmental stage. These results are consistent with those obtained under rainfed conditions by [14, 52, 53]. This greater biomass production in *N. clarazii* than in *N. tenuis* may be due, at least partially, to the well known greater relative growth rates and competitive ability in the first than in the second species [11, 13, 14, 52, 53].

Increases in percentage AM colonization under conditions of high soil plant-available P concentrations resulted in aboveground biomass decreases in the late-seral, highly competitive perennial grass *N. clarazii*. This species has high root length densities [13, 54, 55]. These high root length densities appear to make this species less dependent on mycorrhizal colonization for resource acquisition. Species with lower root length densities were reported to be more dependent on AM colonization for resource acquisition, such as P, than species with higher root length densities [7]. The relationships between AM colonization percentage and aboveground biomass production were not significant in the earlier-seral, less competitive *N. tenuis* and *S. gynerioides*. These species have a much lower root length density than *N. clarazii* [13]. Covacevich et al. (2005) [15] reported that percentage arbuscular mycorrhizal colonization in wheat was highly depressed in soils with greater than 15 ppm plant-available P. In our study, where soils had between 10 and 60 ppm plant-available P, the relationship between aboveground biomass production and percentage arbuscular mycorrhizal colonization appeared to be parasitic rather than mutualistic in the late-seral, highly competitive perennial grass *N. clarazii*. However, no such relationship was found for the earlier-seral, less competitive perennial grass species *N. tenuis* and *S. gynerioides*.

Water stress can reduce AM colonization in several grass species, which is in part associated to stress intensity [17, 19]. AM colonization significantly decreased in *N. tenuis* when leaf water potentials were reduced in June, a fact that we could not show for *N. clarazii* and *S. gynerioides*. Several studies on the relationships between soil and plant variables have found significant differences for these relationships in only one or some, but not all, study species [13, 56]. They showed that relationships between any of their study variables were species-specific.

A negative correlation was found between percentage mycorrhizae colonization and available soil plant-available phosphorus concentrations in plants of *N. clarazii* during the first sampling dates. We were unable to find this relationship in *N. tenuis* and *S. gynerioides*. The reason because mycorrhizal infection should be greater under phosphorus deficient conditions is not clear. Jasper et al. (1979) [57] have suggested that high carbohydrate level in the roots is an important prerequisite for good mycorrhizal infection. This is consistent with the observation that plants poorly supplied with phosphorus have higher root carbohydrate levels than plants adequately supplied with phosphorus [58]. Jackson (1992) [31] and Caldwell (1994) [32] had already emphasized that linear relationships between soil versus root characteristics should not necessarily be expected. In addition, [33] reported that these relationships are often difficult to identify in natural systems. The result obtained in the late-seral perennial grass suggests a strategy that would allow this species avoid

changes from mutualism to parasitism in the plant-mycorrhizae fungi relationship. Similar results were found in plants of *N. clarazii* by [13].

It has been demonstrated that soil plant-available phosphorus has a direct effect on AM colonization [59]. Correlation coefficients obtained in *N. clarazii* between the dependent and independent variables are greater than those reported by [56] for the relation between available soil plant-available P and colonized root length in *Schizachyrium scoparium*. Cool season grasses, like those in our study, generally have well-developed, highly branched root systems and are only weakly dependent on AM fungi for nutrient uptake [60,61]. In addition to SMC, there are other factors which influence the plant-fungi symbiotic relationship, such as the physiological characteristics of the grasses [17, 62]. This plasticity in the response of AM could be a relevant cause of its persistence and importance in native ecosystems [17]. Plants poorly colonized by AM could receive nutrients through hyphae connections with plants of the same or different species. This type of hyphae connections, for example, contributes to find variations in AM colonization levels through time, and lack of correlation between different variables under field conditions. Allen et al. (1989) [17] found a great difference in the AM activity among years, independently of various water levels and defoliation treatments.

Nassella clarazii showed similar or greater, but not lower, soil plant-available phosphorus concentrations under water stress than in the other soil water regimes. These findings partially agree with the general hypotheses. Also, soil plant-available P concentrations under *S. gynerioides* plants were almost double when plants were exposed to long-term (vegetative + internode elongation) than short-term (vegetative) water stress. Phosphorus mineralization from organic matter depends on soil moisture and temperature [63]. Lower self-diffusion coefficients of ^{32}P have been reported as soil water content decreased [22]. Many authors [23, 24, 64, 65] attributed increases in available soil plant-available P concentrations under water stress to a lower phosphorus uptake as soil water contents decreased. In addition, microbe and root phosphorus can be added to the fresh organic phosphorus pool upon their death and/or incorporation into the soil; subsequent decomposition of fresh organic matter may result in net mineralization of organic phosphorus [26]. Thus, we can envision at least two mechanisms by which soil plant-available Phosphorus concentrations may increase with decreasing soil moisture. First, phosphorus is likely to become more concentrated in solution simply because soil moisture decreases. Second, the entire labile fraction of soil plant-available P may appear to increase relative to moist soils because drying soils result in lower rates of phosphorus diffusion and uptake; in moist soils, uptake depletes labile phosphorus. Finally, microbes and roots may liberate more plant available-phosphorus. Our results are similar to those reported by [66] in a wheat crop under water stress. A continued root turnover in *N. clarazii* and *N. tenuis* under water stress conditions might contribute to maintain high levels of soil available phosphorus [67].

Available soil plant-available phosphorus concentrations were greater under the canopy of the late seral, palatable perennial grass *N. clarazii* than under the canopy of the comparatively earlier seral, palatable *N. tenuis* and the early seral, unpalatable *S. gynerioides* under the reported rainfed and/or water stress conditions. A similar response was found by [68] under rainfed conditions. In their study, soil available phosphorus was greater at sites dominated by *P. ligularis*, a late-seral, palatable perennial grass, than at sites dominated by *S. tenuissima*, an unpalatable tussock grass. This would be the result, at least in part, of differences in litter and root chemical composition between species; litter and root

decomposition was faster in *P. ligularis* (low C:N ratio) than in *S. gynerioides* and *S. tenuissima* (high C:N ratio) [69, 70].

This study demonstrated that morphological (i.e., biomass production) and physiological (leaf water potential, AM colonization) study plant responses were very plastic, and appear to respond to the interaction of biotic and environmental characteristics that we do not yet fully understand. It also demonstrated that biomass production of late-seral, highly competitive perennial grasses, but not that of earlier-seral, less competitive perennial grass species, may not benefit by increasing percentage AM colonization under high soil plant-available P concentrations. The high root length densities of the late-seral perennial grasses may be one of the reasons to explain that increased fungi-host interactions resulted in aboveground biomass decreases in soils with high plant-available P, making parasitic rather than mutualistic the fungi-host association.

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Figure legends

Figure 1. (a) Monthly rainfall, long-term (1987-1996) mean (± 1 s.e.m.) monthly rainfall, and mean monthly potential evapotranspiration [(Thornthwaite: De Fina and Ravelo (1973)], and (b) Absolute minimum and maximum and mean monthly air temperatures to 0.25 m above the soil surface during 1995, 1996 and early 1997. Measurements were taken using a meteorological station located 100 m away from the experimental plots.

Figure 2. Periods of imposition of the different water inputs at the vegetative (V), internode elongation (E) or both (VE) phenological stages in 1995 and 1996. Numbers below horizontal, bold lines are rainfall fallen in the rainfed, and water stress treatments, or rainfall + irrigation in the irrigated treatment. Total annual precipitation is indicated for each year and water level within rectangles. Numbers immediately above horizontal bars represent the beginning and end, respectively, of imposition of any given water level. Black, grey or white horizontal bars represent irrigated, rainfed or water stress conditions, respectively.

Figure 3. Scheme which shows the variables used for calculation of the correlation coefficients among them for each species at the shown sampling dates.

Figure 4. Mid-day leaf water potential (-MPa) on plants of *N. darazii*, *N. tenuis*, and *S. gnerioides* which were exposed to irrigated (I), rainfed (R) or water stress (WS) conditions at the vegetative (V), internode elongation (E) or both (VE) phenological stages during June and September 1996. Values in the Y axis are represented as absolute values. Each histogram is an average of $n=2-4$. Vertical bars represent one s.e.m. Different letters to the left of the comma indicate significant differences ($p<0.05$) among water levels, and those to the right of the comma indicate significant differences ($p<0.05$) among sampling dates.

Figure 5. Annual aboveground biomass [(A), g plant^{-1} 1996] and aboveground biomass produced between 21 December 1996 and 27 February 1997 [(B), g plant^{-1}] on plants of *N. darazii* (Ncl) and *N. tenuis* (Nt) exposed to irrigated (I), rainfed (R), or water stress (WS) conditions at the vegetative (V), internode elongation (E) or both phenological stages (VE). Each histogram is the mean of $n=4-8$. Vertical bars represent one s.e.m. Different letters to the left of the comma indicate significant differences ($p<0.05$) among water levels, and those to the right of the comma indicate significant differences ($p<0.05$) between species.

Figure 6. Aboveground biomass (g plant^{-1}) produced until 27 February 1997 on plants of *S. gnerioides* (Sg) exposed to irrigated (I), rainfed (R), or water stress (WS) conditions at the vegetative (V), internode elongation (E) or both phenological stages (VE). Each histogram is the mean of $n=4-5$. Vertical bars represent one s.e.m. Equal letters above histograms indicate lack of significant differences ($p>0.05$) among soil water regimes.

Figure 7. Annual aboveground biomass [(A), g plant^{-1} 1996] and aboveground biomass produced between 21 December 1996 and 28 February 1997 [(B) g plant^{-1}] versus arbuscular mycorrhizae colonization percentage in *N. darazii*. Each symbol represents a single observation from the pool of data obtained under irrigated, rainfed or water stress conditions.

Table 1. Soil phosphorus concentration (ppm) under the canopy of *N. darazii* (Nd), *S. gnyerioides* (Sg) and *N. tenuis* (Nt) plants exposed to irrigated (I), rainfed (R) or water stress (WS) conditions at the vegetative (V), internode elongation (E) or both phenological stages (VE) during April and October 1996, and February 1997.

	April 1996			October 1996			February 1997		
	Nd	Sg	Nt	Nd	Sg	Nt	Nd	Sg	Nt
I	44.2 ^{a,ab}	36.8 ^{a,a}	30.8 ^{a,a}	43.9 ^{a,a}	43.7 ^{a,a}	33.2 ^{a,a}	42.6 ^{a,a}	38.8 ^{a,a}	39.5 ^{a,a}
R	40.3 ^{a,a}	36.9 ^{a,a}	30.8 ^{a,a}	48.2 ^{a,ab}	43.3 ^{a,a}	32.7 ^{a,a}	40.8 ^{a,a}	39.0 ^{a,a}	40.1 ^{a,a}
WS-V	49.6 ^{a,b}	34.3 ^{a,a}	34.5 ^{a,a}	40.9 ^{a,a}	36.3 ^{a,a}	29.0 ^{a,a}	41.4 ^{a,a}	28.2 ^{a,a}	34.0 ^{a,a}
WS-E	43.1 ^{a,ab}	26.5 ^{a,a}	34.7 ^{a,a}	45.9 ^{a,a}	32.0 ^{a,a}	32.5 ^{a,a}	38.9 ^{a,a}	31.7 ^{a,a}	31.8 ^{a,a}
WS-VE	37.7 ^{a,a}	47.3 ^{a,a}	33.7 ^{a,a}	55.0 ^{a,b}	36.3 ^{a,a}	46.8 ^{a,b}	44.5 ^{a,a}	35.1 ^{a,a}	34.8 ^{a,a}

Each value is the mean of n=2-8; Different letters to the left of the comma indicate significant differences ($p < 0.05$) among soil water levels; Different letters to the right of the comma indicate significant differences ($p < 0.05$) among sampling dates.

Table 2. Soil phosphorus concentration (ppm) under the canopy of *N. darazii* (Nd), *S. gnyerioides* (Sg) and *N. tenuis* (Nt) plants exposed to irrigated (I), rainfed (R) or water stress (WS) conditions at the vegetative (V), internode elongation (E) or both phenological stages (VE) during June and September 1996.

	June 1996			September 1996		
	Nd	Sg	Nt	Nd	Sg	Nt
I	38.4 ^{a,a}	42.0 ^{a,a}	34.7 ^{a,a}	37.8 ^{a,a}	35.0 ^{b,a}	35.1 ^{a,a}
R	40.2 ^{a,a}	39.6 ^{a,a}	35.7 ^{a,a}	41.2 ^{ab,b}	38.2 ^{b,b}	32.1 ^{a,a}
WS-V	42.1 ^{a,a}	40.4 ^{a,a}	35.1 ^{a,a}	43.6 ^{ab,b}	23.9 ^{a,a}	30.1 ^{a,a}
WS-E	ND	ND	ND	46.8 ^{b,b}	30.7 ^{ab,a}	31.7 ^{a,a}
WS-VE	48.7 ^{a,a}	50.3 ^{a,a}	36.1 ^{a,a}	47.7 ^{ab,a}	44.6 ^{b,a}	34.9 ^{a,a}

Each value is the mean of n=2-8; Different letters to the left of the comma indicate significant differences (p<0.05) among soil water levels; Different letters to the right of the comma indicate significant differences (p<0.05) among species; ND: Not Determined.

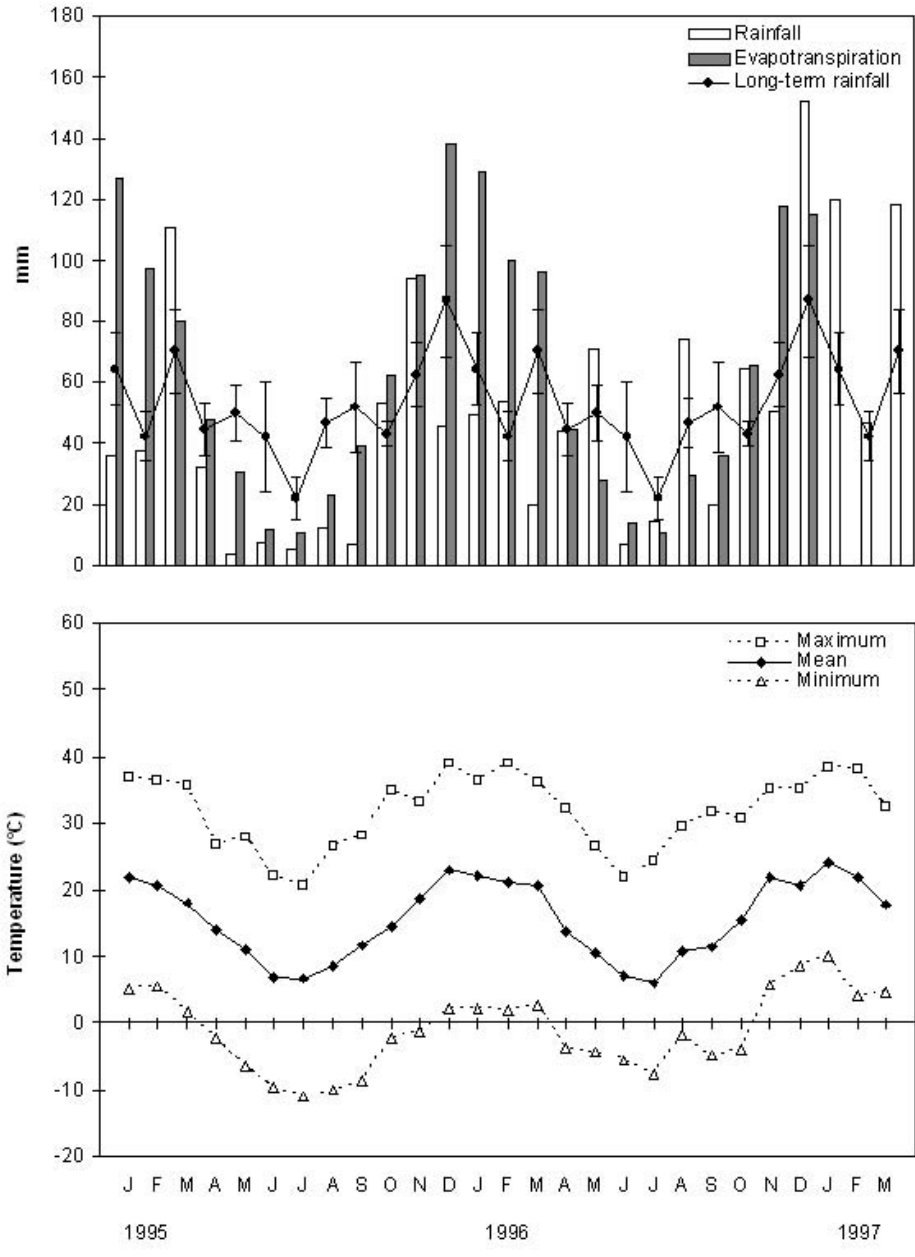
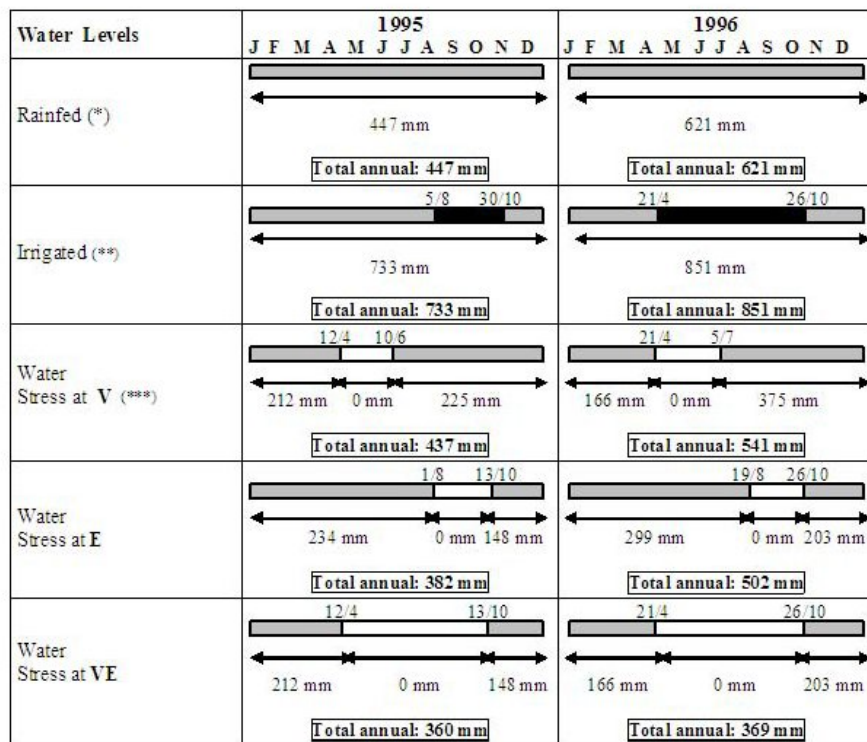


Figure 1






(*) Rainfed  (**) Rainfall+Irrigation  (***) Water Stress 

Figure 2

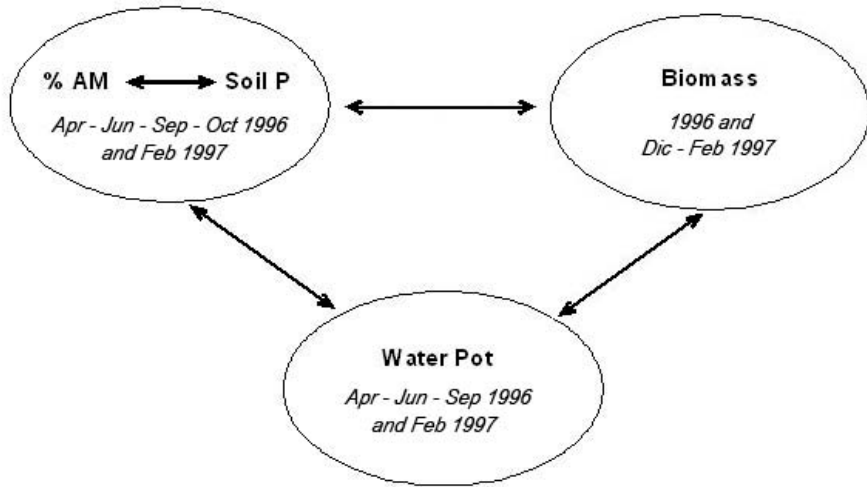


Figure 3

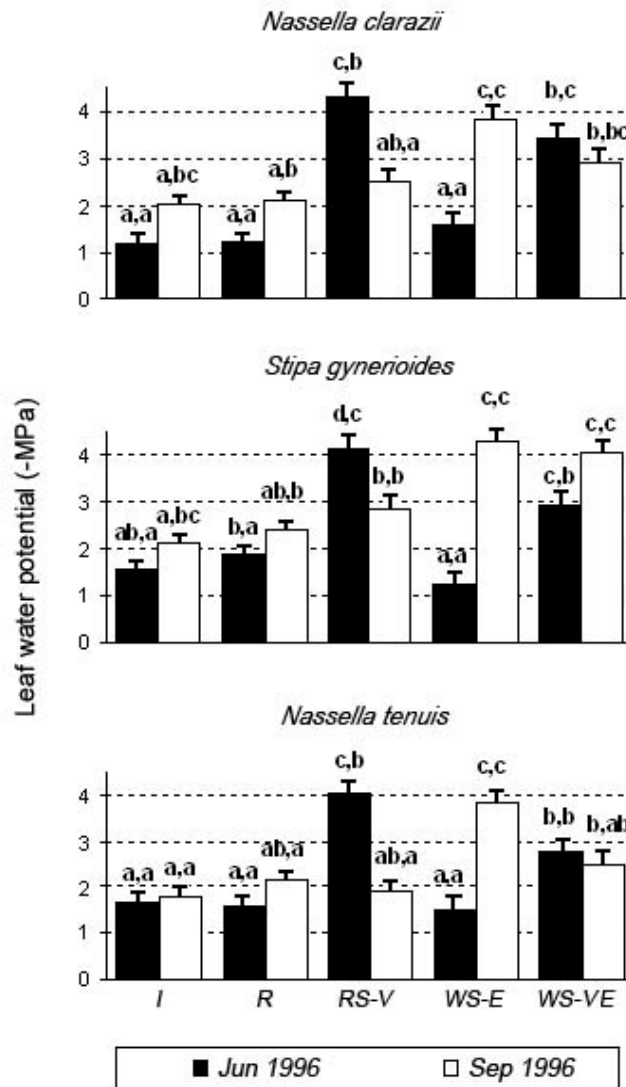


Figure 4

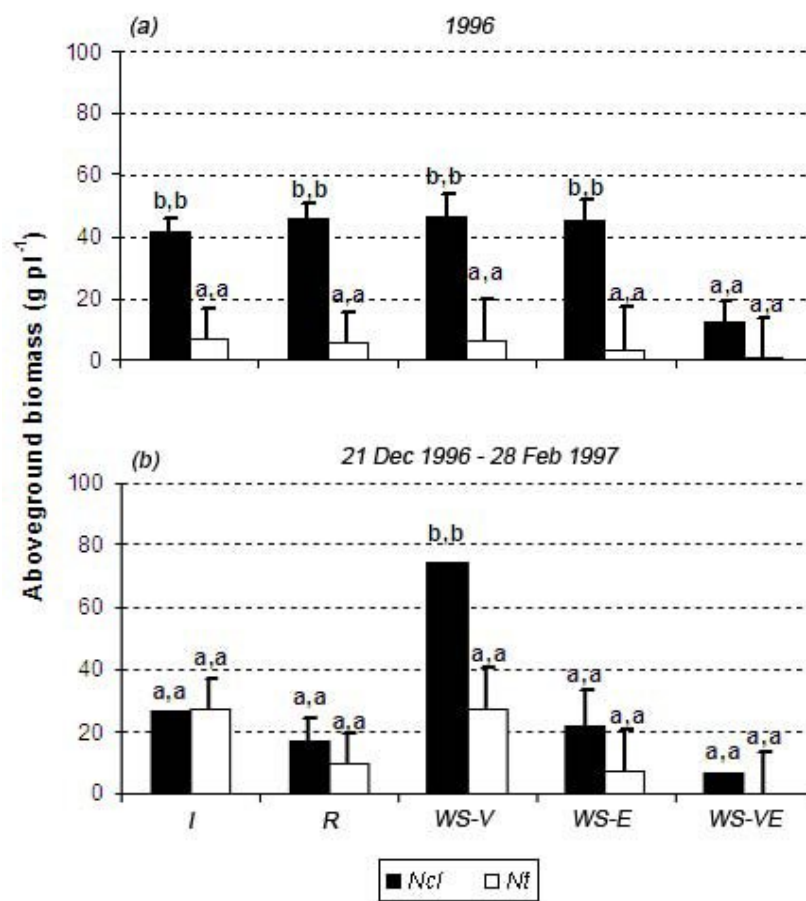


Figure 5

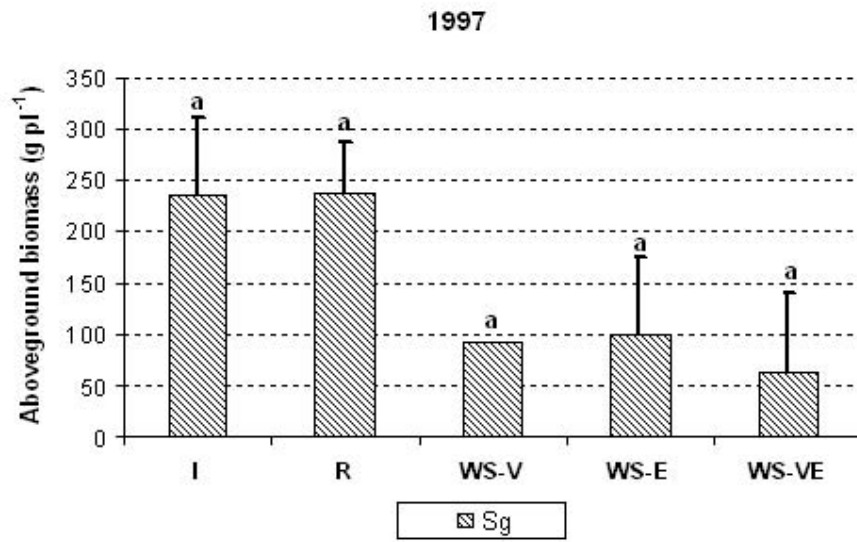


Figure 6

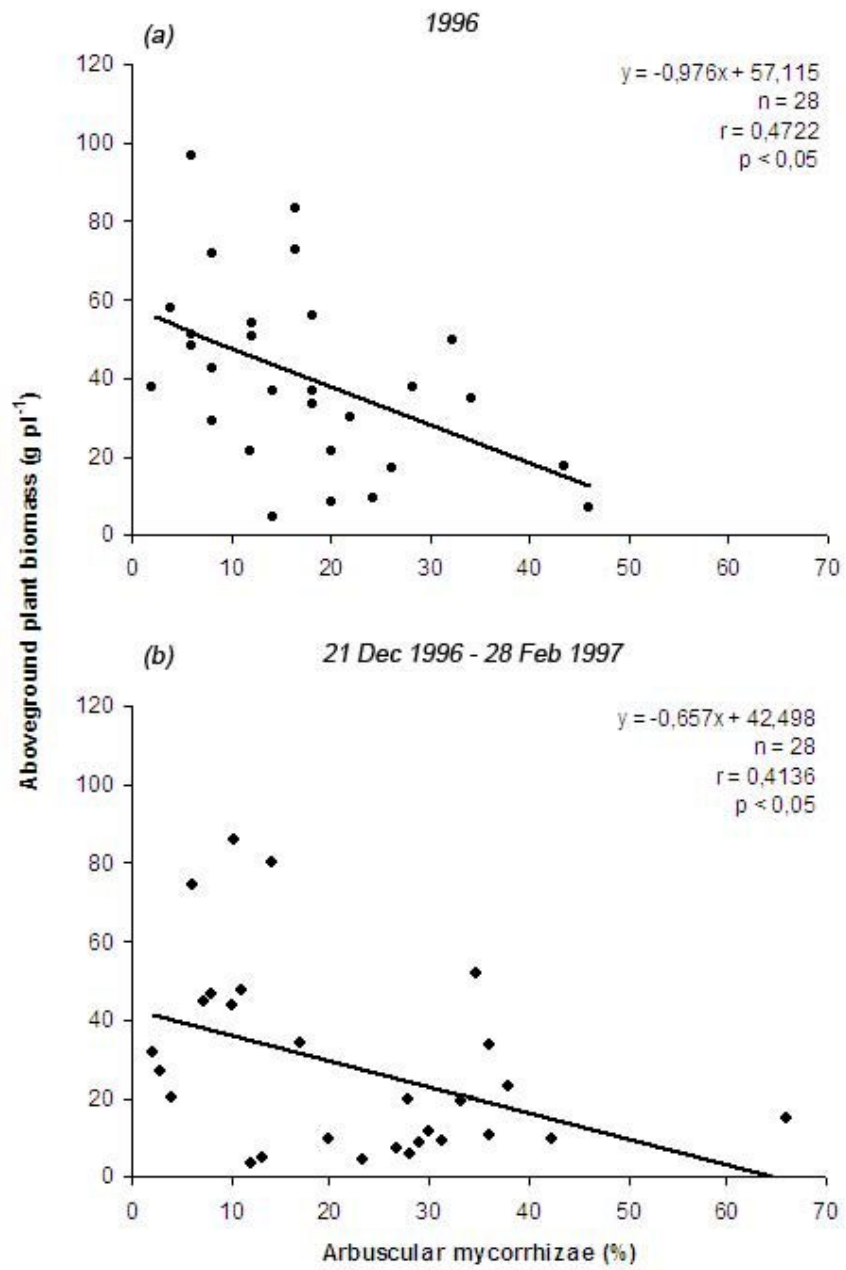


Figure 7