Defoliation Frequency Effects on Shoots and Roots of *Poa ligularis* in Arid Patagonia, Argentina

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ABSTRACT

Poa ligularis is an important forage, perennial bunchgrass in Patagonian rangelands. We hypothesized that (1) root biomass in P. ligularis is not reduced by increased defoliation frequency, as carbon would be allocated similarly above- (for leaf and shoot regrowth) and belowground (to maintain the water uptake necessary for shoot regrowth), and (2) root survival is greater with increasing soil depth in the various defoliation treatments because shallow soils are characteristically more water-limited depending on seasonal precipitation. Five defoliation frequencies (plants defoliated once, twice, or three, four or five times, plus 1 non-defoliated control) were applied in a representative grassland of the Occidental District in Patagonia during each of two consecutive growing seasons. Plants were defoliated, leaving 5 cm stubble, whenever regrowth reached 10 cm height. Root disappearance and appearance (using a root color scale), and root spatial distribution in the soil, were determined every 3 cm-soil depth increments up to 30 cm in the soil profile using a root periscope. The root color scale allowed us to develop equations to calculate root increase and mortality rates during each study period. Shoot biomasses per plant were not affected by any defoliation frequency during 2002/03. However, plants defoliated three or more times annually showed a lower shoot biomass than the non-defoliated controls during the second year of successive defoliations. Root dynamics was not affected by the study defoliation frequencies. Both hypotheses were confirmed by the results. Our findings suggest that (1) plant vigor on P. ligularis is most often favored by no more than two defoliations annually, immediately before internode elongation to 5 cm stubble height each, and (2) root field studies should be performed using short- rather than long-sampling increments into the soil profile because changes in root dynamics occurred at a scale of a few centimeters within it.

Keywords: Perennial grasses, root dynamics, root mortality, root recruitment.

INTRODUCTION

While aboveground growth responses to gradients of disturbances are well-documented (4, 17, 30, 56), our understanding of the variability in root dynamics is incomplete. Specifically, how do changes in root dynamics vary across the rooting profile as a function of grazing history?.

It is widely known that defoliation frequency can affect plant growth in perennial grasses. Growth is affected by shoot production, and/or growth and dynamics of the root system (6). The potential capacity of leaf area replacement is partly determined by the number and physiological activity of the apical and intercalary meristems which remain after a defoliation event (6). Factors which diminish the availability, and/or growth capacity of these meristems [i.e., through increases in defoliation frequency: Newton and Hay (1992) in *Trifolium repens* L.] might compromise persistence of affected rangeland plants (6).

Even though grazing can affect root biomass, its effects depend on the study soil depth. For example, Kitchen et al. (2009) showed that simulated grazing (mowing) in annually-burned plots reduced root biomass in the top 10 cm of the soil compared to unmowed treatments, but biomass did not vary

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between mowing treatments at depths greater than 10 cm. Differences in root biomass between grazed and ungrazed areas can reflect differences in species composition (29). However, if species composition is similar across sites, differences in allocation of root biomass by soil depth most likely reflect reduced allocation of carbon belowground due to repeated defoliation of the aboveground canopy (39). *Poa ligularis* Nees ex. Steud. is a native, perennial grass species in arid and semiarid rangelands of Argentina (12). This species is highly competitive and tolerant of grazing (31). Most grazing-tolerant grass species preferentially allocate carbon to aboveground tissues to rapidly replenish the photosynthetic surface area lost to herbivores (6). However, other grazing-tolerant species can maintain an unabated carbon allocation to roots after grazing (7).

Weaver (1968) reported that perennial grasses in tallgrass prairie can be rooted deeply in the soil profile. The production of deep roots has long been speculated as a mechanism to avoid drought (16). However, Nipper et al. (2012) concluded that deep roots do not serve as a mechanism to avoid the negative consequences of drought on various C_4 grasses in a tallgrass prairie. They suggested that one possible function of deep roots is a survival mechanism to persist through extremely adverse drought conditions, by transporting the minimum amount of water necessary to support a reduced aboveground canopy, and maintain the turgidity of meristematic tissues in belowground organs. Several works have reported a low ecological plasticity in depth of water uptake for various deep-rooted C_4 grass species (2, 54). Other studies showed, however, a greater flexibility to swich water sources in the soil profile on various C_3 herbaceous species (55). Despite the importance of root distribution in the soil profile, our understanding of the variability in root dynamics across the entire rooting profile as a function of defoliation frequency is incomplete. This is very important because the larger the water-absorbing surface of the root system, the greater the rate of water uptake (42). Additionally, Flemmer et al. (2002), using a similar methodology to ours, demonstrated that root growth, and root appearance and disappearance decreased as plant water stress increased in the C_3 perennial bunchgrasses *Stipa clarazii*, *S. tenuis* and *S. gynerioides* in rangelands of central Argentina.

Growth and functioning of roots are dependent upon the energy provided by photosynthesis (6). After a defoliation event, the root system continues functioning as a carbon sink, depending on a continuous supply from the photosynthetically-active shoots (48). As a result, removal of shoot parts may affect shoot and root growth (6, 21). However, the proportion of shoot carbon that might be invested in either shoot or root tissues can vary with the plant species. For example, *Agropyron desertorum* (Fisch. ex Link) Schult., a grazing-tolerant grass species, invest proportionally more carbon to replace photosynthetic tissues after a severe defoliation than *Pseudoroegneria spicata* (Pursh) Á. Löve, a grazing-sensitive grass species, which maintains root growth at the expense of replacing a photosynthetic canopy (61). Various studies have reported that grass defoliation reduces root biomass (35) and growth (21, 58). Successive defoliations have reduced grass root growth in *Bouteloua gracilis* Vasey, hom. illeg. (5), *Themeda triandra* Forssk. (19), *Aristida ramosa* R.Br. and *Danthonia linkii* Kunth (33). Flemmer et al. (2002) reported that total root number decreased as defoliation frequency increased in three perennial grass species. Other studies, however, did not show a decrease in root biomass, growth or numbers as defoliation frequency increased (7).

Positive correlations between root biomass and plant production highlight the importance of studies on the grass root system (60). Although some research has focused upon the effects of defoliation on root growth in perennial grasses world-wide (68), no studies have been conducted to determine the effects of perennial grass defoliation frequency on the determinants of root system dynamics (i.e., root survival, mortality and increase rates). Also, studies are available about shoot response to defoliation in natural grassland ecosystems; however, the root data are often missing. Therefore, comprehensive root system studies are essential to get a complete picture of plant responses to stress.

Poa ligularis is considered one of the major forages in Patagonia, Argentina (26). Its abundance, vigor, height and degree of defoliation are indicators of Patagonian rangeland condition. The genus *Poa* is spread worldwide (Argentina: 30; Canada: 63, China: 4; Peru: 9, USA: 1). Although species of this genus are likely too different in growth characteristics and natural habitats to extrapolate results to a worldwide-scale, *Poa ligularis* response to various defoliation frequencies under arid conditions in Patagonia is likely informative. *Poa ligularis* is resistant to drought and moderate grazing (43), but has been affected by overgrazing (10). However, the effects of various defoliation frequencies on root growth and dynamics of this species are unkown. The objective of this study was to investigate the effect of various defoliation frequencies on production of shoot and root biomasses, and rates of root

increase and mortality in *P. ligularis*. We hyphotesized that increased defoliation frequency on *Poa ligularis* does not reduce root biomass, as carbon is allocated similarly above- (for shoot regrowth) and belowground (to maintain the water uptake necessary for shoot regrowth). We also hypothesized that root survival is greater with increasing soil depth in the various defoliation treatments because shallow soils are characteristically more water-limited depending on seasonal precipitation.

MATERIALS AND METHODS

Study Site

Studies were conducted at the experimental field of INTA EEA Bariloche (41°10'S 70°41'W, 1000 m.a.s.l., NW Patagonia, Argentina), located 80 km from San Carlos de Bariloche, during three consecutive growing seasons (2002-03, 2003-04 and 2004-05). Climate is arid, cold temperate and is characterized by a slightly positive hydric balance during the winter months, and a large water deficit during the warmest months. Mean annual precipitation is 280 mm (11). Mean temperature of the warmest month is 15°C (January) and of the coldest month is 2.1°C (July) (11). An automatic meteorological station located at the study site provided the climate data during the study period (Figure 1).

Vegetation is a shrubby-gramineous steppe dominated by *Mulinum spinosum* (Cav.) Pers., *Adesmia campestris* (Rendle) Rowlee, *Poa ligularis* and *Stipa speciosa* Trin. & Rupr. Soils are of the xerophilous - Haplargid type (47). Grazing is extensive in this region, and stocking rate used by land managers varies between 1 and 0.25 sheep ha^{-1}. *Poa ligularis* has a long dormancy period from mid-summer (January-February) to July-August (mid- to late winter), during which it appears to be dry. Its main forage production is in spring (September-November). It flowers towards the end of spring (November-December) and seed dispersal occurs during summer (January-February) (32).
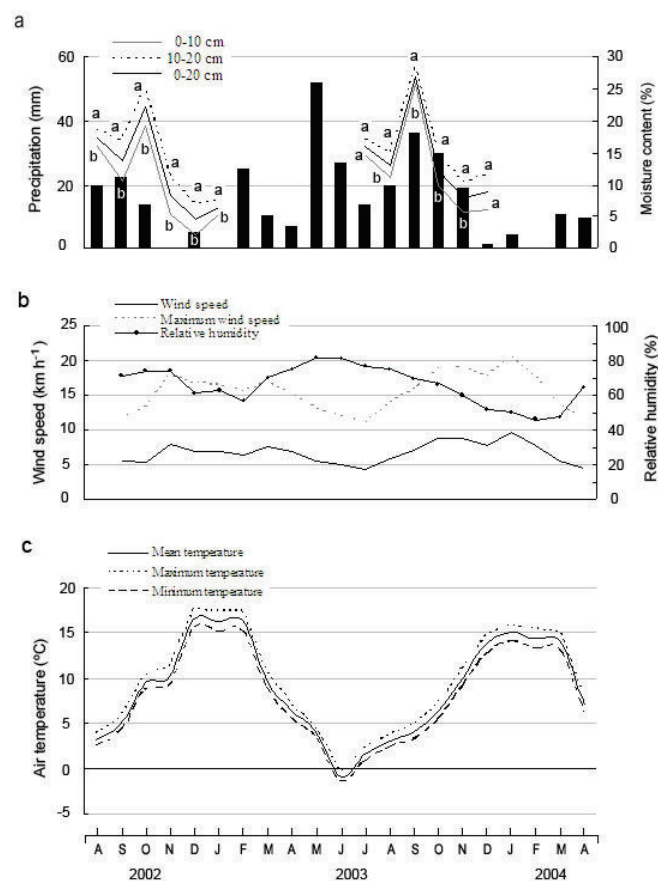

Figure1. *Climate data during the study growing seasons in 2002/03, 2003/04 and 2004/05, (a) Monthly precipitation (black histograms), Lines indicate soil moisture contents at 0-10, 10-20 and 0-20 cm soil depth. Different letters among soil depths indicate significant differences (p<0.05). (b) Monthly average wind speed (solid line), monthly average maximum wind speed (dotted line) and Monthly average air relative humidity (solid symbols), (c) Monthly average mean temperature (solid line), and monthly average minimum (large dashed line) and maximum (small dashed line) temperatures.*

Measurements

Soil Moisture Content. Soil moisture content was determined gravimetrically (8). During each of the study growing seasons, 20 samples were randomly taken once monthly using an auger (10 cm diameter): 10 samples were obtained from a depth of 0 to 10 cm, and the other 10 from 10 to 20 cm depth from the soil surface.

Shoot Biomass. Within an exclosure (1 ha) to domestic livestock and small mammals, 160 plants of *P. ligularis* (80 for each study growing cycle) were selected. Prior to study initiation, during the dormant season, all 160 plants were clipped to 5 cm stubble on 8 March 2002. This allowed detemination of subsequent shoot dry matter production during the growing seasons. Thereafter, diameter (mean ± 1 SE, n; $40.0 ± 0.3$ cm, 80) of the basal area and distance to its nearest neighbor ($8.4 ± 0.4$ cm, 80) were measured on half of these plants. Within each growing season, the 80 randomly-chosen plants were assigned to 5 groups (n=16 per group). Half of the plants on each group remained non-defoliated (Control; 8 plants) or they were defoliated (n=8 plants) to one of 5 defoliation frequencies. Defoliated plants were cut 1, 2, 3, 4 or 5 times using scissors, leaving 5 cm stubble from the soil surface, whenever regrowth reached 10 cm height (see Table 1 for a detailed description of the experimental design). Time intervals between the defoliations varied from 30 to 51 days depending on timing, frequency and study year. During the second growing season, first-year defoliation frequencies were attempted to determine cumulative effects of defoliation. However, during the second growing season, regrowth allowed only 3 out of the 5 study defoliation frequencies, because plants assigned to 4 and 5 defoliations were unable to reach 10 cm height after the third defoliation. Biomass of non-defoliated plants was determined at the same time plants were defoliated once, twice, or 3, 4 or 5 times leaving 5 cm stubble. Thereafter, they were dried 72 h in an oven at 70°C and weighed (g dry matter plant^{-1}).

Table1. *Experimental design to study the effects of various defoliation frequencies [non-defoliated controls, plus plants defoliated once (1), twice (2), three times (3), four times (4) or 5 times (5)] on root and shoot growth (2002-03; 2003-04), and root dynamics (2002-03; 2003-04; 2004-05), in plants of P. ligularis. The number of evaluated plants on each sampling date varied with the parameter evaluated.*

Defoliation frequency	1		2		3		4		5	
Plant number: Shoot and Root biomasses	16		16		16		16		16	
	8 U	8D	8 U	8D	8 U	8D	8 U	8D	8 U	8D
Plant number: Root Dynamics	10		10		10		10		10	
	5U	5D	5U	5D	5U	5D	5U	5D	5U	5D

Abbreviations are as follows: *D= Defoliated; U= Non-defoliated.*

Root Biomass. After shoot harvesting, plants were destructively harvested and brought to the laboratory. Extraction of defoliated and non-defoliated plants from the soil was conducted using a shovel to obtain a 0.5 x 0.5 x 0.5 m (soil volume: 0.125 m^3) soil block. Plant basal area diameters and minimum distances between the target plants and their nearest neighbors (given above) made us confident that most of the root mass in that block came from treatment plants. Roots were washed from soil at the laboratory using a 60 mesh screen (73). Root biomass production (g dw plant^{-1}) was determined on each plant after drying root biomass 72 h in an oven at 70°C.

Root System Dynamics. Within the same exclosure used for the shoot and root biomass studies, 150 plants of *P. ligularis* (50 for each study growing cycle; 2002-03, 2003-04 and 2004-05) were randomly selected and marked. These plants were exposed to the same treatments and manipulations than those used in the root biomass study. Within each growing cycle, 10 plants were assigned to each of 5 defoliation treatments (non-defoliated controls and plants defoliated once, twice, and three, four or five times). Each group of 10 plants contained five plants to be defoliated, and five plants to be used as non-defoliated controls. Because plants assigned to the 4 and 5 defoliations were unable to reach 10 cm height after the third defoliation during the second study year, only the cumulative effects of defoliating once, twice or three times could be evaluated during the second growing season.

A root periscope similar to that used by Becker et al. (1997) allowed direct observation of root appearance (a measure of growth) and disappearance, and root spatial distribution in the soil (28, 71). Glass tubes 0.5 m long with 3.5 cm internal diameter were buried at the plant periphery using an angle (15 to 20° with respect to the vertical: 3) such that the bottom part of the glass tubes reached

approximately the center of the plant crown. Holes were made using an auger to 30 cm soil depth. Fifteen centimeters of each tube were left above the soil surface and covered with dark tape and a piece of PVC pipe to exclude light. Roots exposed to light can have their growth inhibited (44) and their cortex can suberize more rapidly (13). Prior to placement, circles perpendicular to the glass tubes were engraved around them at 3cm-length-increments up to reaching 30 cm soil depth, except the portion above the soil surface. Thereafter, root observations were conducted down to that soil depth. More than 70% of the root system biomass is located in the first 30 cm of soil for various native perennial grasses at the study site (26, 65).

The root periscope was used to map roots at each sampling time, and detect root disappearance and appearance using a root color scale. Sampling times were: 4 October, and 5 December 2002; 31 January, 14 September, and 19 November 2003; 21 January, 25 February, 15 October and 19 December 2004, and 18 February 2005. Four color categories were considered: (1) transparent white (more recent roots), (2) light brown, (3) brown, and (4) dark brown (a result of initiation of root cortex cell death). Color data were used to determine daily percentage root increase (Ri) and mortality (Rm) rates during each study period. We developed equations to estimate root increase, survival, recruitment and mortality rates as follows:

Root increase rates (Ri):

$$Ri = Sr + Rr,$$

where Sr is the percentage root survival per day between consecutive sampling dates and Rr is the percentage of new roots which appeared per day between consecutive sampling dates. Root survival rate (Sr) was determined as:

$$Sr = Survivors(t)/[N(t-1) * L]$$

where Surivors(t) is the number of roots which survived two consecutive samplings, N(t-1) is the number of roots at time t-1 (first sampling during the study period), and L is the period length. Root recruitment rate (Rr) was estimated as:

$$Rr = New(t)/[T(t) * L]$$

where New(t) is the number of new roots at time t (those which appear between the first and second samplings of any study period), T(t) is the total number of roots at time t, and L is the length of the study period. Roots needed to show a white color and a swollen aspect to be considered as new (category 1 in color).

Root mortality (Rm) rates were estimated as:

$$Rm = D(t)/[N(t-1) * L]$$

where D(t) is the number of dead roots at time t (i.e., roots which disappeared between sampling periods), N(t-1) is the number of roots at the previous sampling time (i.e., t-1), and L is the period length. Only roots which disappeared between consecutive sampling times were considered dead, because even though root cortex cells start dying (showing a dark brown color), the stele remains active (22). In addition, water and nutrient uptake occurs in these roots after epidermis and cortex cells have died (24).

Statistical Analysis

Data analysis was conducted using the program Statistica 6.0 (2001; Statsoft, Tulsa, OK, USA), using the General Linear Model (GLM). Within each year, data were analyzed using a nested ANOVA design to evaluate the following factors: Defoliation frequency (5 levels: 1 to 5 defoliations) and Treatment (2 levels: Defoliated and Non-defoliated). This latter treatment was nested within the first one. The test HSD (Honestly Significant Difference) of Tukey (67) was conducted whenever F tests were significant at $P<0.05$. Homogeneity and normality of variances were tested using the Levene (45) and Shapiro Wilks (64) tests. Growth rate (g dw day^{-1} = slope of the relationship) was determined using simple linear regression between biomasses coming from each defoliation frequency and that of controls versus days from study initiation. At the same time, the analysis of assumptions (i.e., normality, homocedasticity and residual independence) involved in the construction of linear models was conducted. Differences between regression lines for each treatment were tested for differences in models by simple linear regression analysis following Neter and Wasserman (1974).

Comparison of root increase, survival, recruitment, and mortality rate was conducted using repeated measures ANOVA. Factors were (1) days between consecutive sampling dates, (2) defoliation treatments (6 levels: T1, T2, T3, T4, T5 and non-defoliated Control), and (3) sampling depths from the soil surface (9 levels: 3-6-9-12-15-18-21-24-27 cm). The sphericity assumption was evaluated using the test of Mauchly (49); when this test did not fit the assumption, the multivariate approach was used through the statistic of Wilks (Wilk´s lambda) (72). The relationship between survival and mortality rates was studied using one-way-ANOVA.

RESULTS

Soil Moisture Content

During the growing season, and at both 0-10 and 10-20 cm depth, the greatest and lowest soil moisture contents occurred in October and December, respectively, during 2002-03, and September and November, respectively, during 2003/04 (Figure 1). Soil moisture content was often greater deeper than shallower with respect to the soil surface level (Figure 1).

Shoot Biomass

During 2002-03, defoliated and non-defoliated plants showed a similar dry matter biomass within each defoliation frequency (Figure 2a). Plants defoliated at least three times had a greater biomass than those defoliated once or twice during the growing season.

During the 2003/04 growing season, the interaction between defoliation frequencies and treatments was significant. Advancement of the growing season increased biomass on non-defoliated controls. Defoliated plants increased dry matter until plants were defoliated twice, when maximum values were reached; these values diminished about 59% when plants were defoliated four or five times.

From the third to the fifth defoliation frequency, dry matter of non-defoliated controls was greater than values on defoliated plants (Figure 2b). Plants that were defoliated either four or five times during 2002/03 only produced dry matter until the third defoliation the next growing season.
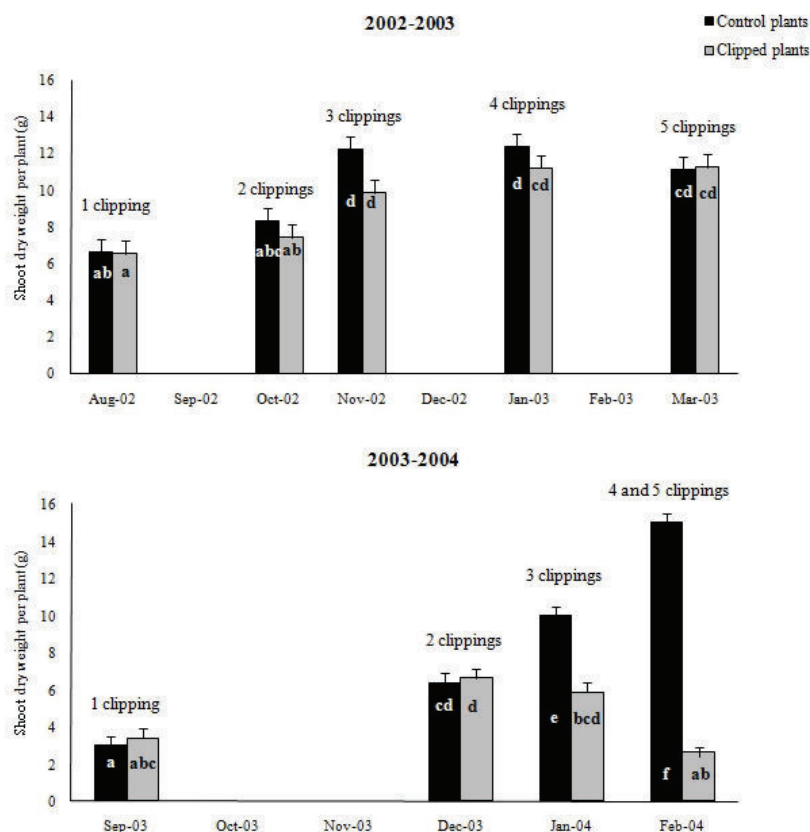

Figure2. *Cumulative, annual shoot dry matter production (g dry matter/plants) for each defoliation frequency (1 to 5 defoliations) on Control (solid histograms) and defoliated (grey histograms) during the study (2002/03 and 2003/04) growing seasons. Each histogram is the mean of n=8. Different letters indicate significant differences between treatments (with or without defoliation) and defoliation frequencies. Vertical bars represent 1 standard error of the mean.*

Shoot Growth Rates

Regression of dry matter on time determined that growth rates of plants defoliated two or three times during the 2002/03 growing season were 20% greater than those on control plants (Table 2). In the second year, plants defoliated one, two or three times showed slopes 40 to 50% lower than those registered on control plants (Table 2). Reductions in slopes were from 70 to 80% on plants defoliated four or five times compared with values on non-defoliated controls (Table 2). Slopes were greater on plants defoliated one, two or three times than on those defoliated four or five times. Slopes within any of the defoliation frequencies were greater in the first than in the second growing season (Table 2). The reduction was 60% for the non-defoliated controls; 80% for plants defoliated one, two or three times; and 90% for plants defoliated four or five times.

Table2. *Slopes, standard errors (S.E.) and confidence intervals, and correlation coefficients (r) of the regression lines between shoot biomass (y) versus time (x) in the various defoliation frequencies and the non-defoliated control during the study years. Within any defoliation frequency, biomasses were accumulated until the last defoliation in that frequency. Each biomass value used in the regression came from n=10. Treatments included various defoliation frequencies: non-defoliated controls or plants defoliated once (1), twice (2), three times (3), four times (4) or 5 times (5).*

Treatments	2002-2003					2003-2004					2002-2003 vs. 2003-2004
	Slope	S. E	Confidence interval		r	Slope	S. E	Confidence interval		r	
			-95%	+95%				-95%	+95%		
1	0.095 ab	0.009	0.076	0.114	0.92	0.015 b	0.004	0.016	0.024	0.84	*
2	0.099 b	0.006	0.087	0.110	0.93	0.020 b	0.002	0.015	0.025	0.88	*
3	0.099 b	0.005	0.089	0.108	0.94	0.016 b	0.001	0.013	0.018	0.92	*
4	0.093 ab	0.004	0.085	0.101	0.94	0.007 c	0.000	0.006	0.008	0.94	*
5	0.087 ab	0.004	0.079	0.095	0.92	0.009 c	0.001	0.007	0.011	0.82	*
Control	0.081 a	0.002	0.077	0.086	0.97	0.032 a	0.001	0.029	0.035	0.96	*

*Different letters in the slope column indicate significant differences in dry matter accumulation with time in the various defoliation frequencies and the non-defoliated control at p<0.05. * significant differences at p<0.05.*
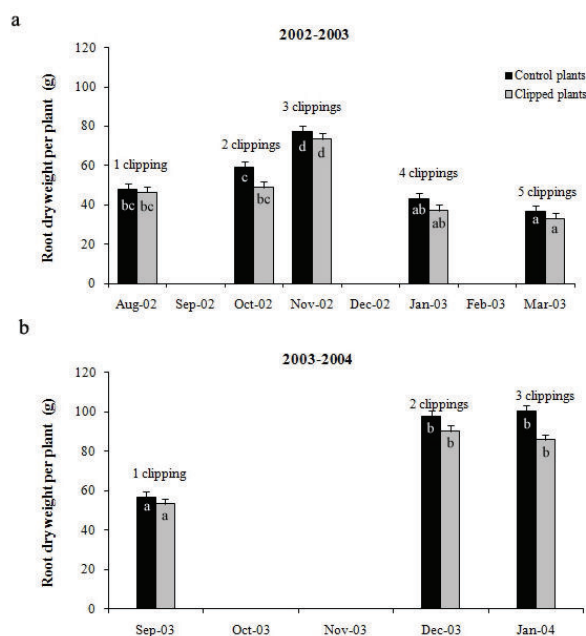
Root Biomass

Figure3. *Biomass of roots (g dry matter/plant) for each defoliation frequency (1 to 5 defoliations) on Control (solid histograms) and defoliated (grey histograms) plants during (a) 2002-2003 and (b) 2003-2004. Each histogram is the mean of n=8. Different letters indicate significant differences between treatments (with or without defoliation) and defoliation frequencies. Vertical bars represent 1 standard error of the mean.*

During 2002-03, the interaction between frequency and defoliation treatments was not significant (p>0.05). Plants defoliated three times during the growing season had the greatest root biomass compared to the other defoliation frequencies in both treatments (with and without defoliation, Figure 3a). Plants defoliated 5 times produced a lower root biomass than those defoliated 1, 2 or 3 times.

Within each defoliation frequency, there were no differences (p>0.05) between defoliated or non-defoliated plants (Figure 3). Timing of defoliation (i.e., either before or after defoliation at any sampling date) did not show significant differences (p>0.05) in root biomass within each defoliation frequency, or between defoliated and non-defoliated plants (data not shown).

The interaction between frequency and defoliation treatments was again not significant (p>0.05) during 2003/04. The greatest root biomass in the second growing season was obtained on plants defoliated two or three times in both defoliation treatments (Figure 3b). Within each defoliation frequency, there were no differences (p>0.05) between defoliated versus non-defoliated plants. Also, within each frequency of defoliation, timing of defoliation had no effect for non-defoliated or defoliated plants (data not shown).

Root System Dynamics

Rates of root survival (0.82±0.09% in 2002/03; 0.50±0.05% in 2003/04; 0.54±0.03% in 2004/05; n=50 in all cases), mortality (1.20±0.05% in 2002/03; 1.20±0.12% in 2003/04; 0.93±0.03% in 2004/05; n=30 in all cases); recruitment (2.20±0.13%: 2002/03; 0.95±0.10%: 2003/04; 0.83±0.30%: 2004/05; n=30 in all cases), and increase (3.00±0.05%: 2002/03; 1.66±0.15%: 2003/04; 1.25±0.02%: 2004/05; n=30 in all cases) were similar (p>0.05) on defoliated and non-defoliated plants. As a result, data coming from both defoliation treatments were pooled for subsequent analysis.

Root Survival Rates

During 2002/03, the interaction between sampling dates and soil depths was not significant (p>0.05). Root survival rates were similar (p>0.05) among sampling dates (0.80±0.10, n=50) and soil depths (0.82±0.06, n=50).
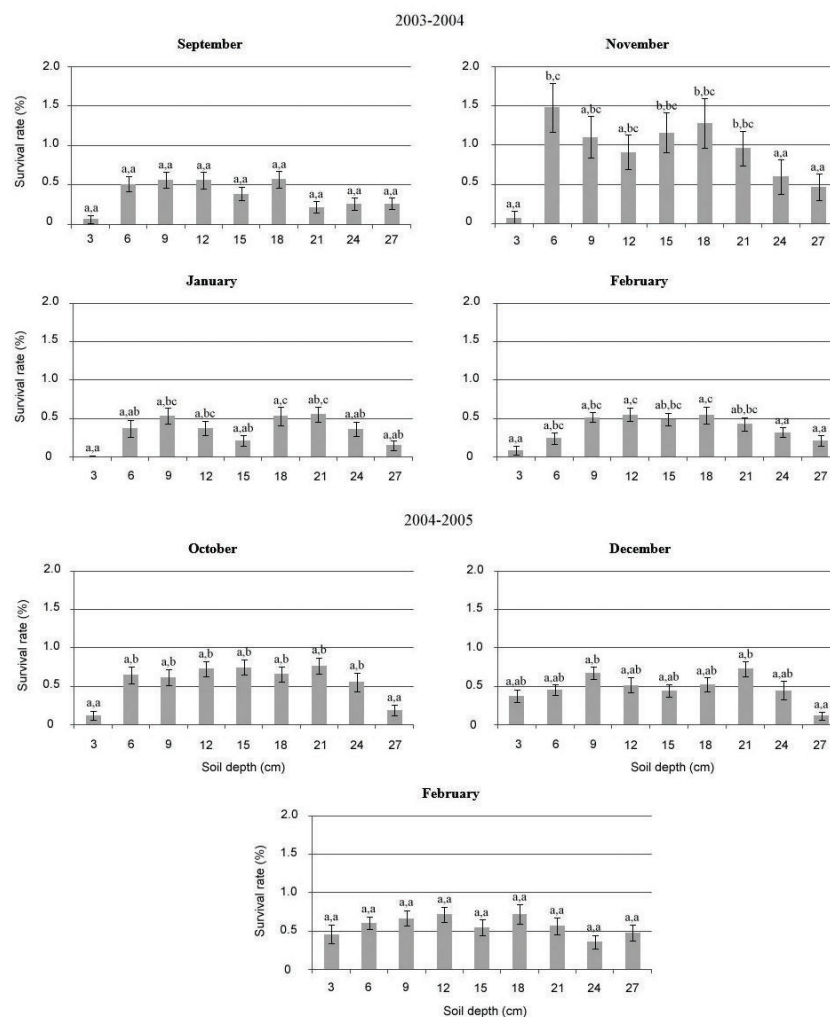

Figure4. *Daily percentage survival rate in the number of roots at the various sampling dates and soil depths during 2003/04 and 2004/05. Each histogram is the mean of n=10. Different letters on the histograms indicate significant differences among dates within each soil depth (first letter) or soil depths within each sampling date (second letter). Vertical bars represent 1 standard error of the mean.*

During 2003/04, there was an interaction between sampling dates and soil depths. In September, root survival rates were similar (p>0.05) among soil depths (Figure 4). However, root survival rates were lower at 3, 24 and 27 than at 6, 9, 12, 15, 18 and 21 cm soil depth during November. In January, such rates were lower at 3 than at 9, 12, 18 and 21 cm soil depth. Root survival rates in February were lower at 3, 24 and 27 than at 6, 9, 12, 15, 18 and 21 cm soil depth (Figure 4). Within each soil depth, root survival rates at 6, 15, 18 and 21 cm soil depth were greatest in November.

The interaction among sampling dates and soil depths was again significant in 2004/05. Root survival rates were lower at 3 and 27 cm than at the other study dephts in October. During December, root survival rates at 27 cm were lower than those at 9 and 21 cm soil depth. Within each study soil depth, root survival rates were similar (p>0.05) among sampling dates (Figure 4).

Root Mortality Rates

During 2002/03, 2003/04 and 2004/05, the interaction between sampling dates and soil depths was not significant (p>0.05). Mortality rates during 2002-03 were similar (p>0.05; overall mean=1.2±0.30%, n=150) in October, December and January. Rates of mortality at 3 cm soil depth (1.4±0.30%, n= 50) were at least 7% greater than those at the remaining study depths.

Root mortality rate in November 2003/04 (2.50±0.80%, n=50) was at least 64% greater than those in September, January and February. Rates of root mortality were at least 150% lower at 3 cm (0.40±0.20%, n=50) than at greater soil dephts.

Root mortality rates in October (1.00±0.20%, n=50) were 20% greater than those in February 2004/2005. Rates of root mortality at 3 cm (0.39±0.10%, n=50) were at least 125% lower than those at greater depths.
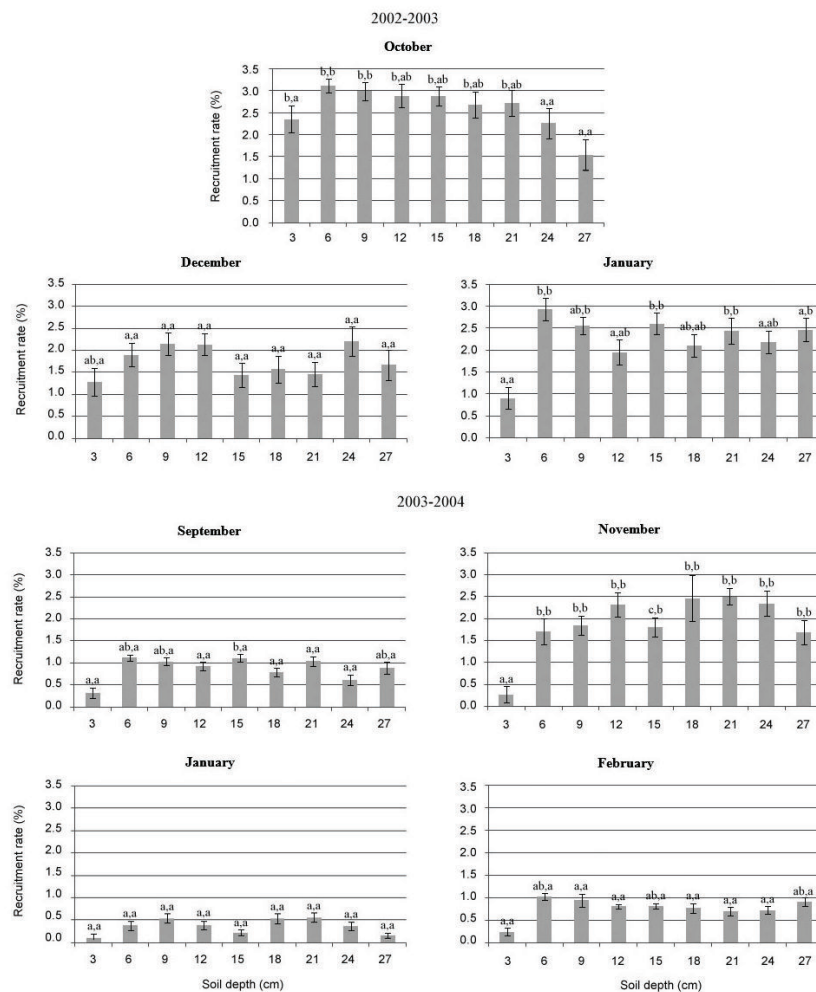
Root Recruitment Rates

Figure5. *Daily percentage recruitment rate in the number of roots at the various sampling dates and soil depths during the study period 2002/03 and 2003/04. Each histogram is the mean of n=10. Different letters on the histograms indicate significant differences among dates within each soil depth (first letter) or soil depths within each sampling date (second letter). Vertical bars represent 1 standard error of the mean*

The interaction among sampling dates and soil depths was significant in 2002/03. Root recruitment rates were greater at 6 and 9 than at 3, 24 and 27 cm soil depth during October (Figure 5). During January, root recruitment rates were lower at 3 than at 6, 9, 15, 21 and 27 cm soil depth. Root recruitment rates decreased from October to January at 3 cm soil depth. Root recruitment rates decreased at all depths between October and December, except at 3, 24 and 27 cm soil depth. Between December and January, root recruitment rates increased at 6, 15 and 21 cm soil depth (Figure 5).

Once again, the interaction between sampling dates and soil depths was significant in 2003/04. During November, the lowest root recruitment rates were found at 3 cm soil depth (Figure 5). In most cases, there were no significant differences ($p > 0.05$) among soil depths in September, January and February. The greates root recruitment rates occurred in November at 12, 15, 18, 21 and 24 cm soil depth. At 6, 9 and 27 cm soil depth, root recruitment rates were greater in November than in January (Figure 5).

Sampling dates and root depths did not interact significantly ($p > 0.05$) during 2004-05. Root recruitment rates in October ($2.50 \pm 0.80\%$, n=50) were at least 64% greater than those in December and February. Root recruitment rates were at least 62.5% lower at 3 cm ($0.30 \pm 0.10\%$, n=50) than at the remaining study soil depths.

Root Increase Rates

During 2002/03, the interaction between sampling dates and soil depths was not significant ($p > 0.05$). Root increase rates diminished by 25% between October and December. In this year, rates of root increase were lower at 3 and 27 ($2.30 \pm 0.50\%$, n=50) than at 6 cm ($3.60 \pm 0.20\%$, n=50) and 9 ($3.50 \pm 0.20\%$, n=50) cm soil depths.
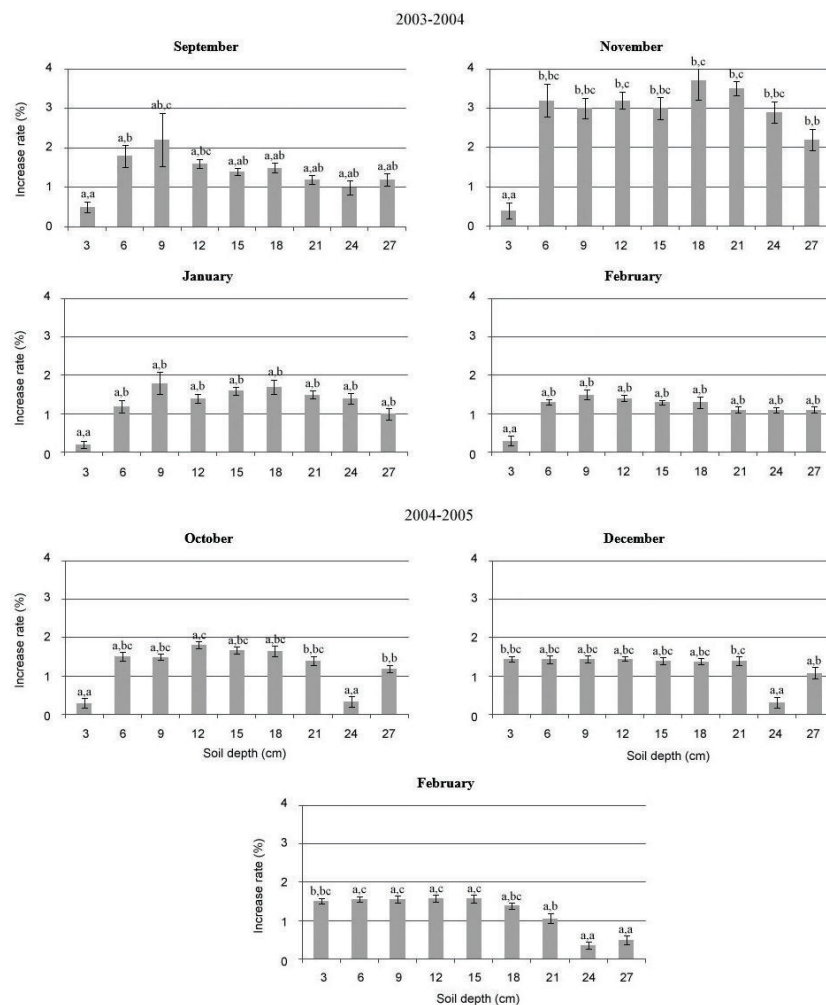

Figure6. *Daily percentage increase rate in the number of roots at the various sampling dates and soil depths during the study period 2003/04 and 2004/05. Each histogram is the mean of n=10. Different letters on the histograms indicate significant differences among dates within each soil depth (first letter) or soil depths within each sampling date (second letter). Vertical bars represent 1 standard error of the mean.*

During 2003/04, both sampling date and depth interacted significantly. In September, root increase rates were lower at 3 than at 6, 9 and 12 cm soil depth. In addition, such rates were greater at 9 than at 24 cm soil depth (Figure 6). During November, January and February, lowest root increase rates, in general, were shown at 3 cm soil depth in comparison to the other study soil depths. Root increase rates at 3 or 27 cm soil depth showed no significant differences (p>0.05) among sampling dates. However, the remaining soil depths showed greater root increase rates in November than at the other sampling dates (Figure 6).

In 2004-05, the interaction between sampling dates and soil depths was significant. Root increase rates were lower at 3 and 24 cm soil depth than at the remaining study soil depth levels in October (Figure 6). The lowest root increase rate during December was observed at 24 cm soil depth. In February, root increase rates were lower at 24 and 27 cm soil depth than at upper soil depth levels. Besides, root increase rates were greater at 21 than at 24 and 27 cm soil depth, but lower than values at 6, 9, 12 and 15 cm soil depth. Root increase rates at 3 cm soil depth increased from October to December and February. At 21 cm soil depth, root increase rates during October and December were greater than those in February. Root increase rates during October were greater than those in December and February at 27 cm soil depth (Figure 6).

Relationship between Survival and Mortality Rates

The relationship between both rates was significantly negative in 2004/05 (y=0.0127 – 0.633x, R^2=45.1%, p=0.002).

DISCUSSION

Soil Moisture Content

Soil moisture content variation during the growing season agrees with that demonstrated for rangelands in central Argentina by Peláez et al. (2001). Increases of soil moisture content with increasing depth are also similar to those reported by Brown (1995).

Shoot Biomass

Defoliation frequency affected shoot biomass of *P. ligularis*. When it was greater than twice yearly, shoot biomass of this perennial bunchgrass was lower on defoliated than on undefoliated plants by the second year of study. Jacobs and Sheley (1999) suggested that a competitive grass community was maintained with 60% or less defoliation in western Montana. These authors also indicated that increasing grazing frequency causes stress to grasses that are similar to increases in intensity. Early season, infrequent defoliation allows grasses time to recover, and defoliation after the grass have matured minimizes impacts (51).

Root Biomass

Biomass of roots showed a seasonal production pattern. During the first growing season, a biomass increase was observed in November (mid-spring) for defoliated and non-defoliated plants. This seasonality in root biomasses during the year has also been observed in other grass species of temperate grasslands (46, 50). Increases in root biomass were corresponded with greater values in the rates of increase, survival and recruitment. Results showed that root biomass was maintained after low (one defoliation during the year) and high (five defoliations during the year) defoliation frequencies in *P. ligularis.* These results disagree with those found by Engle et al (1998), who reported that multiple defoliations reduced root weight more than did single defoliations in the perennial grasss *Adropogon hallii* Hack.

Root biomass increased up to 3 defoliations per year during 2002/03 and 2003/04. This response to defoliation on root biomass has been reported on other grasses of temperate climate (14, 69). Similar results have been reported on various grasses exposed to different defoliation frequencies: *Hyparrhenia filipendula* (Hochst.) Stapf. (15), *Andropogon hallii* Hack. (25), and *Danthonia linkii* (33). Our results differ, however, from those reported by Crider (1955), Davidson and Milthorpe (1966), Jarvis and Macduff (1989), and Engel et al. (1998), where increased defoliation frequencies reduced root biomass.

Root biomass of defoliated and non-defoliated plants appeared to increase from the first to the second studied year. These results could partially be attributed to more favorable conditions during 2003/04 than in 2002/03, related to a wetter spring in 2003 (Figure 1a). Hayes and Seastedt (1987) and Fiala et al. (2009) reported that there was a great root production decline at the surface in soils during drought

periods. Indeed, Weaver (1968) reported that rhizomes of the dominant C_4 grasses persisted for many years despite severly-reduced aboveground production following the prolonged droughts of the 1930's.

Root growth rates in *P. ligularis* were similar between non-defoliated and defoliated plants during 2002/03, 2003/04, and 2004/2005. This indicates that photoassimilates continued to be partitioned to root growth even under severe defoliation frequencies. This is similar to that reported by Richards (1984) in the grazing-sensitive *Pseudoroegneria spicata.* In this species, root growth continued after a severe defoliation to the expense of mobilizing less photoassimilates for photosynthetic canopy re-establishment. This highlights an important factor which most likely contributes to the persistence of *P. ligularis* in rangelands of Central and Southern Argentina.

Poa ligularis was able to sustain root growth maintaining high shoot growth rates during the first study growing season (2002/03), when plants were moderately defoliated (up to three defoliations). Defoliated plants had similar root biomass, root increase rates, recruitment rates and survival rates than control, non-defoliated plants; thus, it was observed a seasonal variation in the magnitude of these parameters, irrespectively of defoliation frequency. Holding survival and recruitment rates during the study growing seasons secured maintenance of root exploration for soil water and nutrients. The results from this study differ from those of Becker et al. (1997). These authors reported lower rates of root appearance and disappearance after plants of the perennial grasses *Stipa tenuis* Phil. and *Piptochaetium napostaense* (Speg.) Hack. were severely defoliated, late in the growing season under rainfed conditions. Their results differ from ours because they defoliated plants of those perennial grasses only once at each of various phenological stages during each of two growing cycles.

Root Dynamic Responses to Defoliation

A novel approach in the root dynamics study was the developed equations for determining the survival and recruitment rates (to determine the increase rates), and the rates of mortality. Another novel finding highlights the importance of conducting root dynamics studies using short soil-depth-increments in the soil profile up to relatively shallow depths (e.g., 30 cm soil depth). This is because of the (1) changes in root dynamics found with small soil-depth increments within the first 30 cm soil depth in the soil profile, and (2) reported lack of reliance on water sources below 30 cm depth as a result of morphophysiological constraints on various perennial grass species (2, 54, 55, 57).

Control and defoliated plants showed similar root mortality rates during the study growing seasons. These results differ from other studies on root dynamics which reported increased root mortality in perennial grass plants immediately after defoliation (6). These authors attributed this response to reduced canopy photosynthesis following defoliation, and emphasized that these experiments clearly demonstrated the importance of current photoassimilates for the maintenance of root growth and function.

No differences were found between defoliated and non-defoliated plants in root dynamics. These results indicate resistance to defoliation-induced changes in the root system of *P. ligularis*. At the same time, they suggest that the resource uptake from surface soils would not be modified after application of various defoliation frequencies. These results differ from those of Nipper et al. (2012) who found that root biomass and total root length in the top 20 cm of the soil profile were greater on ungrazed than on grazed locations in *Lolium perenne*.

Rates of root increase (recruitment + survival) and mortality during 2002/03, 2003/04 and 2004/05 showed variation with soil depth. Lowest values were in general registered in the first 3 cm soil depth; intermediate values in intermediate soil depths (about 20 cm from the soil surface), and low values at the greatest study soil depths (24 - 27 cm). Rates of recruitment and survival followed in general the same pattern of variation. This pattern of new root production rates in relation to soil depth was reported by Liu and Huang (2001) in *Agrostis palustris* Huds., and Steinke et al. (1996) in *Agropyretum repentis* Felfoldy. Various authors have observed a low root density in the most superficial soil layers, followed by an increase toward the deeper soil depths, and back to low root density values at the deepest soil layers (66). Nippert et al. (2012) reported that root biomass and total root length were highest in the top 20 cm of the soil profile and then declined exponentially with increasing soil depth in a tallgrass prairie. Finally, root increase rates were greater than root mortality rates at each depth during 2002/03 and 2003/04, but January 2003-04, which contributes to explain the root biomass increases until November and February during the first and second study growing seasons, respectively.

High root increase and mortality rates in surface soils highlight the availability of resources at these soil depths (e.g., water via rainfaill) or intense competition for soil resources (17, 36). Prodigious biomass production and root length in surface soils allowed *Zea mays* to quickly respond to rainfall events, and thoroughly scavenge all available soil moisture between rainfall events (74). Small rainfall events are common in arid regions of Argentina (e.g., 31). Physiological performance and growth of C_4 grasses in tallgrass prairie species were also linked to water availability changes in surface soils (56).

Root mortality and survival rates were negatively related in 2004/05. Other studies found similar results on other plant species (62). However, these authors reported a great variation when studying such relationship. They pointed out that their relationship was expected to have poor predictive power: it was attributed to the fact that low root longevity could translate into an either high or low root mortality depending on the number of roots that die, and vice versa (62). In their study, for example, browsed and unbrowsed plots had extremely low, nearly identical root survival rates in winter on a two-year-study, yet mortality of unbrowsed plots averaged 2.5 times that of browsed plots over the same time interval.

Greatest rates of survival, recruitment and increase during the study period occurred during early to mid-spring, when precipitation and/or soil moisture content were adequate, and air temperatures were increasing. Other studies have also reported a pulse-like pattern of grass root growth in response to increases in soil water availability and temperature (41).

The results of this study suggest that the immediate effect of defoliating *P. ligularis* up to two times per year do not affect either shoot or root biomass or root dynamics. This is because shoot and root growth were not affected by defoliation frequency, since defoliated plants had similar values as non-defoliated ones. This root response to the study defoliation frequencies has been shown in other grass species of temperate climate (69). It is interesting to note that root biomass was almost one order of magnitude larger than the shoot biomass, a fact that has been extensively reported on perennial grasses in arid regions (23).

Knowledge of the drivers of root growth, turnover, and distribution remains the least understood realm of terrestrial ecology because of the difficulty of assessing differences to environmental gradients or management practices *in situ*. This study provided evidence that, despite various differences were detected, the similar root dynamics at all depths in the first 27 cm-soil depth (i.e., surface soils) is most likely related to the water and nutrient uptake from surface soils. Nippert et al. (2012) reached a similar conclusion, reporting that deep grass roots (i.e., those deeper than 30 cm soil depth) play a minor role for water uptake because of morphophysiological constraints.

IMPLICATIONS

Our results indicate that plant vigor on *P. ligularis* in dry Patagonia is favored by light to moderate defoliation frequencies immediately before internode elongation: no more than two defoliations annually to 5 cm stubble height each. Becker et al. (1997) demonstrated that defoliation previous to differentiation of the growth apex from vegetative to reproductive was conducive to a much greater root dynamics compared to later defoliations during the growing season in two perennial grasses of Central Argentina. Resting periods immediately prior to apex differentiation from vegetative to reproductive are thus critical (i.e., 3).

Our findings also suggest that studies of root dynamics on perennial grasses should consider sampling at short- rather than long-soil depth increments into the soil profile. This is because of the changes in root increase, survival, recruitment and mortality rates found at a rather small scale of soil depth-increments up to 30 cm soil depth into the soil profile.

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