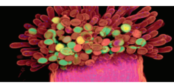
plant biology



Plant Biology ISSN 1435-8603

RESEARCH PAPER

Impact of defoliation intensities on plant biomass, nutrient uptake and arbuscular mycorrhizal symbiosis in *Lotus tenuis* growing in a saline-sodic soil

I. García & R. Mendoza

Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina

Keywords

Arbuscular mycorrhiza; defoliation intensity; *Lotus tenuis*; nutrient uptake; saline-sodic soil.

Correspondence

I. García, Museo Argentino de Ciencias Naturales ''Bernardino Rivadavia'' (MACN), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Ángel Gallardo 470, (C1405DJR), Buenos Aires, Argentina.

E-mail: igarcia@macn.gov.ar

Editor

T. Bisseling

Received: 27 April 2011; Accepted: 7 February 2012

doi:10.1111/j.1438-8677.2012.00581.x

ABSTRACT

The impact of different defoliation intensities on the ability of *Lotus tenuis* plants to regrowth, mobilise nutrients and to associate with native AM fungi and *Rhizobium* in a saline-sodic soil was investigated. After 70 days, plants were subjected to 0, 25, 50, 75 and 100% defoliation and shoot regrowth was assessed at the end of subsequent 35 days. Compared to non-defoliated plants, low or moderate defoliation up to 75% did not affect shoot regrowth. However, 100% treatment affected shoot regrowth and the clipped plants were not able to compensate the growth attained by non-defoliated plants. Root growth was more affected by defoliation than shoot growth. P and N concentrations in shoots and roots increased with increasing defoliation while Na^+ concentration in shoots of non-defoliated and moderately defoliated plants was similar. Non-defoliated and moderately defoliated plants prevented increases of Na^+ concentration in shoots through both reducing Na^+ uptake and Na^+ transport to shoots by accumulating Na^+ in roots. At high defoliation, the salinity tolerance mechanism is altered and Na^+ concentration in shoots was higher than in roots. Reduction in the photosynthetic capacity induced by defoliation neither changed the root length colonised by AM fungi nor arbuscular colonisation but decreased the vesicular colonisation. Spore density did not change, but hyphal density and *Rhizobium* nodules increased with defoliation. The strategy of the AM symbiont consists in investing most of the C resources to preferentially retain arbuscular colonisation as well as inoculum density in the soil.

INTRODUCTION

Plant response to defoliation depends on the intensity and frequency of removal of aboveground biomass, plant species, growing stage and particular environmental conditions, such as the availability of nutrients in soil to sustain shoot regrowth (Paige 1992; Crawley 1997). Reduced root growth after defoliation is linked to the loss of photosynthetic tissue (Davidson & Milthorpe 1966). Plant C balance is modified in such a way that plant C is preferentially allocated to shoots as a physiological adjustment in order to recover the leaf area lost (Gill 2007). Therefore, factors that influence the photosynthetic capacity of plants may affect the arbuscular mycorrhizal (AM) symbiosis (Benthlenfalvay & Dakessian 1984; Allsopp 1998; Barto & Rilling 2010). AM fungi are obligate biotrophs that live symbiotically in the roots of the majority of terrestrial plants. They are involved in taking up and transporting phosphate and other nutrients from the soil to plant roots and, in return, the plant provides the photosynthetically fixed C to its fungal partner (Smith & Read 2008). Root mass affected by defoliation is associated with a mobilisation of nutrients from necrotic roots for the shoot regrowth (Ourry *et al.* 1994; Allsopp 1998). Alternatively, arbuscular mycorrhiza may play an important role in maintaining phosphorus (P) uptake despite loss of root tissue.

The effects of defoliation on roots colonised by AM fungi has yielded controversial results. Some studies showed that defoliation can either positively (Eom *et al.* 2001; Hokka *et al.* 2004; Kula *et al.* 2005), negatively (Gehring & Whithan 1994; Allsopp 1998) or have no effect on AM root colonisation (Walling & Zabinski 2006). The effect of clipping *Bromus inermis* on AM root colonisation was dependent on which AM fungal species were associated with the plant, and which mycorrhizal structure was assessed (Klironomos *et al.* 2004). In this study, intraradical hyphae and arbuscular colonisation were negatively affected by clipping, whereas the production of vesicles in roots and spores in soil was stimulated, albeit to different degrees in different AM fungal species (Klironomos *et al.* 2004). On the other hand, a field study in roots of browsed *Polylepis* trees present in areas with high livestock density showed an increase in AM fungal spore density in soil, a lower proportion of arbuscules and a higher proportion of intraradical hyphae, while the proportion of vesicles and the total lengths of colonised roots did not differ between areas of high and low livestock density (Menoyo *et al.* 2009).

Defoliation that may impact plant growth, AM fungal symbiosis and nutrient cycling is an important factor because the capacity of a forage plant to regrowth after clipping may reflect the ability of this species to grow after grazing in the field. Most of the effects of defoliation on plant–AM fungal

  © 2012 German Botanical Society and The Royal Botanical Society of the Netherlands

symbiosis have been studied in grasses, but few studies have included legumes as host plants.

Lotus tenuis Waldst. & Kit. is a glycophytic naturalised legume much appreciated by farmers because it produces highly nutritive forage for livestock in nutrient-deficient soils (Hidalgo & Cauhépé 1991; Blumenthal & McGraw 1999; Mendoza 2001) and saline-sodic and heavy soils, typically present in lowland grasslands of the Flooding Pampas (García & Mendoza 2008). However, there are few reports relating the effects of defoliation (intensity and frequency) with the growth of *L. tenuis*. It has been reported that 60% and 85% defoliation intensities did not alter shoot biomass of adult plants grown in a glasshouse (Striker *et al.* 2011) or under field conditions (Vignolio *et al.* 2006), respectively. The clipping method differed between these two studies: in the glasshouse experiment, plants were clipped 4 cm above the soil surface, whereas in the field experiment the defoliation was performed leaving a radius of 6 cm of shoot from the centre of the plant, leaving some entire stems from which plants could regrow.

Because *L. tenuis* depends on mycorrhizal symbiosis to grow at low P availability in the soil (Mendoza & Pagani 1997), and a high percentage of root length is colonised at different field sites (Escudero & Mendoza 2005; García & Mendoza 2008), it is interesting to investigate *L. tenuis* responses to a wide range of defoliation intensities, how P and N are mobilised within the plant, and the association with native arbuscular mycorrhizae and *Rhizobium* bacteria in a saline-sodic soil environment. We aimed to test the following hypotheses: (i) *L. tenuis* compensates for shoot biomass removed at low to moderate defoliation intensities; however, plants are not able to compensate shoot biomass clipped at intense defoliations. We predict a lower regrowth under intense defoliation as a result of a drastic reduction of root growth and the adverse saline-sodic environment. (ii) The AM fungal strategy in response to defoliation is to reduce the nutrient transfer structures (arbuscules), the number of entry points and hyphal density in soil, but increase the resistance structures (vesicles) and spore density in soil under a higher defoliation intensity.

MATERIALS AND METHODS

Experimental set up

Soil samples were taken from the top 10-cm layer from a natural grassland located in San Vicente, Province of Buenos Aires (Argentina) in autumn. In this season, the plant community is dominated by *L. tenuis*, *Stenotaphrum secundatum* (Walt.) O.K., *Lolium multiflorum* Lam. and *Paspalum dilatatum* Poir. The soil site was classified as Typic Natraqualf; its physical–chemical characteristics were: pH 8.7; electrical conductivity 5.1 $dS{\cdot}m^{-1}$; exchangeable sodium (Na) 56%; available P 13.5 $mg{\cdot}kg^{-1}$ (Bray I); total C 1.2%; and total N 0.11% (for further details, see García & Mendoza 2007).

Seeds of *L. tenuis* cv. Chaja were surface sterilised and pregerminated in sterile conditions. Thirty closed-bottom pots of 1.6 L were filled with 770 g of air-dry soil sieved through a 2-mm mesh screen. Seven seedlings were transplanted to each pot and the soil surface was covered with 1 cm of sterilised sand to minimise water evaporation. The pots were maintained near field capacity (36%) by daily watering to constant weight. Plants were grown in a greenhouse for 105 days (mean day temperature 30 ± 3 °C, mean night temperature 19 ± 2 °C). The pots were randomised and daily rotated to minimise potential gradient effects. The mean relative humidity was 65–75% and the photoperiod length ranged from 10 to 12 h during the experimental period.

After 70 days of growth, five pots were harvested (initial time) and the remaining pots were subjected to defoliation treatments replicated five times. Defoliation treatment consisted of removing 25, 50, 75 or 100% of the aboveground biomass with respect to the pots harvested at the initial time. For the 100% treatment, the aerial biomass was clipped off 0.5 cm above the soil surface. Plants of five pots were not defoliated and used as control (0% treatment). Defoliated and non-defoliated plants were grown for an additional 35-day period (recovery period), and then the plants were harvested (final time).

Plant yield and analytical determinations in tissue

After each harvest (initial and final time), plant biomass was separated into shoots and roots. The biomass of the clipped shoots was weighed, and later included in the shoot fraction of the corresponding plants at the end of the recovery phase to determine shoot yield. Fresh shoots and roots were divided into two portions: one was oven-dried at 70 °C for 48 h to determine P, N and Na^+ concentrations and content, and the other portion was used to measure total chlorophyll in shoots and AM colonisation morphology and number of *Rhizobium* nodules in roots.

The concentration of total chlorophyll was determined in fresh shoots as described in Lichtenthaler (1987). Dry shoots and roots were digested separately in a nitric-perchloric acid mixture (3:2) to determine P using the molybdovanadophosphoric acid method, Na^+ by standard flame photometry (Benton Jones & Wernon 1990), and in sulphuric acid to determine N using the Kjeldahl method (Jackson 1964).

AM colonisation and root nodulation

Mycorrhizal root colonisation was measured in fresh roots cleared in 10% KOH for 10 min at 90 °C, and stained in 0.05% lactic acid–glycerol Trypan blue (Phillips & Hayman 1970). Twenty-five root segments per plant sample were examined under a microscope at ×200 magnification. The fraction of root length colonised (MC), or root length containing arbuscules (AC), vesicles (VC) and hyphae only (HO) was determined following McGonigle *et al.* (1990). The number of entry points was measured every 3 mm along root fragments at ×200 magnification (Amijee *et al.* 1989) and expressed as the number of entry points (EP) per mm of colonised root. Root length colonised by AM (mc), arbuscules (am), vesicles (vc) and hyphae only (ho) was determined with the line intercept method (Giovannetti & Mosse 1980). *Rhizobium* nodules were counted in whole fresh root systems under a binocular stereomicroscope (×7.5).

AM fungal inoculum

After each the initial and final harvest, soil samples of approximately 250 g were taken from the centre of each pot

to measure AM fungal spore and hyphal densities. Soil samples were kept in plastic bags at 4 °C until processed.

AM fungal spores were isolated from 30 g of soil by a modification of the sucrose gradient centrifugation technique (Daniels & Skipper 1982), and counted under ×35 magnification using a dissecting microscope. Spore density was expressed as the number of spores per gram of dry soil.

The length of external hyphae was determined after they were extracted with a procedure modified from Abbott *et al.* (1984), and measured on 150 fields per filter using the modified grid line intersect method (Giovannetti & Mosse 1980) under ×200 magnification. Hyphal density was expressed as meter of hyphae per gram of dry soil.

Relative plant growth and AM fungal colonisation rates

Relative growth rates (RGR) for shoot (RGRs) and root (RGRr) dry mass of control and defoliated plants during the recovery period (35 days) were calculated as described in Kingsbury *et al.* (1984):

$$\mathrm{RGR}_i = (ln\mathrm{W}tf - ln\mathrm{W}to)/(tf - to) \quad (1)$$

where RGR is the relative growth rate (day^{-1}) and *i* stands for any shoot or root, *tf* is the total period of growth (days) from planting, *to* is the initial period of growth at near field capacity (70 days), W*tf* is dry mass of shoot or root at the end of the whole experimental period (105 days), W*to* is the dry mass of shoot or root at the beginning of the experimental period (70 days), and (*tf*–*to*) is the difference between the initial and the final period of growth (105 days). For each clipping treatment, W*to* of the shoot is the remaining dry mass in each pot after defoliation. For comparing treatments, it was assumed that the daily growth rates were constant over the 35-day recovery period.

An expression similar to equation (1) was used to compute the relative colonisation rate of mc (RCRmc), ac (RCRac), vc (RCRvc) and ho (RCRho) for control and defoliation treatments.

Statistical analysis

Datasets were tested for normality and variance heteroscedasticity with standard methods. Non-normal data were appropriately transformed for comparing treatment means. An analysis of variance was used to test the equality of treatment means. Treatment effects from data that could not be normalised by any of the common standard transformations were tested with the Kruskal-Wallis non-parametric test. Statgraphic 5.0 plus software was used for statistical analyses.

RESULTS

Plant growth

The growth of *L. tenuis* was affected by defoliation intensity. Shoot biomass was significantly affected by defoliation intensity. Defoliation intensities ranging from 25% to 75% did not affect the total shoot biomass yielded by the non-defoliated plants (Fig. 1a). At 100% defoliation, the amount of shoots removed by clipping after 70 days of growth plus that recovered at the final harvest was about 60% smaller than total biomass in either control or 25% defoliation treatment (Fig. 1a). The RGRs of shoots consistently increased from the control to 100% defoliated plants (Fig. 1a).

Defoliation intensity also affected root growth (Fig. 1c). Root biomass decreased as defoliation intensity increased. Root biomass of 100% defoliated plants was significantly smaller than the root biomass produced by the other treatments. This reduction in root biomass was consistently reflected in a decrease in root RGR values; at 75% defoliation the root RGR value was practically zero, and at 100% defoliation it was definitely negative (Fig. 1d).

Phosphorus and N in plant tissue

The mobilisation of P and N in plant tissue was affected by defoliation intensity. The concentration of P in both shoots and roots consistently increased with defoliation intensity

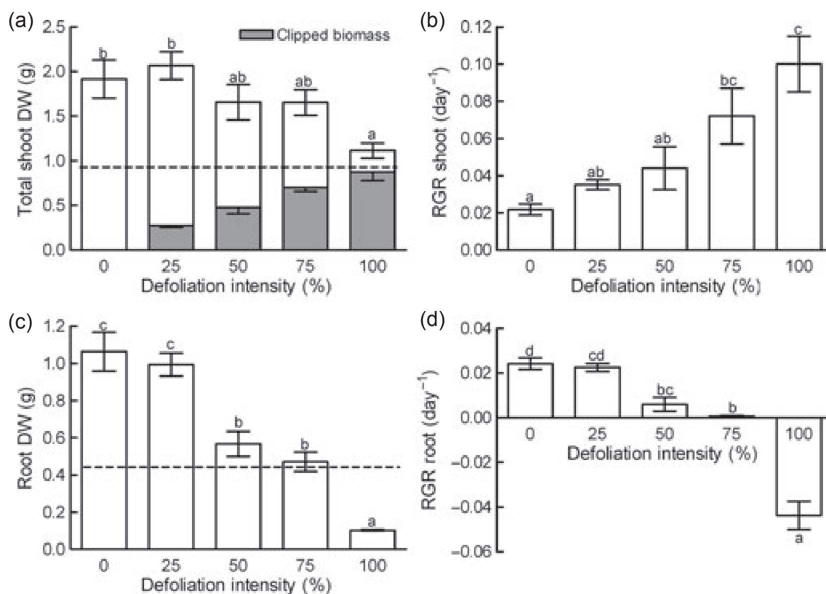


Fig. 1. Biomass of *Lotus tenuis* plants that had been subjected to different defoliation intensities from 0 to 100%. a: Shoot dry weight, b: shoot RGR, c: root dry weight, d: root RGR. The dashed line in a) and c) represents the dry biomass weight after 70 days of growth (initial time) when the defoliation treatments were applied. Grey columns in a) represent the shoot biomass removed (clipped biomass) at different defoliation intensities. Bars denote SE of means of five replicates. Different letters indicate significant differences ($P < 0.05$) between pairs of treatment means according to a Tukey test.

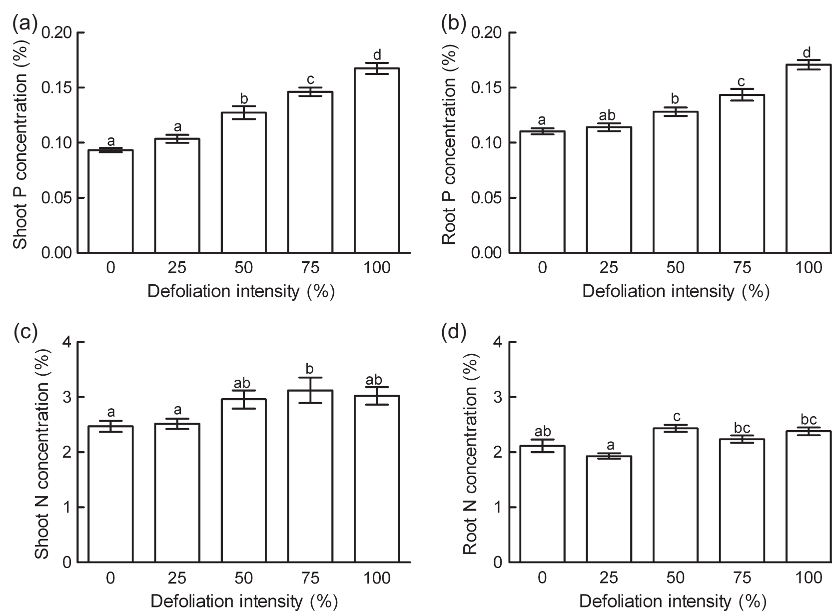


Fig. 2. Concentration of P in shoots (a), roots (b), and of N in shoots (c) and roots (d) of *Lotus tenuis* plants that had been subjected to different defoliation intensities. Bars denote standard errors of the mean for five replicates. Different letters between treatments indicate a significant difference ($P < 0.05$) according to a Tukey test. Shoot P concentrations were log transformed for this analysis.

(Fig. 2a and b). The concentration of N in shoots and roots also increased with defoliation intensity but the effect of the latter was less marked than the effect of P (Fig. 2c and d). The total P content in plant tissue during the 105-day growing period (70 + 35 days) did not change among non-defoliated and 25–75% defoliated plants (Fig. 3a). The total P content at 100% defoliation intensity was significantly lower than the 25% defoliated plants but it did not differ from the non-defoliated plants and the 50–75% defoliation treatments. The total N content was statistically the same among non-defoliated and defoliated plants up to 75% defoliation intensity, and was higher than the total N content at 100% defoliation intensity (Fig. 3b).

Sodium and chlorophyll in plant tissue

Defoliation intensity affected the Na^+ balance between shoots and roots in *L. tenuis* growing in a saline-sodic environment and was associated with a tendency to decrease the chlorophyll concentration. The concentration of Na^+ in shoots showed a marked increase only in 100% defoliated plants with respect to both control and less intensely defoliated plants (Fig. 4a). In contrast, the variation of Na^+ concentration in roots tended to decrease with increasing defoliation intensity from the control to 75% defoliated plants, and for the 100% treatment the Na^+ concentration in root tissues markedly increased (Fig. 4b). The ratio of Na^+ concentration in shoots over roots increased with increasing defoliation intensity (Fig 4c). The total Na^+ plant content consistently decreased with increasing defoliation intensity (Fig. 3c), and the chlorophyll concentration in shoots was statistically the same between non-defoliated and defoliated plants (Fig. 4d).

AM fungal variables and *Rhizobium* nodules

Defoliation intensity affected the association with native arbuscular mycorrhizae and *Rhizobium* bacteria. The intensity of defoliation did not change the proportion of root length colonised by AM (MC), the arbuscular colonisation (AC) and the hyphae-only colonisation indices (HO) (Table 1). The vesicular colonisation index (VC) decreased with increasing defoliation intensity. The total root length colonised (mc), the root length colonised by the different fungal structures (ac, *versus* and ho) and the relative colonisation rates (RCR) decreased with increasing defoliation intensity (Table 1). The largest RCR values were always observed in non-defoliated and 25% defoliated plants. The number of entry points per unit of root length colonised (EP) and the AM fungal spore density in soil did not change with increasing defoliation intensity. The number of *Rhizobium* nodules per unit of root weight significantly increased in the 100% treatment. The hyphal density in soil consistently increased with increasing defoliation intensity (Table 1).

DISCUSSION

The results of this study showed that *Lotus tenuis* plants grown in a saline-sodic environment could compensate the weight of shoot biomass removed by clipping up to 75% of the biomass of non-defoliated plants. This compensation was a consequence of higher rates of shoot growth (RGR) of defoliated compared to non-defoliated plants, and sufficient nutrient availability to sustain shoot regrowth. In general terms, our observations are consistent with previous results observed by Vignolio *et al.* (2006) and Striker *et al.* (2011). However, these two studies differ from the present experiments in several aspects: they clipped adult plants at the reproductive stage and we clipped 70-day-old plants at the vegetative stage; the substrate used for plant growth was better in terms of fertility compared to the present experiment, where a saline-sodic soil was used; and finally, how the plants were clipped in each experiment determined different amounts of remaining biomass able to compensate for the removed material. In our study, when the total aerial biomass was clipped (0.5 cm above the soil surface), the plants were not able to compensate for the growth produced by

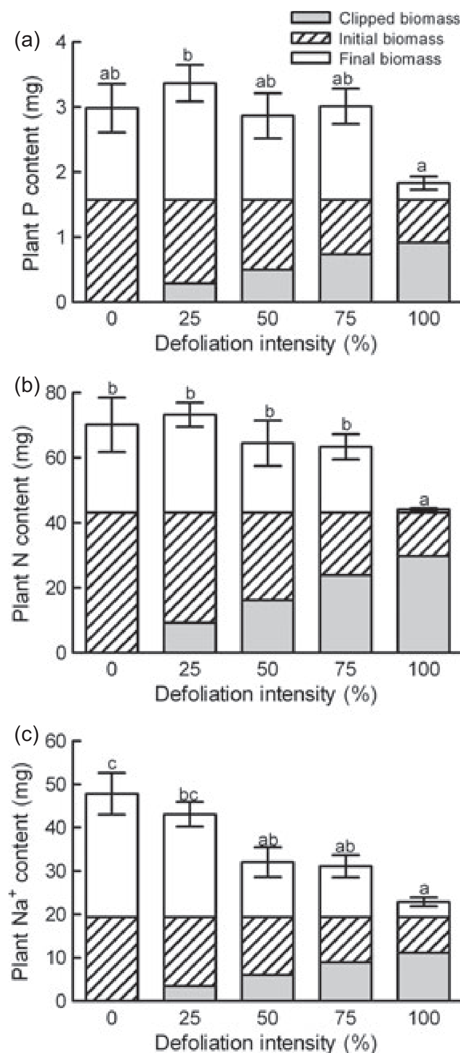


Fig. 3. Total P (a), N (b) and Na^+ (c) content in *Lotus tenuis* plants that had been subjected to different defoliation intensities. The clipped biomass represents the total P, N and Na^+ content of the shoot biomass removed at different defoliation intensities. The initial biomass represents the total P, N and Na^+ content remaining in plants after clipping at different defoliation intensities. The final biomass represents the total P, N and Na^+ content of the plants at the end of the recovery period. Bars denote SE of means of five replicates. Different letters indicate significant differences ($P < 0.05$) between treatments according to a Tukey test.

non-defoliated plants in saline-sodic conditions. The regrowth after defoliation of *L. tenuis* is mainly produced by sprouting of basal auxiliary buds of the old stems (Smith 1962). Intense defoliations determine a marked loss of nutrients, therefore shoot regrowth after drastic defoliation intensities would be dependent of the use of remnant nutrient reserves and the ability of the plant to absorb nutrients efficiently from the soil, rather than the bud sprouting. Hence, the symbiotic associations with AM fungi and *Rhizobium* bacteria are expected to be a key factor in improving nutrient uptake.

Root growth was more affected by defoliation than shoot growth. Both the biomass and the RGR of roots were drastically decreased with increasing defoliation intensities. This effect was also reflected by the decrease in the root/shoot ratio along defoliation intensities: 0.56, 0.48, 0.34, 0.28 and 0.08 for the control and the 25–100% defoliation intensities, respectively. These results suggest that the plants transfer most of the nutrient resources from the roots to the shoots to compensate the demand for regrowth. The loss of root tissue in response to defoliation is ascribed to a decrease in photosynthate production and a reallocation of C resources to the regrowth of photosynthetic tissue. The regrowth of grasses is only dependent on stored reserves for a few days following defoliation (Allsopp 1998). In legume plants, the regrowth additionally depends on C and N reserves allocated in the crown (Ourry *et al.* 1994; Avice *et al.* 1996; Striker *et al.* 2011). This may explain the capacity of *Lotus* plants to regrow under high defoliation intensities.

The P and N absorbed by the plants for growth came from different sources. Moreover, P is fully provided from inorganic soil constituents and soil organic P, while N can be additionally provided through biological fixation. The concentrations of both nutrients in shoots and roots increased with increasing defoliation intensity. Plants defoliated up to 75% of their aerial biomass were able to compensate the total P and N absorbed by the non-defoliated plants. This is associated with an internal recycling of nutrients from necrotic roots to satisfy the demands of shoot regrowth and/or an additional amount of P and N absorbed from the soil and/or N fixed by *Rhizobium*. In agreement with the first part of hypothesis (i), *L. tenuis* compensated for both the shoot biomass and the total P and N removed in low to moderate defoliations, as a result of the higher rates of shoot growth (RGR) of defoliated compared to non-defoliated plants and the sufficiency of absorbed nutrients necessary to sustain shoot regrowth.

Even accepting that *L. tenuis* is a glycophytic legume, its salt tolerance mechanism has not yet been elucidated (Sannazzaro *et al.* 2006, 2007). The primary mechanism of salinity tolerance is centred on the control of Na^+ uptake by the roots and the subsequent distribution of Na^+ in plant tissue to minimise the amount of Na^+ transported to shoots (Plett & Møller 2010). In the current experiment, the concentration of Na^+ in roots was always higher than in the shoots for both control and moderately defoliated plants. Sannazzaro *et al.* (2006), who worked with sensitive and tolerant *L. tenuis* genotypes stressed with NaCl solution, also observed higher concentrations of Na^+ in roots than in shoots. In both studies, the results are consistent with a salinity tolerance mechanism that controls increases of Na^+ in shoot tissue. However, in the present work and at high defoliation intensity, the Na^+ concentration in roots and shoots markedly increased with respect to the other defoliation intensities. It was reported that in plants growing in saline soils, active efflux of Na^+ from the roots to the soil solution may occur at significant rates but may be insufficient to counterbalance the passive unidirectional Na^+ influx from the soil solution to the roots (Jacoby & Hanson 1985; Davenport *et al.* 1997). We propose that in the cases of non-defoliated to moderately defoliated plants, there is a mechanism whereby an increase of Na^+ concentration in shoots is prevented by a reduction in Na^+ uptake by roots from the soil solution and a subsequent reduction of Na^+ transport from roots to shoots through an increase in the amount of Na^+ accumulated by the root

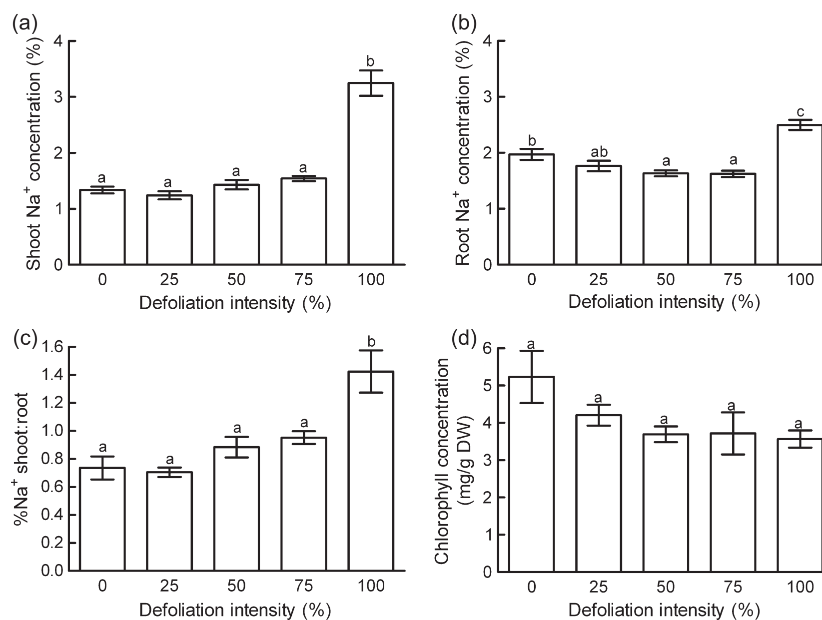


Fig. 4. Concentration of Na^+ in shoots (a) and roots (b), ratio of Na^+ concentration in shoots over roots (c), and shoot chlorophyll concentration (d) in *Lotus tenuis* plants that had been subjected to different defoliation intensities. Bars denote SE of the mean of five replicates. Different letters between treatments indicate a significant difference ($P < 0.05$) according to a Tukey test. Shoot Na^+ concentrations were log-transformed for this analysis.

Table 1. Effect of different defoliation intensities on AM fungal variables and number of *Rhizobium* nodules in roots of *Lotus tenuis* plants. Mean values of five replicates ± SE. For each row different letters indicate a significant difference ($P < 0.05$) between treatments according to the Tukey test. *ln* = log-transformed data.

	defoliation intensity				
variable	0%	25%	50%	75%	100%
AM fungal variables					
MC index	0.94 ± 0.01 a	0.93 ± 0.01 a	0.94 ± 0.01 a	0.95 ± 0.01 a	0.90 ± 0.03 a
AC index	0.66 ± 0.05 a	0.66 ± 0.02 a	0.71 ± 0.02 a	0.77 ± 0.01 a	0.75 ± 0.04 a
VC index	0.19 ± 0.03 c	0.11 ± 0.03 bc	0.08 ± 0.02 bc	0.07 ± 0.01 b	0.05 ± 0.01 a
HO index	0.09 ± 0.02 a	0.16 ± 0.02 a	0.13 ± 0.02 a	0.10 ± 0.01 a	0.09 ± 0.02 a
mc (m)	151.16 ± 10.58 c	123.98 ± 9.35 bc	94.98 ± 9.03 b	89.44 ± 6.19 b	17.91 ± 2.30 a
ac (m)	106.65 ± 13.22 b	87.80 ± 8.44 b	72.50 ± 8.37 b	72.44 ± 4.97 b	15.23 ± 2.47 a
vc (m) *ln*	30.03 ± 5.36 c	14.29 ± 3.62 bc	8.69 ± 2.01 b	7.13 ± 1.24 b	0.97 ± 0.28 a
ho (m)	13.48 ± 2.92 bc	21.89 ± 3.62 c	13.04 ± 1.79 bc	9.69 ± 1.46 ab	1.71 ± 0.23 a
EP (per mm root)	4.88 ± 0.40 a	5.29 ± 0.37 a	6.00 ± 0.40 a	6.15 ± 0.14 a	5.71 ± 0.45 a
Spore density (per g soil)	59.26 ± 5.16 a	61.01 ± 9.10 a	69.23 ± 7.28 a	57.68 ± 5.57 a	56.18 ± 4.38 a
Hyphal density ($m.g^{-1}$ dry soil)	10.43 ± 0.55 a	11.15 ± 0.36 a	12.45 ± 0.20 ab	14.31 ± 1.18 b	14.35 ± 0.51 b
Relative colonisation rates					
RCR mc (day^{-1})	0.024 ± 0.002 c	0.018 ± 0.002 bc	0.010 ± 0.002 b	0.009 ± 0.002 b	−0.037 ± 0.004 a
RCR ac	0.019 ± 0.003 b	0.014 ± 0.003 b	0.009 ± 0.003 b	0.009 ± 0.002 b	−0.036 ± 0.005 a
RCR vc	0.051 ± 0.005 c	0.028 ± 0.008 bc	0.014 ± 0.009 b	0.011 ± 0.005 b	−0.048 ± 0.008 a
RCR ho	0.012 ± 0.006 bc	0.026 ± 0.006 c	0.012 ± 0.004 bc	0.004 ± 0.004 b	−0.045 ± 0.003 a
Rhizobium nodules					
Nodules (per g fresh root)	10.99 ± 1.45 ab	6.00 ± 1.53 a	7.82 ± 2.20 a	4.66 ± 0.92 a	17.63 ± 2.63 b

system. At high defoliation intensity, the salinity tolerance mechanism is altered and the Na^+ concentration in shoots became higher than the concentration in roots.

The increase of Na^+ concentration in shoot tissue at high defoliation intensity was associated with a tendency to decrease the chlorophyll concentration. The leaves of heavily defoliated plants were chlorotic compared to those of non-defoliated or low to moderately defoliated plants. This suggests that salt interferes with chlorophyll synthesis, as reported by Cantrell & Linderman (2001). It is known that Na^+ accumulation in plant cells decreases photosynthesis, thereby reducing transportation of carbohydrates to young leaves and roots (Feng *et al.* 2002), and may affect the capacity of *Lotus* plants to recover the defoliated biomass. The results of plant growth and nutrient uptake at the highest defoliation intensity are consistent with the second part of hypothesis (1), and *Lotus* plants were not able to compensate for clipped shoot biomass as a result of a drastic reduction of root growth and adverse saline-sodic environment.

The effects of defoliation on roots colonised by AM fungi yielded controversial results. Some studies have shown that defoliation can either positively, negatively or not effect AM colonisation. In the present work, defoliation intensity did not affect the fractions of root colonised by AM fungi (MC), arbuscules (AC), hyphae only (HO) or number of *Rhizobium* nodules per unit of root weight, and the colonised root length of these AM morphological structures showed changes associated with a decrease of root mass observed with increasing defoliation. Arbuscules constitute the primary sites of nutrient and C exchange between the symbionts (Smith & Read 2008). The high proportion of roots colonised by arbuscules recorded in this work (ranging from 66% to 77%) suggests that *Lotus* plants and AM fungi may establish a functional symbiosis even when the plants have suffered intensive defoliation in a saline-sodic soil conditions. The vesicular colonisation significantly decreased from 42% to 73% for the 25–100% defoliated plants, respectively, compared to the non-defoliated plants. In addition, both the number of entry points per unit of root length and the spore density in soil did not change during the experiment, whereas the length of the extraradical hyphae increased with increasing defoliation intensity. Vesicle formation is associated with storage structures (García & Mendoza 2007, 2008); it has been reported that the mobilised carbohydrates and lipids are stored in vesicles to form structures like spores (Bentivenga & Hetrick 1992). In the present work, we observed a high proportion of roots colonised by arbuscules but a decrease in vesicle formation with increasing defoliation intensity. Crawford *et al.* (2000) used labelled ^{14}C to show that net C translocation from shoots to roots was very low and almost absent after defoliation, and suggested that the newly assimilated C is used solely for shoot regrowth. Hence, the decrease of C flow to the roots and consequently to the AM fungi may alter the formation of intra- and extraradical fungal structures. In disagreement with hypothesis (2), the results suggest that the strategy of the AM fungal symbiont consists in investing more of the C resources to preferentially maintain the arbuscular colonisation and the inoculum density in soil by exporting C compounds to retain extraradical structures such as spores and the hyphal network. The increase in the external hyphal network would contribute to compensate for the losses of root absorptive area in defoliated plants (Allsopp 1998). This may partly explain why defoliated plants were able to compensate the total P and N content during the regrowth period with respect to non-defoliated plants.

ACKNOWLEDGEMENTS

Thanks are due to Ing. Agr. H. D. Ginzo and Ing. Agr. D. H. Cogliatti for their valuable comments on the manuscript and review of the English text.

REFERENCES

Abbott L.K., Robson A.D., De Boer G. (1984) The effect of phosphorus on the formation of hyphae in soil by the vesicular–arbuscular mycorrhizal fungus, *Glomus fasciculatum*. *New Phytologist*, **97**, 437–446.

Allsopp N. (1998) Effect of defoliation on the arbuscular mycorrhizas of three perennial pasture and rangeland grasses. *Plant and Soil*, **202**, 117–124.

Amijee F., Tinker P.B., Stribley D.P. (1989) The development of endomycorrhizal systems. VII. A detailed study of the effects of soil phosphorus on colonization. *New Phytologist*, **111**, 435–446.

Avice J.C., Ourry A., Lemarie G., Boucaud J. (1996) Nitrogen and carbon flows estimated by ^{15}N and ^{13}C pulse-chase labeling during regrowth of alfalfa. *Plant Physiology*, **112**, 281–290.

Barto E.K., Rilling M.C. (2010) Does herbivory really suppress mycorrhiza? A meta-analysis. *Journal of Ecology*, **98**, 745–753.

Benthlenfalvay G.J., Dakessian S. (1984) Grazing effects on mycorrhizal colonization and floristic composition of the vegetation on a semiarid range in Northern Nevada. *Journal of Range Management*, **37**, 312–316.

Bentivenga S.P., Hetrick B.A.D. (1992) Seasonal and temperature effects on mycorrhizal activity and dependence of cool and warm season tallgrass prairie grasses. *Canadian Journal of Botany*, **70**, 1596–1602.

Benton Jones J. Jr, Wernon W.C. (1990) Sampling, handling and analyzing plant tissue samples. In: Westerman R.L. (Ed.), *Soil Testing and Plant Analysis*. Soil Science Society of America Book Series. Soil Science Society of America, Madison, WI, USA, pp. 389–427.

Blumenthal M.J., McGraw R.L. (1999) Lotus adaptation, use and management. In: Beuselinck P.R. (Ed.), *Trefoil: The Science and Technology of Lotus*. American Society of Agronomy Inc. & Crop Science Society of America Inc, Wisconsin, USA, pp. 97–119.

Cantrell I.C., Linderman R.G. (2001) Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant and Soil*, **233**, 269–281.

Crawford M.C., Grace P.R., Oades J.M. (2000) Allocation of C to shoots, roots, soil and rhizosphere respiration by barrel medic (*Medicago truncatula*) before and after defoliation. *Plant and Soil*, **227**, 67–75.

Crawley J.J. (1997) Plant–herbivore dynamics. In: Crawley M.K. (Ed.), *Plant Ecology*, 2nd edition. Blackwell Science, Oxford, UK, pp. 401–474.

Daniels N., Skipper H. (1982) Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck N.C. (Ed.), *Methods and Principles of Mycorrhizal Research*. American Phytopathology Society, St. Paul, MI, USA, pp. 29–35.

Davenport R.J., Reid R., Smith F. (1997) Sodium–calcium interactions in two wheat species differing in salinity tolerance. *Physiologia Plantarum*, **99**, 323–327.

Davidson J.L., Milthorpe F.L. (1966) The effect of defoliation on the C balance in *Dactylis glomerata*. *Annals of Botany*, **30**, 185–198.

Eom A.H., Wilson G.W.T., Hartnett D.C. (2001) Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. *Mycologia*, **93**, 233–242.

Escudero V.G., Mendoza R.E. (2005) Seasonal variation of arbuscular mycorrhizal fungi in temperate grasslands along a wide hydrologic gradient. *Mycorrhiza*, **15**, 291–299.

Feng G., Zhang F.S., Li X.L., Tian C.Y., Tang C., Rengel Z. (2002) Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza*, **12**, 185–190.

García I., Mendoza R. (2007) Arbuscular mycorrhizal fungi and plant symbiosis in a saline-sodic soil. *Mycorrhiza*, **17**, 167–174.

García I., Mendoza R. (2008) Relationships among soil properties, plant nutrition and arbuscular mycorrhizal fungi–plant symbioses in a temperate grassland along hydrologic, saline and sodic gradients. *FEMS Microbiology Ecology*, **63**, 359–371.

Gehring C.A., Whithan T.G. (1994) Interactions between aboveground herbivores and the mycorrhizal mutualist plants. *Trends in Ecology and Evolution*, **9**, 251–255.

Gill R.A. (2007) Influence of 90 years of protection from grazing on plant and soil processes in the subalpine of the Wasatch Plateau, USA. *Rangeland Ecology and Management*, **60**, 88–98.

Giovannetti M., Mosse B. (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, **84**, 489–500.

Hidalgo L., Cauhépé M.A. (1991) Effects of seasonal rest in above-ground biomass for a native grassland of the Flooding Pampa, Argentina. *Journal of Range Management*, **44**, 471–475.

Hokka V., Mikola J., Vesterg M., Setälä H. (2004) Interactive effects of defoliation and an AM fungus on plants and soil organisms in experimental legume–grass communities. *Oikos*, **106**, 73–84.

Jackson M.L. (1964) *Soil Chemical Analysis*. Prentice Hall, Englewood Cliffs, NJ, USA, pp. 498.

Jacoby B., Hanson J.B. (1985) Controls on Na^{+}-^{22} influx in corn roots. *Plant Physiology*, **77**, 930–934.

Kingsbury R.W., Epstein E., Peary R.W. (1984) Physiological responses to salinity in selected lines of wheat. *Plant Physiology*, **74**, 417–423.

Klironomos J.N., McCune M., Moutoglis P. (2004) Species of arbuscular mycorrhizal fungi affect

mycorrhizal responses to simulated herbivory. *Applied Soil Ecology*, **26**, 133–141.

Kula A.R.R., Hartnett D.C., Wilson G.W.T. (2005) Effects of mycorrhizal symbiosis on tallgrass prairie plant–herbivore interactions. *Ecology Letters*, **8**, 61–69.

Lichtenthaler H.K. (1987) Chlorophylls and carotenoids: pigments of photosynthetic membranes. *Method in Enzymology*, **148**, 350–382.

McGonigle T.P., Miller M.H., Evans D.G., Fairchaild G.L., Swan J.A. (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, **115**, 495–501.

Mendoza R.E. (2001) Phosphorus nutrition and mycorrhizal growth response of broadleaf and narrowleaf birdsfoot trefoils. *Journal of Plant Nutrition*, **24**, 1–12.

Mendoza R., Pagani E. (1997) Influence of phosphorus nutrition on mycorrhizal growth response and morphology of mycorrhizae in *Lotus tenuis*. *Journal of Plant Nutrition*, **20**, 625–639.

Menoyo E., Renison D., Becerra A. (2009) Arbuscular mycorrhizas and performance of *Polylepis australis* trees in relation to livestock density. *Forest Ecology and Management*, **258**, 2676–2682.

Ourry A., Kim T.H., Boucaud J. (1994) Nitrogen reserve mobilization during regrowth of *Medicago sativa* L.: relationships between availability and regrowth yield. *Plant Physiology*, **119**, 1423–1435.

Paige K.N. (1992) Overcompensation in response to mammalian herbivory: from mutualistic to antagonistic interactions. *Ecology*, **73**, 2076–2085.

Phillips J.M., Hayman D.S. (1970) Improved procedure for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions the British Mycological Society*, **55**, 158–161.

Plett D.C., Møller I.S. (2010) Na^+ transport in glycophytic plants: what we know and would like to know. *Plant, Cell and Environment*, **33**, 612–626.

Sannazzaro A.I., Ruiz O.A., Albertó E.O., Menéndez A.B. (2006) Alleviation of salt stress in *Lotus glaber* by *Glomus intraradices*. *Plant and Soil*, **285**, 279–287.

Sannazzaro A.I., Echeverría M., Albertó E.O., Ruiz O.A., Menéndez A.B. (2007) Modulation of polyamine balance in *Lotus glaber* by salinity and arbuscular mycorrhiza. *Plant Physiology and Biochemistry*, **45**, 39–46.

Smith D. (1962) Carbohydrate root reserves in alfalfa, red clover, and birdsfoot trefoil under several management schedules. *Crop Science*, **2**, 75–78.

Smith S.E., Read D.J. (2008) *Mycorrhizal Symbiosis*, 3rd edition. Academic Press, London, UK, pp. 815.

Striker G.G., Manzur M.E., Grimoldi A.A. (2011) Increasing defoliation frequency constrains regrowth of the forage legume Lotus tenuis under flooding. The role of crown reserves. *Plant and Soil*, **343**, 261–272.

Vignolio O.R., Fernández O.N., Castaño J. (2006) Responses of *Lotus glaber* (Leguminosae) cv. Chajá to defoliation in reproductive stage. *Annals Botanici Fennici*, **43**, 284–287.

Walling S.Z., Zabinski C.A. (2006) Defoliation effects on arbuscular mycorrhizae and plant growth of two native bunchgrasses and an invasive forb. *Applied Soil Ecology*, **32**, 111–117.