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Different canopy openings affect underground traits in herbaceous plants of a southern forest in Patagonia

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Abstract

Aims
Forest canopy openings modify the natural environment, producing changes in light quality and intensity, precipitation and temperature. In turn, these changes promote the acclimation of understory species. However, little work has been done on underground responses to those environmental changes. The objective of this work was to determine how *Osmorhiza depauperata*, *Phleum alpinum* and *Poa pratensis* change its root length density and root colonization by mycorrhiza as a function of light availability in a *Nothofagus pumilio* (i.e. lenga) forest harvested following the variable retention prescription.

Methods
We selected three microenvironments in an old growth forest harvested by the variable retention prescription: aggregated retention, dispersed retention with influence of aggregated retention and dispersed retention. A non-harvested primary forest (PF), similar to the harvested one, was used as a control. Every 2 months, from October 2008 to April 2009, we took soil cores from randomly selected plants. From these soil cores, root length density and colonization percentage (CP) by arbuscular mycorrhizae were estimated.

Important Findings
Light availability changed significantly among the microenvironments. In general, root length density was significantly greater in *P. pratensis* than in *P. alpinum* and both species greater than in *O. depauperata*. Light availability increased root length density in all species, although the magnitude of these increases difference among species. Root length density was 187% greater in *P. pratensis*, 101% in *P. alpinum* and 94% in *O. depauperata* in the disperse retention system than in the PF. Mycorrhiza CP was higher in *O. depauperata* than in *P. alpinum* and *P. pratensis*. Also, it was lower in the PF than in the harvested microenvironments. CPs were very low.

Keywords: root length density, mycorrhizal colonization, light shade, understory, Tierra del Fuego

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INTRODUCTION

The forest canopy opening, whether it is from a natural or anthropic origin, produces a modification of the natural environment mainly changing light quality and intensity, precipitation and temperature (Martínez-Pastur *et al.* 2011; Promis *et al.* 2010). Species that are favoured by the canopy openings are those that can acclimate to the new conditions and compete with the other species present in the plant community (Aussenac 2000; Kursar and Coley 1999).

Roots are an essential organ for the acquisition of water and nutrients (Larcher 2003). Soil occupation, of great importance to exploit nutrient resources, depends on traits such as root length (Busso and Bolletta 2007). Root length density is a very precise parameter to estimate the spatial and temporal patterns of water and nutrient acquisition (Yoder *et al.* 1995). This is because fine roots (≤2 mm diameter) are the most active in the acquisition of these soil resources.

In most plant species, the proportion of roots increases with light availability (Bruna and de Andrade 2011; Elemans

2004; McConnaughay and Coleman 1999). Root proliferation and the active acquisition of soil nutrients and symbiotic mycorrhizae consume energy, which comes lastly from photosynthetic organs (Cui and Caldwell 1997). Therefore, light availability limitations (i.e. due to shading) can reduce root length density. On the other hand, mycorrhizae can contribute with a great share of water and nutrients to the plant. The fungus contributes with low mobility nutrients in the soil, like nitrogen (as NH_4^+), phosphorus, copper and zinc (Koltai and Kapulnik 2010).

There are few studies of root length density in understory species (Bakker *et al.* 2004; Fukuzawa *et al.* 2006; McGuire *et al.* 2001). However, they did not work with different light availabilities with the exception of McGuire *et al.* (2001). Reductions in light availability can limit the plant capacity to keep large mycorrhizal associations (Tester *et al.* 1986). Given that tree canopies drastically reduce the amount of light that reaches the understory, the subsequent limitation of carbon for understory plants could negatively affect their colonization by arbuscular mychorrizae.

Recently, a variable retention harvesting technique was implemented in the forests of *N. pumilio* (i.e. lenga) in Tierra del Fuego. This technique creates a variety of microenvironments from a relatively homogeneous primary forest (PF). It allows maintaining the structure and biodiversity of the original forest, while favouring the forest regeneration (Lencinas *et al.* 2008, Lencinas *et al.* 2009, 2011; Martínez-Pastur *et al.* 2009; Martínez-Pastur *et al.* 2011). Nevertheless, there is little information about the requirements, tolerance levels and ecophysiology of the understory species in the forests of southern Patagonia (Martínez Pastur *et al.* 2007a). Modification of the environmental variables in the forests of this region would contribute to determine the optimal conditions for growth of the understory vegetation, and evaluate its competitive ability when competing with the natural forest regeneration. Competitive ability is especially important in species that show a positive response to forest canopy openings (i.e. they can constrain both the establishment and growth of tree seedlings, and therefore have a critical role in determining community structure and ecosystem functioning). Among the species that predominate in the understory during the entire forest management cycle in the southern forests of Tierra del Fuego are *Phleum alpinum* L., *Poa pratensis* L. and *Osmorhiza depuperata* Philippi (Lencinas *et al.* 2011; Martínez Pastur *et al.* 2002).

These species have contrasting growth forms: while *P. alpinum* and *P. pratensis* are grasses, they differ in their vegetative growth form. *P. pratensis* produces a large number of long rhizomes and numerous roots. On the other hand, *P. alpinum* sometimes produces short rhizomes, but there is little detail about its root system. In contrast, *O. depauperata* is a geophyte herb. It has a short rhizome that develops the root system. A previous study showed that *P. alpinum* was more plastic than *O. depauperata* allocating underground resources (Selzer *et al.* 2008). We included *P. pratensis* in this study because it has a great potential to cover large areas and can hamper growth of other species (Bosy and Reader 1995).

The objective of this study was to determine underground acclimation of three species in a lenga forest harvested by the variable retention prescription. We measured differences in root length density and mycorrhiza root colonization, among *O. depauperata*, *P. alpinum* and *P. pratensis*. The working hypothesis is that as light availability increases, root length density and mycorrhizal colonization of the study herbaceous species also increase. However, we expect that *O. depauperata* will have smaller root length density, and will respond less strongly to environmental changes than the two grasses because it is a typical understory species (Moore 1983).

MATERIALS AND METHODS

Study site

The study site was at Los Cerros Ranch in the Isla Grande de Tierra del Fuego (Argentina) (54°18′ S, 67°49′W). An old growth forest was harvested following a variable retention prescription: it consisted of an aggregate retention (a 30-m radius aggregate per hectare; i.e. an island of unharvested forest) and a dispersed retention (10–15 m^2 tree basal area ha^{-1}, homogenously distributed among aggregates; i.e. a harvested area with remnant trees) (Fig. 1).

Climate

It is characterized by short, cool summers and long, snowy winters (Martínez-Pastur *et al.* 2009; 2011). Only 3 months yearly are free of below zero temperatures, and the growing season for lenga is about 5 months (Barrera *et al.* 2000). Martínez Pastur *et al.* (2007b) reported the effect of forest harvesting (Table 1).

In similar forests, under shelterwood forestry, the photosynthetically active photon flux density varied from 1429 (53.1) to 1900 (23) mmol m^{-2} throughout the growing season (Caldentey *et al.* 2008).

Soil

Soils in the study area are shallow, with low to moderate degree of evolution, and can be classified as endoleptic Cambisols. The O horizon is 10 cm depth and has a high content of organic matter. The A horizon is greatly influenced by the organic matter, more or less humified, from the O horizon. Soil texture has been reported as clay loam (Oro Castro, 2014). This study also showed that there were no differences in all soil macronutrients among the different microenvironments except in total P. However, there were no differences in available P. These soils are poor in N (6.2–7.9 g N_{total} kg^{-1} soil) and rich in P (1.4–2.6 g P_{total} kg^{-1} soil).

Experimental design

During the spring of 2008, five aggregates were chosen from a forest harvested 5 years ago, according to the variable retention prescription (Fig. 1). In each aggregate, three microenvironments were selected: (i) inside the aggregate (AR), (ii) in the dispersed retention with influence of the aggregate, i.e. within 30 m from the closest aggregate (DRAR) and (iii) in the

dispersed retention (DR). In addition, five randomly chosen sites were delimited inside an unharvested, PF, which had similar characteristics to the site where the harvesting treatment was applied. Two plants of each of the three study species were randomly selected at each of the study microenvironments ($n = 10$). Unfortunately, some of these plants were lost because of tree falling (over them) during the experiment.

Studied species

Osmorhiza depauperata Philippi (Apiaceae) is a native perennial geophyte, rhizomatous herb (Moore 1983); it has bipinnate leaves. It is antitropically distributed between North and South America (Lowry and Jones 1984). It is usually found growing inside the forest, although it is occasionally found in open spaces (Moore 1983).

Phleum alpinum L. is a native species, often with short rhizomes. It is small sized, glabrous and glaucous. It is a psicromesophyte, adapted to boreal, austral and alpine climates (Moore 1983). As *O. depauperata*, it has an antitropical distribution, but in the Northern Hemisphere, it has a wide circumartic and boreal alpine distribution (Heide and Solhaug 2001). It is usually found growing in open *Nothofagus antartica* forests, *Chiliotrichum* scrubs and montane grasslands (Moore 1983).

Poa pratensis is also a perennial grass, laxly caespitose with long rhizomes; it was introduced from Europe (Moore 1983). It is a mesophyte, sensitive to drought. It is native to Eurasia. This species' litter can inhibit the germination and growth of other species (Bosy and Reader 1995). It is usually found growing in moist grasslands, open scrubs, *Empetrum* heaths, open *Embotrhrium* and deciduous forests (Moore 1983).

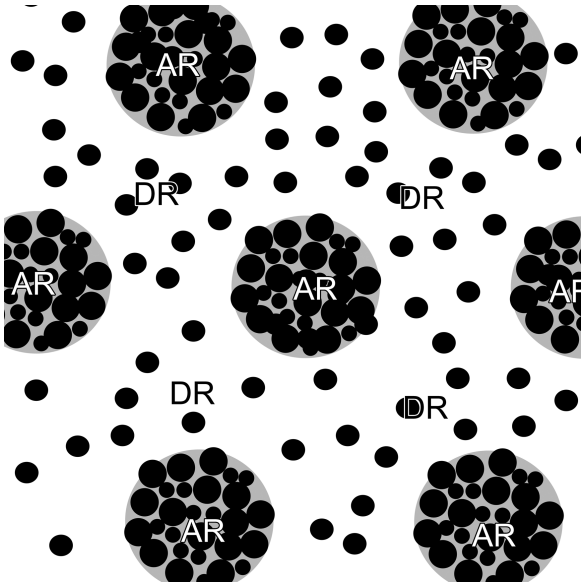

Figure 1: diagram of a forest harvested following a variable retention prescription. AR: aggregated retention, DR: dispersed retention.

Light measurements

Incident light was measured at plant height in each treatment using a ceptometer (AccuPAR LP-80, Decagon Devices, Pullman, Michigan, USA). In February 2009, 30 measurements were taken per light treatment on clear sky days. Results are expressed as percentages of the total incident light outside the forest.

Root length density

From October 2008 to April 2009, the first 10 cm soil depth from the soil surface were sampled using a 2.5 cm diameter corer. Sampling was conducted from the periphery to the centre of each plant at a 30° angle from the vertical to the soil

Table 1: climatic characterization in the primary forest (PF) and harvested stands [aggregated retention (AR) and dispersed retention (DR)]: air (T°C) and soil temperatures (S°C) at 30 cm depth, mean wind speed (m s^{-1}) and rainfall (mm) (reproduced from Martínez Pastur *et al.* 2007a)

Environment	Jul	Agu	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
PF												
T°C	−1.2	0.5	2.7	5.2	7.1	8.1	9.3	8.8	7.5	4.3	1.6	−2.6
S°C	0.3	0.5	1.4	4.0	5.6	6.6	8.0	8.0	7.3	5.3	3.3	1.0
Wind	0.0	0.1	0.3	0.4	0.4	0.4	0.3	0.3	0.6	0.8	0.2	0.2
Rainfall	33.0	37.4	36.8	30.9	36.9	25.8	33.8	40.6	13.9	50.9	38.0	62.3
AR												
T°C	−1.0	0.8	2.7	5.9	7.2	8.2	9.3	9.2	7.7	4.3	1.7	−2.5
S°C	0.1	0.4	1.3	3.9	5.6	7.0	8.2	8.5	7.8	5.6	3.5	0.8
Wind	0.5	0.8	0.9	2.0	1.9	1.7	1.1	1.1	1.7	1.6	0.7	0.4
Rainfall	30.8	36.8	37.2	31.8	36.5	27.2	32.7	39.5	16.1	48.9	42.0	64.3
DR												
T°C	−1.0	0.9	2.7	6.1	7.5	8.7	9.4	9.3	7.9	4.2	1.8	−2.5
S°C	−0.1	0.2	1.3	3.9	5.6	7.4	8.4	10.4	9.0	6.0	3.6	0.4
Wind	1.9	2.2	2.3	3.4	3.2	2.2	1.5	1.6	1.9	2.2	1.7	2.0
Rainfall	46.1	39.0	43.1	37.8	49.6	30.2	48.2	60.6	30.8	52.0	48.4	73.6

surface. Plants were sampled four times, every other month, to avoid affecting plant performance. Soil gravel limited sampling depth. Nevertheless, Boeker (1974) found that 90% of the radical biomass of various turfgrass species and cultivars was concentrated in the first few centimetres from the soil surface. After sampling, soil cores were frozen at −18°C until further processing in the laboratory.

First, soil was separated from roots using a 35-m screen (0.5 mm equivalent). Thereafter, roots were washed very carefully to remove any remaining soil. Roots were subsequently spread on glass surfaces and scanned using a flatbed scanner. Root images were analysed with the Rootedge software (Kaspar and Ewing 1997) to determine the total root length on each sample. Root length density (RLD, cm of root per cm^{-3} of soil) was calculated dividing the total root length on each sample by the soil core volume (49.08 cm^3).

Mycorrhizae

Sampled roots were also used to estimate the colonization percentage (CP) by arbuscular mycorrhiza following Phillips and Hayman (1970). Roots were cut into 20 mm segments, cleared and stained for determination of mycorrhizal colonization at 100–400× magnification (Giovannetti and Mosse 1980). Three fields on each of thirty 20 mm-root segments were scored for presence or absence of hyphae, vesicles and arbuscules, and the mycorrhizal CP for each plant was calculated as follows:

$$CP = CF/TF$$

Where CF is the number of colonized fields and TF is the total number of observed fields. Ten replicates were utilized for each species, sampling date and study site (i.e. AR, DRAR, DR and PF).

Basal area and shoot/tiller number

Basal area was estimated by either using the diameter in plants that were small enough or by measuring the semi-major axis and assuming that it can be approximated to an oval.

We counted the number of shoots (in *O. depauperata*) and tillers per plant in *P. alpinum* or area in *P. pratensis*. Therefore, all number are expressed as numbers per area.

Statistical analysis

Light measurements were analysed with a simple ANOVA, after transforming the variable (x) to $\arcsin(\sqrt{x})$ (Zar 1996). RLD and CP were analysed with three-way mixed model ANOVA with species, sampling dates and study sites as fixed factors, and plants as a random factor. RLD was transformed to $\log(x+1)$, to get rid off heterocedasticity. CP was transformed to $\arcsin(\sqrt{x})$ before statistical analysis (because it was a percentage). Tukey's test was used when significant differences were detected. Pearson correlation test was done on shoot and tiller number and RLD. Additionally, we tested whether RLD/shoot-tiller number ratio differed in species or environments. Back-transformed values are reported in Tables and Figures. The statistical software R (R Core Team 2012) with the packages nlme (Pinheiro and Bates 2000), multcomp (Hothorn *et al.* 2008) and ggplot2 (Wickham 2009) was used to perform all analysis and graphics.

RESULTS

Light measurements

Light intensities differed significantly among study sites ($F_{3;189} = 163.6$; $P < 0.0001$; Table 2). They followed the order: RD > BRA > RA > BP.

RLD

We found multiple interactions: species × study sites ($F_{6;85} = 4.64$; $P = 0.0004$); study sites × sampling dates ($F_{9;176} = 2.95$; $P = 0.0027$), and species × sampling dates ($F_{6;176} = 4.99$; $P = 0.0001$; Table 3). The difference between sites with the highest (DR) and lowest (PF) RLD was greater in *P. pratensis* (187%) than in *P. alpinum* (101%) and *O. depauperata* (94%) (Fig. 2). In *P. pratensis*, RLD was significantly greater in DR and DRAR than in PF and AR (Fig. 2); *P. alpinum* followed the same pattern, but RLD was not significantly

Table 2: light radiation percentages (as compared with values measured outside the forest) on a clear day in February at the different environments

Environment	LCL (%)	Mean	UCL (%)
PF	1.98	2.81 a	3.78
AR	28.35	30.85 b	33.40
DRAR	55.12	57.83 c	60.52
DR	87.99	89.71 d	91.31

Abbreviations: AR = aggregated retention; DRAR = dispersed retention with influence of aggregated retention; DR = dispersed retention, PF = primary forest.
$N = 120$. LCL, UCL: lower and upper 95% confidence limit. Different letters indicate significant differences with the Tukey test ($P < 0.05$).

Table 3: linear mixed effects three-way ANOVA with environment (PF, AR, DRAR, AR), species (*Osmorhiza depauperata*, *Phleum alpinum* and *Poa pratensis*) and month (October, December, February, April) as fixed effects and plants as a random effect

Factor	Root length density ($cm\ cm^{-3}$)	Colonization percentage (%)
Environment (3; 85)	25.68 (<0.0001)	14.06 (<0.0001)
Species (2; 85)	77.44 (<0.0001)	5.17 (0.0068)
Month (3; 176)	36.85 (<0.0001)	39.30 (<0.0001)
Environment × Species (6; 85)	4.64 (0.0004)	1.18 (0.3197)
Environment × Month (9; 176)	2.96 (0.0027)	0.86 (0.5591)
Species × Month (6; 176)	4.99 (0.0001)	0.74 (0.6151)
Environment × Species × Month (1; 176)	0.68 (0.8301)	1.22 (0.2520)

In parenthesis are the degrees of freedom (numerator and denominator) for factors and their interactions. Figures in the same row are F values, and their P values are shown in parenthesis. For each variable, values are the mean of $n = 67–120$.

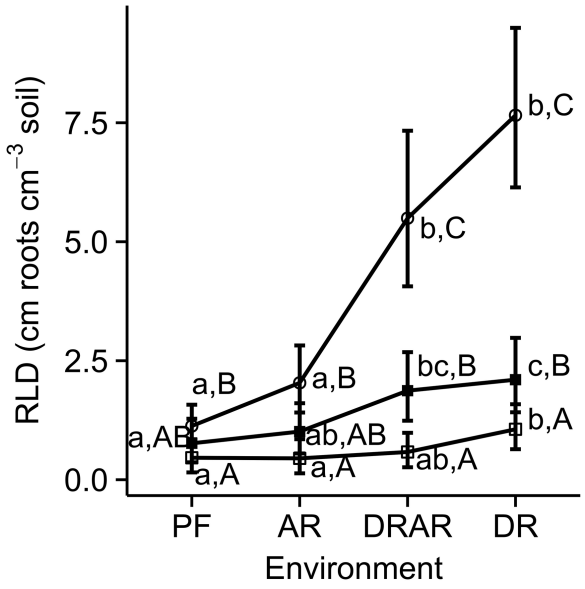

Figure 2: root length density of *Osmorhiza depauperata* (open squares), *Phleum alpinum* (closed squares) and *Poa pratensis* (open circles) during a growing season (from October 2008 to April 2009) in four forest microenvironments. These microenvironments were as follows: PF, primary forest; AR, aggregated retention; DRAR, dispersed retention with influence of aggregated retention and DR, dispersed retention. Lowercase letters represent significant differences among microenvironments and uppercase letters indicate significant differences among species. Tukey test ($P < 0.05$) was used to determine differences among means. Each symbol is the mean of $n = 36–40$. Error bars represent 95% confidence intervals.

greater in DRAR than in AR. In *O. depauperata*, RLD was only greater in DR than at the other study sites (Fig. 2).

In general, RLD was significantly greater in *P. pratensis* (3.36 ± 0.011 cm cm^{-3}) than in *P. alpinum* (1.37 ± 0.016 cm cm^{-3}) and in both of them than in *O. depauperata* (0.62 ± 0.013 cm cm^{-3}) at all study sites. However, differences among species were smaller at PF and AR; RLD differences between *P. alpinum* and *O. depauperata* were not significant in those microenvironments (Fig. 2).

The interaction between species and sampling time was because changes in RLD were greater in *P. pratensis* than in *P. alpinum*, and in both species greater than those in *O. depauperata* ($F_{3;44} = 1.97$; $P = 0.13$; Table 3; Fig. 4). Within each species, RLD values did not change with sampling time. RLD increased with time in AR only in *P. pratensis*: it was significantly greater in April than in October, with intermediate values between these sampling dates ($F_{3;44} = 3.80$, $P = 0.0173$; Table 3; Fig. 3). In *P. alpinum* and *P. pratensis*, RLD changed significantly in DR and DRAR, the sites with the most light (Table 2). At DRAR, RLD of *P. alpinum* increased in December, decreased in February, and then increased again in April. The same pattern followed *P. pratensis* in DRAR and DR. In *P. alpinum*, there was a non-significant increase of RLD in December compared to October in RD; however, RLD of this species at this site was significantly greater in February and April than

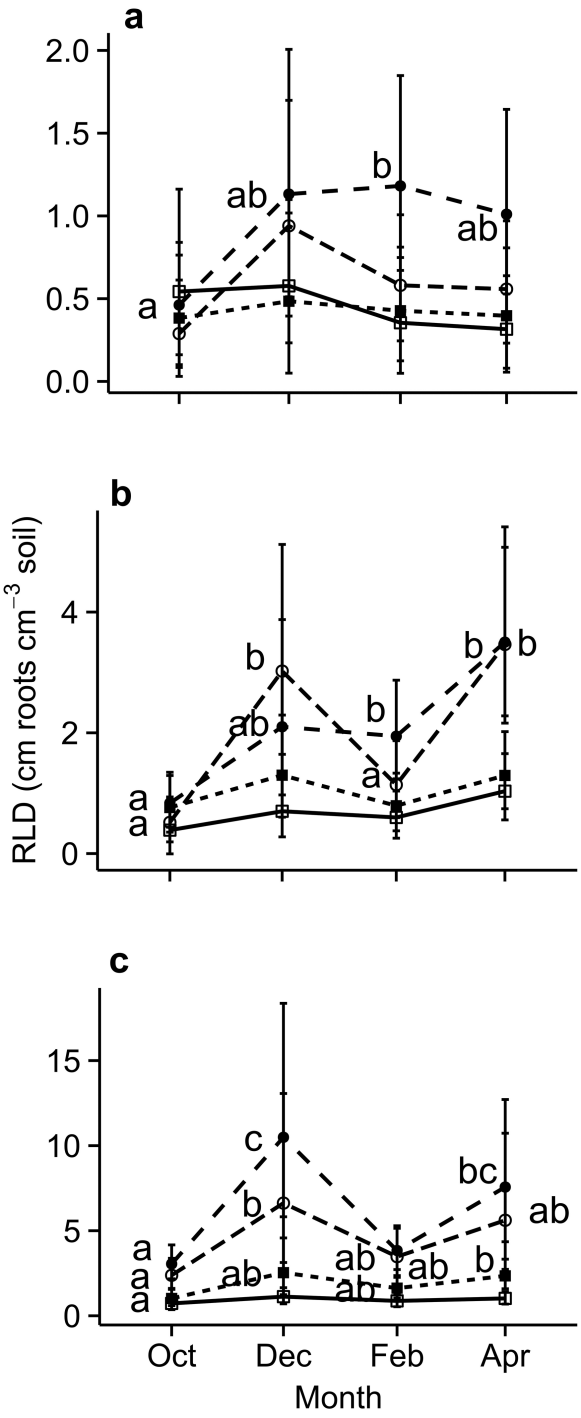

Figure 3: root length density of (**A**) *Osmorhiza depauperata*, (**B**) *Phleum alpinum* and (**C**) *Poa pratensis* during a growing season (from October 2008 to April 2009) in four forest microenvironments: Primary forest (open squares), aggregated retention (closed squares), dispersed retention with influence of aggregated retention (open circles) and dispersed retention (closed circles). Different letters represent significant differences either among months within each species or microenvironments using the Tukey test ($P < 0.05$). Where no differences were found, letters were omitted for clarity. Each symbol is the mean of $n = 8–10$. Vertical bars represent ± one standard error.

in October (Fig. 3). RLD of *O. depauperata* did not increase in DRAR; it increased in DR, but only significantly in February ($F_{3;16}$ = 9.94; P = 0.0006; Table 3; Fig. 3).

Mycorrhiza

All factors influenced CP by arbuscular mycorrhiza (Table 3). Species differed in CP ($F_{2;136}$ = 5.17; P = 0.0068; Table 3). CP was higher in *O. depauperata* than in *P. alpinum* and *P. pratensis* (Table 4). Sampling sites also modified CP ($F_{3;136}$ = 14.05; P < 0.0001; Table 3). CP was significantly lower at PF than at the other sites (Table 4). Sampling date also changed CP ($F_{3.165}$ = 39.05; P < 0.0001) as follows: December > April > October > February; differences between subsequent months were always significant according to the Tukey's test. All effects were additive, given the lack of interaction (P > 0.25).

Basal area and shoot/tiller number

All species increased their basal area in the harvested areas (RD, DRAR) compared with the non-harvested ones (PF, AR, Table 5). *Poa pratensis* had the highest increment in basal area (358%) while *O. depauperata* had the lowest (36%) when comparing with the smallest basal area (in PF) with the biggest (RD).

Shoot or tiller number was significantly correlated with RLD in all species (P < 0.001). *Poa pratensis* had the highest correlation (0.67) while *O. depauperata* had the lowest (0.41) and *P. alpinum* was intermediate (0.55).

We also analysed the RLD to shoot/tiller ratio. Here, we found significant differences of species acclimation to the environments (species × environment interaction: $F_{6:263}$ = 14.657, P < 0.0001). In *O. depauperata* the ratio did not change, in *P. alpinum* it was higher in PF and AR than in DRAR and RD, and in *P. pratensis* it followed PF < AR < DRAR ≈ RD (Fig. 4).

DISCUSSION

RLD

Results supported the first hypothesis. Although the effects were species-specific, increased light availability increased RLD in all species (Fig. 3). Increases in RLD, when comparing DR (the environment with the highest light availability) with PF (the environment with the lowest light availability), were 187% in *P. pratensis*, 101% in *P. alpinum* and 94% in *O. depauperata*. These findings agree with those of Bilbrough and Caldwell (1995), Cui and Caldwell (1997), Hodge (2004) and Jackson and Caldwell (1992), where RLD also increased as shading decreased. Heinemeyer *et al.* (2003) reported lower growth rates on roots of shaded than lighted plants. These findings might help to explain the lower RLD in all species at the PF than at the other sites in our study.

Likewise, other works also found that shading reduces the proportion of radical to total plant biomass (Baeten *et al.* 2010; Tani *et al.* 2001). This reduction could be due to simple allometric relationships; i.e. differences could be simply related to differences in plant size in the different habitats (Wahl 2001). Other authors have also reported that shaded plants have a lower need for water and nutrients, or that shading compromises by itself the plant capacity for nutrient uptake as a result of the reduced carbon supply (Canham *et al.* 1999; Givnish *et al.* 2004).

RLD for *P. pratensis* (1.12–7.65 cm cm^{-3}) was within the range reported for this species by Robinson *et al.* (1999). However, it was lower than that reported by Craine *et al.* (2002) (20.76 cm cm^{-3}) on the same species. We did not found any study to compare RLD values obtained for *P. alpinum*; anyhow, its RLD range (0.76–2.10 cm cm^{-3}) was close to that reported on a related pasture species: *Phleum pratense* (3.96 cm cm^{-3}) (Fort *et al.* 2012). Also, we did not find

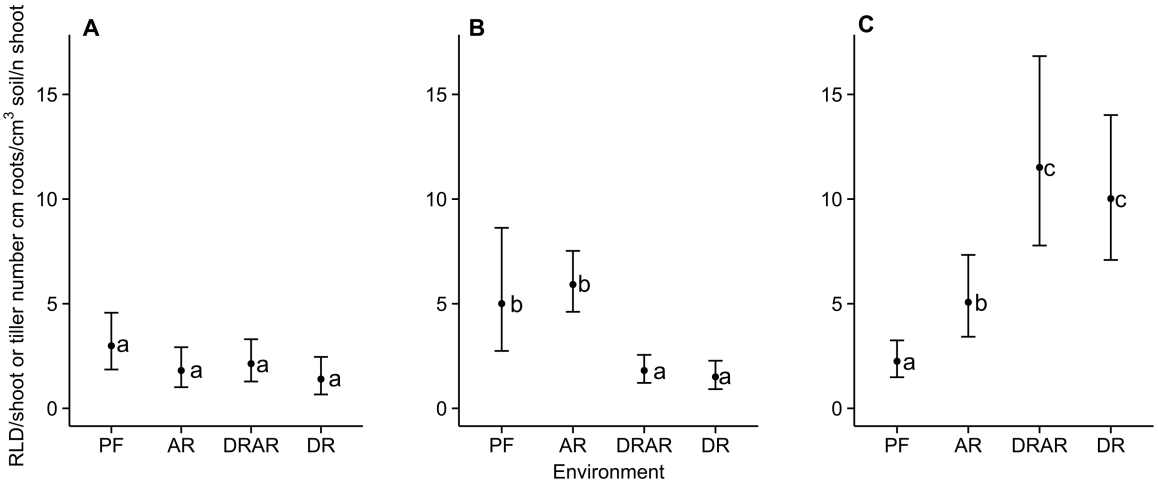

Figure 4: root length density/shoot or tiller number (cm roots cm^{-3} soil n^{-1}) of (**A**) *Osmorhiza depauperata*, (**B**) *Phleum alpinum* and (**C**) *Poa pratensis* plants that grew in four forest microenvironments: PF, primary forest; AR, aggregated retention; DRAR, dispersed retention with influence of aggregated retention and DR, dispersed retention. Different letters represent significant differences either among microenvironments using the Tukey test (P < 0.05). Each symbol is the mean of n = 8–10. Vertical bars represent 95% confidence interval.

Table 4 : root colonization percentages by arbuscular mycorrhizae and 95% confidence intervals for *Osmorhiza depauperata*, *Phleum alpinum* and *Poa pratensis* during a growing season (from October 2008 to April 2009) in four forest environments

Factor	LCL (%)	Mean	UCL (%)
Species			
Osmorhiza depauperata	4.34	6.38 b	8.80
Phleum alpinum	2.61	3.90 a	5.45
Poa pratensis	2.65	3.95 a	5.51
Environment			
PF	1.06	2.07 a	3.42
AR	2.77	4.39 b	6.39
DRAR	4.98	7.14 b	9.70
DR	4.30	6.19 b	8.41
Month			
October	2.40	3.71 b	5.29
December	7.46	9.57 d	11.95
February	0.28	0.88 a	1.80
April	3.28	5.07 c	7.26

Abbreviations: AR = aggregated retention; DR = dispersed retention; DRAR = dispersed retention with influence of aggregated retention; PF = primary forest.
LCL, UCL lower and upper 95% confidence limit. Different letters within each factor represent significant differences using the Tukey test ($P < 0.05$). Each value is the mean of $n = 36–40$.

Table 5: basal area (mean ± standard deviation; cm^2) of *Osmorhiza depauperata*, *Phleum alpinum*, and *Poa pratensis* plants that grew in four forest environments

Environment	*O. depauperata*	*P. alpinum*	*P. pratensis*
PF	15.81 ± 6.91 a	58.16 ± 51.84 a	83.75 ± 10.35 a
AR	14.48 ± 8.50 a	95.06 ± 50.65 ab	73.25 ± 30.38 a
DRAR	34.02 ± 32.74 b	92.62 ± 76.77 ab	173.73 ± 62.47 b
DR	37.36 ± 25.23 b	145.49 ± 74.55b	460.52 ± 57.27c
F(p)	3.12 (0.037)	3.10 (0.038)	158.8 (<0.0001)

Abbreviations: AR = aggregated retention, DRAR = dispersed retention with influence of aggregated retention; DR = dispersed retention; PF = primary forest.
Different letters within each factor represent significant differences using the Tukey test ($P < 0.05$). Each value is the mean of $n = 10$. F(p) Simple ANOVA F value and its probability. Different letters represent significant differences between treatments.

similar studies for *O. depauperata* nor for closely related species. However, we found studies on cultivated species of the same family (Apiaceae), as *Daucus carota* L. and *Pastinaca sativa* L. RLD in *O. depauperata* (0.46–1.05 cm cm^{-3}) was similar to that found in those cultivated species (0.73 and 0.51–1.84 cm cm^{-3}, respectively: (Greenwood *et al.* 1982; Kristensen and Thorup-Kristensen 2004).

In all study microenvironments, RLD of the study species varied following a similar pattern: *P. pratensis* > *P. alpinum* > *O. depauperata*. Only when light availability was the lowest (i.e. at the PF), we failed to find significant differences between *P. alpinum* and the other species. On average, RLD on *P. pratensis* was 70.93% greater than in *P. alpinum* and 206% than in *O. depauperata*.

Our results suggest that differences of RLD among the target species could be partially due to differences in their growth forms. *Poa pratensis* always produces many superficial rhizomes whereas *P. alpinum* only sometimes produces short ones (Craine *et al.* 2002; Moore 1983). *Poa pratensis* and *Phleum pratense* differ in RLD because they have a different percentage of fine roots (i.e. it is greater in *P. pratensis* than in *P. pratense;* 99% versus 59%, respectively: Craine *et al.* 2002; Fort *et al.* 2012). A similar relationship could happen with *P. alpinum,* because *P. pratense* is very similar morphologically and closely related to it (Moore 1983).

The differences among the grass species and *O. depauperata* could be due to the clonality of the grass species. In grasses, each rhizome and tiller grows its own radical system (Derner and Briske 1999a). *Osmorrhiza depauperata,* however, grows its root from a fibrous rhizome (Lowry and Jones 1984). This species showed a greater CP, and a lower RLD, than the grass species (Table 4). Species with less root length per soil volume may be more dependent on mycorrhizal colonization for nutrient acquisition than those with greater root length densities (Koide and Li 1991). Greater root length densities and association with mycorrhizal-forming fungi can contribute to plant nutrient increase. However, these are often alternative strategies for nutrient acquisition by plants (Kothari *et al.* 1990). Determination of the relative contribution of these mechanisms to plant competitive ability constitutes an interesting and difficult research challenge (Derner and Briske 1999b).

The temporal RLD patterns shown in AR could be due to changes in plant size (Selzer 2014). On the other hand, phenological changes could explain the patterns found in *P. pratensis* and *P. alpinum*. In the grass species, most plants grown in DR and DRAR flowered and fructified, whereas few did that in PF and AR (Selzer 2014). Likewise, changes in RLD were noticeable in those microenvironments (i.e. DR and DRAR), where they reached a peak in December (Fig. 3). Other studies found a similar response in cultivated species (e.g. sorghum and sunflower). This was because most of their roots grew just before flowering (Cheng *et al.* 1990). In perennial grass species, tillers that grow early in the growing season most often flower and end its developmental cycle during the same growing season. In contrast, those that start growing late in the growing season overwinter and resume their growth in the following growing season (Briske and Richards 1995). Therefore, as a unit, tillers behave as individual annual plants. Then, the higher RLD values of *P. alpinum* in April on DRAR could be due to the growth of overwintering tillers.

In *O. depauperata*, the stem dies after fructification; however, the rhizome, the organ the roots grow out from, overwinters (Selzer 2014). This could explain the differences on the RLD changes during the growing season among *O. depauperata* and the grass species.

As it is expected from theory, the positive correlation between RLD and shoot/tiller number reflects that RLD increased with the number of shoots or tillers per area in all species. It is interesting that *P. pratensis* showed the highest correlation between these variables and it also showed 103% increment in RLD/tiller ratio (Fig. 4). On the other hand, *P. alpinum* also showed a relatively high correlation. However, the ratio decreased 47%. This is unexpected from theory as it should increase because evapotranspiration is higher in sunnier environments (McConnaughay and Coleman 1999). We believe that this reflects different strategies. While *P. alpinum* invests more on tillers, *P. pratensis* does so in roots that hamper other species roots (Schmid and Bazzaz 1992; Fort *et al.* 2012). In contrast, *O. depauperata* seems to be more conservative as it did not change its RLD/shoot ratio among microenvironments.

Mycorrhiza

Previous works have already shown that *P. pratensis* and *P. alpinum* have arbuscular mycorrhiza (Barni and Siniscalco 2000; Kempel *et al.* 2010; Read and Wandter 1981; Vare *et al.* 1997), although it has not been always the case for *P. pratensis* (Wang and Qiu 2006). Despite CPs have been within the range reported for some studies in *P. pratensis* (Kempel *et al.* 2010; Read and Wandter 1981), they are lower than those determined in other studies (Barni and Siniscalco 2000). CP values in *P. alpinum* are much lower than those reported for this species in northern Fennoscandia (Vare *et al.* 1997). We did not found any previous report on the mycorrhizal levels of *O. depauperata*, except for a previous study which only indicated that *O. chilensis* forms mycorrhiza (Fontenla *et al.* 1998). Compared to other species of the family Apiaceae, CPs in *O. depauperata* were lower than values found on *Daucus carota* L. (33–66%: Tawaraya 2003) and *Coriandrum sativum* L. var Caribe (11–43%: Schroeder and Janos 2004). Therefore, our work does provide the first record on the mycorrhizal status of *O. depauperata*.

In *O. depauperata*, CPs were 62% greater than those in *P. pratensis* and *P. alpinum*. This difference could be due to contrasting root architecture among these species. In general, roots of the species dependant on the mycorrhizal symbiosis are thick, fibrous and have few radical hairs; this is because the fungal partner is the one which performs most of the soil resource uptake (Koltai and Kapulnik 2010). On the other hand, plants that are not dependent on mycorrhizae have thinner radical systems with lots of radical hairs. Lower RLD in *O. depauperata* than in *P. pratensis* and *P. alpinum* suggests that its radical system is less ramified (Fig. 2). The mycorrhizal dependency is negative in *P. pratensis* (Wilson and Hartnett 1998); this means that this species shows depression in growth when it is infected by the mycorrhizal fungus (Tawaraya 2003). We did not found any literature for *P. alpinum*, although Clapperton and Reid (1992) reported the same phenomenon than that in *P. pratense*. This matches the low mycorrhizal dependency found in C_3 grasses (Wilson and Hartnett 1998). Also, we did not find any study on mycorrhizal dependency on *O. depauperata*. However, a study on *Daucus carota* L., a cultivated species of the same family than *O. depauperata*, showed a mycorrhizal dependency of 33–99% on this species (Tawaraya 2003).

High soil phosphorus could explain the very low CP in this study. The extra-radical mycelia are very efficient acquiring phosphorus (Koltai and Kapulnik 2010), a low mobility nutrient in the soil and it is the most important factor affecting CP (Olsson *et al.* 1997, 2010; Schroeder and Janos 2004). In general, increments of phosphorus availability decrease the formation of mycorrhizae (Olsson *et al.* 1997, 2010). Forest soils in this study have very high available phosphorus levels (214–404 or 126–192 mg kg^{-1} in the O or A horizons, respectively: Peña-Rodríguez *et al.* 2013). Recently, Oro Castro (2014) has not found differences in available P among microenvironments (i.e. PF, aggregated retention and DR). Furthermore, Olsson *et al.* (1997) experimented with different concentrations of P and found that 100 mg kg^{-1} soil reduced root colonization to almost 0.

Despite we only found significant differences between the PF and the other microenvironments (112–244%), CPs tended to increase with light availability in all species (Table 4). The results we found match those from previous studies (Hayman 1974; Heinemeyer *et al.* 2003; Olsson *et al.* 2010).

This could be the result of various reasons: (i) plants most often have a lower carbohydrate availability under low light conditions (Burner and Belesky 2008; Jackson and Caldwell 1992); thereafter, less carbohydrates should be available for the fungus; (ii) plants were bigger in microenvironments with higher light availability (Selzer 2014), indicating that possibly there was a plant size effect; (iii) a change in the root biomass ratio under changing light conditions (Elemans 2004; Tani *et al* 2001) and (iv) a reduced rate of new root growth in shaded plants (Heinemeyer *et al.* 2003). Furthermore, a recent study found that ectomycorrhizae could diminish the CP by arbuscular mycorrhizae (Becklin *et al.* 2012). Then, it is possible that ectomycorrhizae in *Nothofagus pumilio* might have interfered, and diminished the abundance of arbuscular mycorrhizae, therefore reducing the CPs in the study species.

ECOLOGICAL IMPLICATIONS

Poa pratensis had a high RLD compared with the other species (Fig. 2). This species is a strong underground competitor for resources and can exclude other species roots as other studies show (Bookman and Mack 1982). The low CP means that it can occupy areas in which few or no compatible mycorrhizal fungus are present, such as newly harvested areas. From the basal area data (Table 5) it is clear that it spreads quickly in the harvested area (i.e. RD and DRAR). With favorable conditions it can spread more than 100 cm per year (Shortell *et al.* 2009). Other study species lack this aggressive lateral growth. We think that these traits could pose a risk to native species and forest regeneration.

Understory effects on *N. pumilio* seedlings are not clear. On one hand, seedling survivor is lesser in RD than in AR or PF (Martínez Pastur *et al.* 2013). This work speculates that reason behind is an increased photosynthetic performance in shaded areas. However, seedling growth is greater in DR as *N. pumilio* is tolerant to high irradiances (Martínez Pastur *et al.* 2011). We think that underground competition with understory species, especially *P. pratensis* which can mechanically exclude other species roots (Schmid and Bazzaz 1992), is the driving force behind low levels of seedling recruitments in harvested area. On the other hand, Martínez Pastur *et al.* 2011 found that *N. pumilio* seedling grew more near monocots than dicots. This is unexpected because current data show that monocots had higher RLD than the dicot. Nevertheless, the present study did not attempt to measure RLD of all understory species and it is unclear from Martínez Pastur *et al.* 2011 which species were most abundant in each group.

CONCLUSION

Our data supported the posted hypothesis. Species increased their RLD in response to light availability. Differences found among the study species could be due to their different growth form and fine root proportion. Temporal patterns found in RLD and CP could be the result of the phenology of the study species. CPs also increased with light availability, although we only found significant differences between the primary and the harvested forests. Also, our CP values were lower than those reported in other studies. This could be related to the high phosphorus content in the study soils. Alternatively, it might have been the result of interference or competition with *N. pumilio* ectomycorrhizae, or an alelopatic effect of *N. pumilio* roots on arbuscular mycorrhizae of the study species. In *O. depauperata*, CPs were higher than those in the grass species. *Poa pratensis*, could exclude other species because of its traits.

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