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**EVALUACION DEL EFECTO DE ESPECIES CON ESTRATEGIAS
COMPLEMENTARIAS EN LA DISPONIBILIDAD Y ADQUISICION DE
NUTRIENTES EN SUELOS DEGRADADOS**



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EVALUACIÓN DEL EFECTO DE ESPECIES CON ESTRATEGIAS COMPLEMENTARIAS EN LA DISPONIBILIDAD Y ADQUISICION DE NUTRIENTES EN SUELOS DEGRADADOS

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TABLA DE CONTENIDOS

INDICE DE TABLAS	6
INDICE DE FIGURAS	7
RESUMEN	8
ABSTRACT	9
1. Introduction.....	10
2. Materials and methods	12
2.1 Soil sampling sites, species selection and plant material collection	12
2.2 Experimental setting and design.....	12
2.3 Seedlings morphological and nutritional evaluations	13
2.4 Soil nutrient and chemical analysis.....	15
2.5 Microbial biomass, Leucine aminopeptidase and phosphatase activity	15
2.6 Statistical analysis	15
3. Results	16
3.1 Effect of the combinations of plant species with different strategies of nutrient acquisition on carbon, nitrogen and phosphorus concentrations in soils and functional traits.	16
3.1.1 Effect of assemblages in soil C, N and P	16
3.1.2 Effect of assemblages in functional traits for each specie.....	18
3.2 Effect of plant assemblages on plant growth and traits of the species	22
3.3 Fertilization addition effect on leaf C: N:P stoichiometry.....	26
4. Discussion	27
4.1 Plant assemblages modify soil nutrient availability and functional traits development.....	27
4.2 Plant interactions	27
4.3 Effect of fertilization on C: N:P leaf stoichiometry	30
5. Conclusions.....	31
6. Anexos	33
7. References	36

INDICE DE TABLAS

Table 1. Soil parameters values for burned soil and each assemblage. Mean \pm standard error (n=10). Significant differences according to the Tuckey test at $p < 0.05$ levels were indicated by different letters. *N. obliqua* (NO), *S. cassioides* (SC) and *L. dentata* (LD). STC: soil total carbon, STN: soil total nitrogen, STP: soil total phosphorus, SAN: soil available nitrogen, SAP: soil available phosphorus, SIP: soil inorganic phosphorus, SOP: soil organic phosphorus 18

Table 2. Leaf nutritional content and $\delta^{13}\text{C}$ for each specie in assemblages. Mean \pm standard error (n=10). Significant differences according to the Tuckey test at $p < 0.05$ levels were indicated by different letters. *N. obliqua* (NO), *S. cassioides* (SC) and *L. dentata* (LD). Parenthesis denotes the accompanying specie. Leaf nitrogen content (LNC), Leaf phosphorus content (LPC) and Water use efficiency (WUEi) as $\delta^{13}\text{C}$.20

Table 3. Average ratios of C:N, C:P and N:P of species assemblages under fertilization and no fertilization treatment. Mean \pm standard error (n=10). Significant differences according to the Tuckey test at $p < 0.05$ levels were indicated by different letters. *N. obliqua* (NO), *S. cassioides* (SC) and *L. dentata* (LD). Parenthesis show co-cultivation specie. Capital letters show significant differences between treatments and lowercase letters among assemblages in each treatment ($p < 0.05$)..... 26

INDICE DE FIGURAS

- Figure 1.** Boxplot of microbial biomass carbon (C_{mic}), leucine aminopeptidase (LAP) activity phosphatase activity and phosphatase activity in soils with *N. obliqua* (NO), growing in mixtures with *S.cassioides* (SC) and *L.dentata* (LD) (NO-SC, NO-LD). Thick line is the median, box upper and lower end represent the 25 and 75 percentiles, while the whiskers 5 and 95 percentile. Letters indicate significant differences among treatments ($p < 0.05$)..... 17
- Figure 2.** Performance of NO, SC and LD on relative growth rate of height and diameter (RGRH and RGRD) and total biomass (TB). Bars shows means \pm se (n=10). *N. obliqua* (NO), *S. cassioides* (SC) and *L. dentata* (LD). Parenthesis denotes the accompanying specie. Different letters indicate significant differences between plant assemblages ($p < 0.05$)..... 21
- Figure 3.** Performance of NO, SC and LD on root length density (RLD) and root tips (RT). Bars shows means \pm se (n=10). *N. obliqua* (NO), *S. cassioides* (SC) and *L. dentata* (LD). Parenthesis denotes the accompanying specie. Different letters indicate significant differences between plant assemblages ($p < 0.05$)..... 22
- Figure 4.** Principal component analysis of soil and plant functional traits. Arrows represent the principal component and symbols represent *N. obliqua* (NO) individuals growing in monoculture and co-cultivated with *L. dentata* (LD) and *S. cassioides* (SC). Ellipses correspond to 95% confidence..... 23
- Figure 5.** Principal component analysis of soil and plant functional traits. Arrows represent the principal component and symbols represent *S. cassioides* (SC) individuals growing in monoculture and co-cultivated with *L. dentata* (LD) and *N. obliqua* (NO). Ellipses correspond to 95% confidence..... 24
- Figure 6.** Principal component analysis of soil and plant functional traits. Arrows represent the principal component and symbols represent *L. dentata* (LD) individuals growing in monoculture and co-cultivated with *S. cassioides* (SC) and *N. obliqua* (NO). Ellipses correspond to 95% confidence..... 25

RESUMEN

Muchos proyectos de restauración en suelos post-incendio consideran la plantación mixta de árboles nativos. Sin embargo, interacciones interespecíficas respecto a la adquisición de nutrientes son en general desconocidas, especialmente en sitios con baja disponibilidad de nutrientes, como los suelos afectados por incendios. Se estableció un experimento de mesocosmos considerando tres árboles nativos co-ocurrentes con diferentes estrategias de adquisición de nutrientes en un suelo post incendio. *L. dentata* (LD) con raíces cluster, *S. cassioides* (SC) con nódulos fijadores de N y *N. obliqua* (NO) con asociación de ectomicorrizas. Se evaluaron los efectos del co-cultivo de estas especies en la disponibilidad y adquisición de C y nutrientes del suelo, además en los rasgos funcionales de las plantas. También se incluyó un tratamiento de fertilización para evaluar la neutralización de la competencia interespecífica por los nutrientes. el experimento consideró tratamientos mono-específicos (NO, SC y LD) y en co-cultivo heteroespecífico (NO-SC, NO-LD y SC-LD) en un diseño factorial completo.

Los mayores contenidos promedio de C y N total del suelo se encontraron en SC-LD. Este ensamble también mostró el P total más bajo, lo que sugiere una mayor extracción de P. los mesocosmos mono-específicos de NO mostraron el mayor contenido de nitrógeno y fósforo total, lo que indica una sub-utilización. La adquisición y los rasgos funcionales variaron entre los ensambles de especies. La mayoría de las especies disminuyeron el crecimiento y presentaron valores más bajos en los indicadores de rasgos funcionales cuando se co-cultivan. NO facilita la adquisición de fósforo para las especies vecinas y de N para LD. LD también favorece la adquisición de N en NO. La fertilización tiende a neutralizar las diferencias en la estequiometría foliar observadas en los tratamientos sin fertilizar. Las raíces cluster y ectomicorrizas muestran una adquisición de nitrógeno complementaria, lo que sugiere una co-existencia beneficiosa en las primeras etapas de crecimiento. Este estudio proporciona información valiosa sobre las complejas interrelaciones que se producen durante el cultivo de especies con estrategias radiculares complementarias. Esta información podría utilizarse para diseñar estrategias de recuperación en suelos afectados por incendio.

ABSTRACT

Many restoration projects in postfire soils consider mixed plantation of native trees. However, interspecific interactions regarding nutrient acquisition are generally unknown, especially in sites with low nutrient availability, like fire-affected soils. A mesocosms experiment was established considering three co-occurring native trees with different nutrient acquisition strategies in a post fire soil. *L. dentata* (LD) with cluster roots, *S. cassioides* (SC) with N-fixing nodules, and *N. obliqua* (NO) with ectomycorrhizal association. The effects of co-cultivation of these species on soil C and nutrient availability and acquisition, and plant functional traits were evaluated. A fertilized treatment was also included to assess neutralization of interspecific nutrient competition. The experiment considered monospecific (NO, SC and LD) and in heterospecific co-cultivation treatments (NO-SC, NO-LD, and SC-LD) in a full factorial design.

The highest average total soil C and N contents were found in SC-LD. This assemblage also showed the lowest P total suggesting higher P extraction. The monospecific NO mesocosms displayed the highest available N and total P indicating sub-utilization. Acquisition and functional traits varied across species assemblages. Most species decreased growth and have lower values in functional trait indicators when co-cultivated. NO facilitate phosphorous acquisition for both neighbor species and N for LD. LD also favor N acquisition in NO. Fertilization tend to neutralize differences in leaf stoichiometry observed under unfertilized treatments. Cluster root and ectomycorrhizal species display complementary nitrogen acquisition suggesting beneficial coexistence in early growth stages. This study provides valuable information about the complex interrelations occurring during co-cultivation of species with complementary root strategies. This information could be used to design strategies to recover fire-affected soils.

1. Introduction

Most temperate forests are frequently affected by human-induced fires (Úbeda and Sarricolea 2016). These fires combust the forest floor, decreasing organic carbon and nutrient concentrations in soils (Caldwell *et al.* 2002; Giovannini *et al.* 1988; Simard *et al.* 2001). After fires, nutrients can be eliminated from soils through leaching, runoff, and the convection of ashes (Litton and Santelices 2003). Higher temperatures transform organic nitrogen into inorganic forms, increasing availability (Attiwill and Adams 1993). Phosphorus does not present significant losses by volatilization or leaching. However, organic phosphorus can be transformed into orthophosphate, which can be rapidly taken up by plants (Sharpley and Tunney 2000) and soil microorganisms (Aiori *et al.* 2017). As a result of increased hydrophobicity, there is also a reduction in infiltration and an increment in runoff (Carreiras *et al.* 2014) increasing erosion susceptibility (Fernández *et al.* 2012). These events can also modify soil chemical properties like pH, conditioning nutrient availability (e.g., nitrogen and phosphorus) in the short term (Johnson *et al.* 1998; Turrion *et al.* 2010). Reductions in microbial biomass and enzymatic activity have also been reported (Certini 2005). This effect is relevant as these microorganisms drive biogeochemical processes in terrestrial ecosystems, including the mineralization of occluded nutrients (Mataix-Solera *et al.* 2009).

After a disturbance, under restrictive environmental conditions, niche complementarity and facilitation are regularly found (Reich *et al.* 2012). Through coexistence in community assembly, species with different functional traits may enhance the absorption of limiting available resources, generating a more diverse and productive community (Hector *et al.* 1999; Da Tilman *et al.* 2001). For example, roots of complementary plant species can improve soil stability and structure, improve nutrient acquisition, and promote the growth of neighboring plants (Hallett and Bengough 2013; Van Der Putten 2009).

Species from the *Proteaceae* family, such as *Lomatia dentata*, form cluster roots, with the capacity to adapt to soils where phosphorus is the main limiting factor (Neumann and Martinoia 2002). Cluster roots release organic acids and phosphatases via rhizodeposition (Jones *et al.* 2004), increasing phosphorus availability and improving its acquisition in neighboring plants. These processes are particularly relevant for

phosphorus acquisition in soils derived from volcanic ashes (Zúñiga-Feest *et al.* 2014). In addition, plant nitrogen-fixing bacteria interactions occur in legumes nodules, such as *Sophora cassioides*. This interaction increases nitrogen content in plant tissues (Temperton *et al.* 2007; Tomm *et al.* 1995). This type of interaction can also benefit associated non-legume species that may acquire the excess nitrogen released into the soil (Li *et al.* 2013; White *et al.* 2013). For example, associations with mycorrhizae in *Nothofagus obliqua* (e.g., ectomycorrhiza) greatly expand root exploration, augmenting nitrogen, phosphorus, and water uptake from the soil in exchange for photosynthetically fixed carbon from the plants. (Simard *et al.* 2003). It also allows nutrient exchange between a mycorrhiza-forming and non-mycorrhiza-forming species (Helgason and Fitter 2005), improving plant coexistence (Montesinos-Navarro *et al.* 2012), and defense against pathogens.

Mixed crops of species with different root acquisition strategies have been studied primarily on agricultural ecosystems to increase crop productivity (Brooker *et al.* 2014). These mixed-planted systems reduce interspecific competition by improving soil nutrient availability and acquisition of restricting resources through complementary functional traits (Zhang and Li 2003). However, little is known about the effect that the combination of species could have on nutrient acquisition in soils affected by fires. This information could be relevant for native forest restoration after ever more often wildfires.

The main objectives of this study were to evaluate the effect of assemblages of *L. dentata*, *S. cassioides*, and *N. obliqua* with different nutrient acquisition strategies on i) soil C, N, and P stocks and available fractions, and ii) plant nutrient acquisition and functional traits of plants growing in a post-fire nutrient-depleted soil. We also aimed to evaluate if higher nutrient availability in the soil can reduce interspecific competition. We hypothesized that the co-cultivation of species with different nutrient acquisition strategies increases nutrient availability in post-fire soils, resulting in higher growth and N and P foliar concentration.

2. Materials and methods

2.1 Soil sampling sites, species selection and plant material collection

The experiment was conducted outdoors over two growing seasons at the Centro Tecnológico de la Planta Forestal, Instituto Forestal (36.84°S, 73.13°O), Biobío Region, Chile. The climate is Mesomediterranean, with an average annual temperature and precipitation of 12.5 °C and 988 millimeters (Amigo and Ramírez 1998). The three species selected for this study have complementarity strategies for nutrient acquisition (cluster root bearing, ectomycorrhizal symbiosis, and nitrogen-fixing species) and are naturally associated within their distributional range. *Nothofagus obliqua* (NO) (Nothofagaceae) is a tree species that has a wide distribution (Rodríguez et al., 1983). It is a deciduous species and presents ectomycorrhizal symbiosis (Marín *et al.* 2018). *Sophora cassioides* (SC) (Leguminosae) is a tree species that has a wide distribution (35°S 71°O – 45°S 73°), which is a nitrogen-fixing species. *Lomatia dentata* (LD) is a Proteaceae cluster roots tree species that have a wide distribution (31°S 71°O - 42°S 73°O), occurring in the Mediterranean and temperate forests as an intermediate to late-successional species.

The soil and seeds used for this mesocosm experiment were collected in Cerro Cayumanque (36°42'S, 72°29'O), Ñuble Region. The Cayumanque forests were affected by a devastating human-induced wildfire in the summer of 2012.

Seeds were collected in Feb-Apr of 2015 from Cerro Cayumanque in areas not affected by the fire. The plants were produced between Aug-Nov of 2015 in composted pine bark as substrate. Once plants reached a similar height, between 15 and 25 cm, they were selected and transplanted to 7 L pots (Feb 2016) to be mix into either monospecific or heterospecific mesocosms (see section 2.2).

The soil in the area has been mapped as part of the Treguaco Association, classified as a fine loamy, mixed, thermic Dystric Xerochrepts (Inceptisol). The Treguaco soil has a silty clay loam texture, moderate depths, and complex topography with slopes greater than 45%, moderate to severe erosion, and well-drained (Stolpe 2011). Soil samples were taken in four sites in 2015, representing one relatively well-preserved forest and

three burned forest areas presenting various signs of degradation associated with fire (e.g., charred surface materials, evidence of sheet erosion, low to nil litter coverage, and higher compaction). The chemical characteristics of the soils at four sites were determined before selecting the soil to be extracted for the experiment. The soil chosen for the mesocosm experiment displayed the lowest pH (5.52), total C, and available N (2.06 and 0.17 mg Kg⁻¹, respectively) among all tested sites (Table S1). The samples for the mesocosms were collected in January of 2016 by extracting the first 20 cm of soil in approximately 10m².

2.2 Experimental setting and design

Seedlings were of a similar height (15 and 25 cm) at the time of the transplant. A total of 360 seedlings were planted in 120 pots. Seedlings were co-cultivated as either monospecific or heterospecific pairs. The three monospecific co-cultivation treatments (NO, SC and LD) had two individuals of the same species per pot. Similarly, the heterospecific co-cultivation treatments (NO(SC), NO(LD), SC(NO), SC(LD), LD(NO), LD(SC)) had two individuals per species, resulting in four individuals per pot. Paired seedlings were planted as close to each other as possible to stimulate root interactions. Pots were filled with the burned substrate collected as indicated before. The experimental design was two blocks with ten replicates for each treatment randomly distributed. Thus, each treatment corresponds to 180 plants in total. The experiment was carried out during February-2016 to – January 2018. Irrigation was controlled and applied weekly according to the volumetric water loss registered by previously calibrated soil moisture sensors (ECH2O EC-5; Decagon, Pullman, WA USA) (S. Figure 2) (Dumroese *et al.* 2015). The fertilization scheme (Table S2) was a diluted nutrient solution described by (Hoagland and Arnon 1950).

2.3 Seedlings morphological and nutritional evaluations

Several aboveground growth traits and root traits influencing the acquisition of mineral nutrients, as well as leaf nutrient concentration measurements, were evaluated (Table

S3). In January 2018, seedlings were harvested to determine basal diameter, height, biomass, root acquisition traits, and leaf nutrient concentrations. Once harvested, the seedlings were separated into leaves, shoots, and roots to calculate relative height and shoot diameter growth rates (RGRH and RGRD). The first and last measurements were divided by days between the transplantation and harvesting date. Aboveground and belowground ratio biomass (AGB:BGB) was the sum of aboveground biomass (shoot and leaves) divided by the sum belowground biomass (roots). Total biomass (TB) included the sum of shoot, leaves, and root weight. For specific root length (SRL), root length density (RLD), and specific root area (SRA), the substrate was removed and washed with water to use optical scanner software Winrhizo (Regents Instrument, Quebec, Canada). Finally, root and leaf dry matter content (RDMC and LDMC) was calculated by dividing the oven-dry mass (mg) by its fresh mass (g).

$$SRL = \frac{\text{root length (cm)}}{\text{root biomass (g)}}$$

$$RLD = \frac{\text{root length (cm)}}{\text{soil volume (cm}^3\text{)}}$$

$$SRA = \frac{\text{surface area (cm}^2\text{)}}{\text{root biomass (g)}}$$

For leaf carbon (LCC) and nitrogen (LNC) content and $\delta^{13}\text{C}$ isotopic analysis as a proxy for water use efficiency (WUEi), leaf samples were dried at 65°C until constant weight, and 2.7 ± 0.001 mg of previously milled leaf material weighted in tin capsules on a Sartorius® ME 36 S microbalance and determine via Dumas combustion using a SERCON® EA CHNS analyzer and coupled to a SERCON® 20-20 isotopic ratio mass spectrometer (IRMS) at the *Laboratorio de Investigación en Suelos, Aguas y Bosques* (LISAB) de la Universidad de Concepción. The analytical wheat standard flour. The natural abundance of ^{13}C ($\delta^{13}\text{C}$) was determined by the ratio of $^{13}\text{C}/^{12}\text{C}$ using (Stout and Rafter 1978) formula:

$$\delta^{13}\text{C} = \left(\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{standard}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right) \times 1000$$

Leaf phosphorus content (LPC) was determined by calcination and phospho-vanadium molybdate colorimetry (Sadzawka *et al.* 2006).

2.4 Soil nutrient and chemical analysis

Previous to determining soil total carbon (STC) and soil nitrogen content (STN), soil samples were sieved at 2 mm and dried at 105°C for 48-72 hours, weighted in tin capsules on a Sartorius® ME 36 S microbalance and determined via Dumas combustion using a Sercon EA CHNS analyzer coupled to an isotopic ratio mass spectrometer (IRMS) versus wheat standard SERCON® 20-20 flour and soil standard SERCON®, respectively. Soil available nitrogen (SAN) was considered as the sum of nitrate and ammonium content. Nitrate was determined by the reduction vanadium (III) method (Miranda *et al.* 2001), while ammonium was determined by the Berthelot reaction method described by Forster (1995). For both nitrate and ammonium, samples were analyzed within 48 h after the extraction. All phosphorus analyses were determined using the ascorbic acid method (Watanabe and Olsen 1965). The sodium bicarbonate extraction method was used for available soil phosphorus (SAP) (Sadzawka *et al.* 2006). For phosphorus fractions, soil inorganic phosphorus (SIP) was determined by digestion with NaOH and Na₂EDTA extraction method. Soil total phosphorus (STP) was determined by digestion K₂S₂O₈ and H₂SO₄ according to Bowman and Moir (1993) and Carter and Gregorich (2007) protocols. Soil organic phosphorus (SOP) was determined as the difference between total and inorganic phosphorus. All these analyses were performed at the *Laboratorio de Investigación en Suelos, Aguas y Bosques* (LISAB) of the Universidad de Concepción.

2.5 Microbial biomass, Leucine aminopeptidase and phosphatase activity

Microbial biomass (C_{mic}) was determined only in the two-species combination treatments containing NO, using the chloroform fumigation-extraction method (Vance *et al.* 1987). In brief, chloroform-fumigated and non-fumigated soil samples were extracted in 0.5M K₂SO₄ and filtered, and dissolved C was measured using a TOC/TN Analyzer (multiN/C 2100, Analytik Jena®, Germany). C_{mic} was then calculated as the difference between the C concentrations of fumigated and non-fumigated samples, divided by a conversion factor of 0.45 (Jenkinson *et al.* 2004). These analyses were performed at the Soil Biogeochemistry of the University of Bayreuth, Germany.

2.6 Statistical analysis

For each of the variables, normality and homoscedasticity were verified, using 'Lilliefors' (Kolmogorov-Smirnov) test ('nortest' package (Juergen Gross & Uwe Ligges 2015)) and the Levene's test (('car' package John Fox and Sanford Weisberg (2019)), respectively. When the data did not meet normality and homoscedasticity, the data were transformed to fit the analysis. Then, a one-way ANOVA was performed to determine the effect of co-cultivation on seedling's morphological and chemical parameters and soil variables, with co-cultivation as the main factor. Afterward, a Tukey significance test was carried out to determine which groups of co-cultivation pairs were significantly different (('stats' package R Core Team (2019)). A Pearson correlation coefficient was used to evaluate the linear relationship between edaphic variables (('agricolae' R package (Mendiburu 2010)). To visualize the effect of co-cultivation on seedlings' morphological and chemical parameters and soil variables, a principal component analysis (PCA) was performed for each species, including all variables using the 'vegan' R package (Oksanen et al. 2013). Finally, to evaluate the effect of fertilization and co-cultivation treatment in C:N:P leaf stoichiometry, a two-way ANOVA was performed, with fertilization and co-cultivation assemblages as the main factors. All analyses were performed in R software (version 3.6.0) on the RStudio platform (Version 1.1.383 2009-2017) with $P < 0.05$ as a significance level.

3. Results

3.1 Effect of the combinations of plant species with different strategies of nutrient acquisition on carbon, nitrogen and phosphorus concentrations in soils and functional traits.

The following section only describes the results of the interactions between plants in mesocosms without considering fertilization.

3.1.1 *Effect of assemblages in soil C, N and P*

The presence of vegetation increased pH values and slightly decreased STC and STN compared to original burned soil levels (Table 1). The main change in soil nutrient content

was observed in available phosphorus and nitrogen content, where SAP and SAN increased up to 27 and 11 times, respectively, from burned soil levels (Table 1). Across assemblages, LD, SC, and NO-LD soil showed significantly lower STN and STC, while SC-LD had the highest SAP and SAN, but the lowest STP content. NO soils showed the highest values of STP and SAN in comparison to other assemblages. NO-SC had the highest values of SOP, while LD soils showed a significantly lower content of SOP (Table 1). Four main significant correlation were found between STC-STN ($r = 0.91$, $p = <0.001$), SAN-SAP ($r = 0.36$, $p = 0.036$), SAN-SIP ($r = 0.47$, $p = 0.004$) and pH-STP ($r = -0.53$, $p = 0.0008$).

Finally, microbial biomass did not show significant differences, neither did leucine aminopeptidase activity (Figure 1). NO-SC assemblage showed a significantly lower phosphatase activity (Figure 1) and phosphatase activity related to microbial biomass (Figure 1).

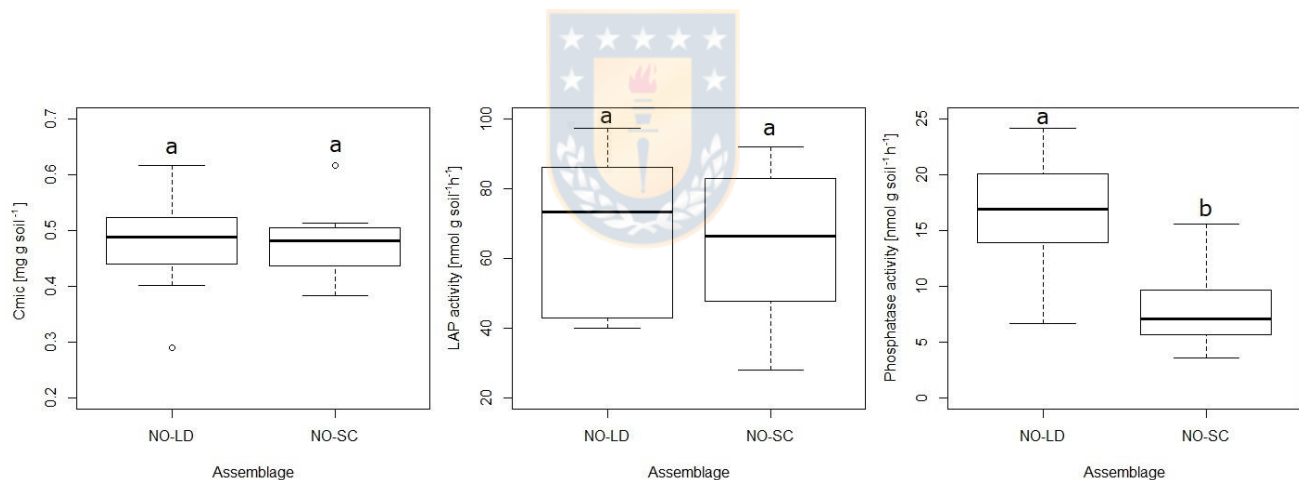


Figure 1. Boxplot of microbial biomass carbon (C_{mic}), leucine aminopeptidase (LAP) activity phosphatase activity and phosphatase activity in soils with *N. obliqua* (NO), growing in mixtures with *S.cassioides* (SC) and *L.dentata* (LD) (NO-SC, NO-LD). Thick line is the median, box upper and lower end represent the 25 and 75 percentiles, while the whiskers 5 and 95 percentile. Letters indicate significant differences among treatments ($p < 0.05$).

Table 1. Soil parameters values for burned soil and each assemblage. Mean \pm standard error (n=10). Significant differences according to the Tuckey test at $p < 0.05$ levels were indicated by different letters. *N. obliqua* (NO), *S. cassioides* (SC) and *L. dentata* (LD). STC: soil total carbon, STN: soil total nitrogen, STP: soil total phosphorus, SAN: soil available nitrogen, SAP: soil available phosphorus, SIP: soil inorganic phosphorus, SOP: soil organic phosphorus

	pH	STC (%)	STN (%)	STP (mg Kg ⁻¹)	SAN (mg Kg ⁻¹)	SAP (mg Kg ⁻¹)	SIP (mg Kg ⁻¹)	SOP (mg Kg ⁻¹)
Original	5.52 \pm 0.08	2.08 \pm 0.14	0.17 \pm 0.007	772.44 \pm 36.81	2.69 \pm 0.30	0.3 \pm 0.13	328.2 \pm 2.24	444.24 \pm 7.84
NO	6.32 \pm 0.22a	2.41 \pm 0.15ab	0.67 \pm 0.006ab	258.82 \pm 19.10a	31.95 \pm 5.61a	6.43 \pm 1.11a	149.48 \pm 20.40a	43.72 \pm 17.99ab
SC	6.57 \pm 0.08a	2.11 \pm 0.37b	0.61 \pm 0.02b	174.79 \pm 25.37ab	21.15 \pm 2.22b	5.85 \pm 1.29a	128.99 \pm 43.85a	48.27 \pm 7.70ab
LD	6.41 \pm 0.31a	2.16 \pm 0.17b	0.63 \pm 0.01b	155.43 \pm 19.46ab	19.99 \pm 3.85b	8.06 \pm 1.43a	165.96 \pm 32.87a	37.47 \pm 11.15b
NO-SC	6.39 \pm 0.15a	2.28 \pm 0.17ab	0.66 \pm 0.10ab	211.69 \pm 12.86ab	20.38 \pm 2.45b	7.27 \pm 0.58a	155.44 \pm 22.35a	66.25 \pm 23.68a
NO-LD	6.70 \pm 0.11a	2.06 \pm 0.20b	0.58 \pm 0.008b	176.48 \pm 22.24ab	20.08 \pm 6.38b	6.52 \pm 1.06a	122.24 \pm 18.77a	54.23 \pm 14.50ab
SC-LD	6.59 \pm 0.08a	2.62 \pm 0.20a	0.76 \pm 0.008a	171.16 \pm 6.96b	27.48 \pm 3.30ab	7.61 \pm 1.85a	126.97 \pm 7.12a	43.45 \pm 2.05ab

3.1.2 Effect of assemblages in functional traits for each specie

Nothofagus obliqua

Most *N. obliqua*'s functional traits differed significantly when this specie was grown in monocultures and in association (RGRd ($p = 6.73 \times 10^{-6}$), RGRh ($p = 0.0102$), TB ($p = 3.1 \times 10^{-5}$), LNC ($p = 7.07 \times 10^{-15}$), LPC ($p = 0.00689$) and RLD ($p = 0.0418$)). The presence of neighboring plants had a significant effect in WUEi ($\delta^{13}\text{C}$) ($p = 1.34 \times 10^{-14}$): NO(LD) > NO(SC) > NO (Table 2). In general, NO showed better growth performance and development of functional traits when growing alone (Figure 2). NO showed significantly higher LPC, RLD, RGRd, RGRh, and TB, while LNC had the lowest values when grown in monoculture. The mix of NO with LD had a negative impact on NO's vegetative traits such as LPC, RGRd, RGRh, and TB; it also decreases RLD and increases LPC. Similarly, the with SC decreased NO's LNC, RGRh and TB compared to NO growing alone, while the mix had no significant effect on LPC, RLD, and RGRd (Table 2, Figure 2 and 3).

Sophora cassioides

S. cassioides's WUEi ($\delta^{13}\text{C}$) varies significantly among mesocosms assemblages ($p=0.000337$). $\delta^{13}\text{C}$ was significantly more negative for SC(NO) (Figure 3). Similarly, assemblages of SC had a significant effect in functional traits such as RGRd ($p = 1.8 \times 10^{-6}$), RGRh ($p = 2.56 \times 10^{-5}$), TB ($p = 5.73 \times 10^{-6}$), LNC ($p = 1.35 \times 10^{-5}$), LPC ($p = 0.00789$), RLD ($p = 1.31 \times 10^{-6}$) and RT ($p = 0.00273$) (Table 2, Figure 2 and 3). SC presented higher LNC, RGRd, RGRh, TB, RLD, and RT when growing in monoculture. When adding neighboring plants, SC's leaf nitrogen content (LNC), root functional traits (RLD and RT), and vegetative traits (RGRd) were reduced. SC's TB and RGRh were only affected in combination with NO; nevertheless, the presence of NO had a significantly positive effect on LPC.

Lomatia dentata

Out of 14 traits evaluated in the LD assemblages, LD only displayed a significantly affected in five of them (RGRh ($p = 0.00195$), TB ($p = 7.51 \times 10^{-7}$), LCC ($p = 0.000495$), LNC ($p = 0.00218$) and LPC ($p = 0.0425$)). Unlike NO and SC, LD did not show any effect in root functional traits. LD showed the highest values of RGRh and TB when growing alone. On the other hand, combination with SC had a positive impact on LCC (Table 2), while negative in LNC and RGRh and TB (Figure 2). Finally, WUEi ($\delta^{13}\text{C}$) was significantly different between mixed assemblages LD(NO) and LD(SC) ($p = 0.0177$) (Table 2). The mix of LD with NO had a positive effect on LD's LNC and LPC while it decreases RGRh and TB.

In sum, only regarding nutrient acquisition, LD boosted nitrogen acquisition but reduces P in NO. Neither LD nor SC altered their P acquisition when co-cultivated. However, LD reduces SC's N acquisition. Similarly, SC diminished N acquisition in NO. On the other hand, NO facilitate P acquisition in both SC and LD. Similarly, NO facilitates N acquisition for SC, but not for LD.

Table 2. Leaf nutritional content and $\delta^{13}\text{C}$ for each specie in assemblages. Mean \pm standard error (n=10). Significant differences according to the Tuckey test at $p < 0.05$ levels were indicated by different letters. *N. obliqua* (NO), *S. cassioides* (SC) and *L. dentata* (LD). Parenthesis denotes the accompanying specie. Leaf nitrogen content (LNC), Leaf phosphorus content (LPC) and Water use efficiency (WUEi) as $\delta^{13}\text{C}$.

Assemblage	LCC (mg g⁻¹)	LNC (mg g⁻¹)	LPC (mg g⁻¹)	WUEi ($\delta^{13}\text{C}$)
<i>Nothofagus obliqua</i>				
NO	443.92 \pm 0.38a	18.75 \pm 0.10b	0.011 \pm 0.002a	-32.3 \pm 0.10a
NO(SC)	447.29 \pm 7.61a	17.04 \pm 0.98c	0.011 \pm 0.001a	-31.85 \pm 0.10b
NO(LD)	447.39 \pm 5.05a	26.48 \pm 1.99a	0.007 \pm 0.004b	-30.6 \pm 0.37c
<i>Sophora cassioides</i>				
SC	431.92 \pm 0.58a	28.92 \pm 0.25a	0.013 \pm 0.003b	-31.31 \pm 0.43b
SC(NO)	424.18 \pm 6.25a	24.78 \pm 2.02b	0.027 \pm 0.016a	-32.08 \pm 0.33a
SC(LD)	417.14 \pm 19.65a	26.10 \pm 1.65b	0.014 \pm 0.002b	-31.48 \pm 0.31c
<i>Lomatia dentata</i>				
LD	427.58 \pm 3.62b	8.42 \pm 0.81b	0.002 \pm 0.0007b	-30.34 \pm 0.47a
LD(SC)	434.50 \pm 3.36a	8.10 \pm 1.32b	0.003 \pm 0.0006ab	-30.64 \pm 0.82a
LD(NO)	429.46 \pm 3.06ab	10.10 \pm 1.26a	0.004 \pm 0.002a	-29.81 \pm 0.32a

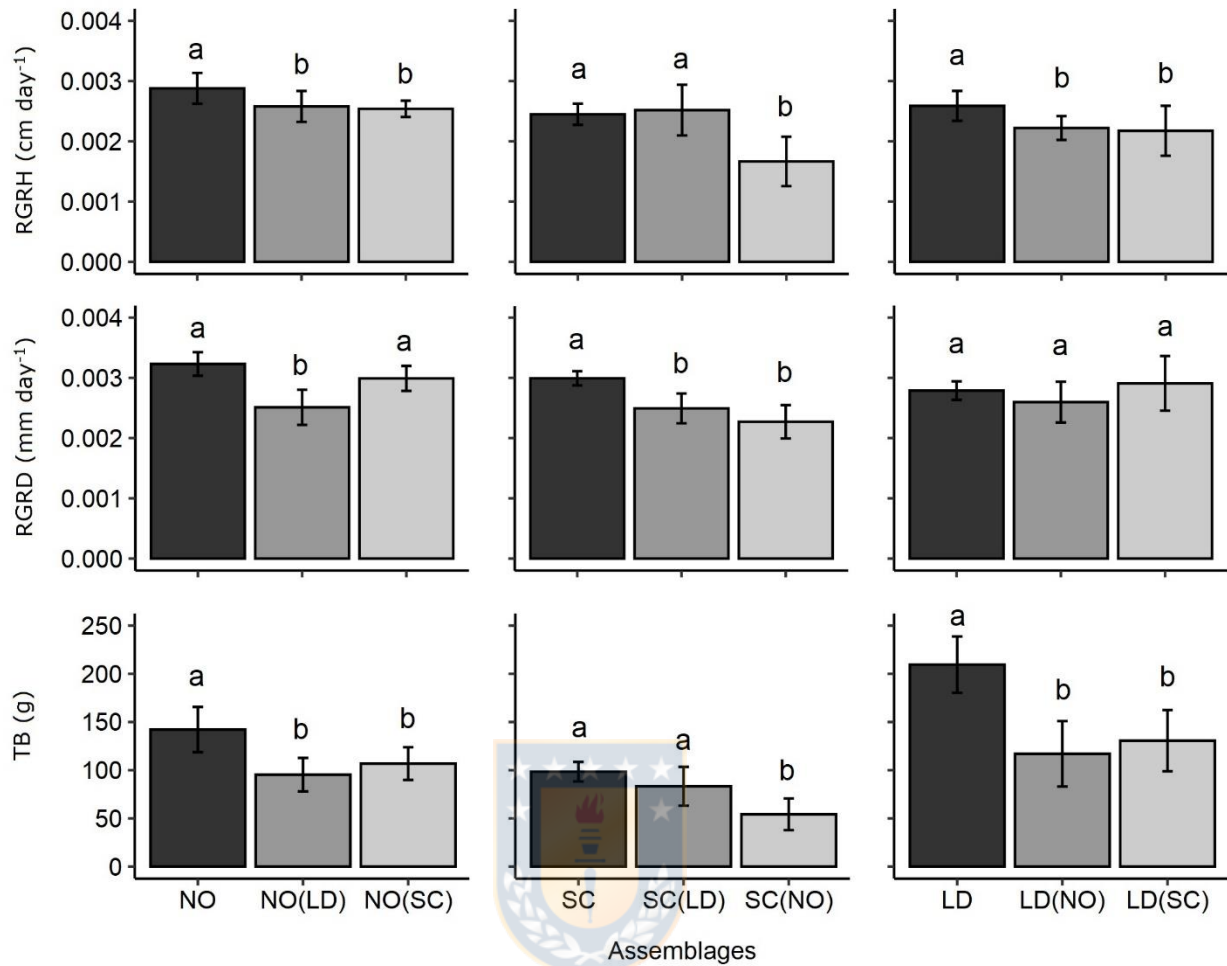


Figure 2. Performance of NO, SC and LD on relative growth rate of height and diameter (RGRH and RGRD) and total biomass (TB). Bars shows means \pm se (n=10). *N. obliqua* (NO), *S. cassioides* (SC) and *L. dentata* (LD). Parenthesis denotes the accompanying specie. Different letters indicate significant differences between plant assemblages ($p < 0.05$).

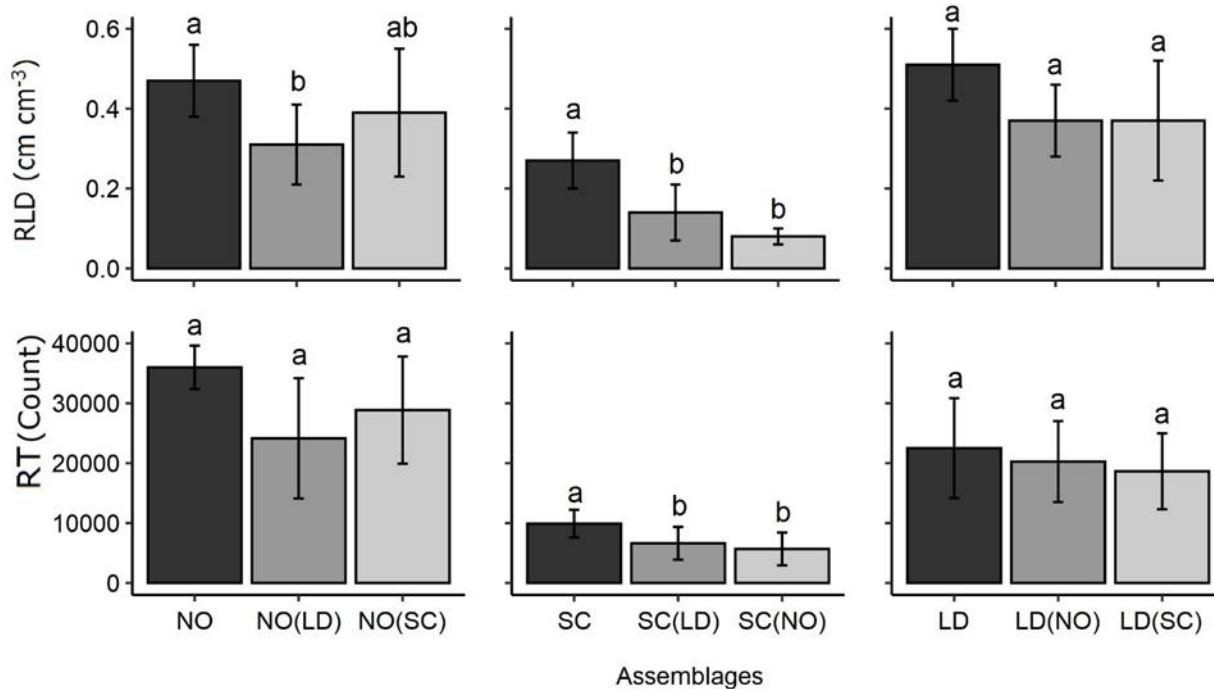


Figure 3. Performance of NO, SC and LD on root length density (RLD) and root tips (RT). Bars shows means \pm se (n=10). *N. obliqua* (NO), *S. cassioides* (SC) and *L. dentata* (LD). Parenthesis denotes the accompanying species. Different letters indicate significant differences between plant assemblages ($p < 0.05$).

3.2 Effect of plant assemblages on plant growth and traits of the species

Response of *Nothofagus obliqua* assemblages

Principal component analysis in *N. obliqua* dataset was explained more than 50% by PC1 and PC 2 (33.8 and 19.4, respectively). PC1, was determined by leaf variables such as WUE ($\delta^{13}\text{C}$), STC, STP and STN (Fig. 4). Meanwhile, PC 2 was related to root functional traits, such as SRA and SRL. RGRd was closely and positively related to STP. It is also important to point out that *N. obliqua* growing in monoculture displayed a significant separation from the assemblage with *S. cassioides*, which is explained mainly by belowground variables such as root functional traits and soil nutrient content.

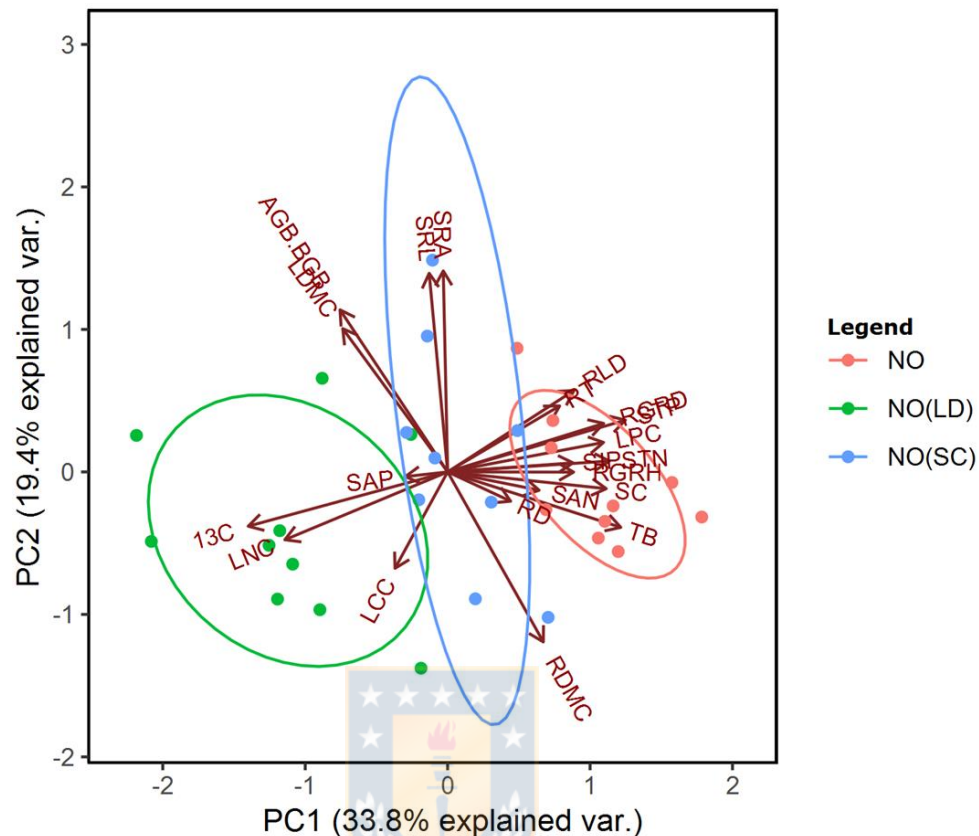


Figure 4. Principal component analysis of soil and plant functional traits. Arrows represent the principal component and symbols represent *N. obliqua* (NO) individuals growing in monoculture and co-cultivated with *L. dentata* (LD) and *S. cassioides* (SC). Ellipses correspond to 95% confidence.

Response of *Sophora cassioides* assemblages

The principal component analysis for *S. cassioides* showed that more than 50% of the variability of the dataset was explain between principal component 1 and 2. Principal component 1 variability (28.8%) was mostly explained by growth and root functional trait, such as relative shoot diameter and root length density, respectively (Fig. 5). It was also explained by soil total phosphorus content, soil inorganic phosphorus and leaf phosphorus content. Principal component 2 variability was explained mainly by root

functional traits such as specific root length and specific root area. The principal component analysis also revealed that *S. cassioides* growing in mixture with *N. obliqua* was clearly different from when it was growing alone. This difference was mostly explained by variables related to soil and plant phosphorus.

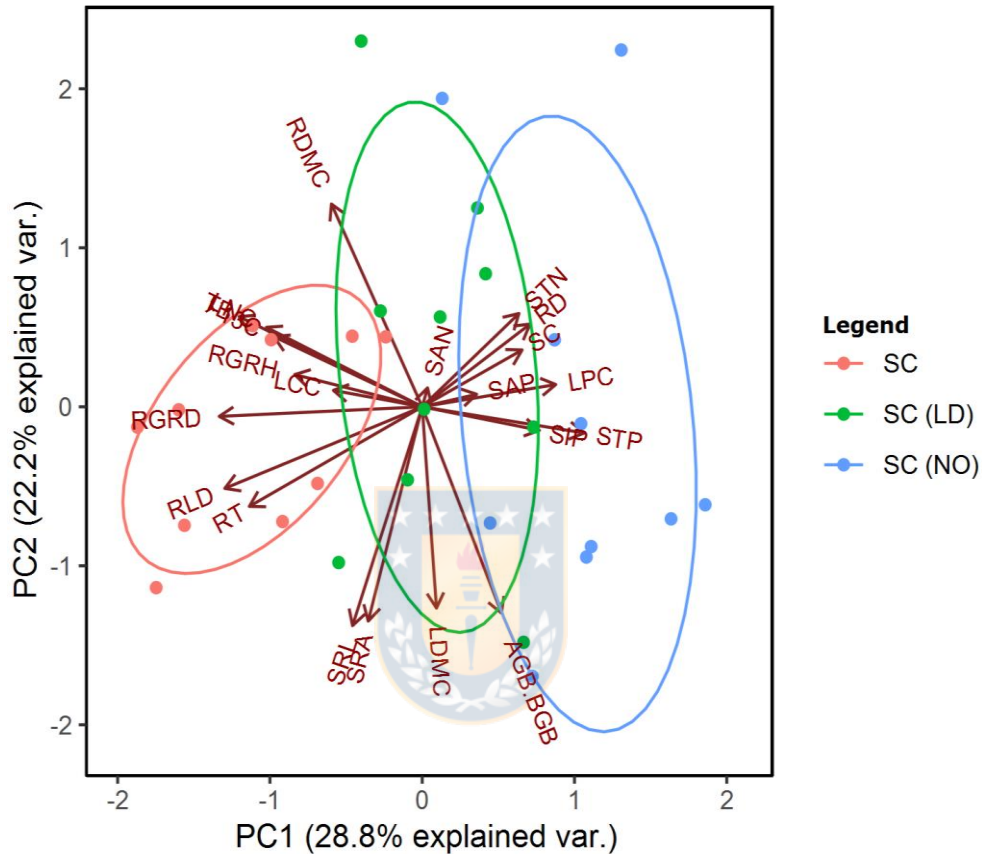


Figure 5. Principal component analysis of soil and plant functional traits. Arrows represent the principal component and symbols represent *S. cassioides* (SC) individuals growing in monoculture and co-cultivated with *L. dentata* (LD) and *N. obliqua* (NO). Ellipses correspond to 95% confidence.

Response of *Lomatia dentata* assemblages

A principal component analysis showed that only 20% of the variability of the whole dataset was explain by principal component 1, mostly by soil total nitrogen content and

soil total carbon, as well as functional root traits such as specific root area and specific root length (Fig. 6). This principal component analysis did not reveal a clear separation between the different assemblages containing *L. dentata*.

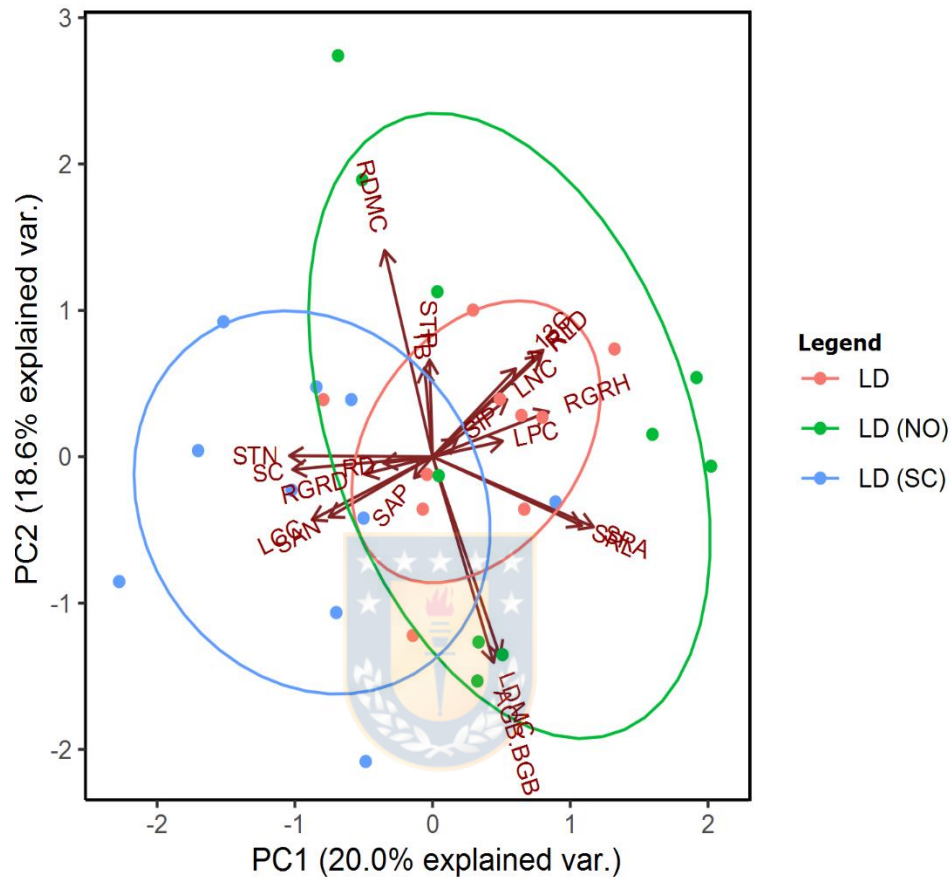


Figure 6. Principal component analysis of soil and plant functional traits. Arrows represent the principal component and symbols represent *L. dentata* (LD) individuals growing in monoculture and co-cultivated with *S. cassioides* (SC) and *N. obliqua* (NO). Ellipses correspond to 95% confidence.

Table 3. Average ratios of C:N, C:P and N:P of species assemblages under fertilization and no fertilization treatment. Mean \pm standard error (n=10). Significant differences according to the Tuckey test at $p < 0.05$ levels were indicated by different letters. *N. obliqua* (NO), *S. cassioides* (SC) and *L. dentata* (LD). Parenthesis show co-cultivation specie. Capital letters show significant differences between treatments and lowercase letters among assemblages in each treatment ($p < 0.05$).

Treatment	Assemblage	C:N	C:P	N:P
<i>Nothofagus obliqua</i>				
Fertilization	NO	15.98 \pm 1.32c	30.38 \pm 1.31b	1.90 \pm 0.15b
	NO(SC)	16.18 \pm 0.70c	49.17 \pm 27.14ab	3.05 \pm 1.73ab
	NO(LD)	14.86 \pm 0.76c	31.92 \pm 12.72b	2.18 \pm 0.97ab
No Fertilization	NO	23.66 \pm 0.16b	40.31 \pm 7.22ab	1.70 \pm 0.30b
	NO(SC)	26.34 \pm 1.83a	42.87 \pm 4.91ab	1.62 \pm 0.13b
	NO(LD)	16.97 \pm 1.31c	84.91 \pm 53.38a	5.14 \pm 3.47a
<i>Sophora cassioides</i>				
Fertilization	SC	16.67 \pm 0.49bc	37.15 \pm 18.55a	2.52 \pm 1.25a
	SC(NO)	13.62 \pm 0.65c	32.31 \pm 12.22ab	2.35 \pm 0.80a
	SC(LD)	14.27 \pm 0.31c	28.87 \pm 2.92ab	2.02 \pm 0.18ab
No Fertilization	SC	14.93 \pm 0.17bc	34.83 \pm 8.74ab	2.32 \pm 0.56a
	SC(NO)	17.24 \pm 1.75a	15.91 \pm 7.30b	0.92 \pm 0.41b
	SC(LD)	16.01 \pm 0.77ab	29.46 \pm 4.22ab	1.84 \pm 0.31ab
<i>Lomatia dentata</i>				
Fertilization	LD	24.02 \pm 4.27c	78.55 \pm 24.76bc	3.38 \pm 1.24a
	LD(SC)	23.76 \pm 3.04c	67.74 \pm 20.96c	2.88 \pm 0.87a
	LD(NO)	20.13 \pm 4.57c	43.62 \pm 15.32bc	2.38 \pm 1.27a
No Fertilization	LD	51.24 \pm 5.12ab	260 \pm 91.72a	5.14 \pm 1.95a
	LD(SC)	55.39 \pm 10.66a	148.79 \pm 36.09bc	2.69 \pm 0.46a
	LD(NO)	43.02 \pm 5.80b	163.40 \pm 84.75ab	3.84 \pm 2.10a

3.3 Fertilization addition effect on leaf C: N:P stoichiometry

As expected, all soils increased their nutrient availability as a result of nutrient addition through fertilization. This increase in soil augmented plant productivity hence nitrogen and phosphorus acquisition.

The analysis of C:N, C:P, and N:P content ratios showed a statistically significant interaction between fertilization treatment and species assemblages ($p = <0.001$).

N. obliqua's C:N ratios were significantly different between all assemblages under no fertilization. In contrast, when fertilization was added, these differences disappear. C:P only showed significant differences between unfertilized and fertilized NO(LD). On the other hand, *N. obliqua*'s N:P was significantly different between assemblages for the fertilized and unfertilized treatment. The most relevant difference was found for NO(LD), which had a much higher ratio (Table 3).

Under the unfertilized treatment, *S. cassioides* display significant differences between assemblages for all three ratios (C:N, C:P, and N:P). *S. cassioides*' in the SC(NO) assemblage display the highest C:N and lowest C:P and N:P ratios. In contrast, when adding fertilization, these differences between assemblages disappeared (Table 3).

Unfertilized *L. dentata* assemblages showed significantly lower C:N in LD(SC) than LD(NO). C:P was substantially higher in LD compared to LD(SC). C:P did not show significant differences between assemblages. When fertilizer was added, all *L. dentata* assemblages significantly decreased C:N ratios compared to unfertilized treatments. C:P only varied for LD growing alone, where the unfertilized monospecific treatment displayed the highest ratio. Finally, N:P did not show significant differences between fertilization treatments or assemblages (Table 3).

4. Discussion

4.1 Plant assemblages modify soil nutrient availability and functional traits development.

The selected burned soils for the mesocosm experiment have a more acidic pH than other nearby affected soils and the unburned forest site (5.52 vs. 6.3-6.7). This low pH could be explained by higher nutrient loss and volatilization of ashes, in addition to the advanced state of erosion in the sampling site (Certini 2005). The pH of the mesocosms soils was raised by approximately one unit after the two years of the experiment in most

assemblages. The lack of differences between monocultures assemblages shows that the pH responded more strongly to the inclusion of vegetation but than to the specific species included. The SC-LD assemblage was the most effective assemblage on accreting C and N in the soils, probably due to synergistic root biomass accumulation product of nodule and luster root development and enhanced root exudates release.

Interestingly, the lowest organic P was found in the LD monoculture. We expected this species to enhance soil P due to cluster root development and organic acid release (Lambers *et al.* 2012). A similar response has been described for another Chilean Proteaceae species that showed a lower P availability in rhizosphere soil than when growing in association with neighboring plants (Piper *et al.* 2019).

We expected that *N. obliqua* would benefit from mixes, taking advantage of excess nutrients that their extensive network of ectomycorrhizal fungi could assimilate. However, the presence of neighboring plants negatively affects *N. obliqua*, especially when mixed with *L. dentata*. The soils (NO-LD) showed lower total and available nitrogen content. Moreover, *Nothofagus* significantly decreased its growth and development. These findings agree with Fajardo and Piper (2019), indicating that *Nothofagus* species did not benefit from the presence of cluster root forming species. Smith and Read (2008) found that cluster rooted species, such as *L. dentata*, promoted available nitrogen uptake and facilitated leaf nitrogen acquisition, but not phosphorus in non-cluster rooted species, such as *N. obliqua*. Higher soil phosphatase activity in NO-LD assemblage indicates that *Nothofagus* is spending more assimilates competing for P. This higher competition for P resulted in a lower LPC in NO. Soils where *N. obliqua* grew with *S. cassioides* as a neighboring plant, presented higher soil organic phosphorus, lower phosphatase activity, and nitrogen availability (SAN) than when growing alone. In this case, *N. obliqua* competes for nitrogen acquisition in the presence of a legume, as expected. Since there was no impact in root traits, this combination of species may represent a lower root activity, hence lower nitrogen fixation and availability, which could be related to lower phosphatase activity (Margalef *et al.* 2017).

We expected that the presence of *S. cassioides* would increase nitrogen availability in soils. However, as highlighted above, SC-LD had the highest STN and STC among all

assemblages. These results indicate that *S. cassioides* and *L. dentata* growing in association, increase productivity, augmenting root exudates production, organic matter accumulation, consequently increasing organic matter bound N and microbial immobilized N. The mix of SC with either NO and LD favored competitive behavior on *S. cassioides* resulting in a reduced nitrogen acquisition at leaf level (LNC), contrary to what has been previously reported (Zúñiga-Feest *et al.* 2018). The reduced N acquisition could be explained by a reduction in root length density (RLD) and root tips (RT) in these assemblages, which directly reduces the capacity to absorb micro and macronutrients (Gilroy and Jones 2000).

We expected that the presence of *L. dentata* would increase nitrogen and phosphorus concentrations in soils (Varughese and Asunción 2014). Monocultures (LD) presented lower SOP due to lower root microbial activity, thus decreasing the organic phosphorus of rapid access for plants (Müller and Bünemann 2014). Lower nutrient content in the soil did not trigger the development of root functional traits associated with nutrients acquisition. The presence of neighboring plants did not significantly affect *L. dentata* in most functional traits evaluated. *N. obliqua* facilitated nitrogen and phosphorus acquisition for *L. dentata*, increasing leaf nitrogen and phosphorus content compared to monoculture. Similar to the results reported by Fajardo and Piper (2019). *L. dentata* redirects its energy to the acquisition of nutrients at the foliar level, spending less energy on total biomass and growth rate. These results contradict previous studies, where the increase of nitrogen in soils could explain a lower concentration of total phosphorus since a higher nitrogen content in the soil allows the plant to increase its growth, hence the need to extract more phosphorus and the ability to solubilize phosphates (Nuruzzaman *et al.* 2005).

4.2 Plant interactions

Our findings showed that each species develop different functional traits, depending on the neighboring species. We observed three types of interactions: Competition, facilitation, and neutral interactions. The main pattern was a decrease in growth and functional traits when a neighboring plant was added, contrary to Bertness and Callaway

(1994). Leaf nutrient content, specifically nitrogen and phosphorus, were the variables that most explained interactions between species. *N. obliqua* and *S. cassioides*, both species, competed for nitrogen acquisition compared to when grown in monoculture. While, *N. obliqua* and *L. dentata* reduced interspecific competition, facilitating each other nitrogen acquisition (Muler *et al.* 2014 ; D Tilman *et al.* 2001), confirming that southern South American Proteaceae can improve nutrient acquisition in neighboring plants. We also found that *N. obliqua* facilitated phosphorus acquisition for neighboring plants.

For LPC, we conclude that *N. obliqua* facilitated leaf phosphorus acquisition for both *S. cassioides* and *L. dentata*. While the presence of *L. dentata* decreased LPC in *N. obliqua*, contrary to Dinkelaker *et al.* (1995), were wheat intercropped with *Lupinus albus*, which has proteoid roots, was able to capture twice as much P and N and more Mn than when grown in monoculture.

As PCA showed, *L. dentata* display the most neutral response to assemblages maintaining traits and growth disregarding neighbor species. *N. obliqua* responded strongly to co-cultivation, especially to the presence of *L. dentata*. Similarly, *S. cassioides* was also affected significantly when growing with *N. obliqua*.

Restoration practitioners may be interested in how we can use these findings from a more practical approach. Here we provided information on how differential complementary root strategies can potentially increase soil nutrient availability and how these belowground interactions modify resource allocation (Clark *et al.* 2012; Reich *et al.* 2003).

4.3 Effect of fertilization on C: N:P leaf stoichiometry

Our hypothesis was proved, where greater availability of nutrients in the soil neutralizes the interspecific competition between the evaluated species in mixed assemblages. The general tendency was a decrease in C:N:P ratios under fertilization treatments.

Due to the enhanced nitrogen and phosphorus availability, there were no significant differences between the foliar nutrient stoichiometry of the assemblages under the

fertilization treatment. It is to be expected that nitrogen and phosphorus acquisition would increase when fertilizer was included. However, these enhance acquisition response was not always significant for some treatments. For example, *S. cassioides* C:N ratio was reduced, but this was most prominent for the co-cultivated treatments. The fertilization effect was more relevant for N than for P for this species, as shown by the increase in N:P when fertilized.

On the other hand, it has been documented that ectomycorrhizal infection decreases in the presence of nitrogen-rich fertilizers (Arnebrant and Söderström 1992), while cluster roots have produced less biomass when treated with fertilizer, besides being sensitive to phosphorus-deficient soils such as volcanic soils (Zúñiga-Feest *et al.* 2010). In our case, by eliminating a possible limitation of nutrients in the soil, the species studied suspended the development of unique root structures, allowing efficient nutrient acquisition for both plants in mixed assemblages, eliminating interspecific competition, and allowing energy to be focused on aerial growth with greater nutrient demand. Thus, the addition of fertilization during the restoration of fire-affected soils could be considered as an effective approach to limit competition in clusters allowing fast growth and soil protection, which is a critical aspect for erosion control and slope stabilization after a fire. However, excessive fertilization could hinder the proper development of root structures that will provide more resilience under future nutrient or water co-limitations. These aspects should be assessed through field experiments as they could be crucial for the long-term success of restoration projects.

5. Conclusions

In general, neighboring plants in co-cultured assemblages showed a notorious decrease in growth and a reduction in most functional traits analyzed. The effect of a given species to neighbor species' nutrient acquisition varied across assemblages and did not only depend on the specific root strategy present in each species. For example, mycorrhizal forming *NO* benefited neighboring plants phosphorus acquisition. However, this did not translate into higher P acquisition for *NO* as *LD* (cluster root) hindered P acquisition for

NO. We also found complementarity for N acquisition (*NO* and *LD*). Opposite to what was hypothesized, co-cultivation of *NO* and nodule forming *SC* hindered N acquisition for both species. As hypothesized, fertilization neutralizes interspecific competition.

Our results showed how the evaluated species adapt their energy investment and functional traits and how these adaptations translate into more effective nutrient acquisition. They also suggest beneficial interspecific interactions that could help soil recovery under post-fire scenarios through plant assemblages. Changes in nutrient acquisition are most likely driven by plant-plant and plant-microbial interactions. Thus, future mesocosms or field studies with native plants should focus on specific microbial-plant associations, such as mycorrhizal fungi, nitrogen-fixing bacteria, and the associated microbiome in different soils types to explore other beneficial belowground interactions.



6. Anexos

Table S1. Chemical variables evaluated for the chosen sites.

Site	NH4 (mg/Kg)	NO3 (mg/Kg)	P Olsen (mg/Kg)	N total (mg/g)	CH (%)	MO (%)	pH
Site 1	6.69	2.22	50.59	1.55	7.62	8.06	6.8
Site 2	1.69	0	33.71	1.5	7.62	7.92	6
Site 3	3.79	1.19	16.51	2.24	26.91	13.08	5.5
Site 4	3.34	0	22.77	4.26	18.58	18.45	6.9

Table S2. Hoagland nutrient solution, compound concentration and salts used in fertilization treatment.

Hoagland nutrient solution		Quimical Compound	
Nutrient	Concentration (ppm)	Salt	Formula
Nitrogen	210	Ferrous Sulfate	FeSO ₄ + 7H ₂ O
Phosphorus	31	Calcium nitrate	Ca(NO ₃) ₂
Potassium	234	Magnesium sulfate	MgSO ₄
Magnesium	34	Monopotassium phosphate	KH ₂ PO ₄
Calcium	160	Potassium Sulfate	K ₂ SO ₄
Sulfur	64	Potassium Carbonate	K ₂ CO ₃
Iron	2,5	Urea	CO(NH ₂) ₂
Manganese	0,5	Manganese Sulfate	MnSO ₄
Boron	0,5	Copper Sulfate	CuSO ₄ + 5H ₂ O
Copper	0,02	Boric acid	H ₃ BO ₃
Zinc	0,05		
Molybdenum	0,01		



Figure S1. Pots inside oven used for calibrating the sensors used to record variations in volumetric moisture content during the calibration period.

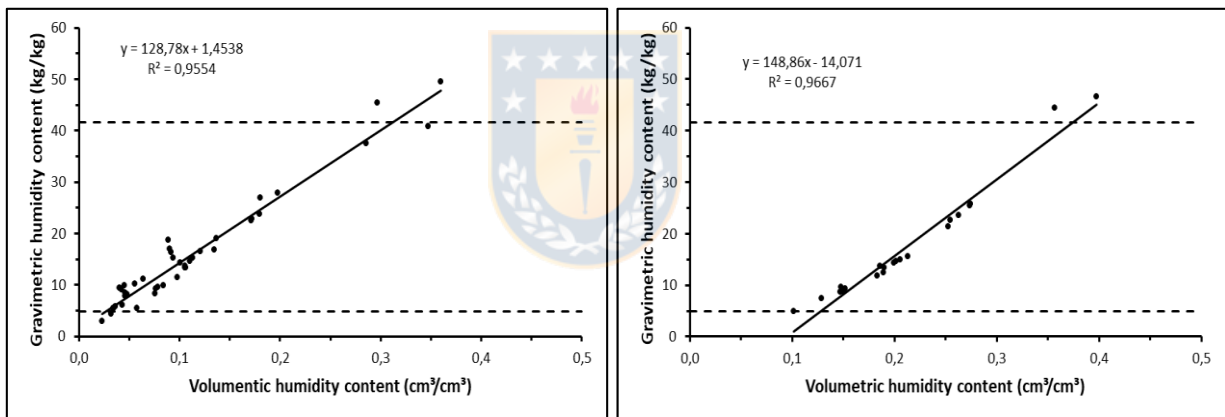


Figure S2. Curve generated to the EM-5 (left) and ET-5 (right) sensors, which presents volumetric and gravimetric moisture content values and determination coefficient.



Figure S3. Plant establishment into pots

No Fertilization		Fertilization	
SC	SC-LD	NO-SC	NO
NO-LD	SC-LD	NO-SC	LD
LD	NO-LD	NO	SC
NO	SC-LD	NO-LD	SC
NO-SC	NO-SC	NO-LD	NO-SC
NO-SC	LD	SC	SC-LD
NO	NO-LD	LD	LD
NO	SC	NO-LD	NO
SC	LD	SC-LD	SC-LD



Figure S4. Pot experiment randomized and fertilization treatment scheme.

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