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Carol Cerda-Peña, Sergio Contreras, A. Huguet

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1 **Intraspecific differences in leaf chemical traits from five common**
2 **evergreen species in contrasting climate conditions (temperature**
3 **and precipitation) from northern Patagonian rainforest (42-44°S)**

4 Cerda-Peña, C.^{1,2,3}, Contreras, S.^{2,3} and Huguet, A.⁴

5
6 ¹Programa de Doctorado en Ciencias Mención en Biodiversidad y Biorecursos,
7 Universidad Católica de la Santísima Concepción, Concepción, Chile

8 ²Departamento de Química Ambiental, Facultad de Ciencias, Departamento de Química
9 Ambiental, Universidad Católica de la Santísima Concepción, Casilla 297, Concepción,
10 Chile

11 ³Centro de Investigación en Biodiversidad y Ambientes Sustentables (CIBAS), Universidad
12 Católica de la Santísima Concepción, Casilla 297, Concepción, Chile

13 ⁴Sorbonne Université, CNRS, EPHE, PSL, UMR METIS, Paris 75005, France

14
15 Orcid

16 Cerda-Peña, C 0000-0002-4213-5021

17 Contreras, S 0000-0001-7192-6810

18 Huguet, A 0000-0002-6124-2922

19
20 Corresponding author: Sergio Contreras scontreras@gmail.com

21 Carol Cerda Peña carol.cerda@ucsc.cl

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36

37

38

39 **Abstract**

40

41 *Background and Aims:* Soil properties and climate influence leaf chemical traits producing
42 intraspecific variation in plants. Studies evaluating their importance in the South American
43 Temperate Forest (STF) species are scarce. This study aimed to evaluate the intraspecific
44 differences in five evergreen species of the rainforest considering two contrasting areas,
45 linking soil and climate with plants traits.

46

47 *Methods:* Soil properties (C_{org} %, N%, C/N, $\delta^{13}C$, $\delta^{15}N$, pH and temperature), climate
48 variables (mean annual precipitation and temperature [MAP; MAT]) and leaf chemical
49 traits (C%, N% and P%, C/N, N/P, $\delta^{13}C$ and $\delta^{15}N$) were measured and compared between
50 two areas in the Northern Patagonia (42°- 44°S). In addition, the relationship of leaf
51 chemical traits with soil and climate was assessed.

52

53 *Results:* Significant differences were found in soil (C_{org} %, C/N and pH; $p < 0.05$) and
54 climate ($p < 0.05$), with MAP identified as the most common factor controlling soil
55 properties (C_{org} %, C/N and $\delta^{15}N$). Intraspecific differences in leaf chemical traits were
56 found between areas, but not in all traits. The most common leaf chemical trait with
57 significant differences was C%. Higher mean C% values were found in the island in plants
58 and soils. High number of correlations ($n=13$ correlations; $p < 0.05$) were found between
59 leaf chemical traits. On the other hand, only MAP was a significant predictor of $\delta^{13}C$ in the
60 leaves.

61

62 *Conclusion:* The leaf chemical traits variability suggests a species-specific response to the
63 soil and climate conditions, with important influence of precipitation as the most common
64 predictor of soil properties and $\delta^{13}C$ in the leaves.

65

66 **Keywords:** Carbon, Nitrogen, Phosphorous, Temperature, Precipitation

67 1. Introduction

68

69 Forest ecosystems have been recognized as one of the most important terrestrial carbon
70 sink due to their absorption capacity of carbon dioxide (CO₂), and their maintenance is
71 essential to mitigate climate change (Bonan 2008; Murdiyarso et al. 2015). However,
72 forests are threatened by climatic events (e.g. drought) (Bréda et al. 2006; Schlesinger et
73 al. 2016), because climate can alter the photosynthetic capacity of the leaves and
74 therefore leaf traits. Among leaf traits, the most studied are the elemental concentration in
75 Carbon (C), Nitrogen (N) and Phosphorous (P). C is the main structural element of leaves
76 (i.e., lignin and cellulose) (Mooney 1972), and its content is related to temperature,
77 affecting photosynthesis and the assimilation of CO₂ (Qaderi et al. 2006; Slot and Winter
78 2017; Dusenge et al. 2019). In addition, δ¹³C in leaves is related to stomatal conductance
79 and water use efficiency (Farquhar and Richards 1984; Roussel et al. 2009), both
80 precipitation and temperatures sensitive processes (Du et al. 2015). N is involved in the
81 production of proteins (Schrader 1984; Tegeder and Masclaux-Daubresse 2018) and P in
82 all the energy molecules (ATP, NADPH) (Lambers and Plaxton 2015; Lambers 2022).
83 Precipitation can affect N and P availability for plants and therefore the leaf concentration,
84 and even the δ¹⁵N in the leaves depending on the chemical form of N uptake (Santiago et
85 al. 2004; Giese et al. 2011; Yan et al. 2020).

86

87 Not only individual elements can change with climate, also the ratio between elements
88 (C/N and N/P) in the leaves. These ratios are key chemical leaf traits (Hobbie and Werner
89 2004; Craine et al. 2015), because plants use these elements in strict proportions to
90 synthesize the biomolecules required for their metabolism (Sterner and Elser 2017). The
91 fact that climatic conditions play an important role in shaping leaf chemical traits (Sardans
92 et al. 2016; Abdala-Roberts et al. 2018; Dong et al. 2020; Du et al. 2020) make useful the

93 measurement of these traits. It allows predicting the forest outcome in response to climate
94 change (Yu and Gao 2011; Trugman et al. 2019) with direct management and
95 conservation implications (Hong et al. 2021).

96

97 To date, leaf chemical traits have been mainly studied interspecifically (i.e. among plant
98 species) at different spatial scales (Poorter and Bongers 2006; Abdala-Roberts et al.
99 2018). Global trends have been proposed like N content increases as a function of leaf
100 mass at higher temperatures and lower precipitation (Wright et al. 2005). Nevertheless,
101 intraspecific variation has been shown to be very high (Siefert et al. 2015; Bloomfield et al.
102 2018; Tautenhahn et al. 2019) and explains even more than interspecies variation
103 considering species turnover along communities (Luo et al. 2016). Then, the lack of
104 information at an intraspecific level likely restricts the use of predictive models trying to
105 understand the fate of forest to climate (Westerband et al. 2021).

106

107 In temperate forest, most studies and reviews evaluating intraspecific variability in leaf
108 chemical traits have been developed with data of Northern Hemisphere forest, and
109 consider temperature and precipitation as the main environmental factors influencing plant
110 characteristics (Ordoñez et al. 2009; Madani et al. 2018; Hecking et al. 2022). The
111 Northern temperate forest (NTF) differs from the Southern temperate forest, because the
112 distribution of the first is affected by enhanced anthropogenic N deposition (Hedin et al.
113 1995; Holland et al. 1999). On the other hand, climatic conditions are also different, with
114 more continentality hence extreme temperature ranges and with more needle-leaved (not
115 broad-leaved) evergreen species in the North than in the South (Armesto et al. 2001).
116 Therefore, information on plants outside the Northern Hemisphere is urgently needed to
117 understand how different forest ecosystems will respond to climate change in these under
118 investigated geographical areas.

119 Extreme climatic events have been observed in the STF over the last years, such as a
120 severe mega drought (2010-2018) with an annual rainfall deficit ranging between 25% and
121 45% (Boisier et al. 2016; Garreaud et al. 2017, 2019), and an increase in the mean annual
122 air temperature (Aranda et al. 2021). This drought has been observed to reduce tree
123 growth in STF (Venegas-González et al. 2019, 2022), and such trend threatens to
124 continue in the coming years (Matskovsky et al. 2021). Changes in precipitation and
125 temperature are expected to occur also in northern Patagonia, of the STF in the evergreen
126 rainforest, south Chile (Araya-Osses et al. 2020).

127

128 However it is required to consider that many others environmental conditions are affecting
129 plants in natural habitats, e.g. their geographical distribution (latitude, longitude, altitude),
130 the radiation levels (Hultine and Marshall 2000; Yang et al. 2016; Abdala-Roberts et al.
131 2016; Sedej et al. 2020), as well as evolutionary process (Flores et al. 2014). It has to be
132 highlighted that soil properties, like soil type (Pensa and Sellin 2003), soil nutrient
133 availability (Ordoñez et al. 2009) also affect the leaf chemical traits. Moreover, some
134 studies have reported that soil properties are more important than climate conditions
135 regarding their influence leaf chemical shifts in plants (Luo et al. 2021). A great influence
136 of the soil properties on leaf chemical traits is especially expected in the STF due to the
137 limited nutrient availability for plants (Borie and Rubio 2003; Redel et al. 2008); attributed
138 to high N loss by leaching in the watershed (Hedin et al. 1995) and the formation of stable
139 complexes between P, Al and Fe attributed to the volcanic origin of soils (Redel et al.
140 2016). Soil nutrient availability is also dependent on climate, as it can affect soil properties
141 like microbial biomass in charge of mineralization, even in the same type of forest (Jafarian
142 et al. 2023). The microbial soil biomass is partly responsible for nutrient availability
143 (Miransari 2011) and is thus considered as crucial for plant species of the temperate forest
144 (Herrera et al. 2020).

145 Previous studies in this forest have found that intraspecific variability accounted for a large
146 proportion of community-level variation in leaf traits (Fajardo and Siefert 2018). STF has a
147 complex orography (Luebert and Pliscoff 2006) and at 42° S begins to fragment in its
148 coastal area, with the larger island called Chiloe island. Most of the studies reporting leaf
149 chemical trait are derived from Chiloe island, because this is a place with scarce
150 anthropogenic disturbances and because large fragments of primary forest still exist
151 (Chacón and Armesto 2006). However previous studies in the area were conducted mainly
152 in litter (Pérez et al. 1991, 1998, 2010; Vann et al. 2002). It is now essential to complete
153 the available data for leaf chemical traits by working directly on leaves from different plant
154 species and by considering the intraspecific variability in the primary forest. It is also
155 needed to extended the geographic coverage of such dataset in the mainland to better
156 reflect the large variability of the environmental conditions in the area.

157

158 The aim of this study was to evaluate potential intraspecific differences of leaf chemical
159 traits in five evergreen species collected along the Chiloe island and mainland (Fig. 1),
160 covering a broad latitudinal zone (42°- 44°S) in the STF. We hypothesized that there will
161 be differences between the leaf chemical traits comparing island and mainland, because
162 these two areas hold different climate conditions with warmer and moderate precipitation
163 in the Chiloe island than in the mainland. We expect to find higher C, N and P content with
164 lower C/N values in leaves for the individuals from the island than for those from the
165 mainland. Such higher C contents may be related to the fact that environmental conditions
166 are less harsh for plant physiology in the island. Regarding the higher N and P, climatic
167 conditions in the island might promote mineralization and therefore enhance nutrient
168 availability (N and P) for the plants. At each sampling site the C, N and P contents of the
169 leaves as well as the ^{13}C and ^{15}N stable isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively)
170 were measured. Precipitation and atmospheric temperature data were determined and

171 bulk soil properties (mean soil particle size, pH, organic C and N content, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)
172 were also measured. This allowed a detailed investigation of the influence of soil
173 properties and climate variables on the leaf chemical traits along the northern Patagonia
174 rainforest.

175

176

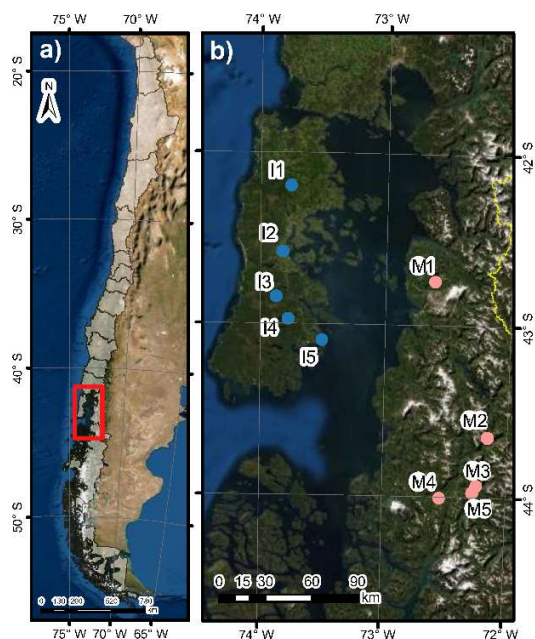
177 **2. Methodology**

178

179 **2.1 Sampling**

180

181 Sampling was performed in January 2018 (austral summer) in the evergreen rainforest of
182 the STF between 42°-44° S and 72°-74° W in Chile (Fig.1). The landscape orography is
183 fragmented in two main terrestrial areas: the Chiloe Island and the mainland, with similar
184 elevation (between 25 to 238 masl). Ten sampling sites (n= 5 in the island and n= 5 in the
185 mainland) were considered because continuous temperature record in soils is available
186 (Tidbit loggers). In each of the ten sites and near the areas where the Tidbit loggers are
187 located (1m), a census of plant species and their abundance was conducted, following a
188 line transect of 100 m length. Based on the census information, the most common species
189 were chosen, accounting a total of five species *Nothofagus dombeyi*, *Amomyrtus luma*,
190 *Chusquea* sp. *Amomyrtus luma* and *Drimys winteri* (Table 1).



191

192 **Fig. 1** a) Location of the study area in Chile. b) Map of the sampling sites. I: island (blue
 193 circles) and M: mainland (pink circles)

194 Table 1. Characteristics of the species studied of the evergreen Rainforest. Leaf life span
 195 as L-life spa

<i>Division/Family/Species</i>	Life form growth	Maximum life span (years)	L- life span (years)	Sites sampled	Individuals sampled
<i>Magnoliophyta</i>					
Nothofagaceae					
<i>Nothofagus dombeyi</i>	Tree	600*	3.5**	2	9
Poaceae					
<i>Chusquea</i> sp.	Bamboo	>10**	NA	8	39
Mirtaceae					
<i>Amomyrtus luma</i>	Tree	200*	3.5*	6	22
Winteraceae					
<i>Drimys winteri</i>	Tree	250*	3.5*	2	11
<i>Pinophyta</i>					
Podocarpaceae					
<i>Saxegothaea conspicua</i>	Tree	>750**	4.2***	4	21
				Total n=	102

196 L-life span: Lusk and Contreras, (2001)*; Hevia, (1991)**; Lusk (2001). Maximum life span: Lusk, (1996)** Lusk
 197 and Pozo, (2002)*; Gonzalez and Donoso, (1998)**.

198

199 At each site, at least three individual per species were collected. All of them were mature
200 individual plants to prevent variability due to different ontogeny stages. The samples were
201 taken with a tree pruner from bottom of the canopy vertical layer of the forest at 6 m aprox.
202 Fully expanded, mature leaves exposed to indirect sun (shade leaves) were selected,
203 stored in kraft paper bags and dried at 50 °C. Superficial soil samples were collected near
204 (0.5 meters) to the ten sites where the temperature records were obtained. The litter layer
205 and biological crust was first removed from the soil. Within each site, three soils replicas
206 were taken, and each individual replica consisted of five subsamples in an area of 1 m² at
207 a depth of 5 cm. The soil samples were frieze-dried (-80°C) and later sieved (<2 mm),
208 removing roots and debris.

209

210 2.2 Plant and soil measurements

211 The leaves were grounded into a fine powder in an electrical grinder (RM 200; Retsch)
212 before chemical analysis. Total C_{org} and N contents (%) (basis dry weight leaf) were
213 measured via dry combustion using EA/IRMS (Thermo Fisher Scientific Delta V
214 Advantage). Total P elemental content was determined by ICP/MS (Agilent 7500-CX) after
215 hot-plate acid digestion (HF, HNO₃ and HClO₄) in Polytetrafluoroethylene (PTFE) vessels.
216 Carbon and nitrogen isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) are expressed in delta notation (‰)
217 relative to the Vienna Pee Dee Belemnite standard (V-PDB) and air, respectively. All leaf
218 analyses were performed at the Alyses platform (IRD – Bondy - France).

219

220 For soil C and N elemental and isotope analysis, the soil was weighed into silver capsules
221 and fumigated with 12M HCl. Once dry, they were wrapped in tin and measured using EA
222 coupled to our Delta Plus XP IRMS (Costech 4010) with carbon and nitrogen isotope
223 values expressed in delta notation (‰) relative to the Vienna Pee Dee Belemnite standard

224 (V-PDB) and air, respectively. The analysis was performed at the Large Lakes
225 Observatory, University of Minnesota-Duluth.

226

227 Soil pH was determined using a pH meter in soil/water ratio of 1:5. Particle size distribution
228 in soils was analyzed using laser diffraction (Mastersizer 3000) and then soil was classified
229 based on the Wentworth scale (Wentworth 1922). Soil temperature data from tidbit loggers
230 (located at 5 cm depth) were used to calculate the mean annual soil temperature (MAST),
231 considering the twelve months before sampling (January 2017 to January 2018). Mean
232 annual precipitation (MAP) and mean annual air temperature (MAAT) were estimated from
233 a monthly reanalysis product available online from the Center for Climate and Resilience
234 Research (CR2), with a climatological grid of 0.05° horizontal resolution, considering
235 available data of the 2018 year for MAP and 2017 for MAAT.

236

237 2.3 Statistical analyses

238 Before any statistical analysis, the data were transformed if needed to obtain normality and
239 homogeneity of the residuals. The mean and standard deviation of soil properties, climate
240 and leaf chemical traits were calculated. A t-student test was performed to evaluate
241 differences between areas (island versus mainland), considering each plant species
242 separately. Then, we conducted a multivariable analysis, through a principal component
243 analysis (PCA), to evaluate multivariate similarities between island and mainland based on
244 soils properties and climate. Data were first centered and standardized (mean=0 and
245 Standard Deviation=1). Another PCA was carried out for leaf chemical traits in order to
246 assess differences among species. To evaluate the influence of MAP and soil properties
247 (pH, MAST, mean grain size) on the soil elemental and isotopic values, a multiple linear
248 regression (MLG) using the stepwise method was conducted. For the MLG a
249 multicollinearity variance inflation factor (VIF) was used considering $VIF < 10$. Then, to

250 understand the correlation among all the leaf chemical traits, independently of the species
251 and considering the whole set of individual plant leaf chemical traits (n=101), a Spearman
252 correlation analysis was performed. In addition, a MLG was conducted considering the
253 mean values of leaf chemical traits by species, to evaluate the relationship between the
254 leaf chemical traits and soil and climatic condition. All statistical analyses were performed
255 using R Studio 1.3.1093 (Rstudio 2020)

256

257 **3. Results**

258

259 3.1 Soil properties and climate variables

260 The soil variables (pH, MPS, MAST and precipitation) differed between the Chiloe island
261 and mainland ($p < 0.01$) (Table 2). The mean annual temperatures were higher in the
262 island (MAST=10.2 °C; MAT=10.6 °C) than in the mainland (MAST=9.9 °C; MAT=8.3 °C).
263 In contrast, the pH and MAP were lower in the island (MAP=1709 mm; pH=4.2) than in the
264 mainland (MAP=3201; pH=4.8), respectively (Table 2). Regarding soil elemental
265 properties, $C_{org}\%$ and C/N showed significant differences between areas ($p < 0.01$; $p < 0.001$,
266 respectively) (Table 2), with mean higher values in the island ($C_{org}\% = 32.9$ and C/N=23.71)
267 than in the mainland ($C_{org}\% = 14.8$; C/N=14.16). Mean values of N%, $\delta^{13}C$ (‰) and $\delta^{15}N$
268 (‰) did not present significant differences between the two areas.

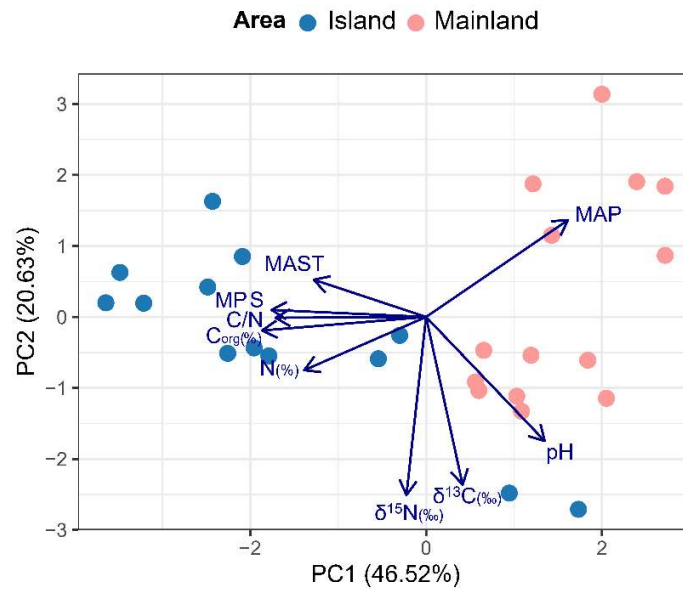
269

270 Table 2. Mean and standard deviation (SD) of the soil properties and climatic parameters
271 in the two areas: island and mainland. Values of the t-student test between the areas are
272 provided. Significant values ($p < 0.05$) are shown in bold. MAST: mean annual soil
273 temperature consider the mean annual record measured directly in soils and MAT: mean
274 air annual temperature.

Variables	Island	Mainland	t/U	P
	Mean ± SD	Mean ± SD		
<i>Climate</i>				
MAT (°C)	10.6 ± 0.3	8.3 ± 0.4	0	<0.001
MAP (mm)	1717 ± 230	3201 ± 450	5.83	<0.001
<i>Soil properties</i>				
MAST (°C)	10.2 ± 0.5	9.2 ± 0.52	3.23	<0.01
pH	4.2 ± 0.6	4.8 ± 0.5	2.85	<0.01
Mean particle size (µm)	149.5	90.5		
Carbon (%)	32.9 ± 16.2	14.8 ± 9.9	3.57	<0.01
Nitrogen (%)	1.3 ± 0.6	1.0 ± 0.6	1.47	0.15
C/N	23.7 ± 6.5	14.2 ± 3.1	4.95	<0.001
δ ¹³ C (‰)	-29.3 ± 2.7	-28.7 ± 0.6	0.83	0.4
δ ¹⁵ N (‰)	0.8 ± 1.9	-0.6 ± 2	1.89	0.06

275

276 The PCA multivariate analysis based on the environmental variables (soils and climate), in
277 the two areas showed that the first two axes of the PCA explain 67.15 % of the total inertia
278 of the dataset (Fig. 2). The first axis explains 46.52% of the total inertia, with similar and
279 higher contributions of C_{org}%, N% and MPS (Table 3). Samples from the island and
280 mainland are well separated along this axis. The second axis explains 20.53% of the
281 variability of the dataset, with a predominant effect of δ¹⁵N, δ¹³C and pH.



282

283 **Fig. 2** PCA ordination of the island and mainland sites based on soil properties and
 284 climatic parameters

285

286 Table 3. Loadings of soil properties and MAP on the first two axes of principal component
 287 analysis of the island and mainland sites based on soil properties and climatic parameters.

288 MAST: mean annual soil temperature, MPS: mean particle size. Variables with loading
 289 higher than the mean on a given PC are shown in bold.

Variable	PC1	PC2
C _{org} (%)	-0.44	-0.04
N (%)	-0.32	-0.17
C/N	-0.40	-0.002
δ ¹³ C (‰)	0.09	-0.56
δ ¹⁵ N (‰)	-0.05	-0.59
pH	0.32	-0.41
MPS	-0.41	0.02

MAST (°C)	-0.30	0.12
MAP (mm)	0.38	0.32

290

291 The assessment of the possible effect of MAP and soil properties into the elemental and
 292 isotopic values of soil, according to the stepwise multiple regression, showed that MAP is
 293 the most common significant predictor of soil dependent variables (Table 4). The
 294 regressions of C/N ($R^2= 0.74$) and $C_{org}\%$ ($R^2= 0.67$), have the highest determination
 295 coefficients. For C/N, MAP and MAST were the best predictors ($p<0.001$) and for $C_{org}\%$ it
 296 was MAP ($p<0.5$) and MPS ($p<0.01$). For all the other elemental and isotopic variables,
 297 only one variable was the best predictor: MPS for N% ($p <0.001$), pH for $\delta^{13}C$ ($p <0.01$),
 298 and finally MAP for $\delta^{15}N$ ($p <0.01$).

299

300 Table 4. Best regression models for multiple regression analyses with the stepwise
 301 method for elemental and isotopic C and N values in soils. The independent variables
 302 were MAP, MAST, pH (water) and MPS. Data transformation are shown as subscript and
 303 partial beta coefficients ($p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$) considered as significant
 304 are shown in bold.

Variable	Beta coefficient						R ²	F	p<
	Intercept	MAP	MAST	pH	MPS				
$C_{org}\%$ (Cochrane-Orcutt)	23.26	-0.006*			0.14***	0.67	26.4	0.001	
N% (Cochrane-Orcutt)	-1.72				0.008***	0.49	11.6	0.001	
C/N	86.5***	-0.005***	-7.001***	-2.46		0.74	23.1	0.001	
$\delta^{13}C$ (‰) (Johnson)	-3.35*	-0.0003		0.95**		0.31	5.76	0.01	
$\delta^{15}N$ (‰) (Cochrane-Orcutt)	0.66	-0.001**		0.89		0.36	7	0.01	

305

306

307 3.2 Leaf chemical traits

308 The C_{org}% is higher than 50% in all species, *D. wintery* presenting the highest values and
309 *Chusquea* sp. the lowest (Table 5). Three of the five species showed significantly different
310 C_{org} content between the island and mainland, with higher values in the mainland than in
311 the island for *D. wintery* (t=4.69; *p* <0.01) and lower ones for *Chusquea* sp. (t=4.49;
312 *p*<0.001) and *S. conspicua* (t=-6.08; *p* <0.001). The N% is higher than 1% for all the
313 species, with *Chusquea* sp. presenting the highest values (~3%). *S. conspicua* is the only
314 species presenting significant N% values between the mainland and the island (t=2.9; *p*
315 <0.05). The P% is around 0.1% for all species, with high CV% in comparison with the other
316 elements (see Table 5 for details). Only *A. luma* present significant differences in P
317 contents between the mainland and the island (t=-4.72; *p* <0.001).

318

319 Whatever the species, the C/N ratio presented higher values in the island than in the
320 mainland. Nevertheless, this difference was only significant for two of the five species:
321 *Chusquea* sp. (t=2.90; *p* <0.01) and *S. conspicua* (t=-3.91; *p* <0.01). Significant differences
322 in N/P between the island and the mainland were only observed for *A. luma* (t=5.87; *p*
323 <0.001). The δ¹³C values present a consistent pattern, with higher values in the island than
324 in the mainland for all species, even though such differences were only significant for
325 *Chusquea* sp. (t=2.76; *p* <0.01) and *S. conspicua* (t=-2.39; *p* <0.05). Finally, there was no
326 specific trend for δ¹⁵N values when considering the different species or sampling location
327 (island vs. mainland).

328

329 Table 5. Mean, standard deviation (SD) and variation coefficient (CV) of the leaf chemical
330 traits of five species: *A. luma* (*Amomyrtus luma*), *D. wintery* (*Drymis wintery*), *N. dombeyi*
331 (*Nothofagus dombeyi*), *Chusquea* sp., *S. conspicua* (*Saxegothaea conspicua*) in the two
332 sampling areas (island and mainland). The *p* values of the t-student or U for Mann Whitney

333 test and the significant values ($p < 0.05$) are shown in bold, from the comparison between
 334 the values of the different variables in the island and mainland.

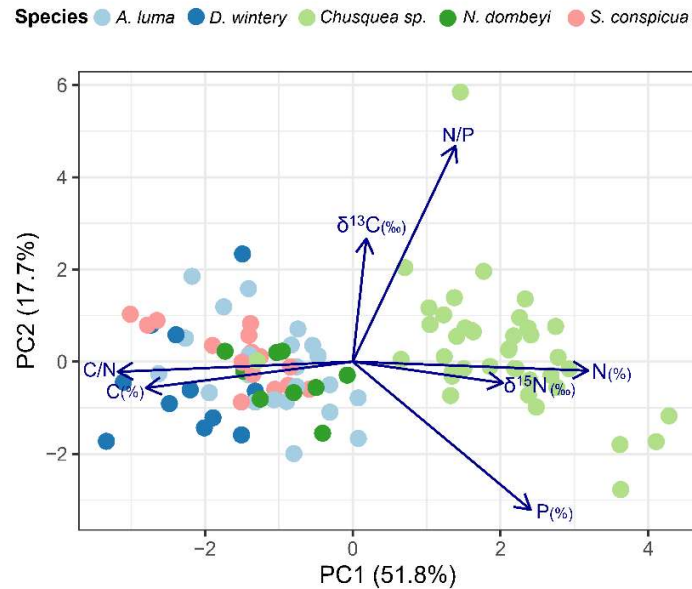
	Island			Mainland			t/U	p
	Mean	SD	CV(%)	Mean	SD	CV(%)		
A. Luma								
C %	58.47	2.61	4.47	59.11	0.73	1.24	65	0.9
N %	1.23	0.25	20.56	1.40	0.31	22.14	-1.38	0.18
P %	0.08	0.02	30.87	0.13	0.03	23.16	-4.72	<0.001
C/N ratio	49.10	9.15	18.65	44.33	10.53	23.75	1.13	0.27
N/P ratio	16.65	2.78	16.67	10.77	1.82	16.93	5.87	<0.001
$\delta^{13}\text{C}$ (‰)	-30.94	1.53	4.95	-31.41	0.82	2.61	0.89	0.38
$\delta^{15}\text{N}$ (‰)	-3.17	3.87	122.22	-4.27	3.42	80.12	0.70	0.48
D. wintery								
C%	62.37	0.59	0.94	64.74	1.05	1.63	4.69	<0.01
N %	1.21	0.30	25.25	1.21	0.24	20.13	0.01	0.99
P %	0.12	0.03	27.27	0.10	0.04	43.13	-0.54	0.60
C/N ratio	54.56	14.50	26.57	55.70	12.92	23.19	0.13	0.89
N/P ratio	10.85	2.57	23.67	14.07	6.94	49.37	1.05	0.32
$\delta^{13}\text{C}$ (‰)	-32.19	1.50	-4.67	-30.70	1.38	4.51	1.70	0.12
$\delta^{15}\text{N}$ (‰)	-7.19	1.57	21.88	-8.44	2.43	28.79	-1.02	0.33
N. dombeyi								
C%	62.21	1.36	2.19	61.22	0.58	0.94	1.35	0.24
N%	1.50	0.18	11.89	1.51	0.26	17.11	-0.03	0.97
P%	0.13	0.04	27.96	0.10	0.03	29.24	1.22	0.26
C/N ratio	41.87	4.60	11.00	41.64	7.30	17.53	0.05	0.95

N/P ratio	11.92	2.58	21.66	14.96	2.10	14.02	-1.9	0.10
$\delta^{13}\text{C}$ (‰)	-30.21	1.14	3.76	-32.03	1.48	4.61	2.09	0.07
$\delta^{15}\text{N}$ (‰)	-4.43	3.49	78.73	-0.93	1.32	142.49	-1.90	0.13
<i>Chusquea</i>								
sp.								
C %	55.24	2.19	3.97	51.97	2.40	4.61	4.49	<0.001
N %	2.75	0.64	23.31	3.11	0.56	18.15	-1.85	0.07
P %	0.16	0.05	31.72	0.17	0.05	32.10	136	0.38
C/N ratio	21.26	6.01	28.28	17.18	2.84	16.52	2.90	<0.01
N/P ratio	18.01	3.84	21.35	19.77	7.76	39.25	186	0.71
$\delta^{13}\text{C}$ (‰)	-29.67	1.67	5.63	-30.86	0.96	3.12	2.76	<0.01*
$\delta^{15}\text{N}$ (‰)	-2.46	2.92	118.45	-0.45	4.13	908.76	150	0.18
<i>S. conspicua</i>								
C %	64.31	1.07	1.66	61.68	0.92	1.49	-6.08	<0.001
N %	1.09	0.09	8.33	1.31	0.14	10.74	2.9	<0.05
P %	0.09	0.02	22.54	0.10	0.02	22.13	0.84	0.41
C/N ratio	59.91	6.2	10.41	47.53	5.14	10.82	-3.91	<0.01
N/P ratio	14.23	1.32	9.26	13.84	2.31	16.66	-0.3	0.76
$\delta^{13}\text{C}$ (‰)	-29.56	0.87	2.93	-30.82	1.45	4.69	-2.39	<0.5
$\delta^{15}\text{N}$ (‰)	-5.97	4.37	73.17	-1.50	1.01	67.31	2.02	0.13*

335

336 The first two axes of the PCA of the leaf chemical traits of all the species explained 69.5%
337 of the variation of the data (Fig. 3). The first axis explained 51.8% of the total inertia of the
338 data, with the four tree species separated from the *Chusquea* sp. bamboo along this axis.
339 The variables having a predominant effect along this axis were N%, C/N and C% (Table

340 6). The second axis explained 17.8% of the variation of the data, with N/P and P% as the
 341 main contributors of the axis.
 342



343
 344 **Fig. 3** PCA ordination of five plants species *Amomyrtus luma*, *Drimys winteri*, *Chusquea*
 345 *sp.*, *Nothofagus dombeyi* and *Saxegothaea conspicua* on the basis of seven leaf chemical
 346 traits

347
 348 Table 6. Loadings of leaf chemical traits for the five plant species along the first two axes
 349 of the PCA. Variables with loadings higher than the mean on a given PC are shown in
 350 bold.

Variable	PC1	PC2
C (%)	-0.43	-0.10
N (%)	0.50	-0.06
P (%)	0.37	-0.55
C/N	-0.50	-0.01

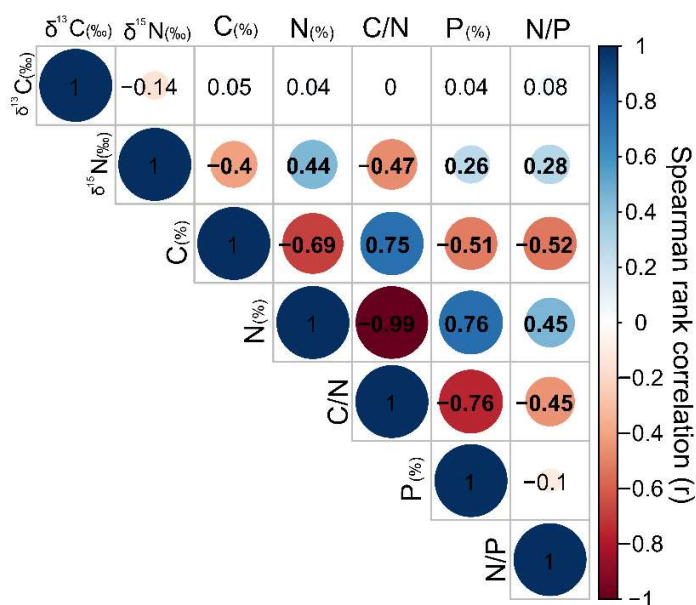
N/P	0.22	0.74
$\delta^{13}\text{C}$ (‰)	0.09	0.34
$\delta^{15}\text{N}$ (‰)	0.30	-0.45

351

352 Bivariate correlations between leaf chemical traits of the five species were observed (Fig.
 353 3). High significant correlations were obtained between N% and C/N ($r=-0.99$), P% and
 354 C/N ($r=-0.76$), C% and N ($r=-0.69$), N and P ($r=0.76$) and C% and C/N (0.75). Only $\delta^{13}\text{C}$
 355 values were not correlated significantly with any other leaf chemical trait.

356

357 Multiple linear regression models between the different leaf chemical traits on the one
 358 hand and soil and climatic properties on the other hand were tested (Table A.1). Only $\delta^{13}\text{C}$
 359 values gave a significant model where MAP was the only significant predictor [$\delta^{13}\text{C}$ (‰) = -
 360 28.72 -0.0008MAP; $F=10.1$, $p<0.001$, $R^2=0.3$].



361

362

363 **Fig. 4** Correlogram among leaf chemical traits for all the individuals (n=101) of the five
364 species: *Amomyrtus luma*, *Drimys winteri*, *Chusquea* sp., *Nothofagus dombeyi* and
365 *Saxegothaea conspicua*. The correlation values are indicated in the circles and the
366 significant values ($p < 0.05$) are shown in bold

367

368 **4. Discussion**

369

370 4.1 Soils of the evergreen STF

371

372 STF is the second largest temperate forest of the world (Donoso 1993). This
373 biogeographical unit that includes the rainforest is a significant carbon sink (Urrutia-
374 Jalabert et al. 2015; Perez-Quezada et al. 2018). In addition, the STF has a high plant
375 endemism due to several geographical barriers (Villagrán and Hinojosa 2005), but
376 unfortunately is highly deforested, especially in its northern and central areas (Echeverria
377 et al. 2006; Heilmayr et al. 2016). Forest soils highly contribute to the distribution and
378 abundance of plant species (Mangan et al. 2010), and are considered as essential to
379 predict future suitable areas for plant species existence (Zuquim et al. 2020). In the STF,
380 most of the information on soils are provided by one organization (Centro de Información
381 de Recursos Naturales - CIREN) related to agricultural activities and working principally in
382 the central area of the STF. In contrast, the physico-chemical properties of the soils in the
383 south of the STF, in the rainforest and in natural areas (i.e. native forest area), are
384 generally unknown, with the few available information obtained by modelling (Padarian et
385 al. 2017). However, C_{org} %, N and P contents as well as the C/N and pH values reported in
386 this study (Table 2) are similar to those reported in previous studies within the northern
387 (Aravena et al. 2002; Perez-Quezada et al. 2021b) and central (Pérez et al. 2005, 2009,

388 2010; Bannister et al. 2021) parts of the island. In the mainland part, almost no information
389 on soils is available (Casati et al. 2019).

390

391 4.1.1 Soil properties between sites

392

393 C_{org} % content in soils of other temperate rainforests are similar (Sun et al. 2004) to those
394 found in this study, even though a high C_{org} % was found in the island (Table 2; Fig. 2a).

395 Plant litter input in soils can contribute, at least partly, to such a difference in C_{org} %
396 contents and C/N between soils of the island and the mainland, because litter affects the
397 soil chemistry (Xu et al. 2013; Bowden et al. 2014). Plant species studied here are the
398 main source of litter in the soil, and the C_{org} % and C/N in their leaves was higher in the
399 island than in the mainland, similarly to what was observed in soils (Table 5). Litter quality
400 is one of the main factors influencing litter decomposition (i.e. physical breakdown of the
401 material). The C/N value is considered as an indicator of the litter quality. High C/N values
402 are reported for Chiloe litter (Pérez et al. 2010), suggesting low litter decomposition rates
403 that may promote C accumulation in the area (Perez-Quezada et al. 2021a). Besides, in
404 Chiloe C_{org} stock in soils has been related to photosynthetically active radiation (PAR) and
405 canopy openness rather than to climatic factors (Perez-Quezada et al. 2021a). The
406 conjunction of litter quality, PAR and canopy openness could explain the high total C_{org} % for
407 Chiloe island soils that has been reported in this study and previous ones, with contents
408 reaching values > 40% (Zarin et al. 1998; Vann et al. 2002; Muñoz Pedreros 2008; Perez-
409 Quezada et al. 2021a). In addition, even if mainland and island sites share part of the
410 species composition, the plant communities inhabiting these two areas are not totally
411 similar, which can also affect the litter and soil C content (Hättenschwiler 2005; Vesterdal
412 et al. 2013). A trend towards a higher N% content in the island than in the mainland was
413 also observed, consistent with higher atmospheric N fixation in northern areas of Chile

414 (Staelens et al. 2005). In general the N% values in the region are high compared to other
415 temperate forests (Perakis and Sinkhorn 2011), which has been attributed to low N
416 mineralization rates reported in the area (Perez et al. 1998).

417

418 Environmental conditions on the island (Table 2) seem more favorable to soil
419 mineralization because of the higher C/N ratio and soil temperatures and the more
420 moderate precipitation (Breuer et al. 2002; Szukics et al. 2010). However the C/N ratio
421 cannot be systematically considered as a reliable qualitative indicator of soil organic matter
422 mineralization (Piñeiro et al. 2006; Ostrowska and Porębska 2015). Some studies reported
423 significant positive correlations between C/N and the abundance of bacterial communities,
424 responsible for mineralization in soils (Luo et al. 2016; Liu et al. 2020). Nevertheless, the
425 microbial abundance is also influenced by pH, with low community composition and
426 biomass at low pH (Bååth and Anderson 2003; Aciego Pietri and Brookes 2009; Ni et al.
427 2021). In this study a lower pH was observed in soils from the island than in those from
428 the mainland, which could at least partly explain the differences in C_{org}% contents and why
429 the expected differences in nutrient availability attributed to mineralization were not found.

430

431 4.1.2 Linking soil to climate

432 In the multiple regression models predicting the soil elemental and isotope properties,
433 MAP was the most common variable followed by MGS (Table 4). MAP was related to C/N,
434 C_{org}% and $\delta^{15}\text{N}$ (‰) and is a key variable affecting the soil properties in the evergreen
435 rainforest. When forests are studied, precipitation is often considered as an indirect
436 measure of water availability for plants (Guswa et al. 2007). However, precipitation is also
437 related to soil moisture (Zhu et al. 2014; Jin et al. 2018) and affects the physical breakup
438 of the soil aggregates (Zhang et al. 2018) and the decomposition of litter and detritus
439 above the soil (Li et al. 2007; Zhou et al. 2018). It can, thus, also influence the soil nutrient

440 availability for plants. Another important soil variable was mean particle size. This was a
441 significant predictor of soil C_{org} % and N%, as previously observed in other types of forest
442 (Hughes et al. 2002). Effects of particle size are important for soil functioning because it
443 affects aeration capacities, the water content (Manns et al. 2014) and other physico-
444 chemical processes that are related to soil organic matter degradation (Grandy et al.
445 2009). As MPS is a significant predictor for soil elemental properties in the present work, it
446 implies that it could be better to work with textural classes in future studies. This is
447 supported by previous worked, which showed that various soil classes tend to be
448 differently related to soil carbon content (Augustin and Cihacek 2016).

449

450 A negative relationship was observed between soil C_{org} % and MAP (Table 4). Thus, the
451 projected decrease in precipitation in the area (Araya-Osses et al. 2020) might affect the C
452 cycling, with an increase in the C_{org} amount in soils from both areas. High C storage by
453 lower litter decomposition rates and higher chemical recalcitrance of organic matter at
454 lower precipitation were reported (Campo and Merino 2016). However, the C cycle was
455 shown to be strongly influenced by the season, with C sink in winter and C release in
456 summer (Perez-Quezada et al. 2018). Models project shorter winters and longer summers
457 in the region (Araya-Osses et al. 2020). In addition, the investigated area is considered as
458 relatively pristine in comparison with the other parts of the STF. The Chiloe Island has a
459 long history of logging (Aravena et al. 2002) and hence increased erosion. Then the
460 synergistic effect of seasonality, climate change and human sourced changes can highly
461 impact the carbon cycling within the forest, especially in the island. More studies on the
462 carbon cycling are needed, as until now previous works in the area mainly focused in the
463 nitrogen cycle (Pérez et al. 1998, 2010; Dixon et al. 2011).

464

465 4.2 Leaf chemical traits

466 4.2.1 Intraspecific variability of leaf chemical traits in the island and mainland.

467 As presented earlier, we found significant differences in leaf traits between sites, but only
468 in some of the traits like C% and C/N, where higher values characterized four of the five
469 species in the island in comparison with the mainland. High C% in leaves relates to low
470 nutritional leaf quality and may act as a defense strategy against insects. This is critical for
471 evergreen species: this strategy could be used for the individuals of the island, which is an
472 area with less precipitation than in the mainland and where usually insect defoliation is
473 higher (Ward and Aukema 2019; Fang et al. 2021). The high production of nonstructural
474 carbohydrates like starch in response to low water availability may also explain the high
475 C% in leaves, as observed in agricultural plants and woody plants (AbdElgawad et al.
476 2020; Du et al. 2020). It should be noted that differences in C/N ratio between the island
477 and the mainland is likely driven by changes in $C_{org}\%$, as the N% is similar between the
478 two sites in the different plants (Table 5). The N% was similar in plants but high values
479 characterized the mainland. The contrary was observed in soils, with high values
480 dominating island soils. This opposite trend could be explained by the fact we measured
481 total N% and not available N%. $\delta^{13}C$ shows similar patterns for the five plant species, with
482 more negative values in the island than in the mainland. This similar pattern may reflect
483 the differences in MAP between both areas (Guo and Xie 2006), because $\delta^{13}C$ values in
484 leaves has been related to water used efficiency (Cao et al. 2020). C3 plants living in poor
485 water conditions are supposed to be enriched in $\delta^{13}C$, in comparison with individuals of the
486 same species living in optimal water conditions (Farquhar and Richards 1984). Less
487 negatives values of $\delta^{13}C$ (‰) with precipitation were also reported in several forests (Van
488 de Water et al. 2002; Kohn 2010; Ma et al. 2012), as observed in this study.

489 It is important to mention that N/P is species-dependent both on the island and mainland.
490 Mean values of N/P were almost all >12.5, consistent with values reported for other plant
491 species in the STF (Seaman et al. 2015); nevertheless, no differences between the island

492 and the mainland were observed (Table 5). The N/P ratio is considered as an indicator of
493 P limitation over N (Tessier and Raynal 2003; Richardson et al. 2004). Even though soils
494 of temperate forests are regularly considered as being N-limited, a previous work in the
495 Chiloe Island (Hedin 1995) showed that there is a strong biotic control of N (Perez et al.
496 2003). In contrast it is assumed that the STF is not P-limited, due to the recent soil
497 geological origin, the inorganic P content, necessary for plant growth, is limited (Redel et
498 al. 2008). This is related to the volcanic origin of the soils (Borie and Rubio 2003). Thus,
499 considering N/P results of the species under study, it is highly recommended to include
500 soil P analyses in further studies in the area.

501

502 4.2.2 Leaf traits among species

503

504 Leaf chemical traits differed from one species to another (Table 5). *S. conspicua* showed
505 the highest number of differences (n traits = 4), followed by *Chusquea* sp. (n traits = 3), *A.*
506 *luma* (n traits = 2), *D. wintery* (n traits = 1), without any significant difference for *N.*
507 *dombeyi*. All the investigated species are evergreen, even though *S. conspicua* is the only
508 gymnosperm species and the only species of the genus, characterized by a narrow spatial
509 distribution compared with the four others trees (Rodriguez et al. 2018). *S. conspicua* also
510 has the highest mean leaf life span (Lusk 2001) among the other species (Table 1).
511 Species with higher leaf life span are characterized by their high leaf toughness (high fiber
512 content and high dry mass to area ratio) (Reich et al. 1991; Onoda et al. 2011; Kitajima et
513 al. 2012). *S. conspicua* characteristics correspond to those of tough leaves as it exhibited
514 higher C% than those observed for the angiosperm species in this study. Species with
515 longer leaf life span as *S. conspicua* has more time to invest in their carbon for tissue
516 construction than species with shorter leaf life span (Chabot and Hicks 1982). *S.*
517 *conspicua* also presents low N% values as previously observed in other conifers of the

518 SFT (N= ~ 1%) (Gallardo et al. 2012). Plant species highly sensitive to environmental
519 changes can be used as biomonitoring species (Karmakar et al. 2021). In the rainforest, *S.*
520 *conspicua* can typically be considered as an appropriate species for monitoring early plant
521 responses to changes in environmental conditions. In addition, *N. dombeyi* is the species
522 with the wider environmental distribution and without significant differences in leaf
523 chemical traits between the mainland and the island (Table 5). Besides *N. dombeyi*
524 showed similar N% and P% values to those reported further north at 37° S (Hevia et al.
525 1999) and at 40°S (Soto et al. 2017). The absence of significant differences between the
526 island and the mainland can be due to the fact that this species has been reported as a
527 plastic species and is able to inhabit in a wide range of soil conditions (Soto et al. 2014). In
528 addition, *Nothofagus* species form ectomycorrhizal mutualistic interactions, which help it to
529 survive in soils with low nutrient amounts (Alvarez et al. 2009; Godoy and Marín 2019).
530 However, it is important to consider that morphological leaf traits in *N. dombeyi* have
531 shown significant differences between nearby sites characterized by contrasting MAP
532 (Diaz et al. 2022). This evergreen species is sensitive to extreme climate conditions with
533 drought-induced mortality reported in the east part of the STF (Suarez et al. 2004).

534

535 Among all the species tested here, *Chusquea* sp. is the most abundant understory species
536 in the STF (Veblen et al. 1977). Furthermore, it is important for the biodiversity of the forest
537 because their leaves are used as material for the nest of many vertebrates (Honorato et al.
538 2016) and as food for rodent (Milesi et al. 2017). The *Chusquea* spp is largely contributing
539 to the biogeochemical recycling of elements within the forest, as their litter represents
540 between 12- 23% of the total litter in old forest of the STF (Gonzalez and Donoso 1999;
541 Vivanco and Austin 2008). When the species studied are compared, the multivariate
542 analysis of leaf chemical traits showed a spatial discrimination between tree species on
543 the one hand and the understory species *Chusquea* sp. on the other hand (Fig. 3). The

544 latter is positively correlated with N% and negatively with C/N and C% (Table 6). The high
545 N content and low C content can be explained by the thickness of the palisade-leaf
546 mesophyll (Wright and Westoby 2002) and fast growth (Poorter et al. 1990). *Chusquea* sp.
547 fulfills this condition with leaves that have low blade thickness (Guerreiro et al. 2013). C%,
548 N% and P% were quantified in the litter of some *Chusquea* species of the STF and the N%
549 was reported to be low (1.17% and 0.5%) and the C/N to be high (43.5) (Veblen 1982;
550 Austin and Marchesini 2012) making the litter derived from these leaves a low quality one.
551 However, in this study, the amount of N in fresh leaves of *Chusquea* sp. is two to three
552 times higher than for the other species, leading to low C/N ratios. The differences in the
553 elemental properties of the fresh leaves and litter of *Chusquea* sp. might be due to nutrient
554 transport from old leaves prior to abscission (Killingbeck 2004). The resorption strategy is
555 typical in species like those in this study to obtain nutrient in new leaves in soils with poor
556 nutrient conditions (Huang et al. 2008; See et al. 2015) as in the rainforest. Such a
557 hypothesis is consistent with the positive relationship observed between nutrient resorption
558 and latitude (Vergutz et al. 2012). High resorption of the plant species of the evergreen
559 rainforest located at 42-44°S can thus be expected.

560

561 4.3 Leaf traits and environmental relationships

562

563 Even though elemental composition of leaves is one of the most typical leaf traits
564 measured worldwide, this is not the case in the rainforest of the STF, where more
565 information is available on physiological leaf traits related to light intensity (Lusk and Pozo
566 2002; Lusk et al. 2008; Lusk and Corcuera 2011; Gianoli and Saldaña 2013). Only a few
567 studies have been interested in the effects of other environmental factors on leaf traits
568 (Fajardo and Siefert 2018, 2019). Here, MAP was shown to be the only and best predictor
569 of $\delta^{13}\text{C}$ in leaves. Nevertheless, the regression model between MAP and $\delta^{13}\text{C}$ of this study

570 reaches a moderate explanation of the data ($R^2=0.3$). The low R^2 imply that other others
571 environmental factors than MAP not considered in this study could contribute to carbon
572 stable isotopes composition of the leaves, like the CO_2 concentration (Caemmerer and
573 Evans 1991). The biochemical processes involved in leaf chemical traits are known to be
574 influenced by variables like temperature and water stress (Niinemets 2001; Meng et al.
575 2015; Zhang et al. 2020). The lack of influence of soil properties and climate variables on
576 the leaf chemical traits in this study differs from results reported in a more global study
577 (Maire et al. 2015) and in a work dealing another type of forests (Meng et al. 2015; Gong
578 et al. 2020). Environmental variables should not be enterally discarded, and we highly
579 recommend to include soil P, based on our results ($N/P >12.5$), as it is a limiting nutrient
580 for the plants. Other variables that might be considered in this type forest are textural
581 classes and other mineral soil compounds, that probably are more important for plants
582 than precipitation as was reported in other temperate forest (Meier and Leuschner 2008).
583 Also in this type of forest with low P availability soil micorrizal fungi are really important for
584 the rainforest, then soil microorganisms may also have a crucial implicaction in leaf
585 chemical traits. Moreover, even that plant species are subjected to different environmental
586 conditions, they develop adaptation mechanisms to multiple stresses (Raza et al. 2020).
587 Then the contrasting environmental, does not necessarily have a significate effect on the
588 leaf chemical traits, like was found for the set of species evaluated (Table A.1). As was
589 said earlier, *N. dombeyi* inhabits a wide range of soils whereas *S. conspicua* and *A. luma*
590 are considered to have lower distribution (Rodriguez et al. 2018). This could explain why
591 soil properties were not found to be significant predictors of leaf chemical traits for all the
592 species.

593

594 However, several significant correlations between leaf chemical traits (Fig. 4) and
595 environmental parameters. The C/N ratio was part of several of the highest correlation

596 among leaf chemical traits (Fig. 4). The relation between C and N of these two elements
597 are directly linked to the plant metabolism, because to synthesize organic molecules like
598 starch (carbon product) or proteins (nitrogen products), organic carbon and energy (He et
599 al. 2006) are required. This high correlation between leaf chemical traits and the low effect
600 of environmental variables on leaf chemical traits reinforce the idea of high biological
601 control in these two areas and a possible great capacity of acclimation or adaptations
602 mechanisms of the individuals of the STF, that for the moment is sufficient to hold the
603 different environmental contrasting conditions in the study area.

604

605

606 **5. Conclusion**

607 This study showed

608 that

609 in the Chiloe Island and in the mainland soil properties ($C_{org}\%$, C/N, pH, MPS) and
610 environmental conditions (MAT and MAP) differ, even when they are separated for no
611 more than 50 km and share similar plant species. Soil between both areas present
612 significant differences in their elemental composition ($C_{org}\%$ and C/N mainly). In addition,
613 MAP is the most common environmental factor found controlling the soil properties ($C_{org}\%$,
614 C/N and $\delta^{15}N$ ‰), thus supporting the importance of precipitation in the biogeochemical
615 cycle of the rainforest. However, the study found different and opposite trends considering
616 the same leaf trait between some plants and areas, a contrasting result from global studies
617 building general trends. Therefore, this study highlights the importance of developing
618 studies at local and regional scales. In addition, the differences above appear to be
619 species-specific with some species, showing differences in one trait and/or another trait(s),
620 with C% as the trait that presents most significant differences in the species. Climatic and
621 soil properties were not good predictors of leaf chemical traits however, leaf chemical traits

622 have a strong correlation between them, especially in their elemental content, suggesting
623 an important biological control on the STF compared to another type of forest. Finally,
624 carbon isotopic values ($\delta^{13}\text{C}$) present a negative relation with MAP.

625

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1129 **Statements & Declarations**

1130

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1140 **Credit author statement**

1141 Carol Cerda-Peña: Conceptualization, Methodology, Writing- Original draft preparation,
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1146

1147 **Data Availability**

1148 The data of the plant leaf chemical traits and soil properties generated during the current
1149 study are available from the corresponding author on reasonable request.