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RESEARCH ARTICLE

Complex bioclimatic and soil gradients shape leaf trait variation in *Embothrium coccineum* (Proteaceae) among austral forests in Patagonia

Gradientes bioclimáticos y edáficos modelan la variación en caracteres foliares de *Embothrium coccineum* (Proteaceae) en los bosques australes de la Patagonia

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ABSTRACT

Patterns of trait variation may be adaptive when vary in relation to an environmental gradient. In particular, leaf traits can affect productivity and competitive ability. We identify patterns of leaf size and shape variation with environmental heterogeneity in one of the most widespread tree species within temperate South America: *Embothrium coccineum* (Proteaceae). We collected leaf specimens and composite soil samples from 35 populations between 38º and 55º S latitude in Patagonia, covering a wide range of mean annual precipitation (MAP) and mean annual temperature (MAT). At each location, we measured nine morphological traits, some of which were cross correlated hence we focus on a smaller number of representative traits. We hypothesized that leaf area (LA), dry mass (DM), and specific leaf area (SLA) would increase, and that leaf shape (SF) would be more elongated, with increasing temperature, precipitation, and soil nutrient availability. We also expected growing season climate to be more closely associated with leaf traits than mean annual metrics. We used bivariate and backward stepwise multiple regressions to analyse the dependence of morphological traits with climatic and edaphic metrics. LA and DM increased with increasing summer rainfall or winter temperature, as hypothesized. Opposite to our hypothesis, LA and DM decreased with increasing summer temperature suggesting that in terms of leaf size, *E. coccineum* may sense summer conditions largely as an increasing aridity stressful gradient. Surprisingly, SLA increased with increasingly warm or dry summers. SF was related positively to MAT and negatively to MAP, suggesting that under more benign western climate regimes *E. coccineum* leaves tend to be elongated. Across sites, LA and DM increased with soil organic carbon and available phosphorus, and decreased with soil nitrogen and exchangeable cations. The opposite pattern was observed for SLA. Biologically meaningful climate metrics and soil nutrient conditions are useful predictors for leaf size and structure in the widespread *E. coccineum*. The SLA patterns probably resulted from lower values in long lasting leaves, in addition to increasing soil nitrogen, so leaves in the south are thicker. Alternatively, it could be consequence from non-isometrical scaling of LA and DM, so larger leaves such as those under oceanic western climates have lower SLA. Patterns of multiple leaf trait variation along complex environmental gradients may become uncoupled from each other, differing from what is suggested in the literature for traits that vary along simple environmental gradients.

Key words: leaf shape, complex gradients, leaf area, dry mass, SLA.

RESUMEN

Los patrones de variación de un rasgo pueden ser adaptativos cuando varían en relación a un gradiente ambiental. En particular, los caracteres foliares pueden afectar la productividad y la habilidad competitiva de las plantas. Identificamos patrones de variación en el tamaño y forma de la hoja con la heterogeneidad ambiental en una de las especies de más amplia distribución del bosque templado de Sudamérica: *Embothrium coccineum* (Proteaceae). Colectamos hojas y muestras compuestas de suelo de 35 poblaciones entre los 38º y 55º de latitud S, cubriendo un amplio rango de precipitación media anual (MAP) y de temperatura media anual (MAT). En cada localidad se midieron nueve caracteres morfológicos foliares, algunos de los cuales están correlacionados entre sí, de manera que enfocaremos en algunos rasgos representativos. Predecimos que el área de la hoja (LA), el peso seco (DM), y el área foliar específica

(SLA) tenderán a aumentar y la forma de la hoja se alargará con el aumento de la temperatura, la precipitación y la disponibilidad de nutrientes en el suelo. También esperamos que el clima de la estación de crecimiento esté más asociado con los rasgos foliares que las métricas climáticas anuales. Utilizamos regresiones múltiples bivariadas y paso a paso reversas para analizar la dependencia de los rasgos morfológicos con el clima y el suelo. LA y DM aumentan con la precipitación de verano o la temperatura del invierno, en concordancia con lo predicho. Opuesto a nuestra predicción, LA y DM disminuyen con el aumento de la temperatura de verano, sugiriendo que en términos de tamaño foliar, *E. coccineum* podría percibir las condiciones climáticas del verano como un gradiente de estrés que incrementa la aridez. Sorprendentemente, el SLA mostró una respuesta opuesta, aumenta con los veranos más calurosos o secos. El índice de forma medido a través del factor de forma (SF) se relaciona positivamente con MAT y negativamente con MAP, sugiriendo que bajo las condiciones climáticas estables del oeste de la distribución de *E. coccineum*, sus hojas tienden a ser alargadas. LA y DM aumentaron positivamente con el carbono orgánico y fósforo disponible en el suelo, y con el nitrógeno y los cationes intercambiables, negativamente. El patrón opuesto se observó para SLA. Las variables climáticas biológicamente importantes y la disponibilidad de nutrientes en el suelo son útiles para predecir el tamaño y estructura de la hoja de *E. coccineum*. Los patrones observados en SLA podrían deberse a valores bajos de esta variable en hojas que perduran más tiempo en la planta, que sumado al aumento del nitrógeno del suelo resulta en hojas más esclerófilas con el aumento de la latitud. Alternativamente, podrían ser consecuencia de la relación no isométrica entre LA y DM, de modo que hojas más grandes, como las que ocurren en los climas con influencia oceánica hacia el oeste, tienden a tener menor SLA. Los patrones de variación en múltiples rasgos foliares a lo largo de gradientes ambientales complejos pueden no concordar entre ellos, difiriendo de lo sugerido en la literatura para rasgos que varían a lo largo de gradientes ambientales simples.

Palabras clave: forma de la hoja, gradientes complejos, área foliar, peso seco, área foliar específica.

INTRODUCTION

Patterns of trait variation may be adaptive when vary in relation to an environmental gradient in different parts of a species' range, and/or functionally related traits vary in coordinated fashion across the gradient (Jonas & Geber 1999). In particular, leaf trait variation can affect water or nitrogen use efficiency, productivity, and competitive ability (Reich et al. 1997). Previous studies have reported that leaves on average are smaller in mass, area, and tend to be rounded as site rainfall, temperature and/or soil nutrient availability decrease (Dolph & Dilcher 1980, Givnish 1984, Hobbie & Gough 2002). Such patterns are typically interpreted as evidence for more conservative leaf trait strategies in suboptimal cold and/or dry environments as compared to optimal warm and/ or moist environments (Abrams et al. 1992, Kubiske & Abrams 1992). More recently Wright et al. (2005), found higher leaf mass per area at hotter, drier sites, at global communities scale. These patterns of leaf size, shape and structure, and their selective control by climate and soils (Cordell et al. 1998, Cunningham et al. 1999, Givnish 1987, Givnish 1988, Nunez-Olivera et al. 1996, Wright et al. 2005) have been recognized for decades. The majority of these studies have been made along environmental gradients representing extreme growing conditions for plant life, such as elevational gradients (Oleksyn et al. 1998) or rainfall gradients in arid zones (Wright et al. 2001). Only a few studies have attempted to account for variation in climate and soil nutrient conditions on leaf traits (Cunningham et al. 1999).

We address these knowledge gaps in the current study, which is, to our knowledge, the first study that considers leaf trait variation within a widespread temperate forest species from southern South America occupying complex latitudinal, longitudinal, elevational, and edaphic gradients. *Embothrium coccineum* J. R. et Forst. occurs in contrasting habitats along its widespread latitudinal distribution of c.20°. The goal of this study was to characterize patterns and possible drivers of leaf trait variation within *E. coccineum* along its range. Our specific hypotheses were (1) that leaf mass, area, and SLA would increase with increasing precipitation or temperature, and (2) that leaf shape would tend to be elongated with increasing precipitation and temperature. For both hypotheses, increasing precipitation and temperature are considered to be associated with benign environments in this ecoregion particularly under more oceanic conditions found on western Andean slopes. In contrast, climates on the eastern Andes are Mediterranean-type where most of the annual precipitation falls in autumn-winter as snow and summers are dry due to the presence of a subtropical high-pressure cell in the south-eastern Pacific (Veblen et al. 1996). Therefore a more or less marked seasonality characterizes southern Andes climates with warm growing season during which leaves reach complete development. As a result, moisture and temperature during the growing season could plausibly be stronger drivers of leaf traits than average annual conditions, and we hypothesize (3) that stronger statistical associations with climate will be found with summer climate rather than annual averages. Additionally, since leaves tend to have larger mass and area, and to be more elongated as soil nutrient availability increases (Hobbie & Gough 2002), we postulate that (4) soil properties should independently influence leaf traits. In particular, both leaf area and mass should increase in co-variation with soil C, N, P and exchangeable cations, as higher levels of these nutrients represent more optimal soil conditions.

METHODS

The species

Embothrium coccineum belongs to a monotypic genus, and has the most widespread range within the austral family Proteaceae. Is a small tree or shrub bearing leaves of variable size and shape. Adults are facultative deciduous, what allows the species to lose leaves before cold or drought episodes, being able to restore them once favorable conditions are reestablished. In cold years the foliage is eliminated in autumn, but at lower altitude the permanence of leaves is almost constant. Under favorable conditions leaves can live up to three years (Alberdi & Donoso 2004). *E. coccineum* grows in mixed forests often associated to open areas and thus it is considered an early colonizer (Alberdi & Donoso 2004). *E. coccineum* is one of the few endemic tree species that span the entire latitudinal range of austral forests, ranging from 35º S to 55° S in Argentina and Chile. Mean annual temperature (MAT) ranges from 10º C at the north to 4º C in austral populations. It occupies a wide longitudinal range following a steep precipitation gradient from areas with > 3500 mm of mean annual precipitation (MAP) in southern Chile going eastward to areas with less than 400 mm in Patagonian steppe. In the southern Andes precipitation is concentrated mostly in autumn-winter as rain and snow. *E. coccineum* inhabits all forest types along the range of austral forests including open woodlands at the forest-steppe ecotone. However, Valdivian rain forests, at the centre of its range, fulfil its optimal conditions. *E. coccineum* is also found along altitudinal gradients from sea level to treeline, which can reach 1500 masl at its northern range (Alberdi & Donoso 2004). Patagonian forests grow mainly on Andisols, characterized by its capacity to retain phosphorus, although soil chemistry also varies along *E. coccineum*'s range. In particular, Proteaceae are characterized by being limited by P, but within austral forests, N appeared to be the more limiting nutrient (Diehl et al. 2004).

Sampling and measurements

We sampled 35 populations of *E. coccineum*, all of them located in open areas, across regional temperature and precipitation gradients (Fig. 1 and Table 1) during austral summer. Ten adult individuals were randomly selected at each location, sampling five terminal unshaded twigs containing fresh foliage. Fully expanded leaves (1750 total) were collected from the 5th spiral of each terminal branch to control for variation in leaf shape due to heteroblasty (Dickinson et al. 1987). Image analyses were performed after detached leaves were fastened to white paper with cellophane tape and scanned with 300 dpi definition. Nine traits were scored on each leaf. Length (L); width (W); area (LA); perimeter; shape factor (SF), defined as 4π x area / perimeter^2 is a unitless measure of an object's circularity, a perfect circle equals 1 whereas a line-shape object will approach zero; anisotropy (A), calculated as A = [perimeter/ Öarea] - 3.54, where 3.54 is a constant for the ratio perimeter and the square root of area in a circle, so would be zero when the leaf is perfectly circular (Rapoport 1982); and width to length ratio (W/L). Leaves were dried at 70º for 48 hours to determine leaf dry mass (DM) and specific leaf area (SLA), calculated as one-sided leaf area per unit of dry mass.

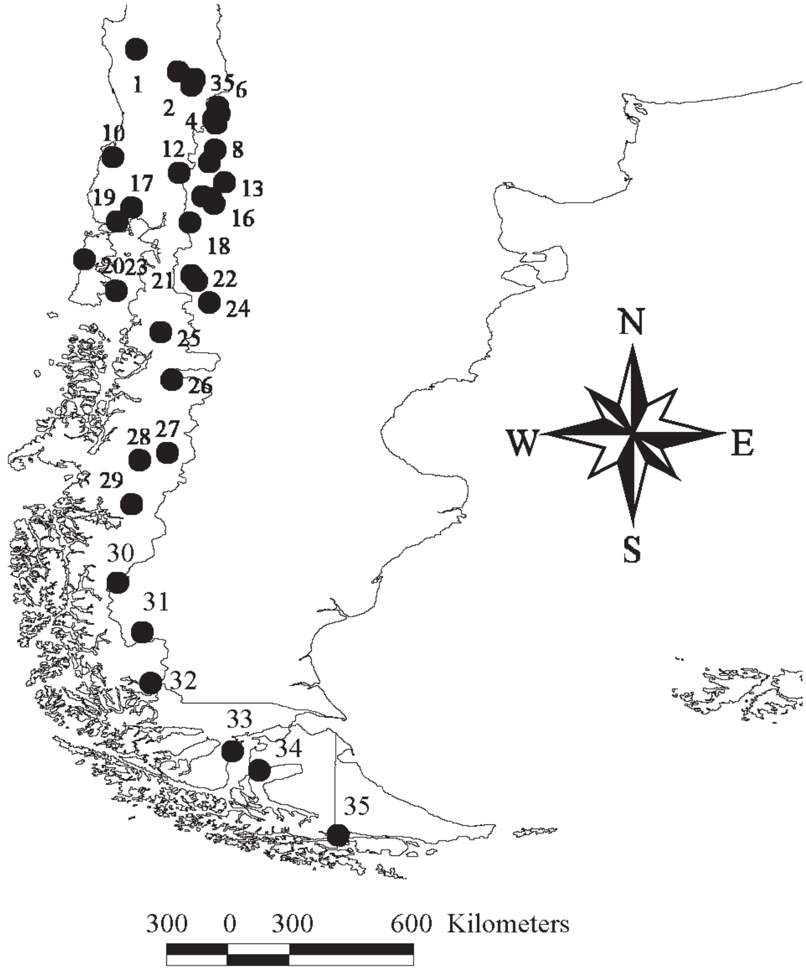

Fig. 1: Map depicting the studied populations of *Embothrium coccineum* along Patagonia. Numbers correspond with full names in Table 1.

Mapa que muestra la ubicación de las poblaciones estudiadas de *Embothrium coccineum* en la Patagonia. Los números se corresponden con los nombres completos de la Tabla 1.

Site climate was described from WorldClim 1.4, a set of global climate layers with a spatial resolution of a square kilometre (Hijmans et al. 2005). Of the available measures, metrics representing annual temperature and precipitation averages (MAT, MAP), and biologically meaningful variables as temperature of the warmest and coldest months (T^o_{min} and T^o_{max}), precipitation of the driest and wettest months (PPT_{min} and PPT_{max}) are presented herein (Appendix 2). T^o_{min} and PPT_{max} represent winter and T^o_{max} and PPT_{min} summer climate conditions, and in all cases are highly correlated with the same metrics expressed over a three-month season, but with lower association with MAT and MAP than the latest. Hence we use T^o_{min} and PPT_{max} as indices of winter, and T^o_{max} and PPT_{min} as indicators of summer climate conditions hereafter.

To characterize soil chemical conditions 5 composite samples were collected 0-15 cm depth after litter removal. Soil samples were air dried, and analysed according to Page et al. (1982) by the Soil Laboratory at CRUB-Universidad Nacional del Comahue. The following analyses were carried out: organic carbon by Walkley-Black wet digestion; total nitrogen by semi-micro Kjeldahl; phosphorus extracted in 0.5 N $NaHCO_3$ (1:10), and exchangeable cations (K and Ca+Mg) extracted in 1N NH_4OAc. (Appendix 3).

TABLE 1

Geographic location and average leaf traits for 35 populations of *E. coccineum*.

Ubicación geográfica y valores promedio de caracteres foliares en 35 poblaciones de *E. coccineum*.

	Population	Latitude S	Longitude W	Altitude masl	Leaf size L (cm)	Leaf size W (cm)	Leaf size LA (cm^2)	Leaf size Per (cm)	DM (g)	SLA (cm^2g^{-1})	Leaf shape A	Leaf shape SF	Leaf shape W/L
1	Nahuelbuta	37º 49’	72º 54’	630	6.21	1.22	4.88	17.04	0.06	84	4.72	0.25	0.23
2	Curacautín	38º 19’	72º 01’	593	5.40	1.46	5.04	14.49	0.04	113	2.89	0.34	0.29
3	Manzanar	38º 28’	71º 40’	816	5.68	1.77	6.42	18.29	0.08	86	3.70	0.31	0.32
4	Conguillío	38º 37’	71º 44’	515	4.13	1.52	4.19	12.20	0.04	115	2.46	0.39	0.37
5	Ñorquinco	39º 07’	71º 16’	1063	3.80	1.64	4.17	13.74	0.04	102	3.06	0.43	0.44
6	Quillén	39º 22’	71º 14’	978	6.72	1.95	8.21	19.21	0.05	171	3.14	0.33	0.30
7	Tromen	39º 34’	71º 26’	1049	5.60	1.52	5.74	16.83	0.06	99	3.63	0.31	0.29
8	Huechulafquen	39º 44’	71º 20’	1097	2.73	1.20	2.19	7.38	0.02	102	1.46	0.52	0.45
9	Lolog	40º 01’	71º 22’	927	5.89	1.46	5.43	15.12	0.04	130	3.03	0.31	0.26
10	Alerce Andino	40º 10’	73º 26’	660	7.20	2.37	11.34	20.25	0.12	94	2.44	0.39	0.36
11	Villarino	40º 27’	71º 34’	846	4.23	1.47	4.24	11.55	0.04	103	2.14	0.43	0.35
12	Antillanca	40º 30	72º 08’	800	5.73	1.95	7.72	15.72	0.05	109	2.18	0.42	0.35
13	Traful	40º 42’	71º 06’	704	3.30	1.28	2.76	8.61	0.05	109	1.73	0.47	0.40
14	Puerto Blest	41º 08’	71º 48’	782	5.65	2.22	8.50	14.67	0.08	109	1.57	0.53	0.40
15	Bariloche	41º 08’	71º 18’	800	3.29	1.16	2.58	9.44	0.06	109	2.37	0.39	0.36
16	La Paloma	41º 10’	71º 15’	900	3.55	1.40	3.34	9.14	0.02	134	1.49	0.51	0.40
17	Lahuen Ñadi	41º 25’	73º 02’	84	8.52	2.56	13.83	30.57	0.16	84	4.63	0.24	0.31
18	Manso Inferior	41º 35’	71º 46’	713	4.27	1.57	4.66	11.08	0.06	109	1.68	0.48	0.37
19	Senda Darwin	41º 55’	73º 34’	67	7.50	2.35	11.69	23.35	0.16	72	3.39	0.29	0.32
20	Cucao	42º 38’	74º 05’	20	7.50	2.55	12.39	24.90	0.19	64	3.64	0.27	0.34
21	Lago Verde	42º 43’	71º 44’	520	5.87	2.11	8.48	16.98	0.10	81	2.29	0.42	0.37
22	Limonao	42º 51’	71º 37’	520	7.91	2.44	13.86	21.69	0.12	116	2.37	0.39	0.35
23	Quellón	43º 05’	73º 36’	144	9.59	2.41	15.07	32.63	0.22	68	4.92	0.20	0.26
24	Corcovado	43º 31’	71º 33’	378	4.96	2.02	6.46	13.85	0.08	83	2.05	0.43	0.42
25	La Junta	43º 58’	72º 14’	170	8.15	2.57	14.39	20.52	0.18	80	1.95	0.44	0.33
26	Mañihuales	44º 58’	72º 09’	200	5.83	2.20	8.73	14.61	0.12	70	1.43	0.52	0.39
27	Fachinal	46º 34’	72º 15’	360	6.87	2.38	10.97	16.66	0.15	73	1.72	0.48	0.36
28	Río Leones	46º 44’	72º 51’	190	7.58	2.73	13.72	19.06	0.16	84	1.69	0.47	0.36
29	L.Vargas	47º 41’	72º 02’	20	5.57	2.53	10.07	14.72	0.14	73	1.14	0.59	0.46
30	Chalten	49º 26’	72º 58’	800	8.41	2.91	16.35	20.21	0.11	147	1.50	0.50	0.35
31	Perito Moreno	50º 27’	73º 01’	350	6.36	2.42	10.86	18.13	0.18	62	2.21	0.42	0.39
32	Pto. Natales	51º 34’	72º 37’	600	4.91	2.25	7.59	12.66	0.10	73	1.07	0.61	0.47
33	Pta. Arenas	53º 02’	70º 50’	130	3.89	2.06	5.48	10.15	0.07	74	0.83	0.66	0.53
34	Bahía Inútil	53º 27’	70º 15’	80	4.67	2.35	7.62	12.35	0.12	66	0.97	0.63	0.51
35	Lapataia	54º 52’	68º 33’	70	4.32	3.62	6.36	12.51	0.14	47	0.99	0.62	0.47
	Mean				5.72	2.04	8.08	16.18	0.09	94	2.29	0.43	0.37
	(CV)				(0.22)	(0.18)	(0.34)	(0.31)	(0.54)	(0.30)	(0.51)	(0.29)	(0.20)

L = length, W = width, LA = leaf area, Per = perimeter, DM = dry mass, SLA = Specific leaf area, A = anisotropy, SF = shape factor, W/L = width to length ratio. First column numbers agree with the ones in Figure 1.

Statistical analyses

We calculated average site values and coefficient of variation (CV) for morphological traits (Table 1). Most leaf traits were approximately normally distributed across the data set. SLA, and some soil condition variables, nitrogen and phosphorous, were log-transformed to achieve normality. Linear correlations between geographic location, soil chemical conditions, and climatic data among sites were carried out using sites as replicates. We also investigated bivariate and backward stepwise multiple regressions to analyse the dependence of morphological traits with environmental, edaphic, and climatic variables separately, as suggested by Wiemann et al. (1998), reporting results after Bonferroni correction. Results were nearly identical using forward stepwise regression (data not shown).

RESULTS

Climate and geographic gradient

Variation in climate metrics was complex in relation to geographic location (Table 2). Given that the altitude of sites occupied by *E. coccineum* decreased with increasing latitude towards the south, the north-south MAT gradient was not as steep as it would be otherwise (Spearman correlation, r = -0.67, n = 35; P < 0.05). Also, T^o_{max} was correlated with altitude but increased, rather than decreased due to the fact that higher elevations sites are located further north, i.e. under relatively warmer climates. PPT_{min} decreased with altitude (r = -0.66, n = 35; P < 0.05).

Across the studied range of latitude and longitude, MAT and MAP both increased to the north and to the west, although these patterns were weak, probably influenced by sites 34 and 35, further southeast (Fig. 1). Stronger geographic patterns exist for seasonal climate metrics. Particularly, PPT_{min} decreased while T^o_{max} increased to the east (Fig. 2). Also, T^o_{max} and PPT_{max} increased to the north (Fig. 2). The temperature of the coldest month (T^o_{min}) was unrelated to latitude and decreased towards the east (Table 2). Thus the temperature range, difference between coldest and warmest months, often considered an index of continentality, was greater to the north and to the east. However, among all sites, T^o_{max} (summer temperature) was not correlated with either winter temperature, T^o_{min} or summer rainfall, PPT_{min}. Thus, latitude and longitude directions (north-south and east-

TABLE 2

Spearman correlation indexes matrix including geographical locators, soil conditions, and climatic variables.

Matriz de índices de correlaciones de Spearman incluyendo ubicación geográfica, condiciones de suelo y variables climáticas.

	Lat	Long	Alt	C	N	P	Cations	T^o_{min}	T^o_{max}	PPT_{min}
Long	0.10									
Alt	-0.67*	-0.35*								
C	0.46*	0.03	-0.24							
N	0.49*	-0.03	-0.15	0.93*						
P	0.51*	-0.40*	0.01	0.34	0.39					
Cations	0.18	-0.50*	0.16	0.51*	0.53*	0.68*				
T^o_{min}	0.03	0.50*	-0.54*	-0.13	-0.21	-0.52*	-0.48*			
T^o_{max}	-0.71*	-0.35	0.51*	-0.33	-0.27	-0.38	-0.04	0.06		
PPT_{min}	0.33	0.67*	-0.66*	0.06	0.01	-0.25	-0.42*	0.80*	-0.19	
PPT_{max}	-0.59*	0.39*	0.10	-0.27	-0.32	-0.81*	-0.62*	0.57*	0.45*	0.50*

* *P* < 0.05. Lat = S Latitude, Long = W Longitude, Alt = Altitude, C = organic carbon, N = nitrogen, P = extractable phosphorus, and Cations = exchangeable Ca, Mg and K , T^o_{min} and T^o_{max} = temperature of the coldest and warmest months of the year, PPT_{min} and PPT_{max} = precipitation of the driest and wettest months of the year.

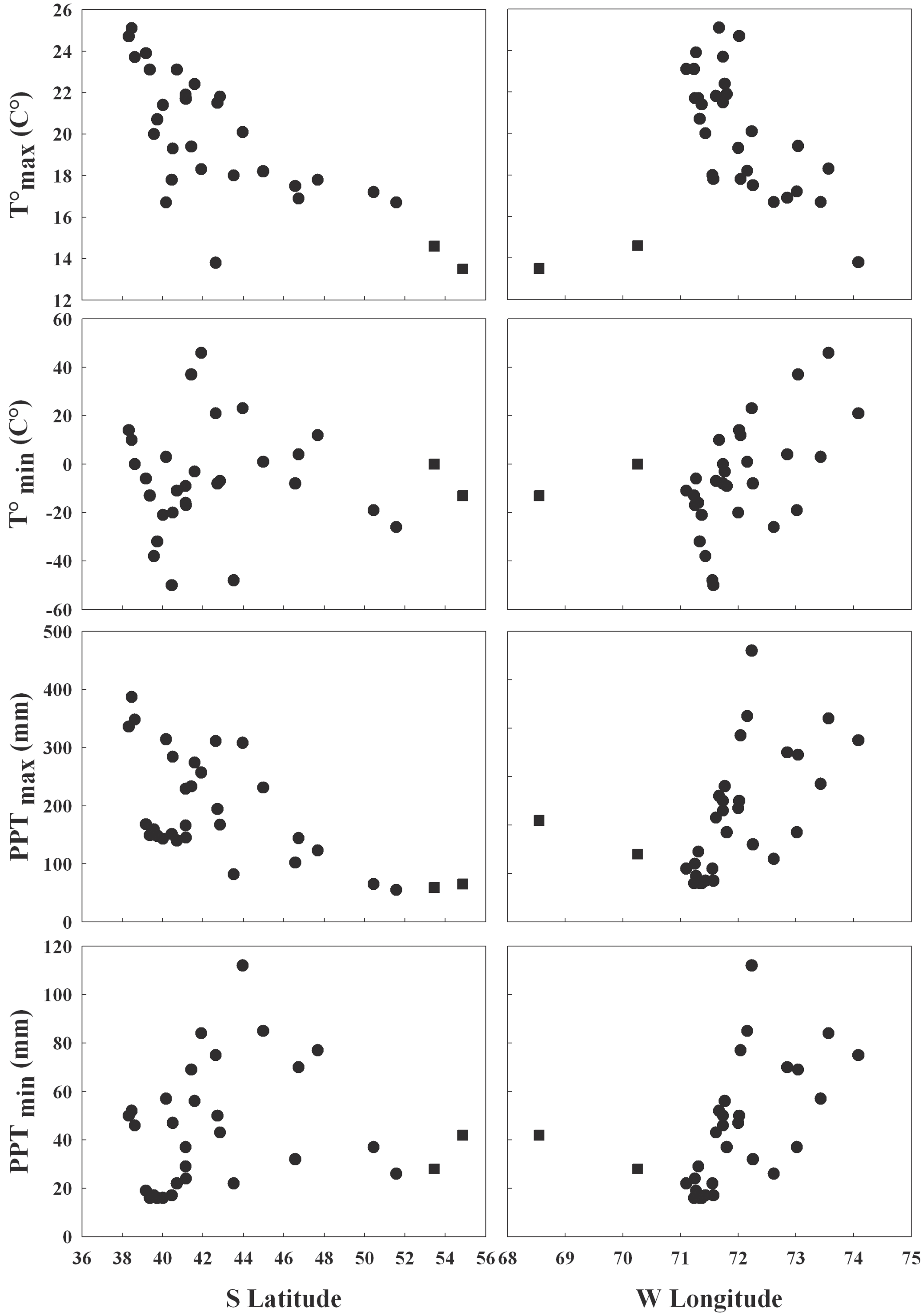

Fig. 2: Bivariate relation between T^o_{max}, T^o_{min} = temperatures of the warmest and coldest months of the year, and PPT_{min} = precipitation of the driest month of the year, along both S Latitude and W Longitude in the studied populations. Filled squares represent populations 34 and 35.

Relación bivariada entre T^o_{max}, T^o_{min} = temperaturas de los meses más cálido y más frío del año, y PPT_{min} = precipitación del mes más seco del año, en el gradiente latitudinal y longitudinal en las poblaciones estudiadas. Los cuadrados negros representan las poblaciones 34 y 35.

west) tended to be somewhat complex in terms of climate variables (Fig. 2), and did not represent any simple gradient when considering all sites inhabited by *E. coccineum*. Hence, we largely discuss leaf traits in relation to climate metrics, since, as shown below, such responses were generally consistent regardless of geographic position.

Leaf traits and climate

Morphological variables (Table 1) were cross correlated (Appendix 1). Hence, we focus on a smaller number of leaf characters that are representative of sets of tightly correlated traits.These are leaf area (LA), dry mass (DM), specific leaf area (SLA), and shape factor (SF).

LA and DM decreased with summer temperature, T^o_{max} (r = -0.45 and -0.61, n = 32; P < 0.05) and increased with winter temperature, T^o_{min} (r = 0.50 and 0.52, n = 32; P < 0.05) (Fig. 3). Similarly, LA and DM both increased with summer rainfall, PPT_{min} (r = 0.65 and 0.70, n = 32; P< 0.05) (Table 3). Although, LA and DM were significantly related to both T^o_{max}, negatively, and PPT_{min}, positively (multiple regression model R^2 = 0.55 and 0.74, n = 32; P< 0.05, for LA and DM, respectively), neither was so their association (Table 2). These results indicate that both indicators of leaf size varied in relation to key attributes of summer climate (temperature and rainfall). These attributes were not related across *E. coccineum*'s range, and thus represent separate axes. LA and DM were not significantly related to MAT, MAP or PPT_{max} (Table 3).

SLA increased with increasing T^o_{max} (r = 0.73, n = 32; P< 0.05) (Fig. 3) and decreased with PPT_{min} (r = -0.42, n = 32; P < 0.05) (Table 3). These SLA patterns resulted from somewhat different responses of leaf LA and DM to the environmental gradients. For instance, the proportional decrease in leaf DM with increasing T^o_{max} was larger than the proportional decrease in LA; i.e., with decreasing leaf size DM decreases disproportionally fast compared to LA. Based on log-log analyses, the relationship between these variables resulted: DM = -2.07 + 1.14* LA, r = 0.83, n = 35, P < 0.05 (Fig. 4). SLA was not significantly related to MAT, MAP, PPT_{max}, or $T°_{min}$.

Leaf shape by means of the shape factor SF also reflects climate influences on *E. coccineum*. SF significantly increased with MAT (r = 0.61, n = 32; P < 0.05), and decreased with MAP (r = -0.44, n = 32; P < 0.05). However, SF was unrelated neither to summer temperature and rainfall nor to winter temperature (Table 3).

Leaf traits and soil conditions

Soil chemical metrics were weakly correlated with climate (Table 2) and thus are considered separately. Soil chemical conditions influenced *E. coccineum* leaf size, shape, and structure (Table 4). In multiple regression models both LA and DM decreased with soil N and cation exchange capacity while increased with soil C and P available phosphorous, (R^2 > 0.30, n = 26; P < 0.05). SLA increased with N and decreased with soil C and P (R^2 = 0.39, n = 26; P < 0.05). SF was unrelated to soil conditions.

DISCUSSION

Leaf metrics and structure of the widespread *E. coccineum* are shaped by prevailing climate and soil conditions of austral forest in South America. Leaf traits seem to be responsive to favorable conditions for optimal growth to a facultative deciduous, i.e. mild winters and moist summers, avoiding freezing and drought of Mediterranean climates. As hypothesized, leaf size by means of LA and DM are larger in relatively warmer winter-wetter summer climates, as in the Valdivian rain forest. However, they were smaller in relatively warmer summer climates, as in North-Western Patagonia. Why did leaf size of *E. coccineum* decreases with summer temperature but increases with winter temperature? On average, variation among sampled sites did not represent a simple gradient from relatively colder to relatively warmer conditions with homogeneous moisture. The prevailing climate is part of complex regional gradients of temperature and rain instead. On the eastern Andes continental locations are characterized by cool and either dry or wet winters coupled with warmer and drier summers. Plants from more arid environments or under colder climatic conditions generally have smaller and rounder leaves in response to stressful conditions (Abrams et al. 1992, Givnish 1984, Kubiske & Abrams 1992). Consistent with this pattern, leaves of *E. coccineum* were smaller and rounder as winters were increasingly cold or summers increasingly dry such as those on the eastern slopes of the Andes. Also, leaves were smaller as summers were increasingly warm as those of northern latitudes, suggesting that in terms of leaf size, *E. coccineum* may sense summer temperature largely as an increasing aridity stressful gradient. In contrast, sites with more stable climate regimes tend to occur to the west, nearer to the Pacific Ocean where *E. coccineum* leaves tend to be elongated.

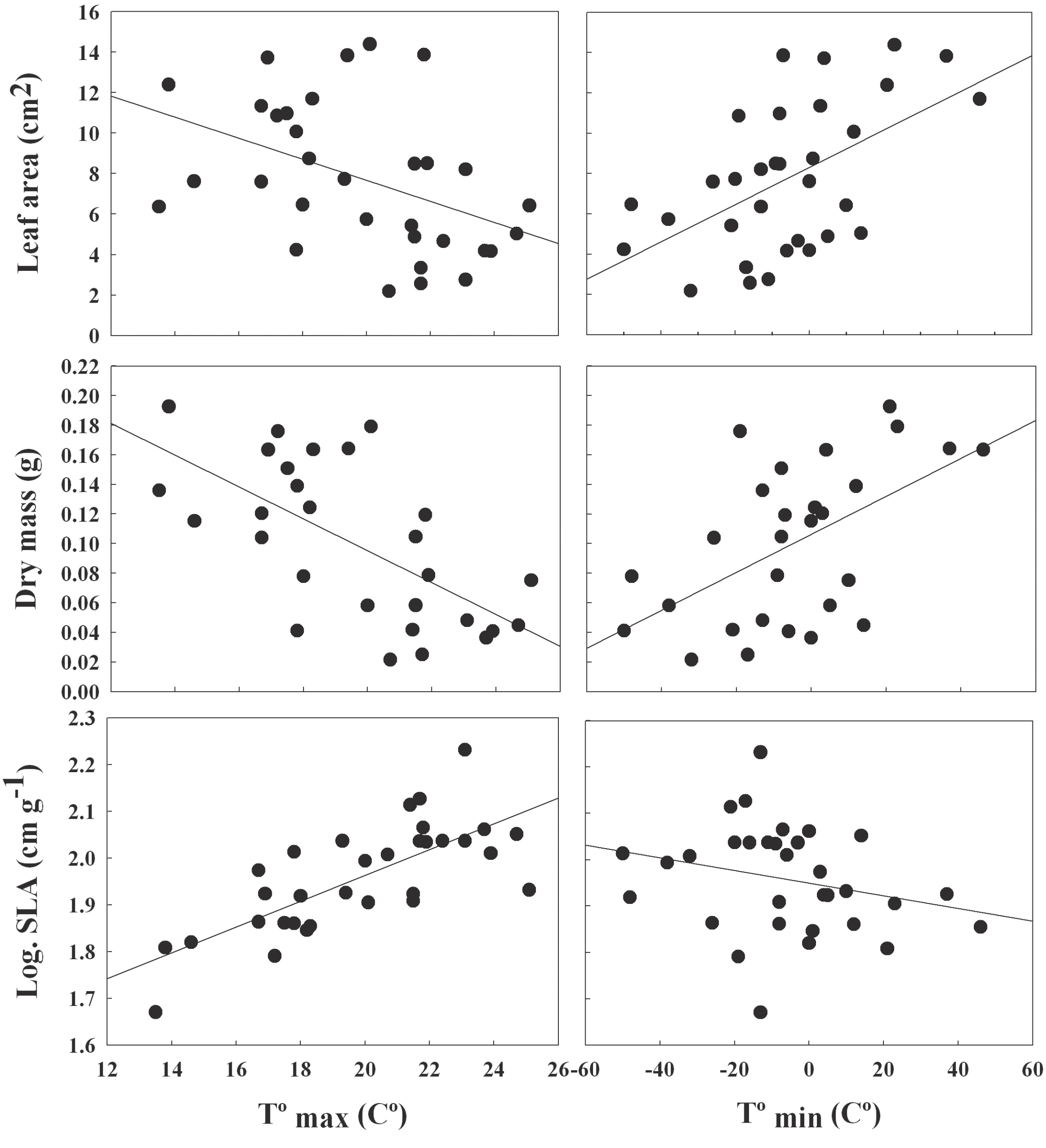

Fig. 3: Bivariate relation between leaf area, leaf dry mass, and specific leaf area (SLA), and T^o_{max}, T^o_{min} = temperature of the warmest and coldest months of the year for the 35 studied populations.

Relación bivariada entre área foliar, peso seco y área foliar específica (SLA), y T^o_{max}, T^o_{min} = temperaturas de los meses más cálido y más frío del año para cada una de las 35 poblaciones estudiadas.

TABLE 3

Spearman correlation indexes between selected climate conditions and leaf traits.

Índices de correlación de Spearman entre variables climáticas y caracteres foliares.

	LA	DM	SLA	SF
MAT	0.16	0.21	0.15	0.61*
MAP	0.29	0.26	0.04	-0.44*
T^o_{min}	0.50*	0.52*	-0.28	-0.27
T^o_{max}	-0.45*	-0.61*	0.73*	-0.29
PPT_{min}	0.65*	0.70*	-0.42*	-0.07
PPT_{max}	0.09	-0.04	0.24	-0.57*

* $P < 0.05$. MAT and MAP = mean annual temperature and precipitation, T^o_{min} and T^o_{max} = temperature of the coldest and warmest months of the year, PPT_{min} and PPT_{max} = precipitation of the driest and wettest months of the year, LA = leaf area, DM = leaf dry mass, SLA = Specific leaf area, SF = shape factor.

TABLE 4

Multiple backward stepwise regression analyses for the dependence of leaf traits with soil chemical conditions. Only significant results are shown.

Análisis de regresiones múltiples “backward stepwise” entre caracteres foliares y condiciones químicas del suelo. Solo se muestran los resultados significativos.

Trait	Independent variable	Significant β	R^2
LA	CPNCations	0.4980.586-0.447-0.440	0.317*
DM	CPNCations	7.6390.575-7.267-0.456	0.401*
SLA	CPN	-0.817-0.4130.819	0.387*

* $P < 0.05$ after Bonferroni correction. We report only significant βetas as the relative contribution and slope to the multiple regression. LA = leaf area, DM = leaf dry mass, SLA = Specific leaf area, C = organic carbon, P = extractable phosphorus, N = nitrogen, Cations = exchangeable Ca, Mg, K.

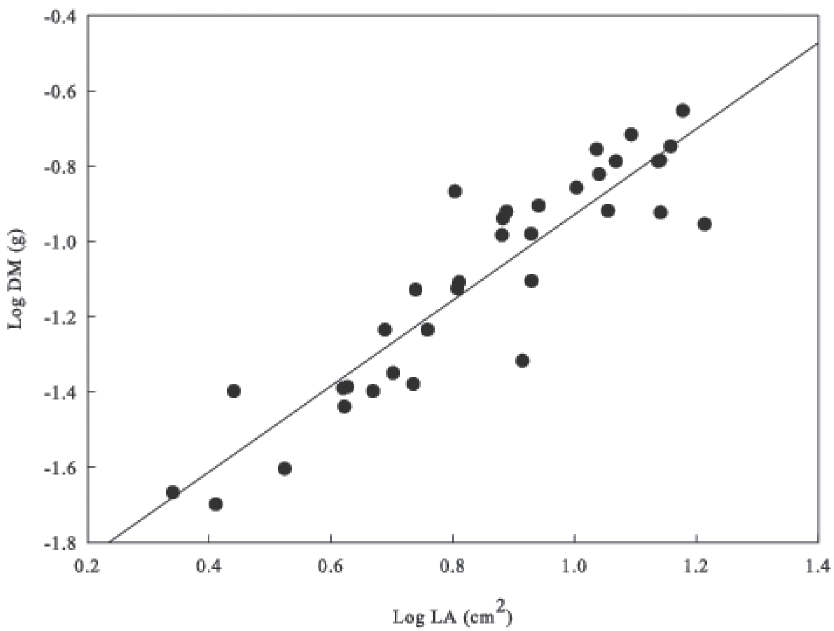

Fig. 4: Bivariate relation (in logarithmic scale) between DM (dry mass) and LA (leaf area) (r = 0.83, $P < 0.05$) for the 35 studied populations.

Relación bivariada (en escala logarítmica) entre peso seco y área foliar (r = 0.83, $P < 0.05$) de cada una de las 35 poblaciones estudiadas.

Physiologic variation in characters that control carbon dioxide reception and water loss is correlated in general with environmental gradients of water, light and nutrients, suggesting that natural selection is the main responsible evolutionary mechanism for the physiologic diversification (Caruso et al. 2006). In this study, SLA responded in opposite fashion to LA and DM to both summer rainfall and temperature gradients, increasing with higher summer temperatures but decreasing with higher summer rainfall. This uncoupling is unusual, as typically one would think dry or warm summers should be reflected by smaller size and lower SLA, i.e. greater sclerophylly (Wright et al. 2005). *E. coccineum*, can be facultaive deciduous, particularly at the northern, i.e. warmer and/or drier portion of its range while in the south leaves tend to be more persitent (Escobar et al. 2006). Literature suggests that leaf life span is related to lower SLA, i.e. long lasting leaves are thicker (Reich et al. 1998, 1999). This would explain contrasting responses for different leaf traits, i.e. leaf size and SLA, in *E. coccineum* that are often closely coupled, resulting in large and thick leaves in colder climates, i.e. southward. *E. coccineum* leaves tend to be thicker with increasing altitude and in sites where the range of intraanual variation in temperature is wide. These sites with important thermal widths along the year represent typical situations of continental climate. Results suggest that these leaves would be more coriaceous as an adaptive response to conditions of climatic stress due to low temperatures and an adaptation to conditions of shorter growing season or with increasing radiation (Givnish 1987). Alternatively, it is possible that the unusual SLA patterns result from stronger responses of leaf size and mass than of SLA to climate gradients. Average DM scales more than isometrically with LA in *E. coccineum*. Thus, as overall leaf size increases, area does not keep pace with mass, so larger leaves have lower SLA. This scaling exponent > 1 of mass to area explains why SLA decreases with increasingly warm or dry summers, despite increasing LA and DM shifts along those gradients, so larger leaves such as those under oceanic western climates have lower SLA.

Additionally, leaf traits are influenced by soil properties. The co-variation of leaf area and mass with soil C and P may represent more optimal soil conditions as previously observed in other species (Hobbie & Gough 2002). As for other Proteaceae, *E. coccineum* seems to be limited by P. This result is opposite to other species from Patagonian forests, where N appeared to be the key limiting nutrient (Diehl et al. 2004). However, soil N is positively linked to SLA. Plants with leaves surviving longer than one season conserve their nitrogen (Larcher 1995). As a result, *E. coccineum* growing under increasing nitrogen conditions such as in high latitudes may consist of sclerophyllous leaves.

Regarding climate responses, variation in leaf size and structural traits were closely associated with seasonal climate metrics rather than annual averages, deviating from predictions based on results along simple gradients or world wide studies (Wright et al. 2005). Especially interesting was the uncoupling of SLA from leaf area and leaf dry mass, and their opposite responses to summer temperatures and rainfall.

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APPENDIX 1

Spearman correlation matrix including 9 leaf traits of *Embothrium coccineum*. DM = dry mass, SLA = Specific leaf area, L = length, W = width, Per = perimeter, A = anisotropy, F = shape factor, W/L = width to length ratio. Marked correlations are significant at *P < 0.05.

Matriz de correlación de Spearman incluyendo 9 rasgos foliares de *Embothrium coccineum*. DM = peso seco, SLA = área foliar específica, L = longitud, W = ancho, Per = perímetro, A = anisotropía, F = factor de forma, W/L = razón ancho/longitud. Las correlaciones marcadas son significativas a *P < 0.05.

	DM(g)	SLA(cm^2g^{-1})	L(cm)	W(cm)	Area(cm^2)	Per(cm)	A	SF
SLA	-0.67*							
L (cm)	0.75*	-0.13						
W (cm)	0.78*	-0.54*	0.58*					
Area (cm^2)	0.85*	-0.25	0.93*	0.77*				
Per (cm)	0.73*	-0.20	0.93*	0.50*	0.83*			
A	0.15	0.07	0.48*	-0.20	0.18	0.68*		
SF	-0.13	-0.19	-0.50*	0.24	-0.20	-0.65*	-0.95*	
W/L	-0.06	-0.34*	-0.55*	0.23	-0.23	-0.55*	-0.79*	0.89*

APPENDIX 2

Climate conditions of studied populations of *Embothrium coccineum*. MAT and MAP = mean annual temperature and precipitation, T^o_{min} and T^o_{max} = average temperature of the coldest and warmest months of the year, PPT_{min} and PPT_{max} = average precipitation of the driest and wettest months of the year. NA = not available. Data from Worlclim.

Condiciones climáticas en las poblaciones de estudio de *Embothrium coccineum*. MAT y MAP = temperatura y precipitación anual promedio, T^o_{min} y T^o_{max} = temperatura promedio de los meses más fríos y cálidos del año, PPT_{min} y PPT_{max} = precipitación promedio de los meses más secos y más húmedos del año. NA = no disponible. Datos de Worlclim.

Nº	Population	MAT (ºC)	MAP (mm)	T^o_{min} (ºC)	T^o_{max} (ºC)	PPT_{min} (mm)	PPT_{max} (mm)
1	Nahuelbuta	8.5	1511	0.5	21.5	29	289
2	Curacautín	10.0	1976	1.4	24.7	50	336
3	Manzanar	9.7	2269	1.0	25.1	52	387
4	Conguillío	8.5	2027	0	23.7	46	348
5	Ñorquinco	8.6	901	-0.6	23.9	19	168
6	Quillén	7.9	791	-1.3	23.1	16	149
7	Tromen	5.3	870	-3.8	20	17	159
8	Huechulaufquen	5.9	801	-3.2	20.7	16	148
9	Lolog	7.2	756	-2.1	21.4	16	143
10	Alerce Andino	7.0	2160	0.3	16.7	57	314
11	Villarino	4.1	819	-5.0	17.8	17	151
12	Antillanca	6.6	1627	-2.0	19.3	47	284
13	Traful	9.0	771	-1.1	23.1	22	140
14	Puerto Blest	8.7	1163	-0.9	21.9	37	229
15	Bariloche	8.2	931	-1.6	21.7	29	166
16	La Paloma	8.1	854	-1.7	21.7	24	145
17	Lahuen Ñadi	10.5	1716	3.7	19.4	69	233
18	Manso Inferior	9.2	1816	-0.3	22.4	56	274
19	Senda Darwin	10.6	1890	4.6	18.3	84	257
20	Cucao	7.2	2169	2.1	13.8	75	311
21	Lago Verde	8.8	1248	-0.8	21.5	50	194
22	Limonao	9.0	1090	-0.7	21.8	43	167
23	Quellón	NA	NA	NA	NA	NA	NA
24	Corcovado	5.2	585	-4.8	18	22	82
25	La Junta	10.1	2300	2.3	20.1	112	308

APPENDIX 2 (continuation)

Nº	Population	MAT (ºC)	MAP (mm)	T^o_{min} (ºC)	T^o_{max} (ºC)	PPT_{min} (mm)	PPT_{max} (mm)
26	Mañihuales	8.2	1653	0.1	18.2	85	231
27	Fachinal	7.8	727	-0.8	17.5	32	102
28	Río Leones	8.0	1252	0.4	16.9	70	144
29	L.Vargas	8.8	1165	1.2	17.8	77	123
30	Chalten	NA	NA	NA	NA	NA	NA
31	Perito Moreno	7.1	602	-1.9	17.2	37	65
32	Pto. Natales	6.5	459	-2.6	16.7	26	55
33	Pta. Arenas	NA	NA	NA	NA	NA	NA
34	Bahía Inútil	6.4	561	0	14.6	28	59
35	Lapataia	5.6	674	-1.3	13.5	42	65

APPENDIX 3

Soil chemistry of studied populations of *Embothrium coccineum*. C = organic carbon, N = nitrogen, P = extractable phosphorus, and Cations = exchangeable Ca, Mg and K. NA = not available.

Química del suelo en las poblaciones de estudio de *Embothrium coccineum*. C = carbono orgánico, N = nitrógeno, P = fósforo extraíble, y Cations = Ca, Mg y K intercambiables. NA = no disponible.

Nº	Population	C %	N %	P (mg kg)	Cations (cmol kg)
1	Nahuelbuta	1.79	0.11	1.28	1.69
2	Curacautín	3.35	0.22	0.46	6.46
3	Manzanar	2.11	0.13	0.37	1.99
4	Conguillío	0.31	0.02	0.55	0.10
5	Ñorquinco	3.81	0.22	1.10	4.40
6	Quillén	11.01	0.69	6.67	19.03
7	Tromen	3.44	0.22	3.11	3.97
8	Huechulafquen	1.76	0.13	1.83	2.76
9	Lolog	8.62	0.57	5.75	12.98
10	Alerce Andino	NA	NA	NA	NA
11	Villarino	2.56	0.16	1.37	4.04
12	Antillanca	NA	NA	NA	NA
13	Traful	1.21	0.10	4.84	5.80
14	Puerto Blest	5.52	0.37	0.85	2.41
15	Bariloche	NA	NA	NA	NA
16	La Paloma	NA	NA	NA	NA
17	Lahuen Ñadi	1.33	0.06	0.09	0.10
18	Manso Inferior	NA	NA	NA	NA
19	Senda Darwin	9.55	0.45	1.74	7.45
20	Cucao	7.61	0.29	0.09	0.35
21	Lago Verde	11.80	0.67	18.81	10.98
22	Limonao	7.22	0.39	5.02	9.07
23	Quellón	9.54	0.56	0.73	2.66
24	Corcovado	5.54	0.52	44.38	14.66
25	La Junta	6.44	0.87	1.01	0.39
26	Mañihuales	7.44	0.49	1.86	2.36
27	Fachinal	4.78	0.41	3.72	14.62
28	Río Leones	1.16	0.05	26.38	0.40
29	L.Vargas	4.90	0.25	12.01	8.22
30	Chalten	NA	NA	NA	NA
31	Perito Moreno	NA	NA	NA	NA
32	Pto. Natales	11.99	0.88	5.75	4.34
33	Pta. Arenas	NA	NA	NA	NA
34	Bahía Inútil	NA	NA	NA	NA
35	Lapataia	13.66	0.65	22.66	11.19