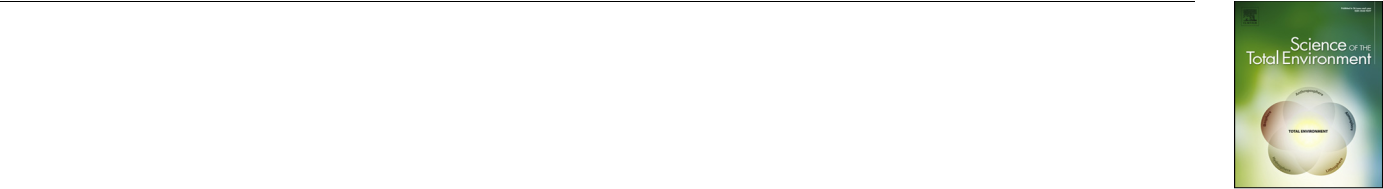
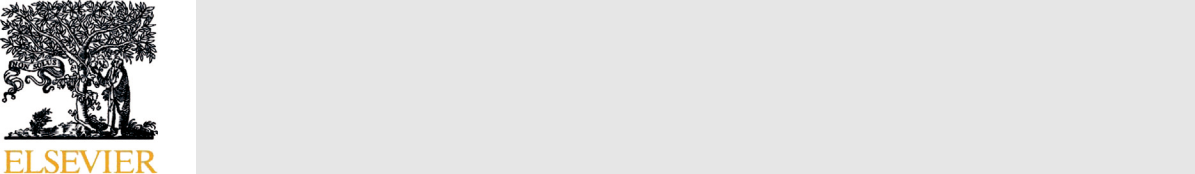
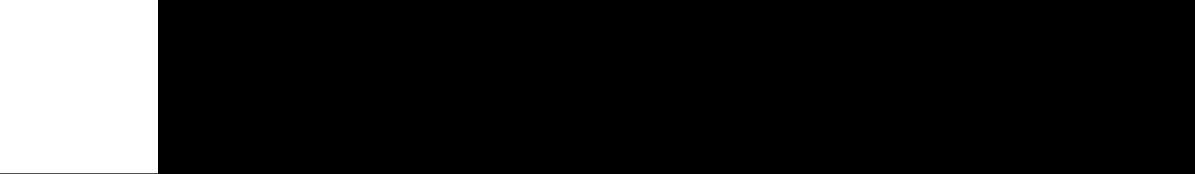
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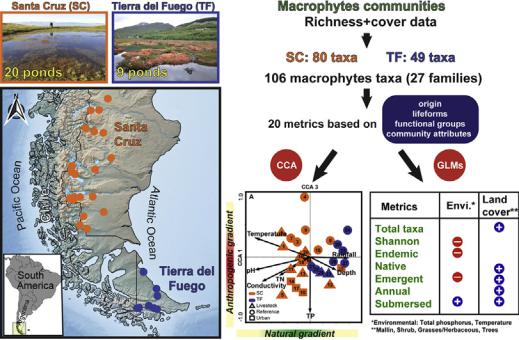
Macrophyte regional patterns, metrics assessment and ecological integrity of isolated ponds at Austral Patagonia (Argentina)

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HIGHLIGHTS GRAPHICAL ABSTRACT

* Macrophyte assemblages are mainly driven by natural factors (temperature, rainfall and pH).
* Higher richness of endemics at insular than at continental region
* Richness of emergent and endemic macrophytes are indicators of pond an-thropogenic disturbance.
* In a 100 m buffer, land cover features in-fluenced macrophytes metrics.
* Small isolated ponds (b0.1 ha) stand as significant habitats for macrophytes.



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Anthropogenic and natural changes are threatening pond ecological integrity in Patagonia and tools for bioas-sessment are required. Macrophytes are good candidates to determine the conservation status of ponds; never-theless, metric selection procedures should be founded on an adequate knowledge of plant ecological responses. We assessed the main environmental constraints driving variation in macrophyte assemblages, and trophic sta-tus at 29 ponds located at the continental and insular Patagonia region. We screened 20 potential macrophyte metrics as indicators of pond condition that included origin (native, endemic, exotic), lifeforms (annual/biannual, perennial), functional groups (submersed, emergent, floating-leaved, landforms), and community attributes. A set of 106 taxa were recorded, and richness per site (10 species) was unexpectedly high for a cold temperate area, reinforcing the value of isolated ponds as habitat for macrophytes in the Patagonian landscape. Natives dominated most assemblages; exotics were present at 24 ponds, contributing with high cover (N45%) at 15% of them. Macrophyte assemblages were driven by natural factors over anthropogenic ones, with temperature, rain-fall, pH, conductivity and nutrients explaining most variation in patterns. However, pond eutrophication symp-toms (high phosphorous concentration and chlorophyll a) were associated with extensive cattle grazing (manure and trampling) and urbanization (runoff). Generalized linear models captured natural variables (tem-perature, alkalinity) as most powerful explaining richness measures. Models also indicated that both richness of emergent and endemics were negatively affected by total phosphorous increases. Land cover factors: grasses/ herbaceous, mallín and trees (%) in 100 m buffer around ponds appeared as additional ecological drivers of mac-rophyte patterns, particularly of submersed (N50%) and native richness (36%). Natural and anthropogenic gradi-ents were overlapped, making it difficult to generalize our conclusions. Further studies are needed to test the

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performance of the macrophyte metrics selected here, which are a vital tool for the conservation of the most aus-tral ponds in South America.

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1. Introduction

Aquatic macrophytes are considered good indicators of freshwater ecosystem status, being key elements in the functioning and dynamics of lentic environments. Macrophyte influence in community and ecosystem-level processes is very pronounced in small shallow lakes and ponds ([Zimmer et al., 2003](#page1); [Nicolet et al., 2004](#page1)). As primary pro-ducers, they are relevant in nutrient dynamics and cycling ([Asaeda](#page1) [et al., 2000](#page1)) often regulating physicochemical conditions in the water column. They provide habitat, shelter, breeding areas and food re-sources to other aquatic and terrestrial species ([Chambers et al., 2008](#page1)).

Macrophytes have become increasingly significant as monitors of aquatic waterbodies, and numerous studies revealed that their re-sponses to environmental stressors can be predictable and diagnostic of ecosystem changes (e.g., [Ruiz et al., 2011](#page1); [Beck et al., 2014](#page1)). Sub-mersed life forms respond rapidly to changes derived from anthropo-genic disturbances, being used as indicators of water quality ([Oertli](#page1) [et al., 2005](#page1); [Sass et al., 2010](#page1); [Bornette and Puijalon, 2011](#page1)) and eutrophi-cation ([Zimmer et al., 2003](#page1); [Penning et al., 2008](#page1)). Other attributes of macrophytes appear useful in reflecting the effects of physical, chemical, biological, and climatic changes in the surrounding aquatic environ-ment ([Cronk and Fennessy, 2001](#page1); [Pereira et al., 2012](#page1); [Alahuhta et al.,](#page1) [2018](#page1); [Fu et al., 2019](#page1)). For instance, emergent and landform associations have been successfully used to detect land use influence resulting from silviculture and cattle grazing ([Hernández et al., 2015](#page1)). Higher cover of exotics has been linked with agricultural land uses ([Stuber et al., 2016](#page1)), whereas measures of macrophyte diversity have been shown to reveal the adverse effects of increases in urban land cover ([Akasaka et al.,](#page1) [2010](#page1)).

Most macrophyte metrics are based on different attributes of com-munities including richness, specific tolerance values, conservation sta-tus, guild structure or vegetative abundance ([Rothrock et al., 2008](#page1); [Poikane et al., 2018](#page1)). Different countries from Europe ([European Envi-ronmental Agency, 2018](#page1)), North America ([U.S. E.P.A, 2016](#page1)) and Australia ([Schallenberg et al., 2011](#page1)) have a good background of knowl-edge regarding species distribution, lifeforms, and origin. This knowl-edge allowed establishing environmental-related policies using macrophytes as biological indicators. Aquatic plant communities have been included in protocols of water assessment in Europe ([Ruiz et al.,](#page1) [2011](#page1); [Willby et al., 2012](#page1)), Australia ([Roberts et al., 2017](#page1)), and USA ([U.S. EPA, 2016](#page1)). Certainly, efforts have been made to understand the causes of natural variation of macrophyte species at a regional scale in order to produce assessment protocols ([Penning et al., 2008](#page1); [del Pozo](#page1) [et al., 2010](#page1); [Alahuhta et al., 2013, 2017](#page1)).

Currently, although previous advances in the ecological assess-ment of Patagonia wetlands (which are colloquially known as “mallines”) ([Epele and Miserendino, 2015](#page1); [Manzo et al., 2019](#page1)), there has been limited empirical research exploring macrophytes communities of the southern Patagonia. Evidence that aquatic plants could make useful tools for pond bioassessment in the re-gion have been provided by [Hauenstein et al. (2008)](#page1); they found that macrophytes displayed consistent responses to eutrophica-tion, with allochthonous taxa dominating assemblages and hemicryptophytes indicating strong human impact (La Araucanía, Chile). For the northern Patagonia [Kutschker et al. (2014)](#page1) found a strong correspondence between macrophyte community composi-tion and intensity of grazing pressure at isolated wetlands, with total species richness, richness of natives and aquatic plant cover-age significantly decreasing towards most disturbed sites.

According to [Crego et al. (2014)](#page1), much of Patagonia is altogether un-protected from anthropogenic degradation, thus wetland areas become vulnerable to disturbance. For more than a century, extensive sheep farming has been the dominant land use in the region, having remark-able environmental consequences ([del Valle et al., 1998](#page1)). Today, grazing by livestock (i.e., sheep, cattle and horses) is still the most widespread land use at arid and semiarid areas, were mallines represent 30 to 40% of the forage supply and are essential as water sources ([Gaitán et al.,](#page1) [2011](#page1)). Even though governmental agencies have concern about pond integrity; most guidelines used for wetland assessment are based on en-vironmental criteria (e.g., physicochemical) rather than biological ones ([DPN Santa Cruz, 2004](#page1); [DPN Tierra del Fuego, 2016](#page1)). Therefore, metrics -an individual measurement or combinations of data types to describe a particular property (e.g., species richness, species cover by growth form, etc.) for a site- or biotic indexes -a combination of metrics used to gen-erate a single score to describe a particular property (condition or stress) for a site- ([U.S. EPA, 2016](#page1)), are not considered in protocols of water quality. Hence, a first step to produce more precise and accurate biological indicators requires reliable information of aquatic plants dis-tributions across environmental gradients ([Beck et al., 2010, 2014](#page1)).

We investigated the responses of different metrics to natural and an-thropogenic gradients, based on functional groups or habits (emergent, floating leaves, submersed and landforms), origin (endemic, native and exotics), lifeforms (perennial, annual) and community attributes (rich-ness, diversity). This included local environmental features (physico-chemical) and land cover patterns in a 100 m buffer area around ponds. Accordingly, our main objectives were to: 1) examine the distri-bution of macrophyte assemblages and their relationships with envi-ronmental gradients (e.g., climatic, physicochemical) at ponds in Austral Patagonia, including the continental (Santa Cruz, SC) and insular region (Tierra del Fuego, TF); 2) assess the efficacy of macrophyte met-rics as measures of pond environmental condition, providing a concep-tual basis for future index development. Main hypothesis is that macrophytes from southern South America would be driven primarily by natural factors (i.e., climate). Concerning regional patterns we expect more diverse assemblages in continental Patagonia (SC) rather than at Tierra del Fuego Island (TF), while in terms of richness, endemics would prevail at the insular region. Our study also provides a baseline for pond conservation and management on a remote region that may be susceptible to water impairment through climate change ([Crego](#page1) [et al., 2014](#page1)) and ecosystem degradation by human pressures ([Newbold et al., 2015](#page1)).

2. Methodology

2.1. Study area

Austral Patagonia is located in the southernmost portion of South America ([Fig. 1](#page1)). The study area includes both Santa Cruz (SC) and Tierra del Fuego (TF) political provinces (continental and insular area, respectively). The climate of the continental area is wet and cold in the western zone, with a mean annual temperature of 5 °C. Precipitation ranges from 3000 to 500 mm, with a decreasing trend towards the east side. In the central zone, mean temperature ranges from 5 °C to 12 °C, and precipitation is lower than in the cordillera (Andean Mountains) (700 to 200 mm). The eastern zone exhibits a temperate and arid cli-mate (mean annual temperature: 12 °C to 20 °C), and annual precipita-tion barely reaches 300 mm ([ANIDA, 2015](#page1)). The climate of the insular area is cold temperate in the south and temperate oceanic in the

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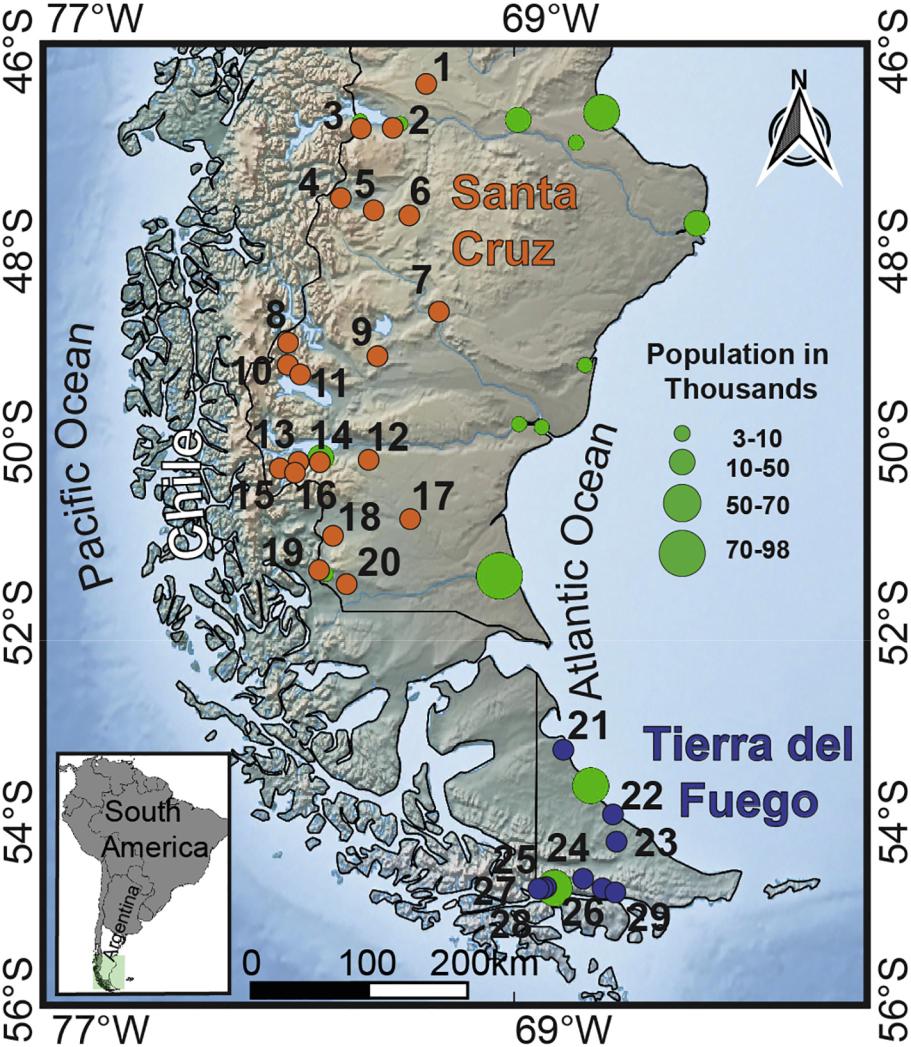


Fig. 1. Locations of studied ponds in the continental (Santa Cruz Province: orange dots) and insular (Tierra del Fuego Province: blue dots) region in the Patagonia Austral. Cities and population are shown (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

north, with a mean annual temperature of 5 °C, and a precipitation range from 600 mm to b300 mm, decreasing from the southwest to the northeast ([Rabassa et al., 2006](#page1)).

The noticeable west to east precipitation gradient has resulted in two main phytogeographical provinces: the Sub-Antarctic Forest and the Patagonian Steppe. The Sub-Antarctic Forest is dominated by ever-green (Austrocedrus chilensis, Nothofagus dombeyi and Maytenus boaria) and deciduous trees species (Nothofagus pumilio and Nothofagus antarc-tica). The shrub and herbaceous strata are characterized mainly by Chusquea culeou, Berberis microphylla, Lomatia hirsuta, Schinus patagonicus, Diostea juncea, Fuchsia magellanica, Alstroemeria aurea, Mutisia spinosa and Mutisia decurrens. The Patagonian Steppe is charac-terized by xerophytic forms, dominated by an herbaceous-shrub-like steppe (Azorella prolifera, Pappostipa spp., Senecio spp., Adesmia sp., Nassauvia sp., Chuquiraga sp. and Poa sp.) ([Tell et al., 1997](#page1)).

2.2. Site selection

Sampling was conducted during summer season (between Decem-ber 2013 and January 2014) in a set of 29 isolated ponds located in wet-lands ([Fig. 1](#page1)). From the pool of sites, 20 corresponded to the continental area (SC) and nine were placed in the insular area (TF). Ponds presented similar hydroperiod conditions (temporary: longer than eight months), as checked in satellite imagery and tested in the field. The sampling

protocol attempted to ensure the following aspects: a) a reduction in the natural variability of communities in relation to seasonal changes,

1. providing enough differentiation of communities across a natural gradient, and c) feasibility from a logistic point of view ([U.S. EPA,](#page1) [2002](#page1)). According to previous works carried out in Patagonia, summer is an appropriate season to study aquatic biota and a time of the year when the wetlands present enough water ([Kutschker et al., 2014](#page1)).

To assess ponds macrophyte assemblages in the vast study area, our fieldwork was extensive and exploratory in nature, and land use condi-tions in the adjacent areas were different according to pond placement. Some water bodies were located in protected areas (National Parks, for-est biome) or without direct disturbances (Reference sites: 3, 4, 7, 8, 9, 15, 21, 22, 24, 25, 26, 27 and 28), but a great number of sites were sub-jected to extensive grazing mainly by sheep (Livestock sites: 1, 2, 5, 6, 11, 12, 13, 16, 18, 20, 23 and 29). None of the sampled ponds were used for disposal of treated urban effluents, but some of them were placed at open areas (parks) surrounding an urban matrix (Urban sites: 10, 14, 17 and 19) ([Fig. 1](#page1)).

2.3. Environmental characterization

Morphometric and physicochemical characteristics on each wetland were evaluated ([Table 1](#page1)). Mean annual temperature and mean annual precipitation (from 1970 to 2000) were obtained from digital maps,

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Table 1

Environmental descriptors measured at 29 Patagonian ponds at wetlands (Santa Cruz and Tierra del Fuego Provinces, Argentina). Mean, standard deviation and minimum and maximum values are consigned.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variables | Santa Cruz | | | |  |  | Tierra del Fuego | | |  |  |
|  |  | | | |  |  |  | | |  |  |
|  | Mean ± SD | | | | Min-Max |  | Mean ± SD | | | Min-Max |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Climatic features |  |  |  |  |  |  |  |  |  |  |  |
| Mean annual temperature (°C) | 6.47 ± 1.56 | | | | 3.72–9.37 | 4.47 | | ± 0.67 | | 3.94–5.79 |  |
| Mean annual rainfall (mm) | 421.55 | | ± | 302.54 | 123–1192 | 517.67 ± 83.32 | | | | 388–612 |  |
| Water temperature (°C) | 14.59 | ± 3.74 | | | 8.1–22.2 | 13.69 | | | ± 2.79 | 10.7–20.1 |  |
| Geomorphological features |  |  |  |  |  |  |  |  |  |  |  |
| Altitude (m a.s.l) | 437.05 | | ± | 191.92 | 166–777 | 67.12 | | | ± 57.39 | 2–174 |  |
| Area (ha) | 0.2 ± 0.32 | | | | 0.01–1.2 | 0.06 | | ± 0.06 | | 0.01–0.17 |  |
| Width (m) | 33.69 | ± 35.91 | | | 5.8–151.55 | 34.2 | | ± 14.66 | | 15–55.4 |  |
| Length (m) | 49.9 ± 32.51 | | | | 7.83–111.95 | 20.76 | | | ± 17.1 | 3.69–61.85 |  |
| Depth (m) | 0.4 ± 0.3 | | |  | 0.13–1.45 | 0.75 | | ± 0.24 | | 0.41–1.19 |  |
| Physicochemical features |  |  |  |  |  |  |  |  |  |  |  |
| Conductivity (μS/cm) | 310.31 | | ± | 321.67 | 14.81–1220 | 149.58 ± 135.08 | | | | 20.9–414 |  |
| TDS (mg/l) | 190.28 | | ± | 203.14 | 7.3–783 | 87.88 | | | ± 73.2 | 12.7–212 |  |
| Salinity (‰) | 0.2 ± 0.21 | | | | 0–0.8 | 0.09 | | ± 0.08 | | 0–0.2 |  |
| DO(mg/l) | 12.61 | ± 4.43 | | | 2.47–21.5 | 12.16 | | | ± 3.87 | 4.33–19.29 |  |
| DO (%) | 128.85 | | ± | 52.11 | 26–232 | 113.85 ± 32.34 | | | | 38.8–145.4 |  |
| pH | 6.66 ± 1.57 | | | | 4.48–9.92 | 4.73 | | ± 1.04 | | 3.12–5.86 |  |
| TN (μg/l) | 463.39 | | ± | 140.23 | 261.19–773.19 | 340.52 ± 71.6 | | | | 249.19–455.19 |  |
| NO3− + NO2−(μg/l) | 165.65 | | ± | 652.35 | 0.3–2936.25 | 7.29 | | ± 10.22 | | 0–30.27 |  |
| NH4+ (μg/l) | 40.79 | ± 52.92 | | | 0.96–228.09 | 16.12 | | | ± 8.31 | 7.34–28.83 |  |
| TP (μg/l) | 132.63 | | ± | 119.97 | 6.12–400.45 | 74.18 | | | ± 23.5 | 40.15–118.21 |  |
| SRP (μg/l) | 47.8 ± 84.91 | | | | 0–372.48 | 10.08 | | | ± 8.47 | 0–23.81 |  |
| DIN (μg/l) | 206.44 | | ± | 645.39 | 8.02–2937.2 | 21.62 | | | ± 15.66 | 7.34–59.09 |  |
| TN/TP | 8.52 ± 11.87 | | | | 0.8–55.48 | 5.03 | | ± 1.9 | | 2.57–7.96 |  |
| DIN/TP | 3.84 ± 14.1 | | | | 0.1–63.65 | 0.34 | | ± 0.31 | | 0.09–1.1 |  |
| TSS (mg/l) | 60.63 | ± 175.1 | | | 0–718 | 0.89 | | ± 1.97 | | 0–6 |  |
| Biological features |  |  |  |  |  |  |  |  |  |  |  |
| Chlorophyll a (mg/l) | 6.82 ± 12.26 | | | | 0–44.25 | 2.26 | | ± 2.96 | | 0–7.48 |  |
| Aquatic plant cover (%) | 81.25 | ± 23.34 | | | 10–100 | 83.89 | | | ± 29.14 | 10–100 |  |
| Eutrophication indexes |  |  |  |  |  |  |  |  |  |  |  |
| TSITN | 42.76 | ± 4.20 | | | 35.08–50.74 | 38.62 | | | ± 3.01 | 34.39–43.09 |  |
| TSITP | 68.61 | ± 14.74 | | | 30.26–90.56 | 65.58 | | | ± 4.75 | 57.39–72.97 |  |
| TSIChla | 30.83 | ± 24.89 | | | 0–67.78 | 23.41 | | | ± 22.83 | 0–50.33 |  |

TDS, total dissolved solids; DO, dissolved oxygen; TN, total nitrogen; NO3− + NO2−, nitrate plus nitrite nitrogen; NH+4, ammonium; TP, total phosphorous; SRP, soluble reactive phos-phorus; DIN, dissolved inorganic nitrogen; TSS, total suspended solids; TSI, trophic state index; Chla, chlorophyll a.

WorldClim database version 2 ([Fick and Hijmans, 2017](#page1)), using the soft-ware open source QGIS 3.4. At the sampling moment, we measured length and width (laser distance measurer), pond area, depth (cali-brated stick), water temperature (°C), specific conductance (μS/cm), total dissolved solids (mg/l), pH and dissolved oxygen (mg/l) (Hach SensION 156 multiparameter probe). For better comparisons, the sites were sampled at the same time each day (between 10 and 12 a.m.). In order to determine nutrient concentrations, water samples were col-lected below the water surface, kept at 4 °C and transported to the lab-oratory. Total phosphorus (TP) was estimated after an acid digestion with potassium persulfate (120 °C for 1 h). Soluble reactive phosphorus (SRP) was determined as Molybdate reactive P according to standard analytical procedures (424-F Standard Methods, [APHA, 1998](#page1)); nitrates (NO3–N) were reduced to nitrites using a cadmium column reduction; nitrites (NO2–N) were determined by diazotization (418-F Standard Methods, [APHA, 1998](#page1)); ammonium (NH+4–N) was assessed by the in-dophenol blue method; total organic nitrogen (TON) was determined by the Kjeldahl method ([APHA, 1998](#page1)). Total nitrogen (TN) was esti-mated as the sum of nitrites, nitrates and total organic nitrogen.

Chlorophyll a analysis was performed as a proxy of phytoplankton; to do so, 500 ml of water were taken from the water column and filtered at each site in Whatman® Grade GF/F Glass Microfiber filters (pore size 0.7 μm) that were cooled and transported in dark containers to the lab-oratory. All samples were frozen until analyzed. Chlorophyll a was extracted from filters in 90% acetone, and measured spectrophotometri-cally with correction by phaeopigments, according to standard methods ([APHA, 1998](#page1)). Total suspended solids (TSS) were assessed gravimetri-cally from water samples (2000 ml plastic bottles). Suspended particles

were measured by filtering through pre-dried and pre-weighed 47-mm filters. Differences between the final and initial weights of dried filters (105 °C for 4 h) were obtained.

Trophic state index (TSI) based on three parameters - TP, TN and chlorophyll a - were calculated ([Carlson, 1977](#page1); [Kratzer and Brezonik,](#page1) [1981](#page1)). According to the values reached by the TSI, four categories can be distinguished ([Carlson and Simpson, 1996](#page1)): oligotrophic (TSI b 30), mesotrophic (TSI: 30–60), eutrophic (TSI: 60–90) and hypereutrophic (TSI N90). TSI was used following the extensive work of [Epele et al.](#page1) [(2018)](#page1), that showed consistent results using this index to evaluate the trophic level at Patagonian ponds.

2.4. Land cover assessment

Land cover variables were assessed as the percentage cover within a concentric circle of 100 m buffer around the pond ([López et al., 2013](#page1)). This attempted to incorporate information about the ecological integrity of pond surroundings, as well as possible sources of disturbances explaining in situ environmental conditions (e.g., water quality). The buffer size selection was based on a large scale study previously con-ducted in Patagonia ([Epele et al., 2018](#page1)), in which consistent responses to land use practices were evidenced.

The adjacent land cover categories used at first were: bare soil (lack-ing vegetation soils), rocks, grasses/herbaceous (dry soils), peatlands, mallín (hydrophilic vegetation on saturated soil), shrubs, trees and urban (including roads and buildings). High spatial resolution imagery concurrent with field sampling was employed as a base map, which were obtained from Google Earth Pro, 2015 (accessed on April 2016)

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and SPOT 5 satellite (2.5 m spatial scale resolution). The cartographic work was performed using the software open source QGIS 3.4 ([QGIS](#page1) [Development Team, 2018](#page1)) and, hence, SPOT imagery was georeferenced to the Universal Transverse Mercator coordinate system. In the classification procedure, parametric decision rules of “minimum distance” were used to separate features. This is an iterative process whereby homogeneous pixels were converted from individual raster pixels to a polygon until the entire study area was completed. Finally, we validated the classification system with ground survey information. After inspection of cover data (distribution, zeros), only six categories emerged consistently and these were retained for further analyses:

1. bare soil, 2) grasses/herbaceous, 3) mallín, 4) shrubs, 5) trees, and
2. urban.

2.5. Macrophyte collection procedure

Quantitative macrophyte samples were gathered from all studied ponds. This survey was accomplished in order to include all functional groups present in a site: emergent (plants rooted, morphologically adapted to growing in a water-logged or submersed substrate), floating-leaves (plants rooted in the substrate with floating leaves), and submersed (plants with photosynthetic tissue entirely submersed) ([Cronk and Fennessy, 2001](#page1)). Following [Luque (1997)](#page1) criteria, landforms (terrestrial vascular plants growing in the surrounding matrix of the wetland) were also included.

At each site, three transects (15 m) were established. Transect di-mension was chosen taking into account that the average pond area of the pool data was 0.15 ha. Each transect was located from the center of the pond to the shoreline according to one of eight possible direc-tions: South, North, East, West, South-East, South-West, North-East and North-West. Transect direction was randomly selected, without re-placement. Macrophyte community was then assessed in ten circle units (0.25 m2) located equidistantly (1.5 m) along each transect ([Gage and Cooper, 2010](#page1)). Species richness and percentage coverage of all species were obtained. Voucher specimens were removed by hand and stored in plastic bags. Material was subsequently conditioned for laboratory identification. In addition, digital pictures were taken for de-tails of live specimens. The species were observed with a LEICA MZ6 ste-reomicroscope and identified using regional bibliography ([Correa,](#page1) [1978–1999](#page1)). Species were also classified as native, endemic and exotic following the Catalogue of the Vascular Plants from the Southern Cone ([Zuloaga et al., 2008](#page1)).

2.6. Metric calculation

In addition to the macrophyte inventory, a set of measures were se-lected to evaluate community responses to possible stressors following existing protocols used at cold-temperate areas and included in water framework directives ([Willby et al., 2012](#page1); [U.S. EPA, 2016](#page1); [Roberts](#page1) [et al., 2017](#page1)). Macrophyte metrics included measures of taxonomic rich-ness, abundance, Shannon diversity, and percentage of taxonomic com-position. Moreover, richness and cover (i.e., quantitative data) of macrophytes regarding to origin (endemics, native and exotics), func-tional groups (emergent, landform, floating-leaves and submersed) and lifeforms (perennial and annual/biannual) were calculated for each pond ([Gage and Cooper, 2010](#page1); [Beck et al., 2014](#page1); [Kolada et al.,](#page1) [2014](#page1)). As a result, 20 measures were obtained.

2.7. Statistical approach

To examine main environmental gradients across the studied sites two Principal Component Analysis (PCA) were performed. PCA's are valuable procedures to detect structure in the relationships among var-iables, which were posteriorly used as explanatory variables in further analysis. First PCA (a) was based on physical (climatic and geomorpho-logical features), chemical (pH, conductivity and nutrients) and

biological parameters (chlorophyll a). Collinearity among variables was checked prior the PCA (a) with a Spearman correlation matrix; as a result the initial set of 26 variables was reduced to 16 variables (vari-ables with magnitudes greater than a pre-selected threshold of ±0.5 were removed). The second PCA (b) was conducted in order to assess the possible influence of land cover patterns in structuring local envi-ronmental measures. The land cover categories and data were those resulting from the analysis of 100 m buffer area around each pond. All variables, except pH and chlorophyll a, were log-transformed using log (x + 1) before analysis (PCA's) in order to reach the normality assumption.

Mann Whitney non parametric tests were performed to check re-gional differences of macrophyte community descriptors between insu-lar (TF) and continental region (SC).

Quantitative macrophyte species data were log (x + 1) transformed and analyzed via Detrended Correspondence Analysis (DCA) to assess the biological turnover within each data set. Gradient lengths obtained for all organism groups (N3.0 SD units) indicated the use of a unimodal model; therefore Canonical Correspondence Analysis (CCA) was chosen to evaluate species environmental relationships ([ter Braak and](#page1) [Smilauer, 1998](#page1)). CCA is a technique of direct gradient analysis, and iden-tifies an environmental basis for community and site ordination by de-tecting the patterns of variation in composition that can be best explained by the environmental variables. The CCA ordination was per-formed with 106 macrophyte taxa and 16 environmental variables. A stepwise selection procedure (forward and backward selection) was employed to select the best model and environmental variables that sig-nificantly (α b 0.05) explained the variance in pond macrophyte assem-blages, which uses a permutation-based test. A Monte-Carlo permutation test (999 permutations) was run on the first axis eigen-value and the “trace” (sum of all canonical eigenvalues) to test the sig-nificance of the environmental and pressure effects under analysis ([ter](#page1) [Braak and Smilauer, 1999](#page1)).

We analyzed effects of environmental and land cover variables on macrophyte metrics using generalized linear models (GLM). Two sets of separate models were run to predict metrics that included: 1) the en-vironmental variables, and 2) the land cover variables as fixed effects. The response variable total taxa, endemic, native, native+endemic, emergent, submersed and annual were modeled assuming a Poisson distribution of errors and log link function, while cover (proportional data) of endemic, emergent and submersed with a Binomial family and logit function. Shannon index was modeled with a Gaussian family and identity link function ([Crawley, 2007](#page1); [Zuur et al., 2009](#page1)). The ex-planatory variables included in the analysis (first set of models) were annual mean rainfall, annual mean temperature, area, altitude, depth, conductivity, dissolved oxygen, chlorophyll a, pH, ammonium and total phosphorus as environmental variables, and shrubs, mallín, trees and grasses/herbaceous as land cover variables (second set of models). Models were evaluated with an automatic backward stepwise selection procedure. To supplement parameter evidence of important effects, the model parameters were bootstrapped and confidence intervals limits (CL) were calculated. Explanatory variables with CL including zero were excluded from the final model. To avoid collinearity between ex-planatory variables, only terms with variance inflation factors ≤4 were allowed. The percentage of explained deviance by each model was cal-culated. Statistical analyses were performed using R software, Version 3.2.3 ([R Core Team, 2016](#page1)), boot ([Canty and Ripley, 2014](#page1)), and car ([Fox](#page1) [and Weisberg, 2011](#page1)) package, in RStudio software v.1.0.136 ([R Studio](#page1) [Team, 2016](#page1)).

3. Results

3.1. Environmental and land cover attributes of ponds

Ponds were small (0.01–1.2 ha) and shallow (0.13–1.19 m). Values of pH showed large spatial variability, ranging from 3.12 to 9.92, but

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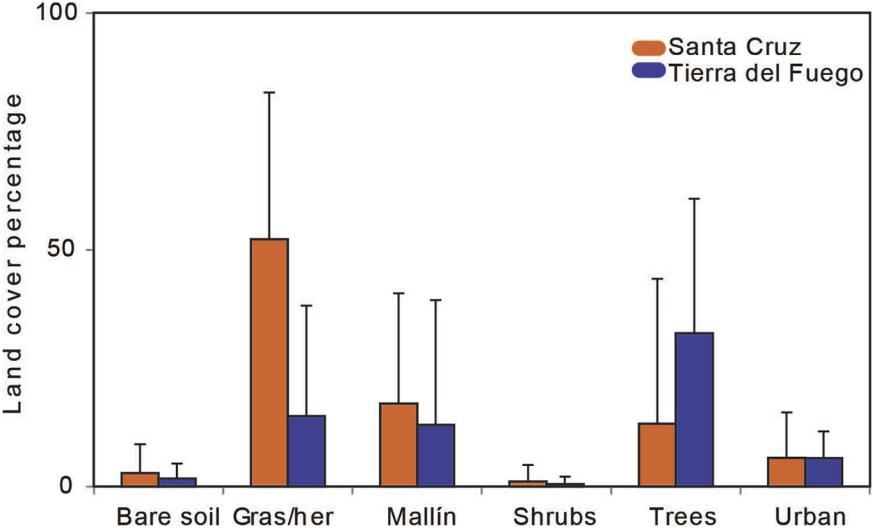
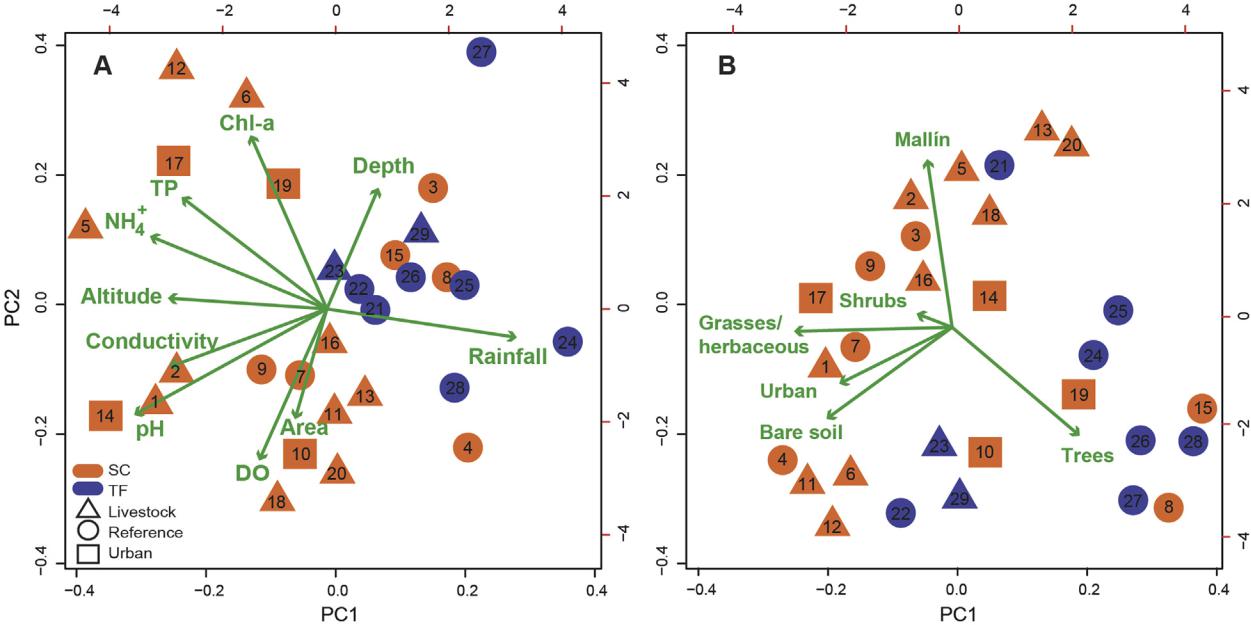


Fig. 2. Average of main land covers per category (+1Standard Deviation) in a 100 m buffer at the 29 studied ponds, Patagonia-Argentina.

ponds from the insular area were acid (b5.86), while ponds from the continental area were generally neutral or alkaline (65% of sites with values N6.2). On average, sites showed similar DO values and were well oxygenated, with 85% showing values between 8 and 21.5 mg/l. Conductivity decreased from the north to the south, with ponds located in the continental area recording double the values of those located in the insular area (310.31 and 149.58 μS/cm, respectively). Regarding nu-trient concentrations, ponds showed a large spatial variability, with the maximum values recorded at ponds in the continental area. Here, aver-age values of total nitrogen (463.39 μg/l), total phosphorous (132.63 μg/

1. and ammonium (40.79 μg/l) doubled average values of the insular area. In addition, most ponds displayed similar chlorophyll a concentra-tion (N75% with values b6 mg/l) and aquatic plant cover (mean: N80%) ([Table 1](#page1)). Eutrophication indexes showed that TSI based in TP content and in chlorophyll a discriminated well among trophic categories, but for TSITN sites where all mesotrophic (Appendix A). Values resulting



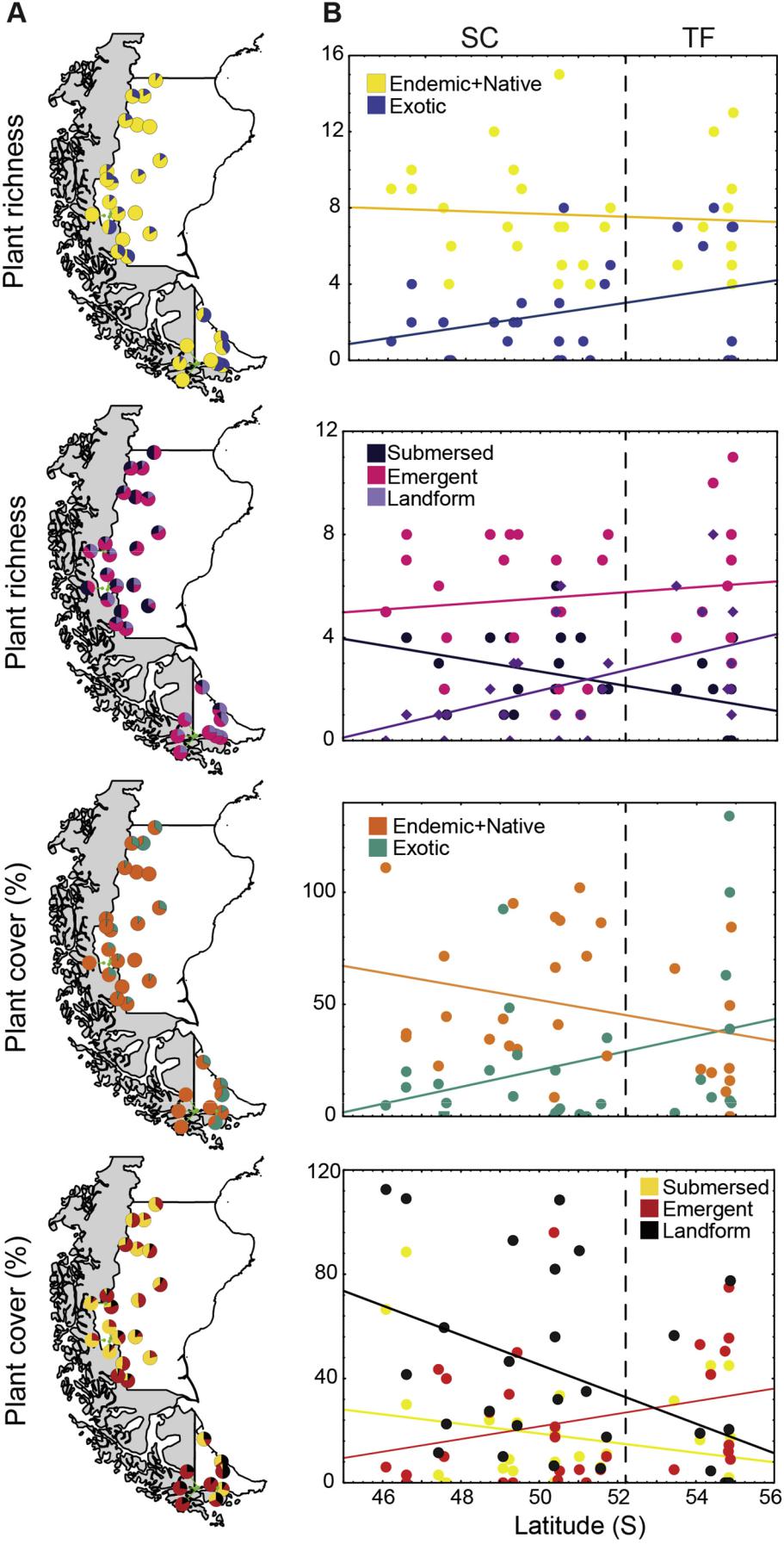
from TSIChla indicated that 15 were mesotrophic, 12 oligotrophic, and 2 eutrophic. Instead, TSITP showed that six ponds were mesotrophic and 22 were eutrophic, and one hypereutrophic ([Table 1](#page1)).

Dominant land cover categories were grasses/herbaceous, mallín and trees, showing some differences between continental (SC) and in-sular regions (TF) ([Fig. 2](#page1)). Mean values of percent cover of grasses/her-baceous were 52.2% and 14.9% for SC and TF, respectively. Conversely, percent cover of trees was lower at SC (13.3%) than at TF (32.4%) (Mann Whitney test, pb0.05). Mallín cover percent was quite similar at both regions with 17.5% and 13% for SC and TF, respectively. The bare soil, shrubs and urban categories showed a lower contribution ([Fig. 2](#page1)).

The first and second axes of the PCA (a) explained 32% and 19% of environmental variability of the studied sites, respectively ([Fig. 3](#page1)a). A clear distinction of sites according to their distribution in the latitudi-nal/climatic gradient was highlighted on axis 1, with ponds from SC

Fig. 3. Principal component analysis of a) physical, chemical and biological environmental variables, and b) land cover pattern in adjacent areas (100 m buffer) at 29 ponds in the continental (Santa Cruz Province: blue) and insular (Tierra del Fuego: orange) region in the Patagonia Austral. Land uses: Reference, circle; Livestock, triangle; Urban, square. DO: dissolved oxygen, NH+4: ammonium, Chl-a: chlorophyll a, TP: total phosphorus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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(continental) being placed on the left and those of TF on the right-side of PCA axis (i.e., positive loadings: mean annual rainfall; high negative loadings: conductivity, pH, ammonium and altitude). The PCA axis 2 separated sites regarding to size and oxygen content (i.e., positive load-ings: depth; negative loadings: pond area and dissolved oxygen). An eu-trophication gradient was evidenced, as vectors of TP and chlorophyll a pointed out towards the same direction. Thus, ponds having high levels of chlorophyll a and TP were located on the upper left corner of the di-agram, whereas those displaying lower TP contents and chlorophyll a were grouped on the lower right corner ([Fig. 3](#page1)a).

For the land cover PCA (b) first and second axes explained 31% and 23%, respectively ([Fig. 3](#page1)b). PCA axis 1 displayed a forest/steppe gradient, with ponds having higher grasses/herbaceous, urban and bare soil cov-erage being placed on the negative side, and ponds displaying higher proportion of trees grouped on the positive end. The PCA axis 2 was re-lated to the proportion of hydrophytic vegetation (i.e., mallín cover).

3.2. Macrophyte assemblages

A total of 106 macrophytes taxa were identified and corresponded to four plant divisions: Bryophyta, Charophyta, Chlorophyta and Spermatophyta, the latter being represented by 27 families, 67 genera and 103 species (Appendix B). The most important families were Poaceae (21 taxa), Cyperaceae (15 taxa), Juncaceae and Ranunculaceae (9 taxa each one), accounting for 50% of the total species. According to the habit, 49% were emergent, 35% landforms, 15.1% submersed and 0.9% were floating-leaves. From the total inventory 39.8% were en-demics, 36.9% natives, and 23.3% exotics. The majority of macrophytes were perennial (77.4%) and the rest (22.6%) were annual-biannual.

A total of 80 taxa were present at SC whereas 49 taxa at TF, with 23 taxa being common at both provinces. At SC the most frequent taxa were Myriophyllum quitense (13 sites), Eleocharis pseudoalbibracteata (10 sites), and Juncus sp. (10 sites); whereas at TF Carex magellanica and Alopecurus pratensis were present at N50% of the sites. Local richness at ponds ranged between 4 and 18 at SC, and from 4 to 20 at TF. In terms of richness, the proportion of submersed, emergent and landforms displayed important variations ([Figs. 4 and 5](#page1)). At SC, landforms domi-nated assemblages at 40% of the sites, whereas emergent dominated at 25% of the sites. In term of richness, most of TF ponds were composed of landform-dominated assemblages (55% of sites), and emergent dom-inated assemblages only at two sites (ponds: 27, 28). At each pond, plant cover by habit displayed a different pattern than highlighted by richness; for example submersed dominated 45% of sites at SC, whereas emergent at 55% of ponds at TF ([Fig. 4](#page1)). Latitudinal trends were appar-ent for some macrophyte groups, with positive responses for exotic (richness and cover) and landforms (richness). A significant decreasing latitudinal trend was observed for endemic+native (cover), landforms (cover), and submersed (richness). As expected, endemics (richness) were significantly higher at TF than at SC ([Figs. 4, 5](#page1), Appendix C).

Native species were well represented at both SC and TF provinces. Most endemics recorded were landforms (Gunnera magellanica, Geum andicola, Polypogon australis, Plantago barbata and Colobanthus quitensis), and other were emergent (Schoenoplectus californicus and Ra-nunculus maclovianus). A higher degree of exotics was observed when analyzing plant cover, with ranges from 0 to 88% at SC and from 0 to 45.5% at TF ([Fig. 5](#page1)).

3.3. Macrophytes and environmental gradients

Results of the CCA (first three axes) are summarized in [Table 2](#page1) and shown in [Fig. 6](#page1) (Axes: CCA1 and CCA3). The CCA3 was plotted (instead CCA2) to display sites and macrophytes assemblages associated with TP

gradient. The environmental variables selected in the analysis are repre-sented in the biplot by arrows, which point in the direction of maximum change in the value of the associated variable ([Fig. 6](#page1)a, b). The species-environmental correlations were: 0.95, 0.91 and 0.90 for the first, sec-ond and third axis respectively, indicating a strong relationship with the environmental variables selected. Monte-Carlo tests were signifi-cant for all axes considered ([Table 2](#page1)).

The strongest explanatory factors of macrophyte patterns were physical (climatic) and chemical variables, and the ordination explained 65.7% of the species environmental relationships ([Table 2](#page1)). CCA axis 1 reflected the distribution of species and sites along a natural gradient. It was defined mostly by climatic drivers (temperature, rainfall), physi-cochemical (pH, conductivity) and other local variables as depth. CCA axis 2 highlighted the existence of an anthropogenic gradient, as vari-ables TN and TP increased to the positive end of CCA axis 2. Finally, TP was shown to be negatively associated to the third axis. Thus, ponds lo-cated in warmer areas (2, 3, 7, 1) and those with higher conductivity values (5, 14, 11) were located in the upper left quadrant of CCA; whereas those ponds with lower pH (acidic), exposed to more humidity (rainfall), with low conductivity were located to the positive end of CCA axis 1 (24, 28, 25, 27, 26) ([Fig. 6](#page1)A). Those ponds with high loads of TP were associated to the negative extreme of CCA3 (10, 12, 14, 17), and on the opposite side of the axis, those ponds displaying low levels of TP were placed towards the positive extreme (4, 9, 24, 2).

The location of macrophyte assemblages along the above described gradients is presented in [Fig. 6](#page1)b. Eleocharis macrostachya, E. melanostachys, Erythranthe glabrata, and Chara sp. characterized ponds with high temper-ature. Distichlis spicata, Veronica anagallis-aquatica, Boopis australis and Polypogon australis featured ponds with high salinity and high TN levels. This assemblage was placed on the left lower quadrant of CCA. On the pos-itive side of CCA 1 axis, the species Primula magellanica, Gentianella magellanica, Marsippospermum grandiflorum, Sphagnum sp., Luzula alopecurus, Trisetum spicatum ssp., phleoides, Drosera uniflora, Carex magellanica, Ranunculus repens, Anthoxanthum odoratum and Gaultheria pumila were located. These taxa characterized assemblages at ponds with very acidic waters and low conductivity values, such as those from TF. Geum andicola, Potentilla anserina and Arenaria serpens peaked at sites with high TP concentration and where grouped on the negative side of CCA3; whereas Centaurium pulchellum, Limosella australis, Juncus stipulates and Juncus burkartii were abundant (high cover) at ponds with lower TP concentration, being grouped on the positive end of same axis.

3.4. Macrophyte metrics assessment

GLM approaches showed that TP was a significant environmental pre-dictor of species associations, indicating a negative effect on five metrics (Shannon index, endemic cover, endemic richness, native+endemic rich-ness and emergent richness; [Table 3](#page1)). In addition, model parameters showed a positive effect of mean annual temperature on submersed rich-ness and cover, and a negative effect on endemic cover ([Table 3](#page1)). Regard-ing land cover variables, GLMs indicated a positive effect of grasses/ herbaceous, mallín and trees on native richness, submersed richness and submersed cover (36, 58 and 53% explained deviance, respectively).

4. Discussion

4.1. Macrophyte diversity at isolated ponds: insular vs. continental area

Ponds in Austral Patagonia sustained an unexpectedly rich assem-blage of 106 macrophyte taxa, which was higher than that reported in few comparable studies conducted at temporary ponds in the region. In an extensive survey in Patagonia northwest [Bran et al. (2004)](#page1)

Fig. 4. Macrophyte metrics according to habitat (submersed, emergent and landform) and origin (Endemic, native and exotic) recorded at 29 sites at continental and insular Patagonia Austral. a) Relative contribution per site based on richness and cover measures. b) Multiple scatterplot displaying latitudinal patterns of metrics. Significant trends (pb0.05): submersed richness, landform richness and landform cover.

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Fig. 5. Boxplots indicating mean values of macrophyte metrics based on richness, lifeforms (annual and perennial), origin (native and endemic) and structure (Shannon diversity). Data recorded at 29 sites at continental and insular Patagonia Austral. (\*) Significant differences according to Mann Whitney test (p b 005).

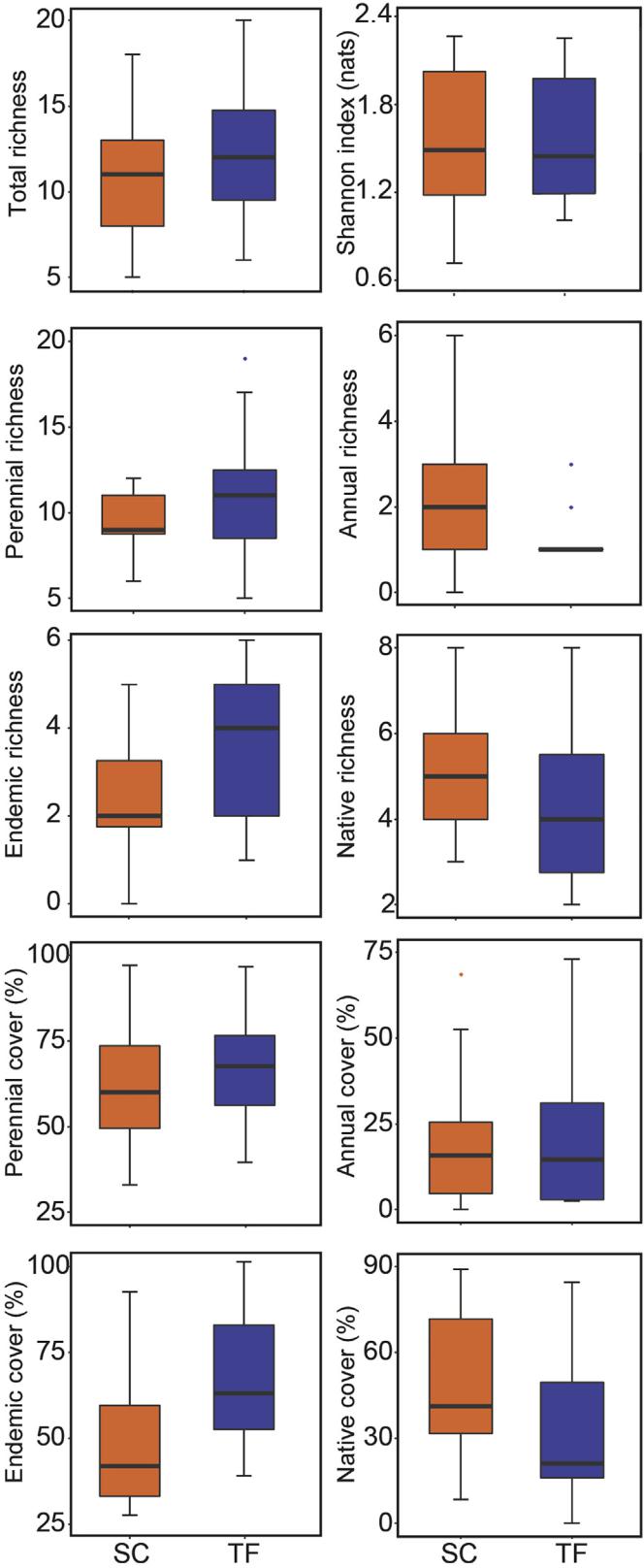


Table 2

Canonical Correspondence Analysis (CCA) results for 29 Patagonian ponds of Santa Cruz and Tierra del Fuego Provinces (Patagonia Argentina). Eigenvalues and loadings for constraining variables for the first three CCA axes are consigned; F-ratio statistics and p-values are listed for the first axis, and for all the axes combined.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Environmental features | Axis |  |  |  |
|  |  |  |  |  |
|  | CCA1 | CCA2 | CCA3 |  |
|  |  |  |  |  |
| Mean annual temperature | −0.88 | −0.07 | 0.30 |  |
| Mean annual rainfall | 0.61 | −0.07 | −0.01 |  |
| Conductivity | −0.73 | −0.38 | −0.36 |  |
| Depth | 0.39 | −0.44 | −0.19 |  |
| pH | −0.84 | 0.15 | −0.19 |  |
| Total nitrogen | −0.46 | 0.56 | −0.27 |  |
| Total phosphorus | −0.01 | 0.34 | −0.83 |  |
| Eigenvalues | 0.68 | 0.43 | 0.36 |  |
| Species-environment correlations | 0.95 | 0.91 | 0.90 |  |
| Cumulative percentage variance | |  |  |  |
| Of species data | 8.6 | 14.1 | 18.6 |  |
| Of species-environment relation | 27.8 | 45.5 | 60.1 |  |

Monte-Carlo permutation test

First canonical axis: F = 1.98, pb0.0009

All canonical axes: F = 1.34, pb0.0001

found 70 taxa (130 ponds), whereas [Kutschker et al. (2014)](#page1) recorded 50 taxa (30 ponds). Besides, [San Martín et al. (2011)](#page1) reported 86 taxa (Chilean Patagonia), but the study included also other freshwater envi-ronments (Appendix D). Global richness was lower to that observed at ponds in other comparable temperate areas, for example [Nicolet et al.](#page1) [(2004)](#page1) in England (182 species on 72 sites), [Pätzig et al. (2012)](#page1) in Germany (132 species on 276 sites), and [Svitok et al. (2018)](#page1) in central Europe (127 taxa, 92 sites) (Appendix D), however those studies assessed more sites.. It is probable that the wide range in environmental conditions here documented at both large and local scales contributed to explain the observed macrophyte richness. In the regional context, alien species contribution was higher than expected (N23%), but compa-rable to that found by [Hauenstein (2006)](#page1) in Chilean inland waters (20.1%). Considering that our dataset included disturbed sites and dis-turbances can favour aquatic invasions ([Svitok et al., 2018](#page1)) this proba-bly enlarged the overall biodiversity detected.

As expected, a significant higher richness of endemics was found at the insular than at the continental area, which is consistent with re-ported at other isolated regions as New Zealand ([Lacoul and](#page1) [Freedman, 2006](#page1)). However, total richness did not differ between re-gions, and at both areas (insular and continental) assemblages were dominated by native species ([Bran et al., 2004](#page1); [San Martín et al., 2011](#page1); [Macchi, 2017](#page1)).

The mean local richness was 10 species, a relatively high value for small sized ponds in cold temperate areas (Appendix D). This outcome reinforces the idea that isolated ponds are valuable habitats for aquatic plants particularly in a region in which aridity is pronounced ([Kutschker](#page1) [et al., 2014](#page1); [Manzo et al., 2019](#page1)).

4.2. Drivers of macrophyte assemblage along environmental gradients

As revealed in our multivariate analysis, and as occurring at large scale approaches in undeveloped areas ([Wiegleb et al., 2015](#page1); [Svitok](#page1) [et al., 2018](#page1)), the most important gradient governing macrophyte spe-cies distribution was defined by climatic factors (natural); that is, re-lated with spatial location of sites on the latitudinal gradient (North-South). In addition, the rainfall gradient, which regulates the ecohydrological functioning (water availability) of Patagonian wetlands ([Chimner et al., 2011](#page1)), was also highlighted in the CCA. Nevertheless, the second and third axes highlighted other environmental drivers, probably from anthropogenic origin, and were related to nutrient con-tent (mainly phosphorous and nitrogen).

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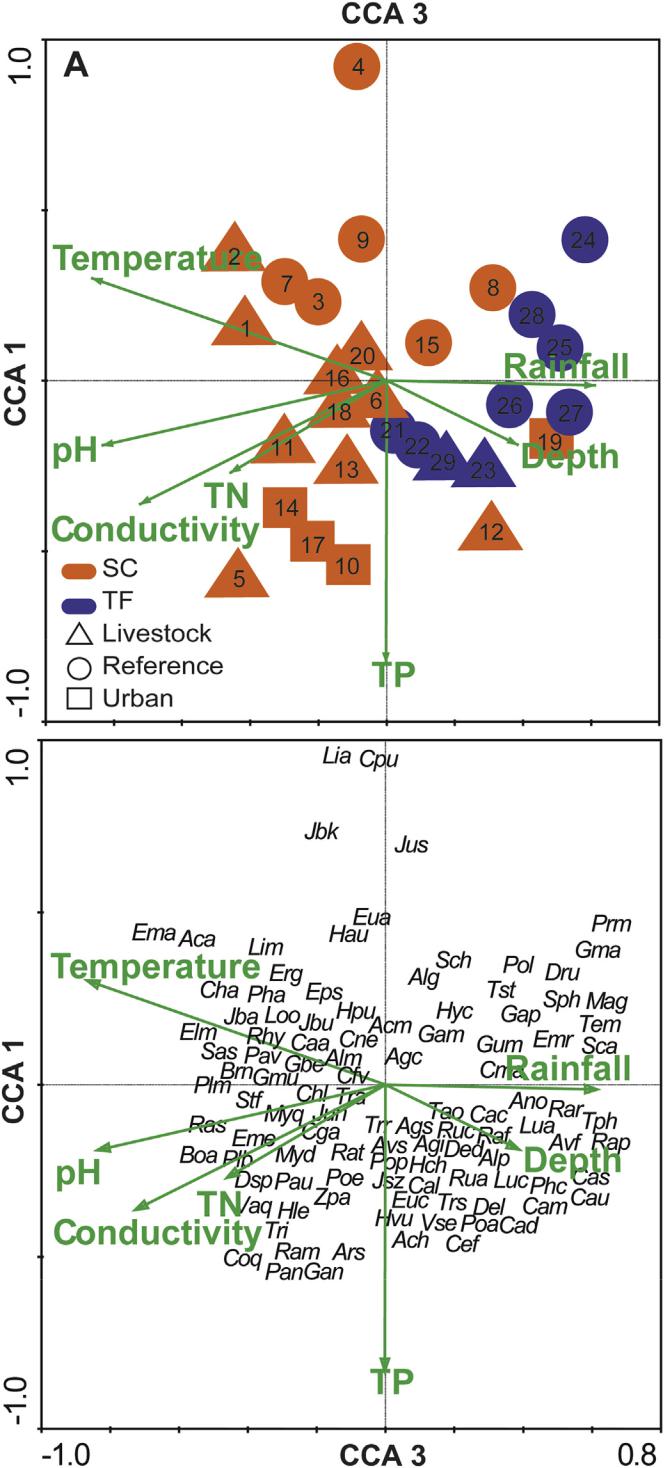


Fig. 6. Ordination diagram (Canonical Correspondence Analysis) based on abundance data of 106 macrophyte taxa and environmental variables (arrows) of 29 ponds in the continental and insular region of Patagonia Austral. a) Biplot of 29 sites and environmental variables. b) Biplot of macrophyte taxa and environmental variables. Full names of taxa codes are given in Appendix B. Ponds are: Santa Cruz, orange; Tierra del Fuego, blue. Land uses: Reference, circle; Livestock, triangle; Urban, square. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Physicochemical factors usually appear as significant predictors of species richness of plant life and growth forms at isolated ponds in tem-perate regions ([Zimmer et al., 2003](#page1); [Pätzig et al., 2012](#page1)). [Clausen et al.](#page1) [(2006)](#page1) found that differences in chemical conditions allowed the

typifying of wetlands at Torres del Paine (Patagonia, Chile), where fea-tures such as conductivity, pH, and minerals were key drivers of macro-phyte assemblages. In our analysis we found that pH was a strong predictor (range: 3.12–9.92) and played an important role in species ar-rangement. According to [Chimner et al. (2011)](#page1) hydrological, chemical and vegetation parameters varied with total precipitation at Patagonian wetlands. The authors indicated that precipitation gradient ruled water chemistry at ponds; with pH, conductivity and cation concentrations all increased as the precipitation decreased. In our work, all peat-lands were grouped together (CCA), these were dominated by Sphagnum magellanicum and featured as very acidic, with poor nutrients and low oxygen contents that are main singularities of the “turberas” (peat bogs) ([Van Breemen, 1995](#page1)). Ponds having very alkaline water (N9) were dominated by submersed as Chara sp., Ranunculus spp. and the ubiquitous Myriophyllum quitense. Other large-scale spatial studies con-ducted in the Northern Hemisphere ([Della Bella et al., 2008](#page1); [Willby](#page1) [et al., 2012](#page1); [Lindholm et al., 2019](#page1)) also demonstrated the prevailing role of pH in shaping macrophyte composition at ponds.

As mentioned, disturbance was linked to total phosphorous (TP) and chlorophyll a contents, highlighting an eutrophication gradient. Indeed, TP discriminated well among different land uses. Mineralization of or-ganic matter in soils and land use associated with cattle breeding appear as important phosphorous sources impacting waterbodies ([Mugni et al.,](#page1) [2005](#page1)). We considered that moderate to high concentrations of TP were at least in part attributed to livestock presence ([Golluscio et al., 1998](#page1); [Epele et al., 2018](#page1)). Livestock tend to spent more time next to the waterbodies for foraging and watering. This animal behavior results on dejection (urine and feces) occurring more in nearby water than at other sites, leading nutrient level to increase at waterbodies ([Epele](#page1) [and Miserendino, 2015](#page1); [Horak et al., 2019](#page1)). A higher availability of this nutrient surely promoted an increase of planktonic chlorophyll a ([Sosnovsky and Quirós, 2006](#page1)). On the other hand, the highest concen-trations of nutrients (e.g., TP, SRP) occurred at urban ponds, where we suspect that runoff played a major role, since no ponds operated for ef-fluent disposal.

4.3. Macrophyte metrics indicators of anthropogenic disturbance

An assessment index should effectively distinguish between signal (e.g., shift in community composition from stressors) and noise (e.g., natural variation across climatic gradients) ([Beck et al., 2014](#page1)). We found that the relationships between macrophyte based metrics and the environment were quite complex. In particular, to discriminate between nutrient sources along a large scale gradient, as the present study observed, appears to be a challenging task ([Epele et al., 2018](#page1)). Nevertheless, we found six metrics based on richness, cover and com-munity structure that responded significantly to increases in TP con-tents (disturbance variable). Three of them retained TP as the unique predictive variable, but with a moderate explanatory power (24–32% deviance).

Shannon index decreased with TP increases, indicating structural community changes as a result of possible eutrophication processes or disturbances. One disadvantage is that calculation of Shannon index re-quires a higher effort than taxonomic composition measures ([Kanninen](#page1) [et al., 2013](#page1)). Besides, some authors found some limitations of the use of this metric since highest macrophyte diversity can occur in mesotrophic or slightly eutrophic ecosystems ([Della Bella et al., 2008](#page1)). Thus, we rec-ommend the use of endemic and emergent richness metric, since might be equally informative of water quality conditions at ponds, but easier to calculate ([Mackay et al., 2010](#page1); [Hernández et al., 2015](#page1)).

Invasive species can be indicators of pond disturbance ([Stuber et al.,](#page1) [2016](#page1)), indeed measures of richness and coverage of exotics are included in bioassessment protocols as in United Kingdom ([Willby et al., 2012](#page1)), Australia ([Roberts et al., 2017](#page1)) and in United States ([U.S EPA, 2016](#page1)). However, we did not found significant relationships of richness or cov-erage of exotics measures with documented environmental variables. In

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the literature at a regional level, [Bran et al. (2004)](#page1) stated that exotics in-creased at wetlands associated to livestock development, but according to [Kutschker et al. (2014)](#page1), exotics did not vary consistently with the level of disturbance related with livestock existence. Recently, [Urrutia](#page1) [et al. (2017)](#page1) examined the distribution of exotics macrophytes and indi-cated that alien species appears affecting freshwater ecosystems

functioning (Chile). More studies are crucial to improve our knowledge regarding this matter.

Our findings also sustain the premise that out of the helophytes, emergent rather than landform groups would be advantageous as indi-cators, as richness responded consistently to TP increases, and reflected the diminution of suitable areas on the aquatic-terrestrial interphase.

Table 3

Results of Generalized Linear Models explaining the effects of environmental and land cover variables on different macrophytes metrics studied at 29 Patagonian ponds. Explanatory var-iables, parameter estimates (β) (±Standard Error), and confidence intervals (CL) are shown. Explanatory variables with CL including zero were excluded from the final model. NM: Any satisfactory model was obtained.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Environmental variables | |  |  |  |  |  |  | Land cover variables | |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Model | Explanatory | β±SE | z | p value | CL: | CL: | Explained | Explanatory | | β±SE | z | p | CL: | CL: | Explained |  |  |  |
|  |  | variables |  | value |  | lower | upper | deviance | variables | |  | value | value | lower | upper | deviance |  |  |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  | |  |  |  |  |  |  |  |  |  |
|  | Total taxa | NM |  |  |  |  |  |  | (Intercept) | | 2.3 | 37.79 | b2e−16 | 2.14 | 2.47 | 6% |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | ± 0.06 |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  | Shrubs | | 0.03 | 1.98 | 0.04 | 0.005 | 0.15 |  |  |  |  |
|  |  |  |  |  | 7.58e−15 |  |  |  |  |  | ± 0.02 |  |  |  |  |  |  |  |  |
|  | Shannon index | (Intercept) | 1.83 | 15.33 | 1.58 | 2.05 | 26% | NM | |  |  |  |  |  |  |  |  |  |
|  |  |  | ± 0.12 |  |  |  | −9e−04 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | Total | −0.002 | −2.79 | 0.009 | −0.004 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | phosphorus | ± 8e−04 |  | 2.49e−15 |  |  |  |  |  |  |  | b2e−16 |  |  |  |  |  |  |
|  | Endemic richness | (Intercept) | 1.40 | 7.91 | 1.08 | 1.72 | 24% | (Intercept) | | 1.16 | 8.76 | 0.89 | 1.37 | 12% |  |  |  |
|  |  |  | ± 0.18 |  |  |  |  |  |  |  | ± 0.13 |  |  |  |  |  |  |  |  |
|  |  | Total | −0.004 | −2.53 | 0.01 | −0.007 | −0.001 |  | Mallín | | −0.01 | −1.89 | 0.05 | −0.02 | −0.003 |  |  |  |  |
|  |  | phosphorus | ± 0.001 |  |  |  |  |  |  |  | ± 0.006 |  |  |  |  |  |  |  |  |
|  | Native richness | NM |  |  |  |  |  |  | (Intercept) | | 0.52 | 1.17 | 0.24 | −0.91 | 1.25 | 36% |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | ± 0.44 |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  | Grasses/herbaceous | | 0.01 | 2.73 | 0.006 | 0.005 | 0.03 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | ± 0.005 |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  | Mallín | | 0.01 | 2.26 | 0.02 | 0.005 | 0.03 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | ± 0.005 |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  | Trees | | 0.01 | 1.69 | 0.009 | 0.001 | 0.02 |  |  |  |  |
|  |  |  |  |  | b2e−16 |  |  |  |  |  | ± 0.006 |  |  |  |  |  |  |  |  |
|  | Native + endemic | (Intercept) | 2.08 | 18.53 | 1.87 | 2.28 | 38% | NM | |  |  |  |  |  |  |  |  |  |
|  | richness |  | ± 0.6 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | Alkalinity | 6e−05 | 2.13 | 0.03 | −0.001 | −1e−04 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  | ± 3e−05 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | Total | −2−03 | −2.38 | 0.02 | −0.003 | −5e−04 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | phosphorus | ± 8e−04 |  | b2e−16 |  |  |  |  |  |  |  | b2e−16 |  |  |  |  |  |  |
|  | Emergent richness | (Intercept) | 2.05 | 16.91 | 1.83 | 2.25 | 32% | (Intercept) | | 1.68 | 20.19 | 1.51 | 1.85 | 8% |  |  |  |
|  |  |  | ± 0.12 |  |  |  |  |  |  |  | ± 0.08 |  |  |  |  |  |  |  |  |
|  |  | Total | −0.003 | −3.13 | 0.001 | −0.005 | −0.001 |  | Shrubs | | 0.04 | 1.95 | 0.05 | 0.01 | 0.16 |  |  |  |  |
|  |  | phosphorus | ± 0.001 |  |  |  |  |  |  |  | ± 0.02 |  |  |  |  |  |  |  |  |
|  | Submersed | (Intercept) | −0.43 | −0.92 | 0.36 | −1.38 | 0.36 | 28% | (Intercept) | | −1.9 | −2.07 | 0.04 | −3.59 | −0.42 | 58% |  |  |  |
|  | richness |  | ± 0.47 |  |  |  |  |  |  |  | ± 0.92 |  | 5e−04 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  | Grasses/herbaceous | | 0.03 | 3.46 | 0.02 | 0.05 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | ± 0.01 |  |  |  |  |  |  |  |  |
|  |  | Mean | 0.21 | 3.05 | 0.002 | 0.11 | 0.34 |  | Mallín | | 0.03 | 2.8 | 0.005 | 0.01 | 0.05 |  |  |  |  |
|  |  | annual | ± 0.07 |  |  |  |  |  |  |  | ± 0.01 |  |  |  |  |  |  |  |  |
|  |  | temperature |  |  |  |  |  |  | Trees | | 0.03 | 2.47 | 0.01 | 0.009 | 0.04 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | ± 0.01 |  |  |  |  |  |  |  |  |
|  | Annual richness | NM |  |  |  |  |  |  | (Intercept) | | 0.04 | 0.14 | 0.89 | −0.24 | 0.27 | 29% |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | ± 0.27 |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  | Grasses/herbaceous | | 0.01 | 2.85 | 0.004 | 0.006 | 0.02 |  |  |  |  |
|  |  |  |  |  | b2e−16 |  |  |  |  |  | ± 0.004 |  |  |  |  |  |  |  |  |
|  | Endemic cover | (Intercept) | 3.84 | 16 | 1.12 | 6.42 | 52% | (Intercept) | | −0.32 | −0.53 | 0.59 | −1.34 | 0.6 | 16% |  |  |  |
|  |  |  | ± 0.28 |  | b2e−16 |  |  |  |  |  | ± 0.61 |  | 1e−07 |  |  |  |  |  |  |
|  |  | Mean | −0.56 | −16 | −0.94 | −0.24 |  | Grasses/herbaceous | | −0.02 | −1.42 | −0.04 | −0.003 |  |  |  |  |
|  |  | annual | ± 0.035 |  |  |  |  |  |  |  | ± 0.001 |  |  |  |  |  |  |  |  |
|  |  | temperature |  |  | b2e−16 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | Total | −0.02 | −15.9 | −0.035 | −0.01 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | phosphorus | ± 0.01 |  |  |  |  |  |  |  |  |  | b1e−08 |  |  |  |  |  |  |
|  | Emergent cover | NM |  |  |  |  |  |  | (Intercept) | | 0.34 | 5.68 | −0.32 | 0.94 | 8% |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | ± 0.6 |  | b2e−16 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  | Grasses/herbaceous | | −0.01 | −12.1 | −0.02 | −0.003 |  |  |  |  |
|  |  |  |  |  | b2e−16 |  |  |  |  |  | ± 0.01 |  |  |  |  |  |  |  |  |
|  | Submersed cover | (Intercept) | −3.74 | −20.4 | −5.24 | −1.81 | 45% | (Intercept) | | −4.7 | −1.55 | 0.12 | −7.86 | −2.45 | 53% |  |  |  |
|  |  |  | ± 0.18 |  | b2e−16 |  |  |  |  |  | ± 0.3 |  | b2e−16 |  |  |  |  |  |  |
|  |  | Mean | 0.47 | 17.2 | 0.24 | 0.72 |  | Mallín | | 0.06 | 1.5 | 0.03 | 0.1 |  |  |  |  |
|  |  | annual | ± 0.3 |  |  |  |  |  |  |  | ± 0.04 |  |  |  |  |  |  |  |  |
|  |  | temperature |  |  | b2e−16 |  |  |  |  |  |  |  | b2e−16 |  |  |  |  |  |  |
|  |  | Total | 0.007 | 13.96 | 0.003 | 0.01 |  | Trees | | 0.04 | 1.1 | 0.001 | 0.08 |  |  |  |  |
|  |  | phosphorus | ± 5e−04 |  |  |  |  |  |  |  | ± 0.03 |  | b2e−16 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  | Grasses/herbaceous | | 0.06 | 1.6 | 0.03 | 0.09 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | ± 0.03 |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

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Since helophytes contributed largely to the species pool and have a key functional role in aquatic environments, in concordance with studies at similar regions ([Alahuhta et al., 2013](#page1); [Rääpysjärvi et al., 2016](#page1)) we rec-ommend their inclusion in bioassessment protocols. As examples, existing guidelines in UK ([Willby et al., 2012](#page1)) and Spain ([Ruiz et al.,](#page1) [2011](#page1)) included them as a key part of impacts assessment of hydromorphological pressures.

Here we found that a higher ecological integrity of pond surround-ings would buffer water quality threats (e.g., nutrients). Examination of the influence of pond land cover patterns in a 100 m buffer as predic-tors of macrophyte metrics revealed that the most meaningful models were obtained for both: macrophytes richness and cover measures (higher deviance explained). Submersed and natives plants were shown to be positively associated with a greater cover of grass/herba-ceous, mallín and trees. According to [López and Fennessy (2002)](#page1), the in-tegrity of the buffer zone is interpreted as a measure of environmental factors that maintain and control plant communities at wetlands. In Pat-agonia, many direct and indirect negative effects are expected by live-stock existence, like nutrient increases through excretes (fecal and urine) ([Sauer et al., 1999](#page1)), the alteration of pond shoreline conditions by trampling and foraging ([Ciari, 2010](#page1); [Peña and Campos, 2012](#page1)), with consequent sedimentation and soil compaction ([del Valle et al., 1998](#page1)). Despite studies from other regions of the globe demonstrate the impor-tance of wetlands buffer zone ([Akasaka et al., 2010](#page1); [Sass et al., 2010](#page1); [Stuber et al., 2016](#page1)); at present there are no actions for wetlands conser-vation in Patagonia ([Epele and Miserendino, 2015](#page1)). It is crucial to estab-lish management guidelines for pond conservation in order to maintain their ecological roles ([Biggs et al., 2017](#page1)). In this sense, our results sup-port that at least 100 m buffer zones should be well-conserved to keep ponds integrity.

5. Conclusion

Isolated ponds in the Patagonian Austral region stand as valuable aquatic habitat for macrophytes, many of them endemics. We found that the main constraints in species distribution and abundance depended greatly on climate and physicochemical factors: temperature, rain and pH. Future climate change scenarios in the area, predict an in-creasing trend in temperature and a decreasing pattern in precipitation ([Magrin et al., 2014](#page1)), meaning that at a regional scale profound conse-quences on biodiversity patterns of macrophytes can be expected. A dis-turbance gradient was identified, with total phosphorus explaining additional variation in aquatic plant distribution. Metrics based mostly in macrophyte richness appear to be consistent assessment tools, with considerably less effort when it comes to surveying. Alien species are frequently included in protocol assessment; however in our work this group did not appear reliable indicators of disturbance and should be used with cautiousness. Although most study sites displayed good water quality conditions, grazing at wetlands even in extensive modal-ities imposes a high pressure on pond surrounding areas leading to deg-radation. Future studies should be directed to develop long-term monitoring plans in order to assess changes of aquatic plant biodiversity facing climate change and probably an intensification of land use practices.

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CRediT authorship contribution statement

L.M. Manzo:Conceptualization, Data curation, Formal analysis, In-vestigation, Methodology, Visualization, Software, Writing - original draft.M.G. Grech:Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Software, Supervision, Validation, Writing - review & editing.L.B. Epele:Conceptualization, Investigation, Method-ology, Visualization, Supervision, Validation, Writing - review & editing. A.M. Kutschker:Data curation, Methodology, Supervision, Validation,

Writing - review & editing.M.L. Miserendino:Conceptualization, Meth-odology, Funding acquisition, Investigation, Project administration, Re-sources, Writing - original draft, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influ-ence the work reported in this paper.

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