

# Patterns and mechanisms underlying ecoregion delineation in North American freshwater plants

Running title: Ecoregions of freshwater plants

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## 23    **ABSTRACT**

24    **Aim:** The regionalized patterns of biodiversity distributions are actively studied in terrestrial and  
25    marine ecosystems, but much less is known on the geographical patterns of ecoregions founded on  
26    freshwater taxa. Here, we studied, for the first time, how well existing freshwater ecoregions  
27    describe the geographical distribution of inland water plants.

28    **Location:** Greenland, continental Canada and USA

29    **Taxon:** Freshwater vascular plants of all taxa and multiple functional groups (i.e., growth forms).

30    **Methods:** Using newly available fine-grained data on freshwater plant distributions, we studied how  
31    ecoregions founded on fish are suitable for freshwater plant regionalization across North America.  
32    Specifically, we calculated internal homogeneity and distinctness among neighboring ecoregions in  
33    relation to species replacements and richness differences. We also explored how a complex suite of  
34    ecogeographical characteristics affect ecoregion delineation of freshwater plants using spatially  
35    explicit regression routines.

36    **Results:** We found a clear geographical patterning of ecoregion robustness for North American  
37    freshwater plants, with communities being more internally homogeneous and more similar to one  
38    another in Polar and Subtropical inland waters. The degree of internal homogeneity and ecoregion  
39    distinctness were almost equally driven by species replacements and richness differences.  
40    Considering different life forms, ecoregion delineation performed best for emergent and floating–  
41    leaved plants. Finally, within–ecoregion homogeneity and distinctness were best explained by annual  
42    mean temperature and terrain ruggedness, respectively, with mean water alkalinity, ecoregion area  
43    and late Quaternary glacial legacies having supplementary effects.

44    **Main conclusions:** Our findings suggest that selection through climate filtering (e.g., mean annual  
45    temperature) is likely the main mechanistic driver of freshwater plant ecoregions. Geographical  
46    regionalizations founded on a particular organismal group may not be directly applicable for all taxa

but can be a good basis for further adjustments. Our study is a promising starting point for further investigations of geographical delineations for freshwater taxa other than fish.

**KEYWORDS:** Aquatic macrophytes, Ecoregions, Hydrophytes, Inland waters, North America, Regionalization

#### **STATEMENT OF SIGNIFICANCE**

In biogeographical regionalization, a biota is categorized into meaningful geographical units, such as ecoregions. However, ecoregions delineated for a particular group of organisms may not be applicable to other assemblages. We assessed whether ecoregions founded on fish are suitable for freshwater plants across North America. Internal homogeneity and ecoregion distinctness were almost equally driven by species replacements and richness differences, and best explained by annual mean temperature and terrain ruggedness. Our findings suggest that ecoregions defined by fish distributions did not show consistent robustness for freshwater plants in North America. This study is a promising starting point for further investigations of geographical delineations for different freshwater taxa, having also applied value in conservation planning and management.

#### **CONFLICT OF INTEREST**

There is no conflict of interest.

## 70 INTRODUCTION

71 Biogeographical regionalization, which refers to classification of biotas into meaningful geographical  
72 units, is one of the central objectives in biogeography and ecology (Ficetola et al., 2017; Kreft & Jetz,  
73 2010; Smith et al., 2018). In these biogeographical units, such as biomes and ecoregions, taxonomic  
74 composition ought to be maximally homogenized inside their boundaries (i.e., species composition  
75 across an entire region is relatively consistent), while showing highest differences among neighboring  
76 units (i.e., communities existing in different ecoregions are relatively dissimilar) (Bailey, 2004;  
77 Divisek et al., 2016; Holt et al., 2013). Regionalization helps us to understand fundamental  
78 biogeographical patterns, but also helps inform vital applied perspectives related to establishment of  
79 science-based conservation plans designed to protect regions, habitat types and taxa (Divisek et al.,  
80 2016; Droissart et al., 2018; Ennen et al., 2020). A common approach in conservation is to identify  
81 biodiversity hotspots and ecoregions, where protection and restoration efforts are deliberately focused  
82 (Bernardo-Madrid et al., 2019; Dinerstein et al., 2017). However, delineation of geographical units has  
83 mostly focused on terrestrial taxa (e.g., Dinerstein et al., 2017; Ficetola et al., 2017; Higgins et al.,  
84 2016; Holt et al., 2013), whereas freshwater ecoregions have received less attention (but see Abell et  
85 al., 2008; Ennen et al., 2020; Matamoros et al., 2016). Moreover, available freshwater ecoregion  
86 definitions are typically founded on well-investigated assemblages, such as fish (Abell et al., 2008;  
87 Matamoros et al., 2016) and amphibians (Dias-Loyola et al., 2008), but they may not mirror  
88 biogeographical units for all freshwater organisms, because diversity patterns and distributions are not  
89 often correlated among taxonomic groups (Ennen et al., 2020; Rolls et al., 2017).

90

91 Recent advances have questioned whether an ecoregion-based conservation approach can effectively  
92 protect biodiversity across regions and taxa (MacDonald, 2005; Smith et al., 2018). Ability of  
93 ecoregions to capture all variability inside them is incomplete because biogeographical units are not

intrinsic properties of the biosphere (MacArthur, 1972). Distinction of ecoregions is also often founded on imprecise evaluation or qualitative expert judgements (Ennen et al., 2020). Furthermore, strength of ecoregion boundaries may vary among regions, organismal groups and taxonomic resolutions (Ficetola et al., 2017; Ennen et al., 2020; Smith et al., 2020). For example, Smith et al. (2020) found that ecoregions based on plants, animals and fungi were more distinct in Tropical zones compared with other land areas. Similarly, ecoregion delineations also became less evident when the number of fish families was increased in the continental United States (Matamoros et al., 2016). These potential challenges in biogeographical regionalization clearly imply that existing ecoregion distinctness founded on limited biota does not necessarily portray biogeographical units for all organismal groups and realms. Because an ecoregion approach can provide a useful tool in defining ecological and phytosociological communities (Blasi & Frondoni, 2011), it is important to assess whether current ecoregion classification schemes are valid for different biotas and ecosystem types, and which mechanisms modulate the robustness of neighboring boundaries for these organisms.

Here, we present an analysis of the descriptive power of freshwater ecoregions across North America (25°N–78°N) at a 50 x 50 km spatial resolution founded on understudied freshwater plants. Benefiting from newly available fine-grained data on freshwater plant distributions (Alahuhta et al., 2020, Vieira et al., 2021) and ecoregion maps (Abell et al., 2008; FEOW), we integrate measures of community dissimilarity (Carvalho et al., 2012) with data on putative ecogeographical mechanisms and plant life forms (Cook, 1999; Sculthorpe, 1967) potentially underlying ecoregion delineation. More specifically, we applied recent ideas of Smith et al. (2020) and used variation in community composition both within and between ecoregions to determine which areas are most internally homogeneous and most heterogeneous with neighboring boundaries. We then explored whether internal homogeneity and cross-boundary heterogeneity were driven by their relationships to species replacements and richness differences, and examined how a complex suite of ecogeographical variables (i.e., contemporary

119 environmental features, current climate, topography, Late Quaternary glacial–interglacial climate–  
120 change velocity and human footprint) and functional groups (i.e., emergent plants, floating–leaved  
121 plants, free–floating plants and submerged plants) affect the robustness of the FEOW ecoregion  
122 classification scheme in North American freshwaters.

123

124 We first hypothesized (**H1**) that the internal homogeneity and cross–boundary heterogeneity from the  
125 FEOW classification scheme would broadly represent freshwater plant distributions across North  
126 America, not least because these ecoregion maps have been shown to explain variation in broad–scale  
127 geographical patterns of multiple freshwater organisms, from invertebrates to reptiles (Abell et al.,  
128 2008, 2011; Petry et al., 2016). We also predicted (**H2**) that ecoregion distinctness would decline with  
129 increasing latitude (Olson et al., 2001), with ecoregions being least internally homogeneous in their  
130 communities (Janzen, 1967), but most dissimilar to one another, in Subtropical areas (Sheldon et al.,  
131 2018; Smith et al., 2020). Based on a previous study on global variation in community similarity of  
132 lake plants (Alahuhta et al., 2017), we also expected (**H3**) that within–ecoregion and across–ecoregion  
133 heterogeneity would be caused by species replacements rather than by differences in species richness.  
134 Freshwater plants often follow clumped species replacement structures uniformly across the world,  
135 indicating that more than one species group (association) is responding concomitantly to the  
136 environment (García–Girón et al., 2020b). These two hypotheses (**H2 & H3**) are based on the insight  
137 that tropical communities have higher spatial turnover than their temperate counterparts due –in part–  
138 to less variable environmental conditions and longer evolutionary history in the absence of major ice  
139 ages at lower latitudes (Alahuhta et al., 2020; Janzen, 1967; Smith et al., 2020). Similarly, we predicted  
140 (**H4**) that contemporary climate would explain a substantial variation in the composition of freshwater  
141 plant ecoregions (Alahuhta et al., 2020; 2021; Chappuis et al., 2012; García–Girón et al., 2020a; Heino,  
142 2011; 2020b; Iversen et al., 2021), with topography, Pleistocene Ice Age legacies, human footprint,  
143 water alkalinity, availability of inland waterbodies and the surface area of individual regions playing

supplementary role (Chappuis et al., 2014; Iversen et al., 2019; Lacoul & Freedman, 2006; Murphy et al., 2019; 2020). This hypothesis originates from the latitudinal diversity gradient and Rapoport's rule, which posit that species occurring at lower latitudes, which have lower climatic seasonality and longer evolutionary legacies, are thermal specialists with narrower ranges (Currie et al. 2004), resulting also in higher species richness in and around the Tropics (Alahuhta et al., 2021; Murphy et al., 2019). By contrast, species at high latitudes are thermal generalists due to higher climatic variability and shorter evolutionary history, both of which have led to lower species richness near to the Poles (Alahuhta et al., 2021; Currie et al., 2004; Murphy et al., 2020). Strong influence of contemporary climate would likely suggest that ecological limits, instead of dispersal, diversification rates or time for species accumulation (Pontarp et al., 2019), are the underlying mechanism driving both species richness patterns and the robustness of ecoregion delineation in freshwater plants. Finally, we hypothesized (**H5**) that ecoregions would be a more robust and useful classification for floating-leaved and submerged plants. This hypothesis derived from the relationships among plant life forms, species-specific tolerance ranges and vagility (García-Girón et al., 2019a; Gillard et al., 2020; Schneider et al., 2018), with emergent and free-floating species likely experiencing lower cross-boundary heterogeneity, potentially leading to less defined boundaries in their distributions.

## **MATERIALS AND METHODS**

### ***Ecoregion delineation***

The map of freshwater ecoregions that we used for our analyses comes from Abell et al. (2008), whose boundaries generally –but not entirely– correspond with those of drainage basins and are roughly equivalent to biomes for terrestrial systems (Abell et al., 2011). At the finest level, the delineation process for North America includes 55 ecoregions and derives principally from the best available presence/absence information of individual freshwater fish species, coded to eight-digit hydrologic

unit codes (HUCs) from NatureServe and published sources. In Canada, separate cluster analyses were conducted on occurrences in each of the nine primary drainage basins, whereas ecoregion delineations in the United States were based on the subregions of Maxwell et al. (1995), with relatively small modifications made following the Endangered Species Committee of the American Fisheries Society (see Abell et al., 2008 for details). A more detailed description of the delineation methodology is available in Abell et al. (2008) and in their primary sources <<https://www.feow.org>>.

174

### 175 *Freshwater plant data and explanatory variables*

We studied the geographical distributions of freshwater vascular plants across North America (from 25°N to 78°N) using a grid of equal-area quadrats, i.e., 50 x 50 km spatial resolution. This dataset is one of the world's few fine-grained repositories of freshwater plant distributions at continental scales, and has already been described previously to produce maps of species richness (Alahuhta et al., 2020) and investigate range size conservatism and range overlap (Vieira et al., 2021). In brief, distribution maps of 180 freshwater plants were digitalized from the Flora of North America (Flora of North America Editorial Committee, 1993–2007) for a study region that was restricted to the main continental areas of the United States and Canada, excluding Mexico and remote islands. We strictly focused on vascular plant species that are strongly associated with freshwater habitats, removing peatland and marine species following Crow & Hellquist (2000), Flora of North America Editorial Committee (1993–2007), Lichvar (2014) and Murphy et al. (2019, 2020). Hence, riparian, shoreline and semi-aquatic plant species were also excluded from our study. Although this freshwater species list only consists of a relatively limited number of all aquatic species found in North America (Chambers et al., 2008), all important freshwater hydrophyte genera and species (e.g. *Ceratophyllum* spp., *Myriophyllum* spp., and *Potamogeton* spp.) are present in the data (Crow, 1993; Crow & Hellquist, 2000; Murphy et al., 2019). Moreover, most of the species used in our study have ranges centered in



192 the Northern Hemisphere (Chambers et al., 2008; Crow, 1993), and species richness patterns at  
193 continental scales follow those seen at global scales (Alahuhta et al., 2020; Murphy et al., 2019).

194

195 We considered ten explanatory variables representative of the ecogeographical determinants that we  
196 thought most likely would influence the descriptive power of ecoregions in freshwater plants (Alahuhta  
197 et al., 2021). These explanatory variables (Supplementary Information Appendix S1) were associated  
198 with contemporary environmental features, human footprint, present-day climate, topography,  
199 instability of glacial-interglacial climate and the surface area of individual regions from the FEO  
200 classification scheme. Here, we used zonal statistics to calculate the mean value for each ecoregion  
201 and variable (*sensu* Smith et al., 2020). Environmental features included proportion of freshwaters at  
202 150 m resolution (presence/absence, Lamarché et al., 2017) and mean water alkalinity at 1/16 degrees  
203 resolution (mequiv l<sup>-1</sup>, Marcé et al., 2015). Proportion of freshwaters determined the availability of  
204 potential habitats for aquatic plants (Jones et al., 2003), whereas alkalinity is a measure of carbon  
205 source that can be utilized during photosynthesis (Iversen et al., 2019). Human footprint was assessed  
206 based on the global Human Influence Index (HII) from the NASA Socioeconomic Data and  
207 Applications Centre <<https://earthdata.nasa.gov>>. This measure combines metrics of eight variables  
208 (i.e., crop land, pasture land, built infrastructure, population density, electric power, roads, railways  
209 and navigable waterways) into a single proxy of recent anthropogenic pressures on biodiversity  
210 (Sanderson et al., 2002). This variable can also indirectly reflect potential distribution of invasive  
211 species (e.g., Meyerson & Mooney, 2007). Current climatic variation indicates not only energy  
212 availability and water level fluctuations for freshwater plants, but also materials leaching from  
213 surrounding lands and potential dispersal events (García-Girón et al., 2020a; Kosten et al., 2009).  
214 These variables (i.e., annual mean temperature, °C; annual total precipitation, mm; temperature  
215 seasonality, °C; and precipitation seasonality, mmm) were averaged for the period 1970–2000 from  
216 WorldClim 2.0 (Fick & Hijmans, 2017), representing both average conditions and their variability

217 across the year. Evidence from recent studies suggests that the extent of mountainous areas is a strong  
218 predictor of freshwater plant diversity (Fernández-Aláez et al., 2018) and rarity (García-Girón et al.,  
219 2021). Here, we calculated terrain ruggedness (m) as implemented in the MERIT–Digital Elevation  
220 Model (DEM) from the Geomorpho90m global dataset (Amatulli et al., 2020), which uses the NASA  
221 Shuttle Radar Topographical Mission (SRTM) to provide topographical variables at 3 arc-second  
222 resolution. Although no consensus still exists on the influence of Late Quaternary history on freshwater  
223 plant diversity (Alahuhta et al., 2020, Murphy et al., 2020), we also calculated the average velocity of  
224 climate change from the Last Glacial Maximum (LGM) to present day (“expressed as dividing the rate  
225 of projected climate change in units of °C per year by the rate of spatial climate variability, i.e. the  
226 temperature differential of adjacent grid cells”, Hamann et al., 2015) from a set of transient simulations  
227 downscaled to c. 1/5 degrees resolution (Sandel et al., 2011). This variable describes the speed at which  
228 species must migrate over the Earth’s surface to maintain constant climatic conditions ( $\text{m yr}^{-1}$ , see  
229 Sandel et al., 2011 for details). Although all simulations have their uncertainties related, for example,  
230 to migration requirements of particular taxa, model complexity and scale issues, major advantage of  
231 this variable is its simplicity and clarity of interpretation (Hamann et al., 2015). All the variables were  
232 rescaled to the 50 km study resolution, as needed.

233

## 234 *Statistical analyses*

235 To determine in what areas ecoregion delineation best describes the underlying variability in  
236 freshwater plant distributions (i.e., ecoregion robustness), we calculated **(i)** within-ecoregion  
237 homogeneity (i.e., ecoregions that are most internally homogeneous) and **(ii)** cross-boundary  
238 heterogeneity (i.e., ecoregions that are highly heterogeneous with nearby areas). First, we measured  
239 the Sørensen dissimilarity values based on species composition for each individual grid cell, and only  
240 averaged the values across the grid cells whose centroids are within the borders of each ecoregion,

241 thereby allowing us to derive a single, ecoregion–level, homogeneity score. Second, we calculated the  
242 Sørensen index between all pairs of ecoregions from a presence/absence community matrix  
243 synthesizing variation in community composition of freshwater plants across North America. Because  
244 our analyses here focus principally on the heterogeneity among nearby ecoregions, we followed Smith  
245 et al. (2020) and subset these pairwise comparisons to include only comparisons among ecoregions  
246 that were fewer than 2,000 km apart. However, since metrics of community distinctness ambiguously  
247 capture spatial turnover when compared across samples with different species richness (Atmar &  
248 Patterson, 1993), we also partitioned the Sørensen index of dissimilarity into its additive fractions (i.e.,  
249 species replacements and species loss; Carvalho et al., 2012), and checked whether internal  
250 homogeneity and across–ecoregion heterogeneity were driven by their relationships to species  
251 replacements and richness differences. We further stratified our analyses by plant life forms (i.e.,  
252 emergent plants, floating–leaved plants, free–floating plants and submerged plants; Cook, 1999;  
253 Sculthorpe, 1967) to test whether the descriptive power of the FEOW ecoregion classification scheme  
254 differed by functional groups (Supplementary Information Appendix S2). We chose to focus on plant  
255 life forms because information was available for all species (Crow & Hellquist, 2000; García–Girón  
256 et al., 2020a; Murphy et al., 2019), and also because these functional categories show differences in  
257 their dispersal biology (García–Girón et al., 2019a; Santamaría, 2002), as well as in their sensitivity to  
258 present–day climate and accessibility to carbon and nutrients from the atmosphere, water and  
259 sediments (Alahuhta et al., 2018; Lacoul & Freedman, 2006).

260 We used spatially explicit regression techniques to examine which explanatory variables contributed  
261 most to community dissimilarity within regions and across ecoregion borders. In order to obtain model  
262 convergence, we trimmed the original number of candidate variables using multivariate linear  
263 regressions. More specifically, we applied forward selection with adjusted  $R^2$  values (adj.  $R^2$ ) and two  
264 stopping criteria (i.e., significant level  $\alpha$  and global adj.  $R^2$ ; Blanchet et al., 2008) to choose statistically  
265 explanatory variables to the models (Borcard et al., 2018). Prior to forward selection, we evaluated

266 statistical dependence among the explanatory variables using bivariate correlations ( $r \geq 0.7$ ; Dormann  
 267 et al., 2013), transformed these predictors and our response variables to get normally distributed  
 268 residuals (Peterson & Cavanaugh, 2019), and converted the explanatory variables to their  
 269 corresponding  $z$ -scores to allow comparison of their slope coefficients. Both linear and quadratic terms  
 270 were used in the analyses, because we expected nonlinear impacts of certain explanatory variables.  
 271 Since Moran's  $I$  coefficients using Bonferroni correction (Cabin & Mitchell, 2000) indicated the  
 272 presence of spatial autocorrelation in the residuals of preliminary multivariate linear regressions  
 273 (Supplementary Information Appendices S3 and S4), we constructed simultaneous autoregressive  
 274 spatial (SAR) models (Cressie, 1993; Haining, 2003). Here, we tested the performance of three  
 275 different simultaneous autoregressive model types (spatial error model SAR<sub>err</sub>, lagged model SAR<sub>lag</sub>,  
 276 and mixed model SAR<sub>mix</sub>) and twenty different neighborhood structures (lag distances between 500  
 277 and 10,000 km) with three model selection criteria: **(i)** minimum residual autocorrelation (minimum  
 278 absolute Moran's  $I$  coefficients), **(ii)** maximum model fit (maximum Nagelkerke's pseudo  $R^2_a$ ), and  
 279 **(iii)** the Akaike Information Criterion (AIC; Kissling & Carl, 2008). Furthermore, we used variance  
 280 partitioning (Borcard, Legendre & Drapeau, 1992) based on the above-mentioned protocol by Borcard  
 281 et al. (2018) to model the dependence of the Hellinger-transformed *species x sites* matrix on within-  
 282 ecoregion homogeneity and cross-boundary heterogeneity. We ran an additional approach based on a  
 283 matrix of orthogonal principal coordinate (PCO) eigenvectors to optimize the association of freshwater  
 284 plant distributions with the FEOW classification scheme (García-Girón, Fernández-Aláez,  
 285 Fernández-Aláez & Alahuhta, 2019b). To do this, we retained as many eigenvectors as required to  
 286 maximize the fit between explanatory and response matrices following Duarte, Prieto & Pillar (2012).  
 287 All statistical analyses were performed in R version 3.6.0 (R Development Core Team, 2018). The list  
 288 of R packages and statistical routines used in this paper is provided in Supplementary Information  
 289 Appendix S5.

## RESULTS

Variance partitioning (Supplementary Information Appendix S6) suggested that FEOW ecoregion maps were representative of broad-scale geographical patterns of freshwater plants in North America. Combining within-ecoregion homogeneity and cross-boundary heterogeneity revealed that total explained variation (adj.  $R^2$ ) was highest for the PCO eigenvector approach (28–84%), followed by the Hellinger-transformed community matrix (18–32%). The contribution of within-ecoregion and across-ecoregion heterogeneity varied slightly for emergent (0.06–0.17, 0.09–0.25, respectively), floating-leaved (0.03–0.04, 0.04–0.11, respectively), free-floating (0.09–0.17, 0.04–0.01, respectively) and submerged plants (0.04–0.05, 0.08–0.23, respectively). However, their joint contributions accounted for the largest fractions of spatial variation in all plant life forms (Supplementary Information Appendix S6). Second, we found a clear geographical patterning of within-ecoregion homogeneity and cross-boundary heterogeneity in North American freshwater ecoregions, and these results slightly varied among plant life forms (Figures 1 and 2). More specifically, ecoregions were found to be more internally homogeneous and more similar to one another in their communities in Polar and Subtropical freshwaters, including the northernmost areas of the Canadian Shield, the Arctic Archipelago and the Neotropical Floristic Province of the United States. Across-ecoregion dissimilarity was strongest in the temperate floodplain and upland freshwaters in and around the Interior Plains, the Great Lakes and Saint Lawrence region, and the Mediterranean chaparral and endorheic basins of the Southwest, extending along the Pacific Temperate Rainforest and the eastern and western flanks of the Rocky Mountains. The degree of internal homogeneity and ecoregion distinctness were almost equally driven by species replacements (0.26 and 0.32, respectively) and richness differences (0.29 and 0.32, respectively). The replacement component contributed most to ecoregion dissimilarity in the Arctic tundra biome, and species gains and losses more strongly differentiated ecoregions in and around the Great Plain Grasslands (Figures 1 and 2).

316 [Figure 1 here]

317

318 [Figure 2 here]

319

320 We also found a strong positive and relatively uniform relationship between the robustness of the  
321 FEOW classification scheme and latitude, with ecoregions being less internally homogeneous and  
322 more dissimilar to one another at temperate latitudinal bands. Conversely, the relative importance of  
323 longitude differed depending on the response variable, with ecoregions being more homogeneous in  
324 the East Coast (Figure 1) and cross-boundary heterogeneity being highest in and around the  
325 Intermountain region. Ecoregions predicted to be dissimilar from one another for one functional plant  
326 group were also likely to be dissimilar for the other life forms. However, once accounting for the  
327 tendency of each life form to have different homogeneity values, ecoregion delineation was more  
328 robust for emergent and floating-leaved plants than for submerged hydrophytes, whereas free-floating  
329 species tended to be more shared across neighboring boundaries (Figure 3). In other words, ecoregions  
330 were more distinct for emergent and floating-leaved plants than they were for submerged and free-  
331 floating hydrophytes.

332

333 [Figure 3 here]

334

335 After forward selection of explanatory variables (Supplementary Information Appendix S3) in  
336 multivariate linear regressions (Supplementary Information Appendix S4), SAR models worked  
337 reasonably well, with Nagelkerke's pseudo  $R^2_a$  ranging between 0.10 and 0.74 (Supplementary  
338 Information Appendix S7). Despite there was considerable variability in how freshwater plants

responded to each individual predictor, some general trends emerged (Figures 4 and 5). We found that within–ecoregion homogeneity was highest in regions with higher average temperatures, lower terrain ruggedness, and higher mean water alkalinity concentrations (Figure 4). However, the relative importance of these explanatory variables differed depending on the plant life form considered, with ecoregion area being the best predictor for emergent (Fisher’s  $Z = 0.47$ ) and free–floating plants (Fisher’s  $Z = -0.34$ ), and present–day climate being most important for floating–leaved (Fisher’s  $Z = -0.21$ ) and submerged plants (Fisher’s  $Z = -0.25$ ). When we examined which variables contributed most to ecoregion distinctness, we found that the three top predictors were the same, i.e., annual mean temperature (Fisher’s  $Z = -0.42$  to  $-0.53$ ) along with topography (Fisher’s  $Z = 0.34$  to  $0.71$ ) and the surface area of individual regions (Fisher’s  $Z = 0.54$  to  $0.62$ ; Supplementary Information Appendix S7), with neighboring boundaries being the most similar to one another at higher annual mean temperatures. There was a strong and positive relationship between increasing terrain ruggedness and increasingly distinct ecoregions. A relationship was also found for late Quaternary glacial legacies, with ecoregions more strongly differentiating emergent plant communities in areas that experienced relatively high velocities of climate change since the LGM. Finally, the surface area of individual regions strongly predicted how well the FEOW classification scheme can describe freshwater plant distributions across North America, with medium–sized ecoregions being the most distinct (Figure 5).

[Figure 4 here]

[Figure 5 here]

## DISCUSSION

Ecoregions can be powerful tools for understanding biodiversity patterns and supporting conservation actions (Dinerstein et al., 2017; Droissart et al., 2018). However, these geographical units are often delineated based on well-known, often charismatic organismal groups, which may not reflect ecoregions for all taxa (Ennen et al., 2020; Rolls et al., 2017). Due to the virtual lack of any previous investigations on freshwater plant regionalization, we applied existing freshwater ecoregions of North America founded on fish (Abell et al., 2008) to investigate community dissimilarity of freshwater plants (all taxa and plant life forms separately) and their underlying ecogeographical mechanisms within and across these ecoregions. We based our study on four hypotheses, which received variable degree of support.

Firstly (**H1**), we expected the internal homogeneity and cross-boundary heterogeneity from the FEOW ecoregion classification scheme to closely mirror the geographical patterns of the phytocoenosis inhabiting North American inland waters. So far, our findings seem to confirm this expectation since both within-ecoregion homogeneity and cross-boundary heterogeneity contributed significantly to explain freshwater plant distributions (adj.  $R^2 = 0.18\text{--}0.84$ ), and their joint effects were also high for all life forms. Interestingly, this proportion of explained variation is similar to the reported roles of different ecogeographical mechanisms structuring diversity and community composition of aquatic plants at large spatial scales (see Alahuhta et al., 2021 for a review), thereby supporting our assumption that ecoregion maps predict distributional patterns of these plants. However, although the adjusted coefficients of determination in variation partitioning were ecologically meaningful, future studies should include efforts to develop an updated regionalization for freshwater plants beyond the FEOW classification scheme founded almost exclusively on fish. Secondly (**H2**), we assumed that ecoregion distinctness declines with increasing latitude (Olson et al., 2001; Sheldon et al., 2018; Smith et al.,



2020). We mostly found support for this hypothesis, although the overall pattern was more hump-shaped than an increasing one, i.e., Polar and Subtropical freshwater ecoregions were more internally homogeneous and more similar to one another in their communities. Thirdly (**H3**), we hypothesized that within-ecoregion and across-ecoregion heterogeneity is caused by species replacements rather than by differences in species richness (Alahuhta et al., 2017; García-Girón et al., 2020b). Contrary to our presumption, these mechanisms explained equal amount of variation in internal homogeneity and cross-boundary heterogeneity. Fourthly (**H4**), we expected climate to have the strongest influence on the variation of freshwater plant ecoregions, followed by other ecogeographical variables (García-Girón et al., 2020a; Murphy et al., 2019). This hypothesis was partly supported by our findings, as ecoregion homogeneity and distinctness were best explained by annual mean temperature and terrain ruggedness, respectively, with alkalinity, area and postglacial velocity of climate change having supplementary effects. Finally (**H5**), we hypothesized that ecoregions are a more robust and useful classification scheme for floating-leaved and submerged plants than for other aquatic macrophytes, because emergent and free-floating species likely experience lower across-region heterogeneity (García-Girón et al., 2019a; Gillard et al., 2020; Schneider et al., 2018), potentially leading to less defined boundaries in their distributions. For this expectation, we evidenced support for floating-leaved (more robust delineations) and free-floating (less robust delineations) plants, but not for emergent (more robust delineations) and submerged (less robust delineations) taxa.

404

405 *Polar and Subtropical ecoregions are most internally homogeneous but less distinct from*  
406 *neighboring boundaries*

407 Most internally homogeneous ecoregions situated in the most southern and northern latitudes. Our  
408 outcome partly follows recent evidence based on a similar study approach gained for a large body of  
409 terrestrial taxa, which ecoregions were most homogeneous at Tropical and Subtropical areas (Smith et

410 al., 2020). Our results also contradict these previous findings that ecoregions are more distinct near the  
411 Tropics. The relatively weak ecoregion distinctness in the southernmost areas of North America  
412 appears to originate from their comparatively lower spatial turnover (Figures 1 and 2). This finding  
413 contradicts Janzen's (1967) original hypothesis that low-latitude taxa have higher turnover than  
414 species communities at high-latitude areas because of more homogeneous climate conditions and  
415 longer evolutionary legacies. However, what drives community resemblance in freshwater plants is  
416 still largely unknown (Alahuhta et al., 2020), and additional insights from studies conducted in areas  
417 outside of North America are needed to provide more empirical foundation for the low across-  
418 ecoregion dissimilarity that we found in and around the Subtropics.

419

420 Interestingly, the found hump-shaped pattern in within-ecoregion homogeneity and cross-boundary  
421 heterogeneity closely mimics that of broad-resolution species richness-latitude relationship for  
422 freshwater plants in North America (Alahuhta et al., 2020) and worldwide (Murphy et al., 2019). The  
423 internal homogeneity of high-latitude ecoregions is speculative though, because the species data used  
424 here are rather limited in the most northern parts of North America, where vascular plants are  
425 presumably substituted by aquatic bryophytes (Heino & Toivonen, 2008). Unexpectedly, species  
426 replacement and richness difference explained equal amounts of variation for ecoregion robustness.  
427 Recent studies have shown that species replacement primarily structures freshwater plants independent  
428 of spatial scale and study region (Alahuhta et al., 2021; 2017; Murphy et al., 2020). However, these  
429 previous exercises utilized an alternative measure of richness difference (i.e., nestedness), which does  
430 not consider overall difference in species richness explicitly (Legendre 2014; Schmera et al., 2020).  
431 This may partly explain different results between our and other studies (see also Alahuhta et al., 2020).

432

433 We further discovered that species replacement and richness difference components varied rather  
434 inconsistently even among neighboring ecoregions with high or low level of ecoregion robustness  
435 (Figure 1 and 2). For example, most temperate ecoregions had both high and low degree of species  
436 replacement and richness difference in adjacent units. Pinto–Ledezma et al. (2018) detected that  
437 species replacement dominated in southern biomes of North America, whereas richness difference (or  
438 nestedness in their case) prevailed in the temperate and boreal biomes for (predominantly) terrestrial  
439 vascular plants. They further found that species richness was higher in biomes characterized by species  
440 replacement and nestedness was more influential in species–poor biomes. These patterns were  
441 arguably caused by historical effects and further by differences in speciation time between southern  
442 and northern biomes (Pinto–Ledezma et al., 2018). Our findings on freshwater plants give no clear  
443 support for their conclusions, as no distinct geographical patterns were generally noticeable for species  
444 replacement and richness difference components vs. species richness, and these two components of  
445 beta diversity were not significantly correlated (species replacement:  $r = -0.02$ ,  $p = 0.91$ ; richness  
446 differences:  $r_s = -0.25$ ,  $p = 0.08$ ). Although species replacements outweighed richness differences to  
447 some extent in the northernmost ecoregions of Canada, no firm conclusions can be drawn from this  
448 due to data limitations at highest latitudes. Historical effects neither had constant influence on  
449 ecoregion of freshwater plants in our models. Moreover, Alahuhta et al. (2020) did not report any clear  
450 distinction between nestedness and turnover in the range sizes of freshwater plants across North  
451 America, instead finding that both processes acted simultaneously. This means that, in addition to  
452 narrow–ranging species being nested within the distributions of broad–ranging species, some narrow–  
453 ranging freshwater plant species are also replaced by broad–ranging species towards high latitudes  
454 (Hausdorf & Hennig, 2003; Tomasovych et al., 2016). These findings, incorporated with the findings  
455 that annual mean temperature was the main determinant, suggest that selection via climate filtering is  
456 probably the main driver of freshwater plant ecoregional composition.

457

458

459 ***Climate and topography are the primary drivers of freshwater plant ecoregions in North America***

460 Ecoregional composition of freshwater plants were mainly driven by current climate (i.e., annual mean  
461 temperature) and terrain ruggedness, which had the highest contributions to internal homogeneity and  
462 cross-boundary heterogeneity, respectively. Temperature affects physiological responses of  
463 freshwater plants (Lacoul & Freedman, 2006), which also suffer from indirect responses to cold  
464 temperatures, such as freezing of surface sediments, ice erosion, limiting light penetration and air–  
465 water gas exchanges resulting from thick ice and snow cover (Nilsson et al., 2012). Although aquatic  
466 ecosystems mitigate extreme atmospheric climate conditions, different present-day climate variables  
467 have been evidenced to influence freshwater plant distributions at broad spatial scales (Alahuhta et al.,  
468 2020; Gillard et al., 2020; Murphy et al., 2019). On the other hand, topographical variation via terrain  
469 ruggedness affected ecoregion robustness of all freshwater taxa, with negative and positive  
470 relationships for within-ecoregion homogeneity and across-ecoregion heterogeneity, respectively.  
471 Alahuhta et al. (2017) discovered that environmental heterogeneity originated from topographical  
472 variation had the highest influence on global beta diversity of lake plants. This observation was further  
473 supported by a global study from six continents combining taxonomic, functional and phylogenetic  
474 information for lake plant metacommunities (García-Girón et al., 2020a). A greater variety of habitats  
475 or resources with greater variation in elevation explained lake plant distributions in their studies, which  
476 is consistent with our findings that nearby freshwater ecoregions become more distinct and less  
477 internally homogeneous in topographically dissected landscapes.

478

479 ***Comparison among plant life forms***

480 Differences in the trends of ecoregion robustness were only modest among different plant life forms,  
481 with communities being less internally homogeneous but more distinct to one another at intermediate

latitudes. Although these overall geographical patterns were relatively similar among the life forms, we found differences in the strength of ecoregion robustness and ecogeographical variables explaining ecoregion delineations. Floating-leaved and emergent plants showed more robust ecoregions than free-floating and submerged plant taxa, which contradicts our expectation that emergent species show less defined boundaries in their distributions. Emergent and floating plants benefit from a more direct atmospheric connection for carbon and oxygen use (e.g., Iversen et al., 2019), and greater light availability (e.g., Hautier et al., 2009). However, climate or alkalinity did not consistently structure ecoregions of different plant life forms, with the exception of submerged and free-floating species, which delineations in terms of internal homogeneity were most robust at higher mean annual temperatures and mean water alkalinity, respectively. Instead, topography and ecoregion area contributed strongest to the ecoregions of floating-leaved and emergent plants. These two variables reflect habitat and ecosystem availability for different species of floating-leaved and emergent plants (García-Girón et al., 2020; Jones et al., 2003). Topographical variation can also indicate a wider range in temperature and precipitation that would support our climate-driven reasoning for these plant groups.

497

Ecoregions of free-floating plants were more robust at high alkalinities, which was expectable considering that these plants primarily uptake carbon in the form of bicarbonate from water (Iversen et al., 2019). Many submerged species similarly depend on bicarbonate for carbon use, but alkalinity had no significant contribution to them. It may be that stronger effect of climate overshadowed water quality contributions on submerged plants. Our findings also suggested that historical effects contributed to cross-region heterogeneity of emergent plants, implying that most diverse ecoregions experience relatively high velocities of climate change since the LGM. However, no similar trend was observed for within-ecoregion homogeneity of emergent taxa, and many of the ecoregions covered by

506 the Cordilleran and Laurentide Ice Sheets showed more similarity to one another than more disparate  
507 ice-free ecoregions.

508

## 509 *Conclusions*

510 Our investigation emphasizes that geographical regionalizations, such as ecoregions, founded on a  
511 particular organismal group may not be directly applicable for all biological assemblages but can be a  
512 good basis for further taxa-specific adaptations. Ecoregions originally developed for fish predicted  
513 relatively well the distributional patterns of freshwater plants in North America, being thus a promising  
514 starting point for further investigations of geographical delineations for other freshwater taxa. We  
515 found that within-ecoregion homogeneity was highest in regions with higher average temperatures,  
516 lower terrain ruggedness, and higher mean water alkalinity concentrations, whereas neighboring  
517 boundaries became more distinct in medium-sized, temperate and topographically fragmented  
518 landscapes that have experienced oscillations of the Laurentide Ice Sheet after the LGM. Both species  
519 replacement and richness difference components were equally important for ecoregions of freshwater  
520 plants but showed no evident geographical trends across the continent. Instead of low-latitude taxa  
521 having higher turnover than their high latitude counterparts due to more stable climate conditions and  
522 longer evolutionary legacies (Janzen, 1967), narrow-ranging freshwater plants were likely not only  
523 nested within the distributions of broad-ranging species, but some narrow-ranging species were  
524 probably also replaced by broad-ranging plant species towards high latitudes. These findings suggest  
525 that selection through climate filtering (i.e., mean annual temperature) is the main mechanistic driver  
526 of freshwater plant ecoregions. This study sets a first step for further assessment and development of  
527 geographical regionalizations not only for freshwater plants but also for other taxa inhabiting inland  
528 water systems. These updated regionalizations can then be used for conserving different biotas in  
529 freshwaters, which are currently among the most threatened ecosystems in the world.

530

## 531 DATA AVAILABILITY

532 Freshwater plant distributions and resampled environmental data are found from Dryad  
533 (<https://doi.org/10.5061/dryad.6wwpzgn0v>).

534

## 535 FIGURE CAPTIONS

536 **Figure 1.** The geographical distribution of within–ecoregion homogeneity for freshwater plant ecoregions in  
537 North America (left panels, i.e., darker colors indicate freshwater ecoregions that are less internally  
538 homogenous), and bivariate maps comparing internal homogeneity with species replacements and richness  
539 differences (center and right panels, respectively). These maps represent the normalized scores (i.e., the lower  
540 the score, the higher the internal homogeneity of individual ecoregions) for **(a)** all freshwater plant species, as  
541 well as for analyses stratified by plant life forms, i.e., **(b)** emergent, **(c)** floating–leaved, **(d)** free–floating and  
542 **(e)** submerged plants. Relationships of response variables with latitude and longitude were evaluated with linear  
543 regressions, accompanied by Akaike Information Criterion (AIC) to assess the importance of linear vs. quadratic  
544 relationships, and only significant relationships are displayed. Solid lines in these plots show the median  
545 estimate along with the 95% credible intervals. Projection was based on ellipsoid WGS 1984.

546

547 **Figure 2.** The geographical distribution of cross–boundary heterogeneity for freshwater plant ecoregions in  
548 North America (left panels, i.e., darker colors indicate freshwater ecoregions that are more different from one  
549 another), and bivariate maps comparing across–ecoregion heterogeneity with species replacements and richness  
550 differences (center and right panels, respectively). These maps represent the normalized scores (i.e., the higher  
551 the score, the more dissimilarity exists among ecoregion boundaries) for **(a)** all freshwater plant species, as well  
552 as for analyses stratified by plant life forms, i.e., **(b)** emergent, **(c)** floating–leaved, **(d)** free–floating and **(e)**  
553 submerged plants. Relationships of response variables with latitude and longitude were evaluated with linear  
554 regressions, accompanied by AIC to assess the importance of linear vs. quadratic relationships, and only

555 significant relationships are displayed. Solid lines in these plots show the median estimate along with the 95%  
556 credible intervals. Projection was based on ellipsoid WGS 1984.

557

558 **Figure 3.** Ecoregion distinctness in relation to geographical distances to neighboring boundaries (2,000 km;  
559 *sensu* Smith et al., 2020). Lines are colored by plant life form and show the median estimate in a solid line along  
560 with the 95% credible intervals (shading), with lower values indicating ecoregions that are more similar in their  
561 communities.

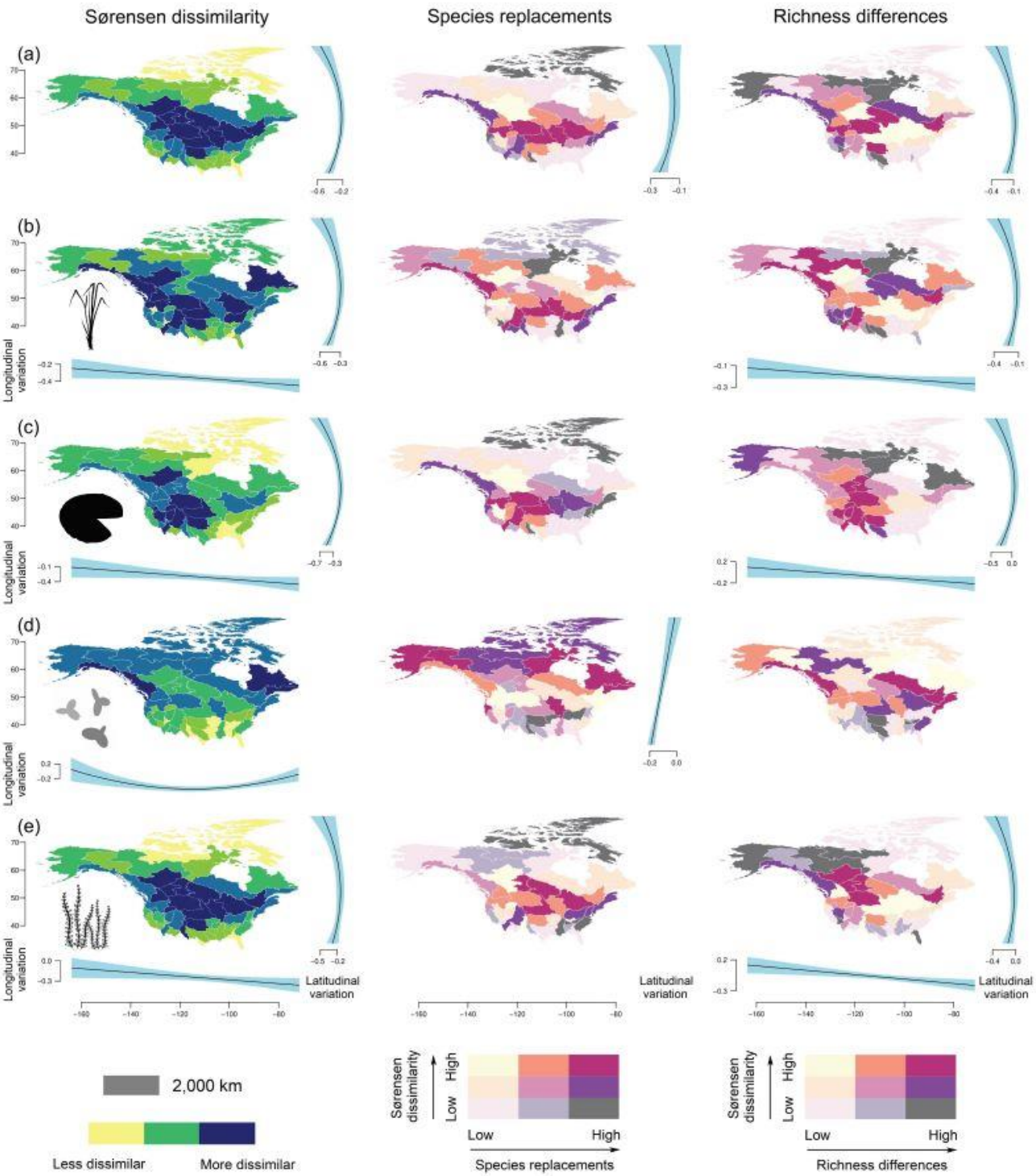
562

563 **Figure 4.** Normalized explanatory variables most strongly related to the internal homogeneity of individual  
564 ecoregions. This graph shows the effect of a predictor variable (x-axis) on the changes in the Sørensen  
565 dissimilarity index (y-axis). Shown are significant explanatory variables based on forward selection  
566 (Supplementary Information Appendix S3) in multivariate linear regressions (Supplementary Information  
567 Appendix S4) and spatially explicit regression models (Supplementary Information Appendix S7). Solid lines  
568 show the median estimate along with the 95% credible intervals (shading). Ecoregions are most internally  
569 homogeneous (i.e., more negative values) in small-sized to medium-sized, warm and flat areas, with their  
570 freshwaters having relatively high mean water alkalinity concentrations

571

572 **Figure 5.** Normalized explanatory variables most strongly related to cross-boundary heterogeneity. This graph  
573 shows the effect of a predictor variable (x-axis) on the changes in the Sørensen dissimilarity index (y-axis).  
574 Shown are significant explanatory variables based on forward selection (Supplementary Information Appendix  
575 S3) in multivariate linear regressions (Supplementary Information Appendix S4) and spatially explicit  
576 regression models (Supplementary Information Appendix S4). Solid lines show the median estimate along with  
577 the 95% credible intervals (shading). Ecoregions become more distinct (i.e., higher values) in temperate,  
578 medium-sized and topographically fragmented landscapes that have experienced oscillations of the Laurentide  
579 Ice Sheet after the Pleistocene.





581  
582 **Figure 1.**

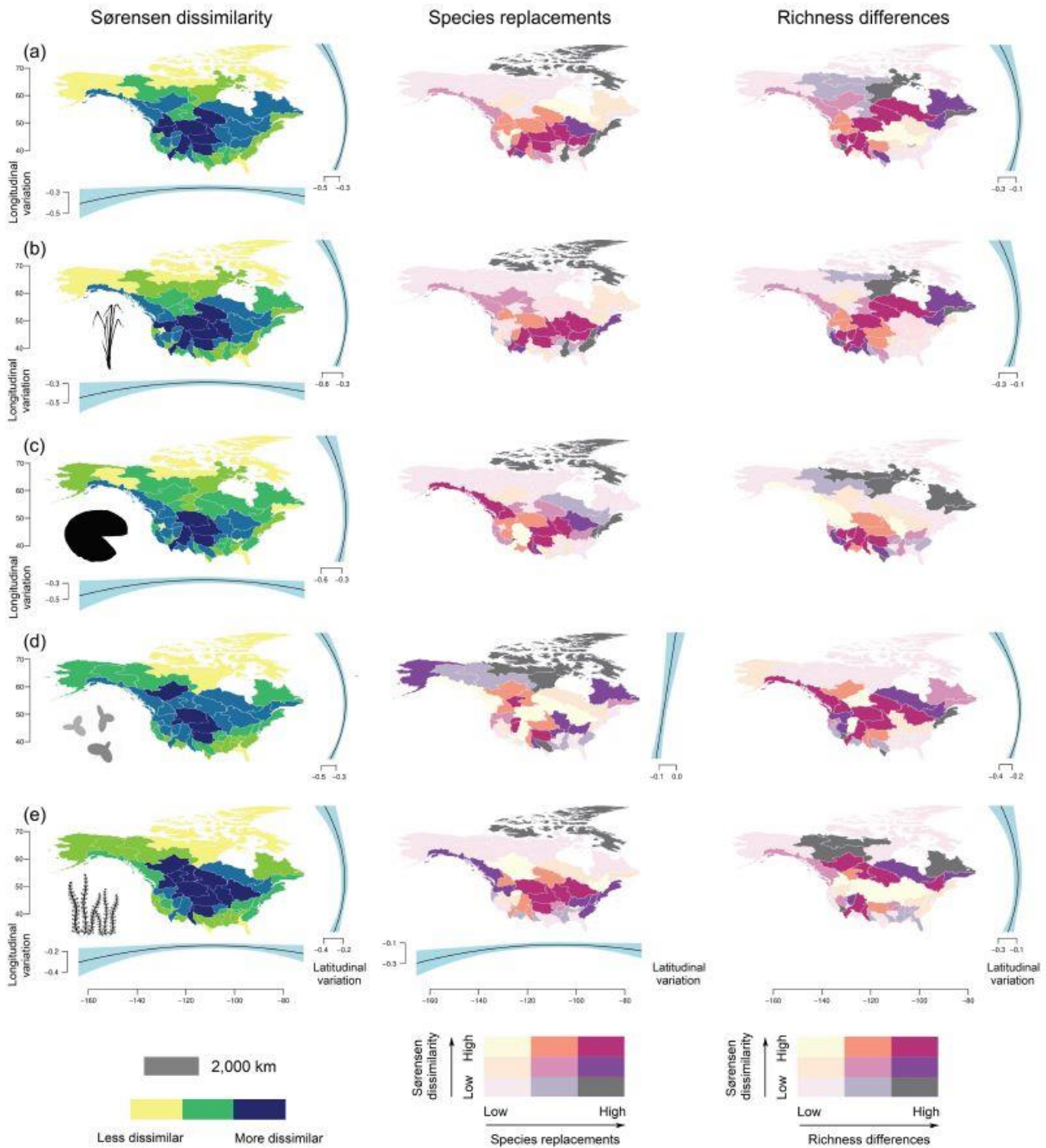
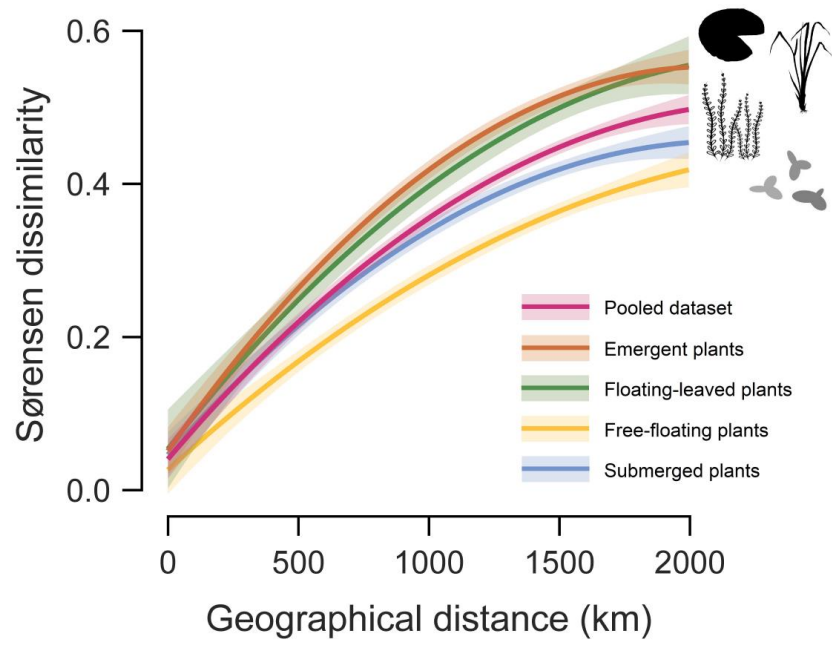


Figure 2.

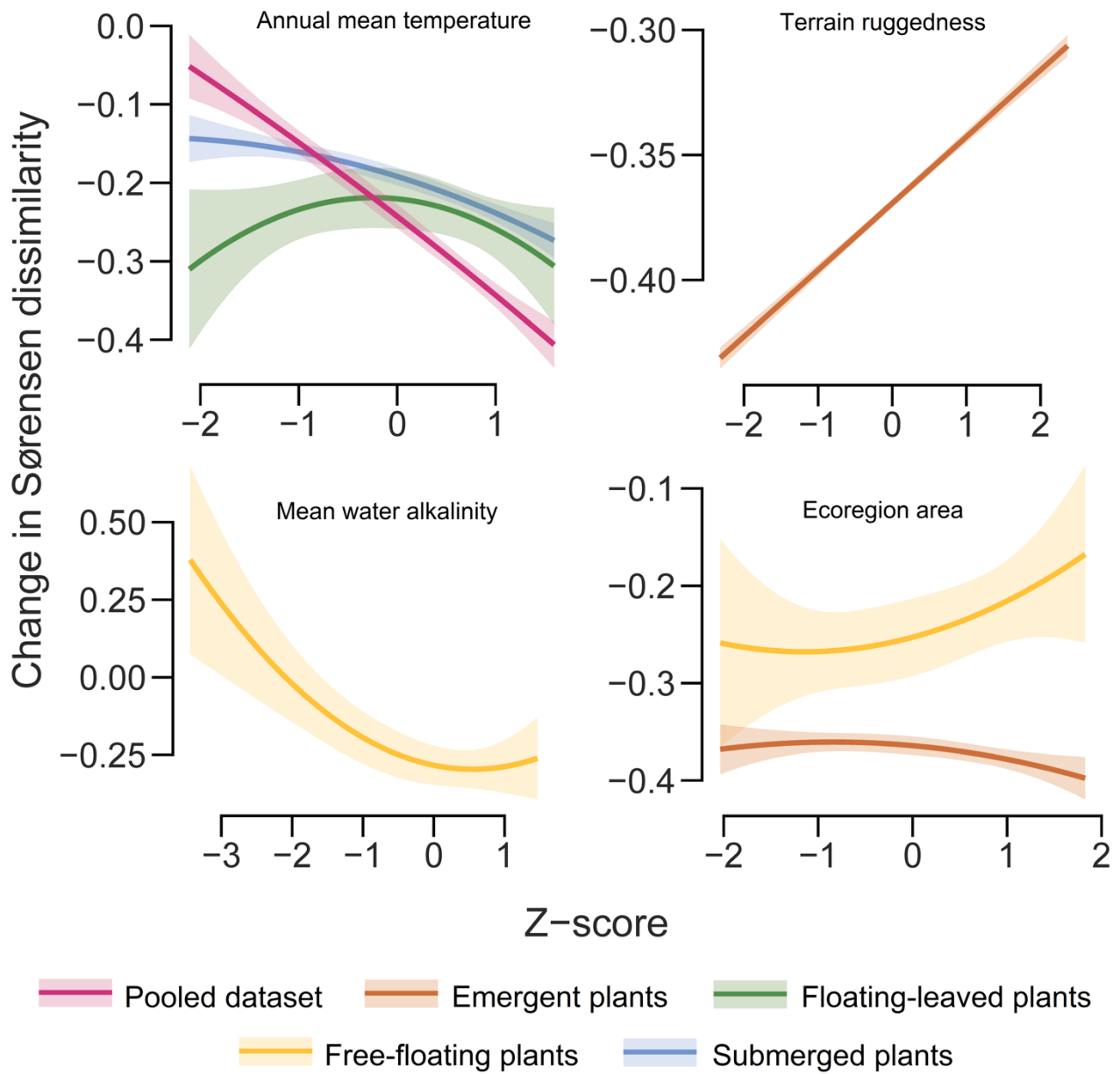


589

590 Figure 3.

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593

594 Figure 4.

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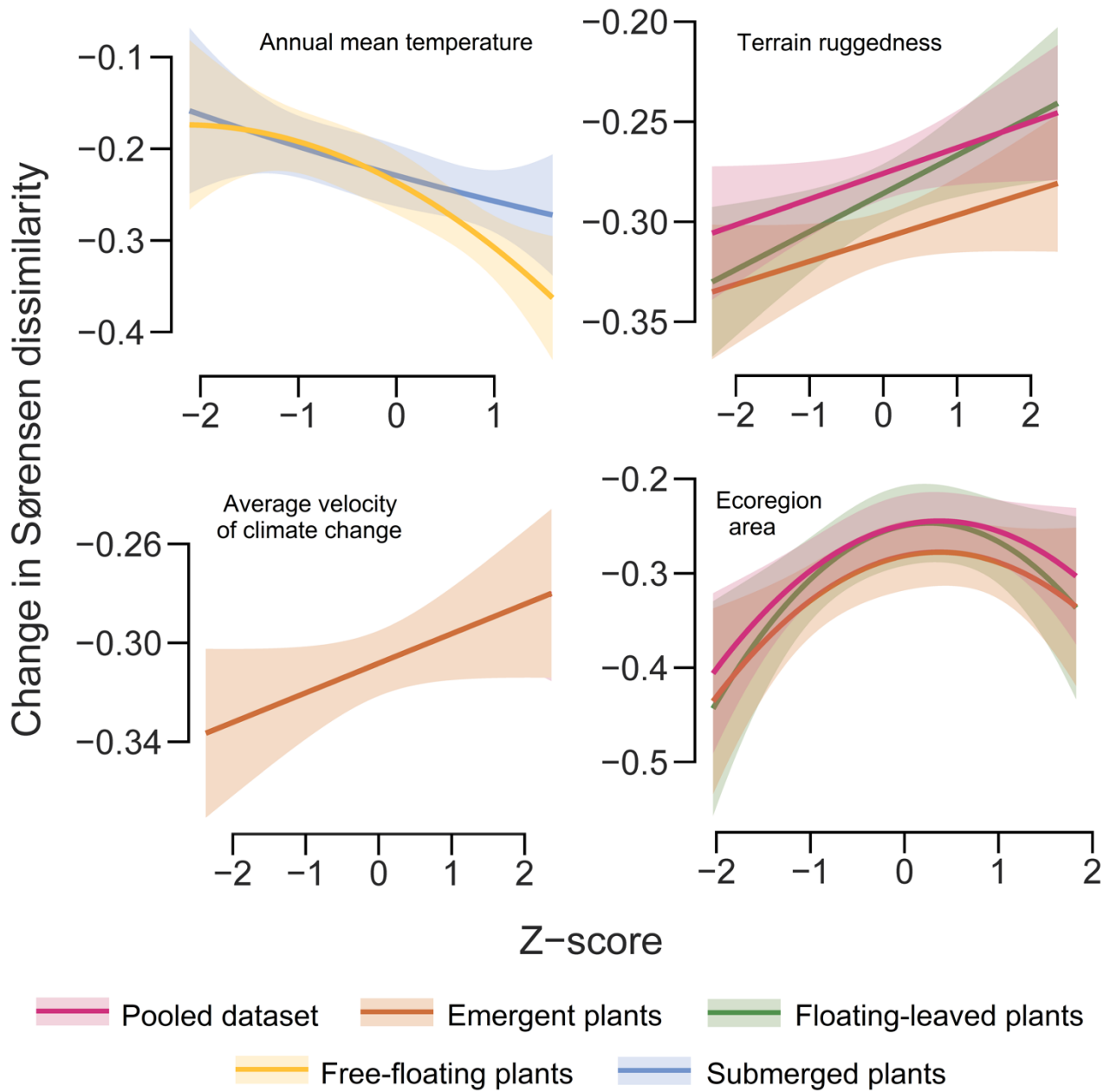


Figure 5.

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788 **BIOSKETCH**

789 Janne Alahuhta heads the Macrophyte Biogeography Lab operating at University of Oulu and Jorge  
790 García–Girón is a postdoc researcher at the Finnish Environment Institute. Both are passionate about  
791 the biogeography of freshwater biotas and have actively collaborated to advance our understanding on  
792 the patterns and processes underlying broad–scale freshwater biotas since the IBS Malaga 2019  
793 Symposium.

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796 JA conceived the original idea, whereas JA and JGG contributed to the study design equally. JGG  
797 processed the data and performed the analysis. JA and JGG were together responsible for the writing  
798 of the manuscript.