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1   **Spatial and environmental drivers of macrophyte diversity and community**

2   **composition in temperate and tropical calcareous rivers**

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Abstract

The hypothesis was examined that sources of variation in macrophyte species richness (alpha-diversity: S) and community composition ("species-set"), attributable to spatial and environmental, variables, may differ in importance between tropical and temperate calcareous rivers (>10 mg $CaCO_3$ L^{-1}). To test this hypothesis geographic, environmental, and aquatic vegetation data were acquired for 1151 sites on calcareous rivers within the British Isles, supporting 106 macrophyte species (mean S: 3.1 species per sample), and 203 sites from Zambian calcareous rivers, supporting 255 macrophyte species (mean S: 8.3 species per sample). The data were analysed using an eigenfunction spatial analysis procedure, Moran's Eigenvector Maps (MEM), to assess spatial variation of species richness and community composition at large regional scale (>10^5 km^2: British Isles and Zambia); and at medium catchment scale (10^4 – 10^5 km^2: British Isles only). Variation-partitioning was undertaken using multiple regression for species richness data, and partial redundancy analysis (pRDA) for community data. For the British Isles, spatial and environmental variables both significantly contributed to explaining variation in both species richness and community composition. In addition, a substantial amount of the variation in community composition, for the British Isles as a whole and for some RBUs, was accounted for by spatially-structured environmental variables. In Zambia, species richness was explained only by pure spatial variables, but environmental and spatially-structured environmental variables also explained a significant part of the variation for community composition. At medium-scale, in the British Isles, species richness was explained by spatial variables, and only for four of the six RBUs.

Keywords: Biodiversity; Macroecology; Spatial scale; Hard-water rivers; Aquatic macrophytes; Landscape; Partitioning of variance; Species richness; Alpha-diversity; Spatially-structured factors.

58 **Footnote**

59 Abbreviations: MEM (Moran's Eigenvector Maps: an eigenfunction spatial analysis

60 procedure which is a generalization of Principal Coordinates of Neighbor Matrices,

61 PCNM); RBU (River Basin Units)

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63 **Highlights**

64 • We analysed the aquatic vegetation of 1354 tropical and temperate river sites
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66 • MEM, multiple regression and pRDA were used to analyse the datasets
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68 • Spatial and environmental variables were both significant driving factors
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70 • Species richness (S) in tropical rivers was only driven by spatial factors
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72 • In temperate rivers S was driven by both spatial and environmental variables

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1. Introduction

Understanding the causes of geographic patterns of species and biodiversity distribution is central to ecology. As with other groups of biota, the spatial distribution of freshwater macrophytes ("aquatic photosynthetic organisms, large enough to see with the naked eye, that actively grow permanently or periodically submerged below, floating on, or growing up through the water surface": Chambers et al., 2008) varies considerably in terms of both species richness and community composition at different spatial scales across the world (e.g. Jones et al., 2003). Recently, considerable progress has been made toward documenting large-scale patterns of species richness (e.g., Hillebrand, 2004), and macrophytes pose no exception to the many suggestions made, for different biota, to try to explain observed geographical and temporal patterns of variation in species richness and community composition (e.g., Hawksworth, 1995; Murphy et al., 2003; Varandas Martins et al., 2013).

Factors potentially influencing macrophyte community distribution, and variation in alpha-diversity, in freshwater systems have been considered at various scales (Hawksworth, 1995). First, there is the large, regional scale (e.g., Murphy, 2002) where these community characteristics are usually primarily driven by geography-related factors (e.g. temperate versus tropical climate: Crow, 1993). Second is medium, or catchment scale, where, for example, hydrological and chemical variation in the system may be important (e.g., Varandas Martins et al., 2013; Spink et al., 1997; Vestergaard and Sand-Jensen, 2000). Third is small scale, related to environmental features of specific habitats and communities, and the biological interactions which go on at this level, such as herbivory and competition (e.g., Lacoul and Freeman, 2006).

Both community composition and diversity are primarily affected by the sum and interactions of the numerous processes occurring at these various spatial scales (Borcard et al., 2004). Modelling spatial patterns in plant communities at multiple temporal and spatial scales can hence be a useful approach to improve understanding

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of community characteristics, and their potential future response to environmental change (Borcard et al., 2004), but has only rarely been carried out previously in river research (e.g., Poff, 1997).

Rivers are hierarchically structured, from source to mouth, meaning that spatio-temporal variation in the species richness and composition of the macrophyte communities which they support is influenced by a combination of local in-stream variables, regional environmental factors, and catchment characteristics. Only a few studies have so far attempted to assess the relationships between environmental factors and richness of aquatic macrophyte assemblages on a large spatial scale (e.g., Rørslett, 1991; Crow, 1993; Baattrup-Pedersen et al., 2006; Chambers et al., 2008). However several previous studies, undertaken at smaller scales, have shown similarities in the main environmental gradients underlying the species-environment model for river vegetation. For instance slope, substrate characteristics, dissolved oxygen and nitrate have all been found to be of varying importance in driving river macrophyte species distribution (Dodkins et al., 2005). Other driving variables that have been identified in this context include calcium concentration and flow regime (Wilby et al., 1998; Varandas Martins et al., 2013).

In this paper we address questions related to how environmental factors varying at medium scales (in this case, within individual river basins of the British Isles, at a unit size of approximately $10^4 - 10^5$ km^2), such as alkalinity and altitude; and factors varying at a regional, large scale (e.g. temperature and precipitation regimes), in both the British Isles and Zambia (each with unit size $>10^5$ km^2), may interact with each other, and with spatial location data (i.e., latitude and longitude of the sampling sites), to help explain observed variation in patterns of river macrophyte species richness and community composition (species presence/absence across sites).

These questions were posed for a closely-defined type of freshwater habitat, namely calcareous ("hard-water") rivers and streams, located within two target regions of the world: one temperate (the British Isles) and the other tropical (Zambia). Hard-

129 water systems are here minimally defined (Tapia Grimaldo, 2013) as $10 - 19.9$ mg L^{-1}

130 CaCO$_3$ concentration ("marginally hard-waters"), through to a maximum of rivers with

131 >200 mg L^{-1} CaCO$_3$ concentration ("very hard-waters").

132 Combined analysis of spatial and environmental factors has hitherto only rarely

133 been applied to aquatic macrophyte communities (e.g., Capers et al., 2009; O'Hare et

134 al., 2012). The inclusion of geographic location as a predictor can help improve

135 understanding of whether species richness and/or community composition is spatially-

136 structured (examples of underlying causal factors which may influence such observed

137 spatial pattern include biological limits upon dispersal in individual species, and climatic

138 constraints on species survival: Borcard et al., 1992).

139 Useful in such combined analyses are approaches based upon eigenfunction

140 spatial analysis, such as Moran's Eigenvector Maps (MEM: Borcard and Legendre,

141 2002; Dray et al., 2006; Griffith and Peres Neto, 2006). MEM can quantify spatial

142 patterns in species data (e.g., variation in richness and community composition) across

143 a range of geographical scales (Borcard and Legendre 2002; Borcard et al., 2004), by

144 generating spatial variables that could also account for unmeasured environmental

145 variables (Peres-Neto and Legendre, 2010). A comprehensive account of the

146 procedure, providing detailed interpretation of the meaning of MEM variables and

147 scales represented by them, is provided by Dray et al. (2006), while Landeiro et al.

148 (2011) also provide a succinct description of the primary characteristics of MEM and its

149 outputs.

150 In this study we examined the hypothesis that sources of variation in

151 macrophyte species richness (alpha-diversity) and community composition, attributable

152 to spatial, environmental, and spatially-structured environmental variables, may differ in

153 importance between tropical and temperate calcareous rivers.

154

155 **2. Methods**

2.1. Data collection

The analysis used data for 1151 sites located on hard-water rivers and smaller streams in the British Isles, supporting a total of 106 macrophyte species, together with a further 203 sites from Zambian calcareous rivers, supporting 255 macrophyte species. Vegetation species richness (S: number of species recorded per site) and community composition (presence/absence data across sites) were assessed per 100 m stretch at each site.

The data were obtained:

(i) by field survey, undertaken by the authors during 2006 - 2012: 54 sites in England, Scotland, and Ireland, and 203 Zambian sites, using an adapted version of the Mean Trophic Rank (MTR) field protocol developed in the United Kingdom to acquire vegetation data for river quality bioassessment (Holmes et al., 1999; WFD-UKTAG, 2014; Kennedy et al., 2015);

(ii) by extraction of information for sites located on hard-water rivers, from a large pre-existing dataset held by the authors (MTR data: collected since 2000 using the standard MTR protocol, as above). This dataset formed the bulk of the British Isles data analysed, comprising 1051 sites; and

(iii) from two older datasets for hard-water Irish and UK rivers, extracted from information in Caffrey (1990), Spink (1992), and Spink et al. (1997) comprising a further 46 sites.

The taxonomic resolution for the data used here was 85% to species level and the remaining 15% to genus level, across the different surveys contributing to the dataset.

Alkalinity (ALK: mg $CaCO_3$ L^{-1}) was measured by standard Gran titration procedure for water samples taken from each site (Neal, 2001). The MTR data set includes information on water alkalinity for the 1051 sites taken from this database. Data for climatic variables, as mean values for 1950 – 2000, for the British Isles and Zambia were obtained from the global climate database Worldclim (Hijmans et al.,

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184 2005; www.worldclim.org/bioclim). These variables were: annual evapotranspiration

185 (EVAP: mm); annual mean temperature (AMT: $^{\circ}$C); temperature seasonality (TS:

186 standard deviation*100); maximum temperature of warmest month (MAXTW: $^{\circ}$C);

187 minimum temperature of coldest month (MINTC: °C); mean temperature of wettest

188 quarter (MTWeQ: $^{\circ}$C); mean temperature of driest quarter (MTDQ: $^{\circ}$C); annual

189 precipitation (AP: mm); precipitation seasonality (PS: coefficient of variation);

190 precipitation of wettest quarter (PWeQ: mm); precipitation of warmest quarter (PWQ:

191 mm); and precipitation of coldest quarter (PCQ: mm).

192   Altitude (ALT: m above mean sea level) was also recorded for each site, along

193 with site coordinates (latitude (LAT) and longitude (LONG): both in decimal degrees).

194 These data were acquired either using GPS equipment in the field, or from large-scale

195 (1:50.000) maps.

196

197 *2.2. Data analysis*

198   Spatial variation of datasets for macrophyte species richness and community

199 composition variation in hard-water streams was evaluated at two spatial extents: (i)

200 regional, large scale ($>10^5$ km^2: British Isles; Zambia); and (ii) medium (catchment)

201 scale (approximately $10^4 - 10^5$ km^2: for the British Isles only), within River Basin Units

202 (RBUs), of which six non-political entities (some crossing national borders) cover the

203 British Isles. RBUs individually comprise sets of River Basin Districts (RBDs),

204 established primarily around the catchments of the major river systems of the British

205 Isles. The six RBUs are: Scotland (Scotland, and Solway Tweed RBDs), Northern

206 England (Northumbria, and North West RBDs), South East England (Anglian, Thames,

207 and South East RBDs), South West England and Wales (South West, Severn, Dee, and

208 Western Wales RBDs), Northern Ireland (North Eastern, Neagh Bann, and North

209 Western RBDs), and Southern Ireland (Western, Shannon, Eastern, South Eastern, and

210 South Western RBDs). Further details and maps showing boundaries of RBDs in the

211 British Isles are available from the websites of the Environment Agency (England and

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212 Wales) www.wildswimming.co.uk/wp-

213 content/uploads/2013/08/River_Basin_District_Map_LIT_8050_75c4b2-724x1024.jpg;

214 Scottish Environment Protection Agency: www.gis.sepa.org.uk/rbmp; and the

215 Geological Survey of Ireland: www.gsi.ie/NR/rdonlyres/780BFC43-AF88-4969-8B08-

216 029840C7FF6F/0/River_Basin_Districts_1.jpg.

217 Because of the lower sample size for Zambia, analysis was undertaken only at

218 regional (whole country) scale for that dataset.

219 To evaluate spatial patterns in species richness and community composition, in

220 separate analyses for the British Isles and Zambia, spatial variables were created using

221 the eigenfunction spatial analysis procedure Moran's Eigenvector Maps (MEM), which

222 is fully described by Borcard and Legendre (2002), Griffith and Peres Neto (2006) and

223 Dray et al. (2006). Before the development of eigenfunction spatial analyses, spatial

224 patterns in biodiversity data were modelled using simple trend-surface analysis (TSA;

225 i.e., a multiple regression analysis allowing for latitude and longitude of the sampling

226 sites or for polynomial expansion of these coordinates: Borcard and Legendre, 2002).

227 The problem with TSA is that it is suitable to model only simple spatial patterns (e.g.,

228 trends and parabolas) and, therefore, more complex patterns of spatial variation, so

229 common in nature, may pass undetected with this method (Borcard and Legendre,

230 2002). Also, the monomials (e.g., Latitude and Latitude^2) are not orthogonal. On the

231 other hand, MEM creates orthogonal explanatory variables (eigenvectors = spatial

232 variables), representing different patterns of spatial relationships between sampling

233 sites, which are potentially able to model complex spatial patterns of a response

234 variable (e.g., species richness; see Fig. 2 of Griffith and Peres-Neto, 2006). These

235 spatial variables are obtained by computing the eigenvectors of a connectivity matrix,

236 which in its turn is derived from the geographical position of the sampling sites (see Fig.

237 1 of Griffith and Peres-Neto, 2006). The first eigenvectors associated with large,

238 positive eigenvalues represent coarse spatial patterns and positive spatial

239 autocorrelation. The last eigenvectors, associated with small eigenvalues, represent

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240   fine spatial structures (Griffith and Peres-Neto, 2006). In short, instead of using simple

241   latitude and longitude (or polynomial expansions of these), some of the eigenvector

242   maps, along with the environmental variables, are used as explanatory variables in

243   statistical models (see below).

244   To model species richness and species presence-absence data (community

245   composition), as functions of spatial and environmental variables, we used multiple

246   regression analysis and partial Redundancy Analysis (pRDA; Legendre and Legendre,

247   2012), respectively. Explanatory variables (both spatial and environmental) were

248   selected for inclusion in the final models using the forward selection procedure

249   proposed by Blanchet et al. (2008). This method consists of first running a global test

250   with all explanatory variables. The forward procedure continues only when this test is

251   significant. The interest of this method is that usual significance levels and adjusted

252   coefficients of determination are other two criteria used, which avoid overfitting.

253   In both cases (multiple regression and pRDA) we used variation-partitioning

254   (Peres Neto et al., 2006) to determine the relative importance of environmental and

255   spatial variables in explaining variation in macrophyte species richness and community

256   composition at each spatial extent in the target locations. This approach split the total

257   variation explained by each analysis outcome into four components: (i) variation

258   explained exclusively by environmental variables (pure environmental variation); (ii)

259   variation explained exclusively by spatial variables (pure spatial variation); and (iii)

260   variation that can be explained by both environmental and spatial variables (shared

261   fraction), also termed spatially-structured environmental variation (Blanchet et al.,

262   2008). The fourth component was residual (unexplained) variation. We used adjusted

263   R^2 (adj-R^2) values, which correct for unequal ratio between number of observations and

264   explanatory variables, to perform the variation-partitioning (Peres-Neto et al. 2006).

265

266   **3. Results**

267   The findings provide evidence for the existence of spatial patterns in both

268   macrophyte alpha-diversity and community composition in temperate and tropical

269   calcareous rivers. There were substantial differences in mean values of alpha-diversity

270   (S) between the British Isles (3.1 species per sample) and Zambia (8.3 species per

271   sample), and also between RBUs within the British Isles (Table 1).

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273   *3.1. British Isles*

274   *3.1.1. Regional/ large scale species richness*

275   Gamma-diversity for macrophyte species recorded from the sampling sites in

276   temperate calcareous rivers of the British Isles comprised 58 emergent, 14 floating, and

277   34 submerged species, giving a total of 106 species. The mean alpha-diversity for

278   macrophytes at sample sites for the British Isles as a whole was 3.1 species per sample

279   (Table 1). Distribution of hard-water river macrophyte diversity across the British Isles is

280   shown in Fig. 1. Only 2.1% of the variation in diversity was accounted for by pure

281   environmental effects (e.g. alkalinity, temperature seasonality: see Fig. 2). Variation in

282   macrophyte species richness was best explained by spatially-structured environmental

283   factors (11.4%), and pure spatial variables (8.8%). These acted primarily at three spatial

284   scales: broad, intermediate and fine, represented by MEMs 4, 20, and 100 (together

285   with a number of MEMs of lesser importance, within these three scale ranges: see

286   Table 1 and Fig. 3).

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288   *3.1.2. Regional/ large scale: community composition*

289   Variation in macrophyte community composition was best explained by pure

290   spatial variables (MEMs), but the variation accounted for was low (5.4%; Table 2).

291   Spatially-structured environmental factors accounted for a further 3.9% of variation,

292   while pure environmental factors (e.g. annual precipitation, minimum temperature of

293   coldest month, precipitation of warmest quarter: see Fig. 2) taken together accounted

294   only for 1.1% of the variation.

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3.1.3. Medium (River Basin Unit) scale: species richness

There were substantial differences (Table 1) in average macrophyte alpha-diversity between RBUs, with southern Ireland having the highest, at 7.1 species per sample and Scotland the lowest, at 2.2 species per sample. Macrophyte richness variation in hard-water rivers within each of the six individual RBUs comprising the British Isles (Table 1) was explained only by spatial variables, and only for four of the six RBUs. Species richness variation in Scotland and Southern Ireland was not accounted for by any of the explanatory variables (environmental or spatial). The proportion of variation explained ranged from 5.9% (for South East England) to 14.4% (Northern England). Environmental and spatially-structured environmental adjusted R^2 values were negligible in all RBUs. Species richness for Northern England and South East England was explained by MEMs representing patterns at intermediate to fine spatial scales. Conversely South West England and Wales, and Northern Ireland retained low-order MEMs indicating broad spatial patterns of diversity in these RBUs.

3.1.4. Medium (River Basin Unit) scale: community composition

In contrast to the results for medium-scale richness within the British Isles, macrophyte community composition variation at medium scale (Table 2) was partially explained by all three sets of variables (spatial, environmental and spatially-structured environmental variation) within individual RBUs, but the relative importance of each differed between RBUs. In Scotland and Northern England, variation in community composition was best explained by spatially-structured environmental variables (6.9% and 4.5% respectively). However in South East England, and South West England and Wales, spatial variables were of primary importance in this respect (accounting for 7.1% and 4.2% of variation, respectively. In both Northern and Southern Ireland spatial variables were of sole importance in explaining variation in community composition. In all RBUs the pure environmental component (e.g. alkalinity, temperature seasonality,

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323 and minimum temperature of coldest month) was always of little or no importance

324 (accounting for zero to 2.8% of variation). In all RBUs with significant spatial patterns,

325 the order of influential MEMs was low to intermediate (e.g., for Scotland: MEMs 3, 1, 4,

326 28: see Table 2), suggesting that spatial patterns of variation in macrophyte community

327 composition are operating mainly at broad scales.

328

329 *3.2. Zambia*

330 *3.2.1. Regional/ large scale: species richness*

331 The total number of macrophyte species recorded from the Zambian sites

332 (gamma-diversity) was 255, consisting of 186 emergent, 18 floating and 51 submerged

333 species. Mean species richness (alpha-diversity) at individual sites in Zambia sampled

334 during 2009 – 2011 was 8.3 species per site, substantially higher than for the British

335 Isles dataset. Macrophyte species richness variation, within Zambian streams (Table 1,

336 Fig.4) was accounted for solely by the pure spatial component, which explained 25.8%

337 of variation. Influential MEM orders were low, indicating broad-scale patterns of spatial

338 variation.

339

340 *3.2.2. Regional/ large scale: community composition*

341 In contrast to the results for species richness, spatial, environmental and

342 spatially-structured environmental variables all influenced the variation in macrophyte

343 community composition observed in Zambian hard-water rivers (Table 2). The spatially-

344 structured environmental component was of greatest importance, explaining 4.6% of the

345 variation. The pure spatial component (with MEMs representing broad-scale spatial

346 patterns) accounted for a further 3.8%, and pure environmental variables explained a

347 further 2.7% of the variation, with both being statistically significant. Environmental

348 variables that best explained the variation observed in macrophyte community

349 composition were annual precipitation, precipitation seasonality, evapotranspiration,

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altitude and alkalinity. Fig. 5 shows the distribution of three of these variables across Zambia.

4. Discussion

Our results suggest that in the tropical calcareous rivers of Zambia only spatial factors were of importance (though quite strongly so) in explaining species richness variation, mainly acting at broad scales. In contrast, although a total fraction of the variation in species richness comparable to that seen for Zambia was explained by variables retained in the final model for the temperate rivers of the British Isles, this was made up not only of pure spatial factors, but also pure environmental (alkalinity, temperature seasonality, maximum temperature of warmest quarter, minimum temperature of coldest quarter, and mean temperature of wettest quarter) and spatially-structured environmental factors, whilst spatial factors operated across a wide range of scales from broad to finer-scale patterns. It is noteworthy that, in both cases, the inclusion of spatial factors in the analysis helped explain a significant proportion of the observed variation for species richness in calcareous river vegetation, demonstrating the importance of spatial processes (e.g., unmeasured environmental variables, dispersal) when analysing large-scale species diversity distributional patterns (see Legendre et al., 2009).

In terms of community composition, differences between the tropical and temperate outcomes are less marked than for the richness outcomes, with all three components (spatial, environmental and spatially-structured environmental) contributing to explain community variation, and a comparable total proportion of variation (ca. 10 – 11%) being accounted for in both target regions. Within this total proportion of variation explained there were minor differences in the importance of each component between the two regions, with spatial factors being of greater importance in the temperate rivers of the British Isles, and the spatially-structured environmental component being most

377 important in tropical Zambian rivers. In both cases spatial patterns operating mainly at

378 broad scales were suggested by the order of MEMs retained as of primary importance

379 in the final models (Table 2).

380   Of the three environmental variables most strongly contributing to the outcomes

381 for variation in regional community composition, annual precipitation was of primary

382 importance in both Zambia and the British Isles. In both cases a further precipitation

383 variable (precipitation of wettest quarter in the British Isles; precipitation seasonality in

384 Zambia) was second in importance. However the third strongest variable was quite

385 different between the target regions, being minimum temperature of coldest month in

386 the British Isles, and annual evapotranspiration in Zambia. This may reflect the

387 importance of cold winter temperatures in potentially stressing vegetation in temperate

388 rivers, and the probable importance of evapotranspiration in contributing to water loss

389 from aquatic systems in tropical rivers, again causing potential stress to river plants as

390 their habitat dries out during the dry season.

391   The overall proportions of variation explained by the analysis of regional-scale

392 species richness and community composition are undoubtedly low (see Tables 1 and

393 2). However, these outcomes are of comparable magnitude to those recorded from

394 variation-partitioning analyses in similar studies elsewhere which have incorporated

395 spatial analysis (e.g. Heino et al., 2009; Astorga et al., 2011; O'Hare et al., 2012; see

396 also Soininen et al., 2014; 2016 for general quantitative reviews).

397   In order to improve the total explained variation it is likely that the inclusion of

398 large-scale data for additional environmental factors (such as river flow regime, nutrient

399 status, pH and other measures of water chemistry, and relevant catchment-scale

400 factors such as land use) that are likely to influence river macrophyte richness and

401 community would be helpful (e.g., Johnes et al., 1996; Kennedy et al., 2015). Such

402 issues notwithstanding, our findings provide evidence to support the suggestion (e.g.,

403 Capers et al., 2009; O'Hare et al., 2012) that large regional-scale patterns in diversity

404 are often strongly related to climate, though we also found that alkalinity and altitude

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405 were useful explanatory variables for community composition distribution (less so for

406 species richness).

407   According to metacommunity theory, a significant environmental fraction

408 provides evidence for the role of niche-based based processes (species sorting) in

409 structuring communities (Leibold et al., 2004). Thus, in general, our results suggest the

410 importance of species sorting processes in structuring local communities, despite the

411 low values obtained for the pure environmental fractions.

412   Comparing the British Isles with Zambia, it is interesting to note that in both

413 tropical and temperate rivers the primary environmental variable explaining community

414 composition variation was annual precipitation. There are strong spatial gradients of

415 annual precipitation in both regions: primarily increasing from east to west in the British

416 Isles, and south to north in Zambia (Figs. 2, 5). These gradients are reflected in

417 changing macrophyte community composition in rivers in both regions, with some

418 examples detailed below.

419   In Zambia, Kennedy et al. (2015), using a dataset which included the data

420 utilised in our study, but also including sites on non-calcareous rivers, found strong

421 evidence that macrophyte community composition in rivers of the northern part of the

422 country (primarily comprising the Bangweulu-Mweru freshwater ecoregion (Abell et al.,

423 2008), which lies in the catchment of the Upper Congo, flowing to the Atlantic) shows

424 substantial differences from rivers in the southern part of the country (in several

425 freshwater ecoregions, but all within the Zambezi catchment, flowing to the Indian

426 Ocean). For example a community type indicated by the presence of *Ottelia exserta*

427 (Ridl.) Dandy, together with a number of less-common (within Zambia) macrophyte

428 species such as *Potamogeton octandrus* Solms., *Aldrovanda vesiculosa* L., and *Ottelia*

429 *cylindrica* (T.C.E.F. r.) Dandy, occurred only in upland calcareous streams of the

430 Bangweulu-Mweru ecoregion in northern Zambia. The same study found that a very

431 different community type, indicated by the presence of *Lagarosiphon ilicifolius* Oberm.,

432 *Ceratophyllum demersum* L., *Azolla filiculoides* Lam. and *Potamogeton schweinfurthii*

433   A. Benn., was characteristic only of sites on rivers located in low-lying valleys of the

434   Zambezi catchment, in the southern part of Zambia.

435   Spatial vegetation trends in calcareous river macrophyte community

436   composition have long been well documented for the British Isles along the well-known

437   east – west precipitation gradient for this region (e.g., Butcher, 1933; Haslam, 1982;

438   Caffrey, 1990; see also Fig. 2). A good example is the calcareous river macrophyte

439   community type dominated by Batrachian *Ranunculus* spp., one variant of which

440   (indicated by *Ranunculus penicillatus* subsp. *pseudofluitans* (Syme) S.D. Webster)

441   tends to occur in more westerly, higher-flow rivers in the wetter parts of Britain, but

442   which is much less common in the more sluggish calcareous rivers characteristic of

443   lower-precipitation areas of eastern England (Holmes and Raven, 2014; see also

444   information on the autecology of this plant, and a map of its British Isles distribution

445   provided   by   the   Online   Atlas   of   the   British   Flora   at:

446   www.brc.ac.uk/plantatlas/index.php?q=node/1476). This illustrates the point that factors

447   such as annual precipitation may not be the primary proximal cause of spatial variation

448   in species distribution and hence community composition. In the case of annual

449   precipitation other factors (such as topography) associated with the discharge and

450   velocity of rivers (as well as a whole suite of other physico-chemical factors) will also

451   strongly influence the ecology of these systems, and hence help determine what

452   species they support. However, it is clear that spatially-structured environmental

453   variables, such as annual precipitation, can act as a strong surrogate for a larger set of

454   factors, in this case associated with flow regime, which influence river vegetation.

455   Overall, variation in calcareous river macrophyte community composition at

456   regional scale in the British Isles, and at catchment scale in Great Britain (but not in

457   Irish RBUs) was generally quite strongly attributable to spatially-structured

458   environmental variables, though different variables were of greater or lesser importance

459   within individual RBUs. Precipitation of coldest quarter was one such variable that was

460   retained in the final model for every one of the RBUs in Great Britain.

461   Species richness variation was attributed to spatially-structured environmental

462   variables at regional level, and this clearly mirrored well-documented climatic gradients

463   which influence rivers in the British Isles and in Zambia. For instance hard-water river

464   macrophyte species richness generally increased along a north-west to south-east

465   gradient in the British Isles and in the opposite direction across Zambia (Figs. 1, 3).

466   Several environmental variables such as temperature seasonality, and maximum

467   temperature of warmest quarter vary spatially along a similar gradient in the British Isles

468   (Fig. 2), while in Zambia precipitation seasonality and annual evapotranspiration show a

469   clear south-west to north-east spatial gradient, mirroring the richness gradient (Fig. 5).

470   In this study we made no attempt to identify what the actual factors were, acting at

471   different spatial scales upon river vegetation, which influenced the richness and

472   community composition outcomes for spatial variation. Our results simply show that one

473   or more such spatial factors, associated with each relevant MEM filter (as listed in

474   Tables 1 and 2), differentially influenced variation in alpha-diversity and/or community

475   composition of the macrophyte assemblages present at river sites in different parts of

476   the British Isles and Zambia. A considerable amount of further work is needed to tease

477   out what exactly is responsible for these observed results, but the observed outcomes

478   are highly likely to be due to spatial structure (as indicated by MEMs).

479

480   **5. Conclusions**

481   Our results suggest that the sources of variation in macrophyte species richness

482   and community composition in hard-water rivers, are, at least in part, spatially

483   organized; implying the presence of spatial structure, termed induced spatial

484   dependence (Peres Neto and Legendre, 2010), i.e. non-random organization across

485   space of either species distribution or environmental processes, or both. Returning to

486   our original hypothesis it is apparent that the variation in both richness and community

487   composition attributable to spatial, environmental, and spatially-structured

488   environmental factors, differs in detail rather than fundamentally, when comparing

489 tropical and temperate calcareous rivers. We suggest that variation in both species

490 richness and community composition for hard-water river macrophytes can (to a small

491 but significant degree) be partially explained by the interaction of environmental and

492 spatial processes (usually, but not always, operating primarily at broad scales) in both

493 temperate and tropical systems. However, the detail of the driving processes (for both

494 alpha-diversity and community composition) differed between tropical and temperate

495 rivers.

496   The principal question arising from the outcomes of this study is whether the

497 observed spatial variation is really mirroring differences in actual spatially-varying

498 environmental drivers of calcareous river vegetation community characteristics, and if

499 so in what way(s)? This question is beyond the scope of this study to address, and

500 emphasises the need to include as wide a range as possible of environmental drivers

501 potentially influencing river plant ecology (e.g., O'Hare et al., 2012), in future studies,

502 but at least our results set out some possible directions for future work to address such

503 issues.

504

516

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653

654 Table 1. Spatial and environmental models explaining macrophyte species richness (alpha-diversity, S: average number of species per sample) variation in

655 the British Isles and Zambia, and for individual River Basin Units (RBUs) within the British Isles only. Order of listing of spatial and environmental variables

656 follows their level of importance in the final model. Probability values (*p*: considered significant at *p*<0.05) are shown respectively for outcomes of analysis of

657 environmental and spatial components, for the whole dataset (Global), and the partitioned dataset (Fractions): *p* Global Environmental (ENV); *p* Global Spatial

658 (SP); *p* Fractions Environmental (ENV); *p* Fractions Spatial (SP). Adjusted R^2 values for partitioned variation are respectively for environmental (ENV),

659 spatially-structured environmental (SSE) and pure spatial (SP) fractions: Adj R^2 Fractions ENV; Adj R^2 Fractions SSE; Adj R^2 Fractions SP. See Methods

660 Section 2.1 for list of environmental variables codes.

661

Region	Environmental variables retained in final model	Spatial variables (MEM) in final model	p Global ENV	p Global SP	p Fractions ENV	p Fractions SP	Adj R^2 Fractions ENV	Adj R^2 Fractions SSE	Adj R^2 Fractions SP	Mean alpha-diversity (S)
British Isles	ALK, TS, MAXTW, MINTC, MTWeQ	4, 20, 100, 6, 16, 21, 8, 525, 166, 99, 23, 383, 42, 39, 101, 438, 135, 102, 320	0.0002	0.0002	0.0002	0.0002	0.021	0.114	0.088	3.1
Scotland	None	None	0.9016	0.6472	-	-	-	-	-	2.2
N England	None	81, 7, 16, 19, 65, 61, 75	0.0810	0.0344	-	0.0002	-	-	0.144	2.5
SE England	None	106	0.3656	0.0054	-	0.0002	-	-	0.059	3.6
SW England and Wales	None	1	0.1078	0.0002	-	0.0298	-	-	0.109	2.8
N Ireland	None	4, 6	0.1888	0.0004	-	0.001	-	-	0.138	3.1
S Ireland	None	None	0.4012	0.5122	-	-	-	-	-	7.1
Zambia	None	9, 7, 3, 8, 2, 1, 21	0.11	0.01	-	0.005	-	-	0.258	8.3

667   Table 2. Spatial and environmental models explaining macrophyte species community composition variation in the British Isles and Zambia, and for

668   individual River Basin Units (RBUs) within the British Isles. Order of listing of spatial and environmental variables follows their level of importance in the

669   final model. See Methods Section 2.1 for environmental variable codes, and caption to Table 1 for key to other abbreviations.

670

Region	Environmental variables retained in final model	Spatial variables (MEM) in final model	p Global ENV	p Global SP	p Fractions ENV	p Fractions SP	Adj R^2 Fractions ENV	Adj R^2 Fractions SSE	Adj R^2 Fractions SP
British Isles	AP, PWQ, MINTC, TS, MAXTW, ALT, ALK, PS, MTWeQ, PCQ, PCQ, AMT, EVAP.	1, 4, 2, 3, 5, 20, 10, 9, 6, 16, 7, 14, 12, 8, 11, 15, 19, 18, 24, 193, 21, 22, 17, 28, 53, 25, 54, 27, 47, 45, 23, 41, 338, 56, 65, 387, 26, 522, 51	0.005	0.005	0.005	0.005	0.011	0.039	0.054
Scotland	ALK, TS, MINTC, MTWeQ, PCQ	3, 1, 4, 28	0.028	0.005	0.018	0.103	0.028	0.069	0.013
N England	MAXTW, ALT, MINTC, TS, MTWeQ, ALK, PS, PCQ, PWQ, AP	1, 6, 4, 11, 9, 14, 3, 13, 7, 2, 15, 52	0.005	0.005	0.005	0.005	0.025	0.045	0.036
SE England	PCQ, MAXTW, PS, ALT, ALK, TS, MTDQ, AP, MINTC	8, 1, 21, 2, 7, 13, 18, 19, 30, 10, 3, 120, 6, 147, 108, 11, 24, 97, 23, 31, 9, 52, 25	0.005	0.005	0.005	0.005	0.013	0.021	0.071
SW England and Wales	PWQ, PCQ, ALT, MAXTW, AP, ALK	2, 1, 8, 6, 47, 4, 37, 89, 3, 5, 130, 7, 94, 67, 54	0.005	0.005	0.005	0.005	0.015	0.023	0.042
N Ireland	None	4, 1, 2	0.082	0.005	-	0.005	0	0	0.041
S Ireland	None	none	0.22	0.65	-	-	0	0	0.031
Zambia	AP, PS, EVAP, ALT, ALK.	1, 2, 7, 4, 34, 6, 41, 32, 24, 39	0.005	0.005	0.005	0.005	0.027	0.046	0.038

Macrophyte species richness per 100m (S)

- ○ 1 - 2
- ◔ 2 - 4
- ◉ 4 - 8
- ● 8 - 13
- ● 13 - 19
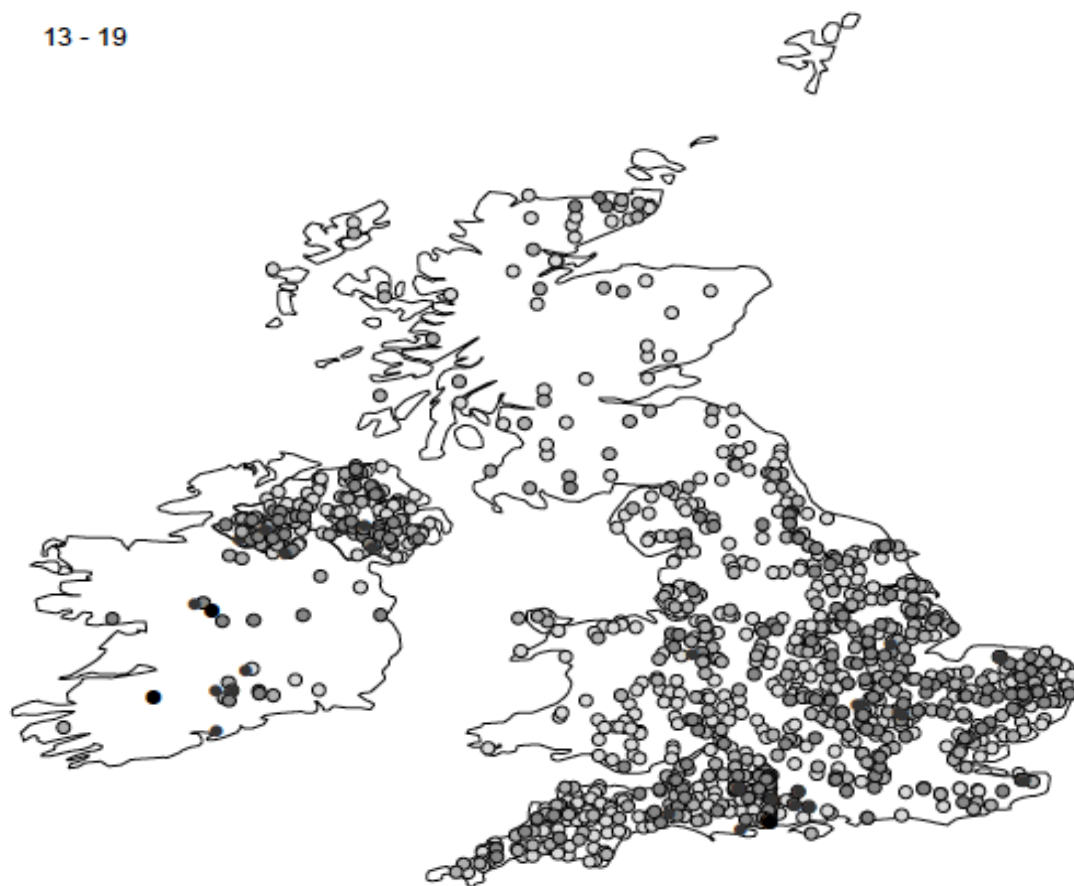
0   75   150   300 Kilometers

ALK mg L^{-1}

- 10 -75
- 76 - 158
- 158 - 240
- 240 - 794
- 794 - 2140

TS (SD * 100)

- 333 – 435
- 435 – 488
- 488 – 546

(a)

MAXTW °C

○ 15 - 19
◉ 19 - 21
● 21 - 23

MINTC °C

○ -2.80 - 0.30
◉ 0.30 - 1.50
● 1.501 - 3.20
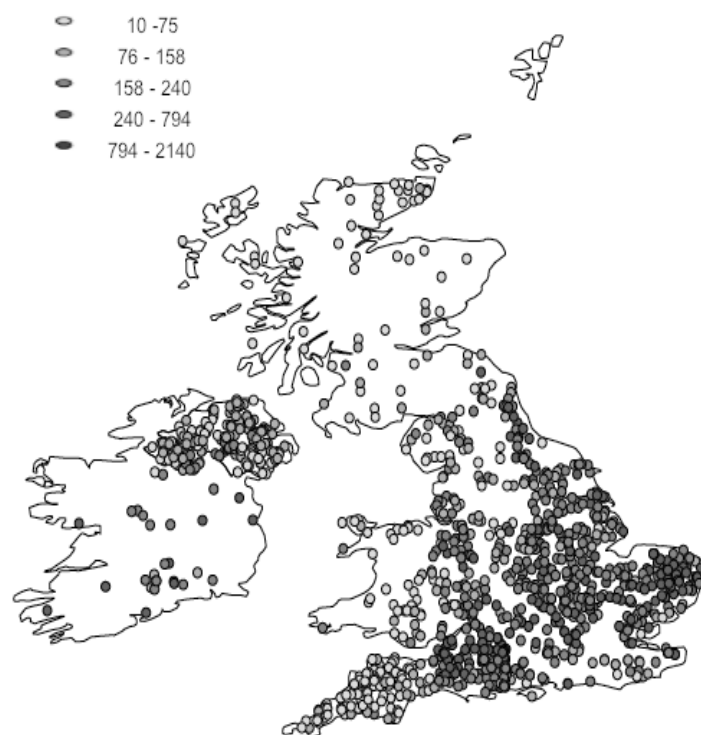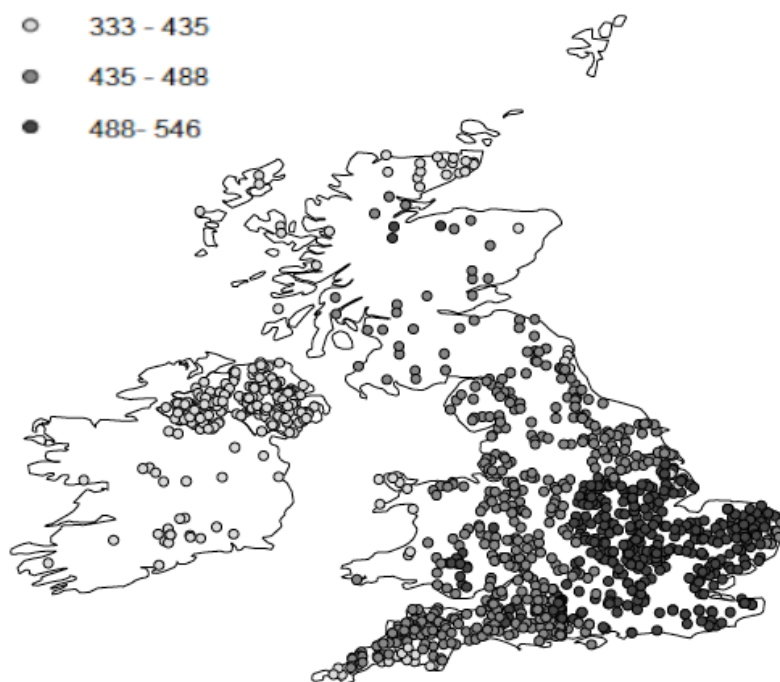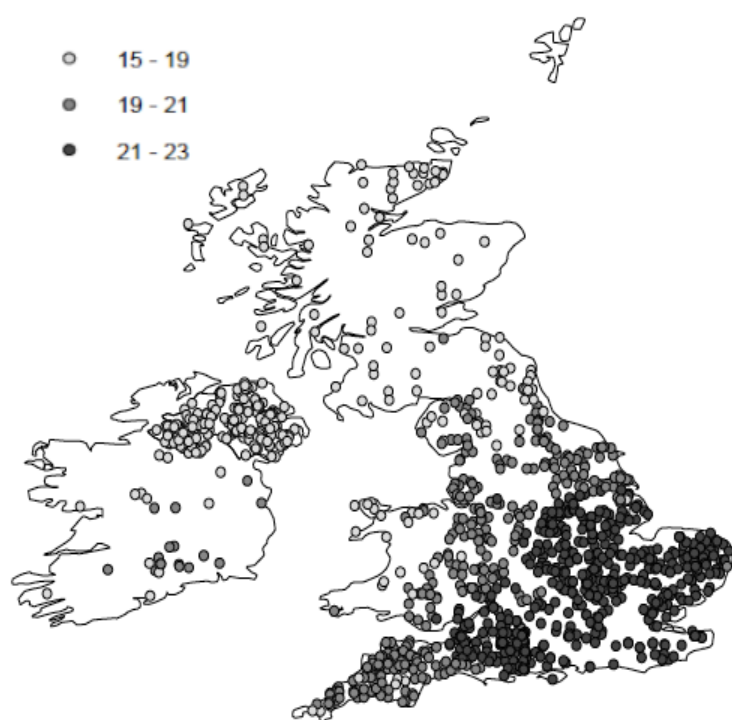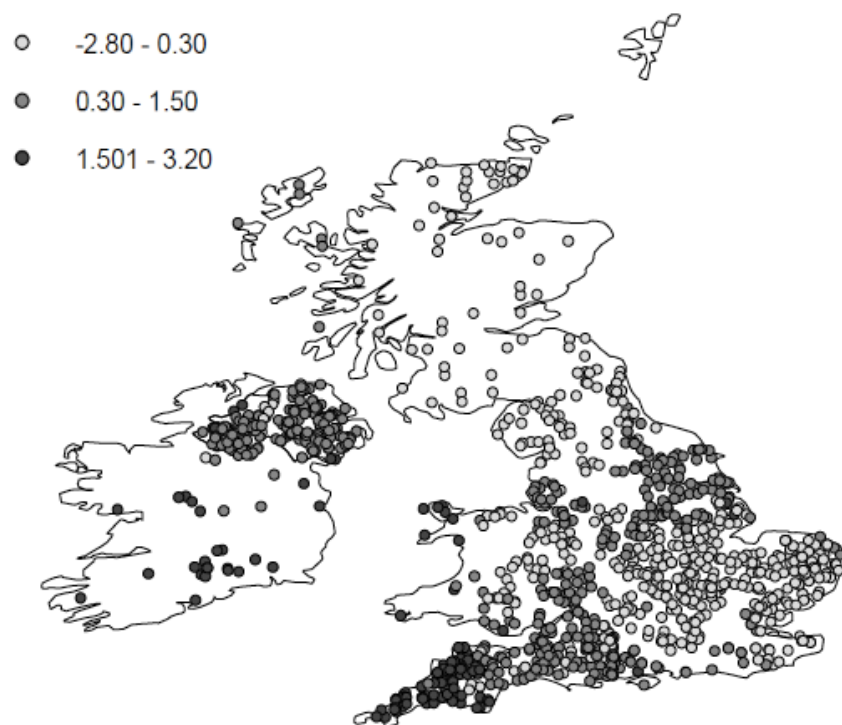
(b)

AP mm
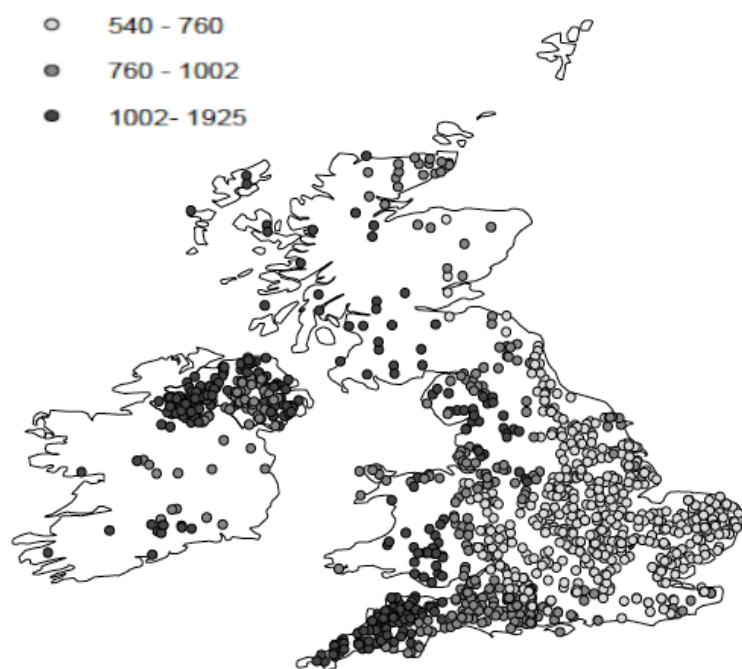
PWQ mm

(c)

ALT m above sea level

- 1.0 - 67
- 67 - 139
- 139 -252
- 252- 489
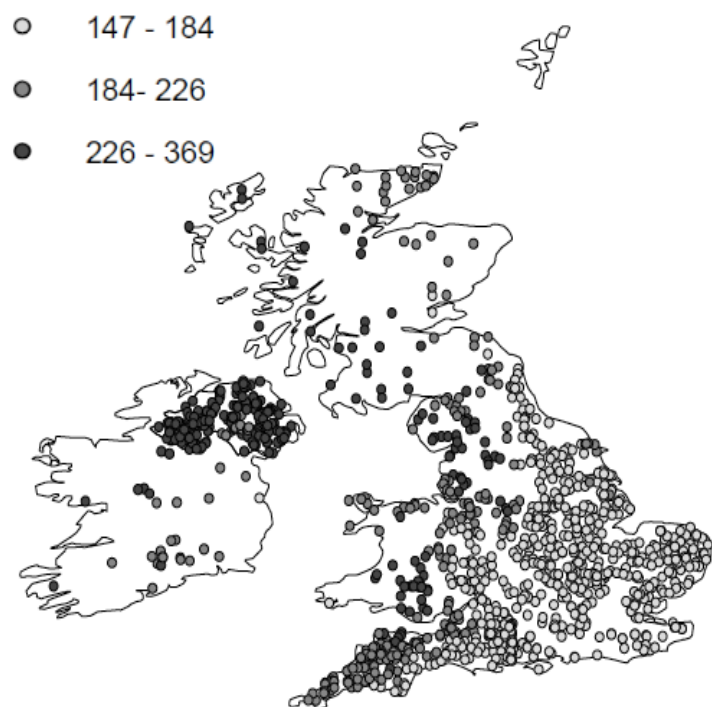
0   75   150   300 Kilometers
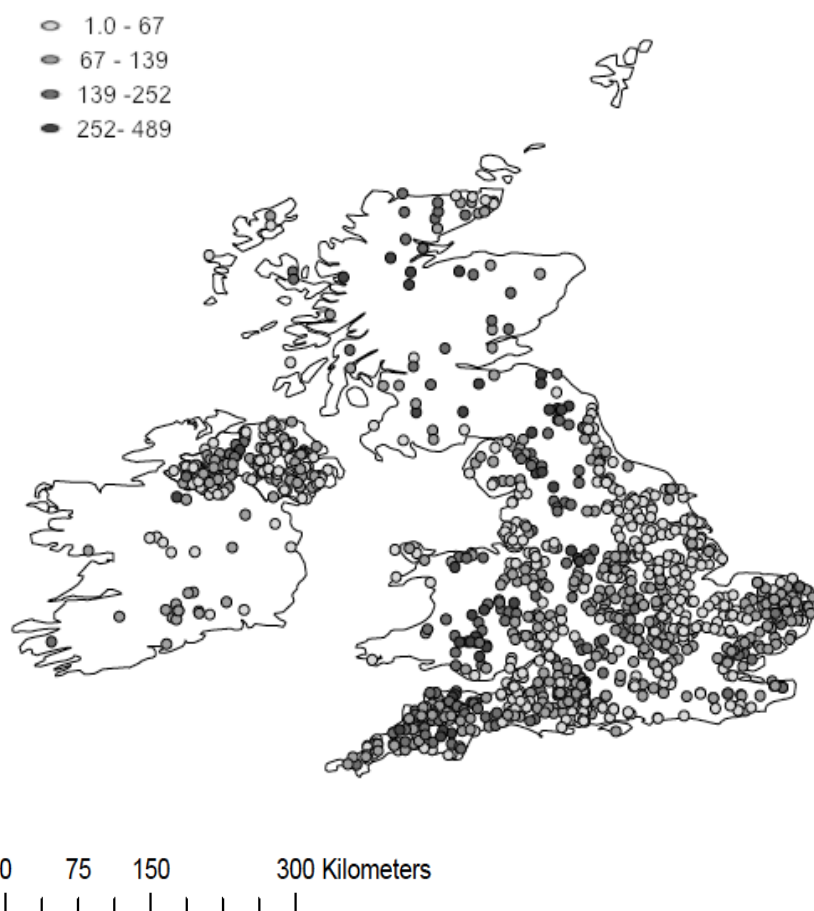
(d)

MEM 4

- ○ -0.47 to -0.04
- ◉ -0.04 to 0.006
- ● 0.006 to 0.39
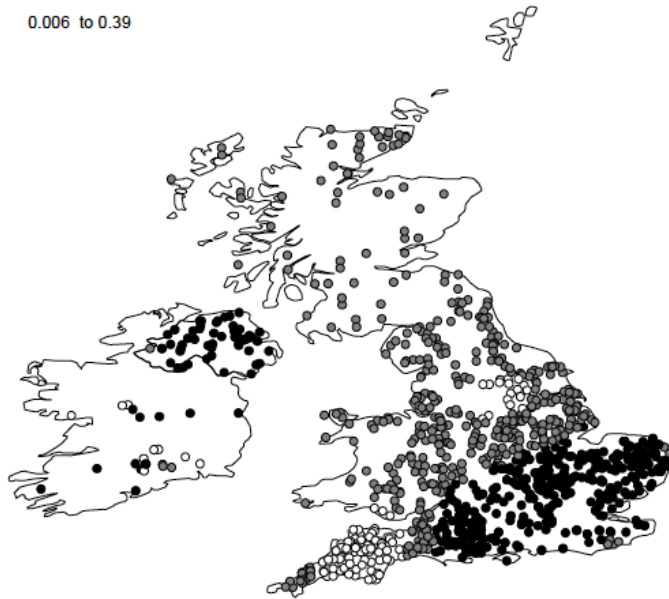
MEM 20

- ○ -0.12 to -0.05
- ◉ -0.05 to -0.001
- ● -0.001 to 0.06
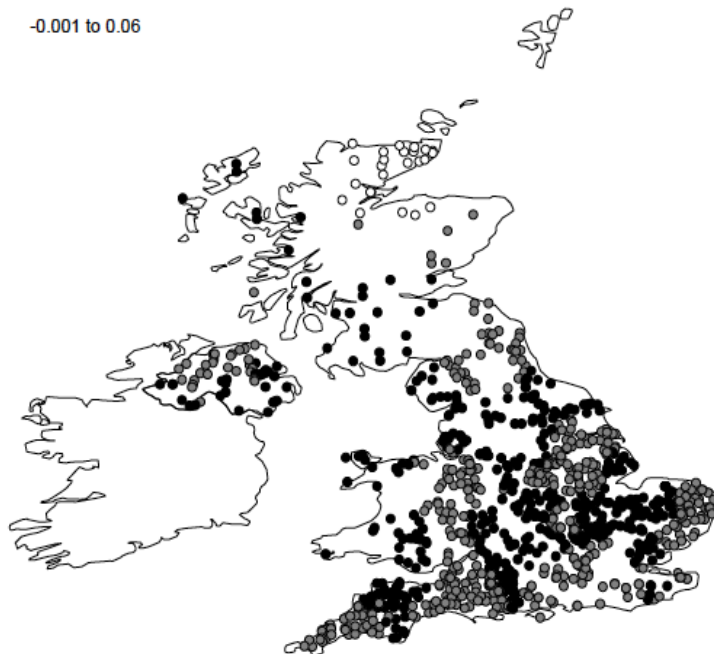
(a)

MEM 100

○   -0.10 to -0.01

◉   -0.01 to 0.01

●   0.01 to 0.11
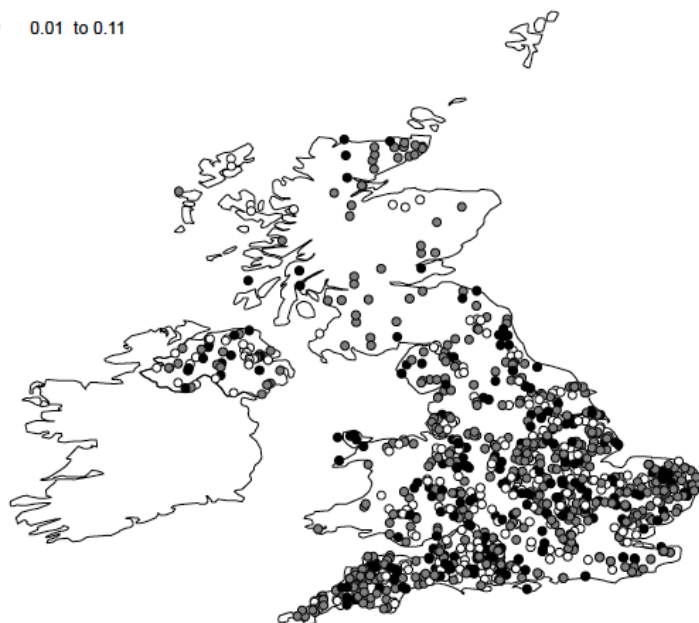
0   75   150   300 Kilometers

(b)

Macrophyte species richness per 100m (S)

○	1-5
◐	5-8
◉	8-13
●	13-21

0   75   150   300 Kilometers

AP mm

○ 658 - 909

◑ 909 - 1164

● 1164 - 1301

PS (coefficient of variation)

○ 99 to112

◑ 112 to 121

● 121 to 128
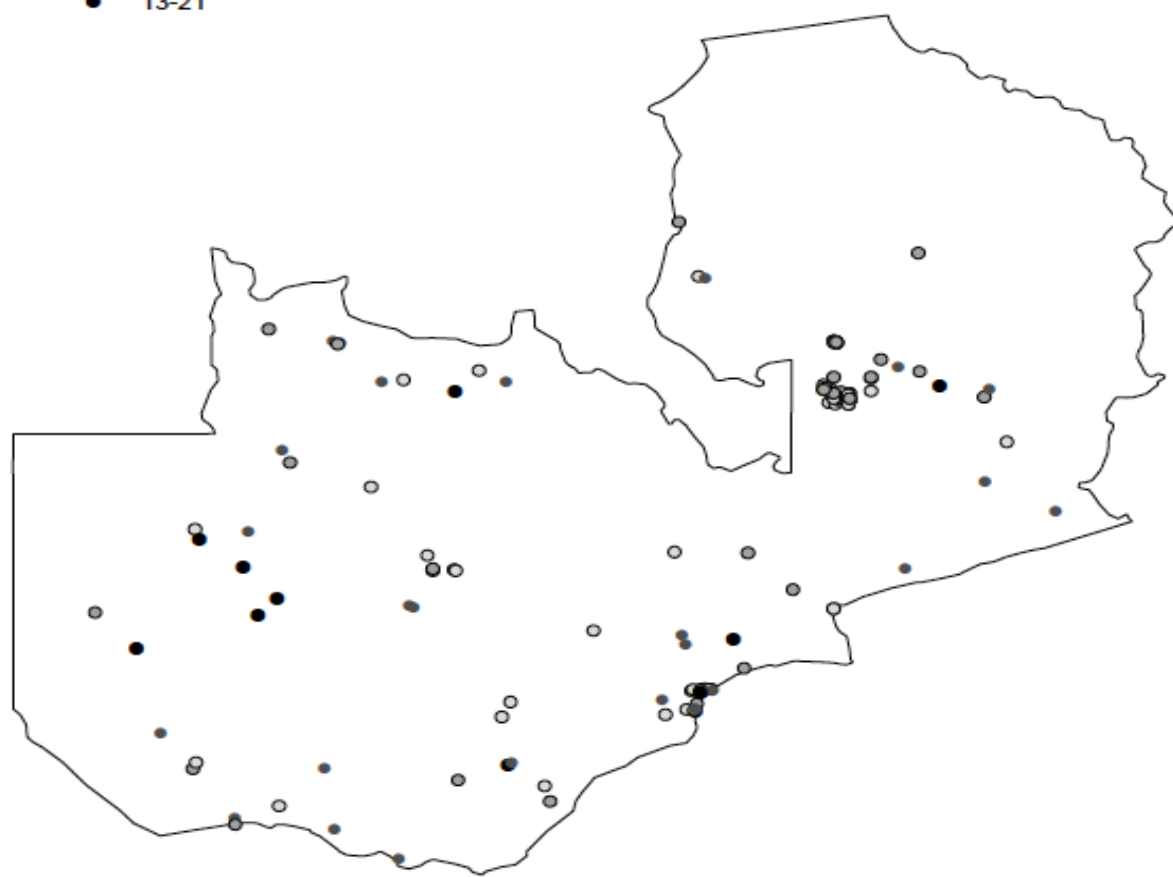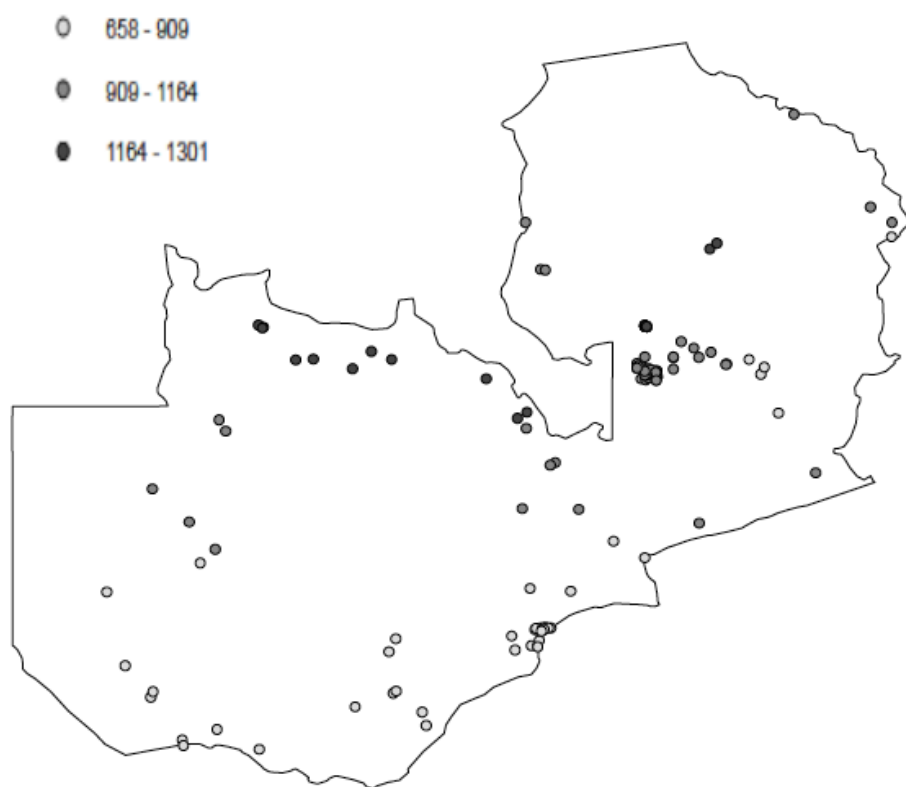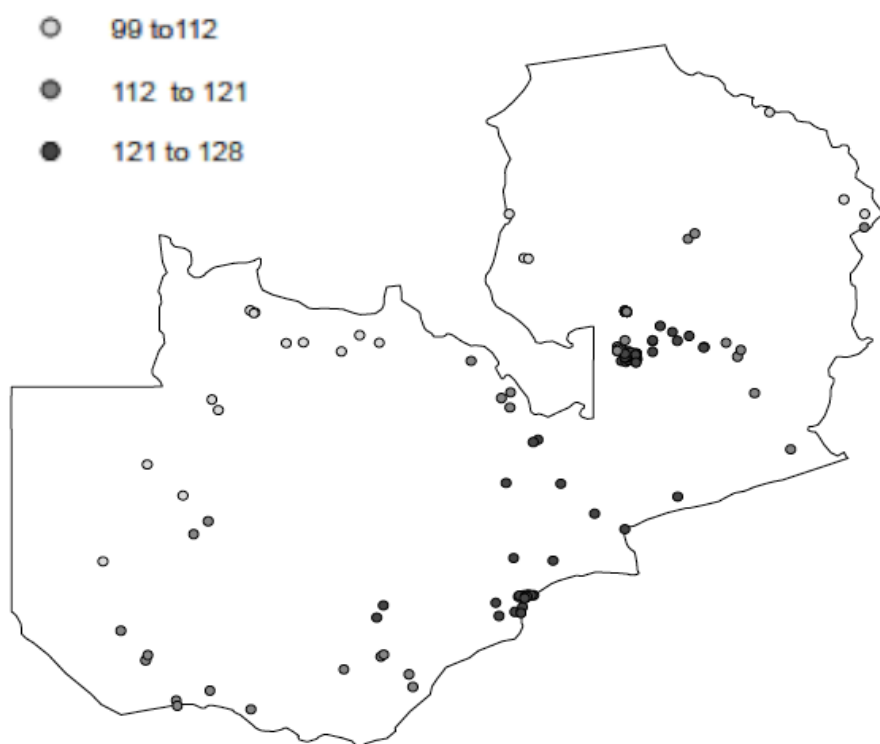
(a)

EVAP mm

- ○ 617 to 707
- ◐ 707 to 900
- ● 900 to 1069

```
0    75    150        300 Kilometers
```

(b)