 DOI 10.1080/03736687.2018.1446484

On the influence of water conductivity, pH and climate on bryophyte assemblages in Catalan semi-natural springs

Authors: M. Bes[1], J. Corbera[1], F. Sayol[2], G. Bagaria[2], M. Jover[3], C. Preece[2,4], F. Sabater[1,4,5], A. Viza[1,5], M. Fernández-Martínez[*1,6]

Affiliations:

[1] ICHN, Delegació de la Serralada Litoral Central, Mataró, Catalonia, Spain

[2] CREAF, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain

[3] Department of Environmental Sciences, Faculty of Sciences, University of Girona, Girona, Catalonia, Spain

[4] CSIC, Global Ecology Unit, CREAF-CSIC-UAB, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain

[5] Department of Ecology, University of Barcelona, Barcelona, Catalonia, Spain

[6] Centre of Excellence PLECO (Plant and Vegetation Ecology), Department of Biology, University of Antwerp, 2610 Wilrijk, Belgium.

***Corresponding author**

Marcos Fernández-Martínez

E-mail address: marcos.fernandez-martinez@uantwerpen.be

Manuscript length: 3540 words

Keywords: Bryophyte ecology, Ecological niche, Fountains, Liverworts, Mosses, Tolerance ranges

Abstract

Bryophytes are some of the most sensitive biological indicators of environmental change. Springs have a significant presence of bryophytes and hence they are ideal habitats for studying their relationship with the environment. We tested whether bryophyte assemblages can be explained with macro-, meso- and micro-ecological variables (i.e., seasonal climate, altitude, water pH and conductivity) sampling bryophytes from 198 semi-natural springs distributed along montane regions in the north-eastern Iberian Peninsula. We tested the influence of environmental variables on bryophyte assemblages in springs using sparse Partial Least Squares (sPLS). Our results show that variability in bryophyte assemblages is explained by seasonal climate (temperature and precipitation from winter, spring, summer and autumn and temperature and precipitation seasonality), altitude and water conductivity. The results obtained by the present study will be useful for predicting bryophyte diversity in springs using simple and easy to obtain variables such as climate, water pH and conductivity.

Introduction

Bryophytes are very sensitive to environmental conditions (Kapfer *et al.*, 2012), mainly because they do not have a well-developed cuticle or epidermis, and hence most of them can absorb water and nutrients through their entire surface. This is why bryophytes are a very interesting group to study the effects of environmental variability and pollution on community composition. In fact, bryophytes have previously been used as bioindicators of air pollution (Slack, 1990; Suren & Ormerod, 1998: Suren & Duncan, 1999) and water quality (Arts, 1990; Vanderpoorten & Klein 1999; Ceschin *et al*., 2012).

Despite the fact that bryophytes are generally more widely distributed than other plants because of their dispersal capacity (Patiño *et al*., 2015; Wang *et al*., 2017), species distribution is very dependent on environmental conditions. The most studied variables include climate (Gignac & Vitt, 1990; Nicholson *et al*., 1996), water availability (Pentecost & Zhang, 2006), and geology (Pentecost & Zhaohui, 2002; Belland, 2005; Callaghan & Ashton, 2008a) which, through their interaction, determine more local and potentially important environmental variables such as water pH (Tyler *et al.*, 2018) and conductivity in springs and streams where bryophytes can establish (Peñuelas & Sabater, 1987; Vanderpoorten & Palm, 1998; Sabater *et al.*, 2015).

One of the habitats that can be dominated by bryophytes is the headwaters of rivers and springs. In these environments, climate, altitude, water pH and conductivity have been suggested to determine which species can become established (Tomaselli *et al*., 2011; Ceschin *et al*., 2012; Tessler *et al*., 2014a; Vieira *et al*., 2014). However, other micro- and mesoscale environmental variables such as substrate or habitat heterogeneity (Suren, 1996; Strohbach *et al*., 2009; Vieira *et al*., 2014), aspect and slope (Pentecost & Zhang, 2006), or disturbance regime of floods and droughts may also be factors explaining variation in bryophyte assemblages in aquatic and semi-aquatic habitats (Suren & Ormerod, 1998; Suren & Duncan, 1999). In similar habitats, such as peatlands, some studies have suggested that bryophyte assemblages depend on gradients of temperature and precipitation (Gignac, 1994), water pH and conductivity (Gignac, 1992) or shade and permafrost (Belland & Vitt, 1995). Other studies carried out in temperate ecosystems of England (Callaghan & Ashton, 2008a), coastal zones of Canada (Belland, 2005), and semi-arid zones of Australia (Eldridge & Tozer, 1997) indicate that the different bryophyte assemblages depended on precipitation, soil pH and geology, showing a clear differentiation between communities located over calcareous and non-calcareous lithologies (Callaghan & Ashton, 2008b; Spitale *et al.*, 2009; Virtanen *et al.*, 2009). Additionally, bryophyte communities have also been suggested to vary along altitudinal gradients (Wolf, 1993; Andrew & Rodgerson, 2003; Bruun *et al.*, 2006; Grytnes *et al.*, 2006; Ah-Peng *et al.*, 2007; Grau *et al.*, 2007; Spitale, 2016), which can be a proxy for differences in topology and radiation amongst others.

Notwithstanding all this research on bryophyte ecology, the study of differences in bryophyte assemblages along gradients of altitude, seasonal temperature and precipitation, water pH and conductivity combined, is still very limited in the region of Catalonia and especially in semi-natural or man-made springs (Corbera *et al.*, 2015). In our study region (Catalonia, NE Iberian Peninsula) the ecology of bryophytes has been poorly studied (but see Peñuelas, 1983; Peñuelas & Sabater, 1987), especially concerning the interaction between macro-, meso- and microecological variables. Hence, the aim of this study was to test to what extent macro-, meso- and microecological variables such as climate (seasonal temperatures and precipitation and their seasonality), altitude, and water pH and conductivity can explain variation in bryophyte species assemblage in springs within a relatively large climate and water chemistry gradient. To achieve our aim, we surveyed springs and their bryophyte communities in montane regions of north-eastern Iberian Peninsula (Catalonia, NE Iberian Peninsula). Based on results provided by previous studies, we hypothesised that water pH and conductivity would play a main role determining bryophyte communities of semi-natural springs, due to the direct contact between the bryophytes and the water, and species differences in tolerance ranges for these properties. In addition, because in the Mediterranean climate, summer is the warmest and driest season of the year, we hypothesised that climate from the summer season (dry and warm season) would also significantly explain variability in species composition of spring bryophyte communities. Altitude was also hypothesised to be an important factor for determining spring bryophyte assemblage as it can reflect differences in topography, radiation and climate.

Materials and methods

Study area and experimental design

We surveyed 198 water springs following a 95 km south-to-north and a 50 km east-to-west gradient along the north-eastern Iberian Peninsula territory (Figure 1), comprising 4 different mountain ranges: the Central Littoral mountain range, Montseny-Guilleries, Lluçanès and Garrotxa. The springs were unevenly distributed across the different mountain ranges: 101 springs occurred in the Montseny-Guilleries, 55 in the Central Littoral mountain range, 32 in Lluçanès and 10 in Garrotxa. Climate also differs amongst these mountain ranges. The Central Littoral mountain range has a typical maritime Mediterranean climate with mild summers and winters, while the climate is Mediterranean humid and sub-humid in Montseny-Guilleries and Lluçanès and Mediterranean pre-Pyreneeal in Garrotxa (Martín-Vide, 1992), being more continental. Lithologically, the springs of the Central Littoral mountain range are located over different types of granite, except for the eastern side which is dominated by metamorphic rocks (Sabater *et al.*, 2015; Fernández-Martínez *et al.*, 2016). In Montseny, springs are located over granite, metamorphic and calcareous rocks while in Guilleries most of them are over granite. Lluçanès is completely calcareous and Garrotxa is divided into calcareous and volcanic rocks. This rich lithology produces a large variability in the characteristics of water from springs, affecting dissolved ions and thus conductivity and pH (Sabater *et al.*, 2015).

Most of the springs we surveyed are small human-made constructions to collect water from the underground and release it through a spout (Figure S1). Some of the springs drained water mines (collecting water from aquafers) while others were built in naturally occurring springs. However, in all of the surveyed springs, flowing water did not receive any sanitary treatment. The morphology of our springs did not vary greatly (for a typical example see Figure S1). Almost all of them contained the same microhabitats such as a spout, wet rock walls, soil ground/walls and a sink where the water makes a little pool. Surveys of the springs were carried out during spring and autumn seasons of the years 2013 and 2015, and although many other springs were surveyed, only those that had water flowing at the time of the survey were sampled and included in this study. We also excluded from analysis any springs whose water flow was controlled by a tap. Therefore, the springs we sampled contained bryophytes that were continuously receiving water during most of the year, apart from springs in which drought had temporarily interrupted water flow. Water pH and conductivity of the flowing water was analysed in the field with pH-meter (ORION, Thermo-Scientific, Waltham), previously calibrated with standard solutions at pH 7 and 10, while electric conductivity was measured using a conductivity-meter (WTW, Xylem Inc., Weilheim) calibrated at 25ºC and with a standard solution of 1314 $\mu S\ cm^{-1}$.

To determine the bryophyte communities (as presence or absence data) we took a sample of the different bryophyte species present in the spring that were either in direct contact with the water flow, receiving water drops or under water (see yellow line in Figure S1). We did not standardise our bryophyte measurements by the total area sampled because, (1) sampled area was lower than 0.9 m^2 for most of the springs, for which differences in scale were minimal, (2) species-area relationship was missing for a subset of our dataset (Figure S2) and, (3) surveying a predetermined area would have made us miss some species present in the springs. Species number per spring was generally very low, most of them having between 1 and 4 species (Figure S3). We used identification keys in Smith (1978 & 1990) and Casas *et al.*, (2001 & 2004) and follow the nomenclature established by Hill *et al*., (2006). Some of the samples could not be identified to species level (3%, 18 out of 538 samples). To study the variation in bryophyte assemblages we used those bryophyte species that appeared in more than 10 springs (13 species in total), to ensure enough replicates per species.

Using the geographical coordinates of the springs, obtained in the field with a GPS, we extracted average seasonal (winter, spring, summer and autumn) temperatures and precipitation, as well as mean annual temperature (MAT) and precipitation (MAP), from the Digital Climatic Atlas of Catalonia (Pons, 1996; Ninyerola *et al*., 2000; available at http://www.opengis.uab.cat/acdc/index.htm) with a resolution of 180 m. Seasonal climate was calculated as the average temperature and total precipitation values of December to February for winter, March to May for spring, June to August for summer and September to November for autumn. Using seasonal climate, we also calculated temperature and precipitation seasonality as the coefficient of variation of seasonal temperatures and precipitation. Altitude was obtained using a GPS in the field.

Statistical analyses

To test whether bryophyte assemblages depend on environmental conditions we performed a sparse Partial Least Squares (sPLS) analysis (Lê Cao *et al.*, 2008), fitted by the regression mode, where water pH and conductivity, seasonal temperatures and precipitation, temperature and precipitation seasonality, and altitude were the environmental matrix of predictors and the binary variables of bryophyte species presence were the response matrix to be predicted. Because the response matrix (species presence) was a binary variable we fitted the model after applying a log-ratio transformation. PLS family methods work by finding the underlying relationships between two matrices (X and Y, predictors and responses) by extracting latent variables to model the covariance structures of the two spaces. PLS models attempt to estimate axes or variates in the X matrix that explains the maximum variance in Y matrix. These methods are particularly useful when the X matrix has more variables than observations, and when high multicollinearity is present among X values. Additionally, sparse PLS methods include a process of selection of the predictor variables to facilitate biological interpretation of the results. The sPLS model was fitted using the “spls” function in *mixOmics* R package (Le Cao *et al.*, 2017). Tuning of the model suggested that extracting more than two variates did not improve the prediction of the response matrix (total Q value saturates at the second variate extracted). To visualise the results of the sPLS analysis, we plotted a Clustered Image Map (CIM), using the “cim” function in *mixOmics*, which shows the correlations between the predictor and the response variables while clustering both based on their similarity on the multivariate space.

To look for significant differences of the variables selected by the sPLS analysis among species, we performed analyses of variance (ANOVA) in which we related the environmental variables selected in the sPLS as a function of the species. This analysis allows us to estimate ranges of tolerance for the species and to test whether different species share their ecological niche or not. Differences amongst species were tested using the post-hoc Tukey HSD test. These analyses were done with the 82 springs in which at least one of the most frequent species was found. All ANOVAs accomplished the required statistical assumptions of normality, homoscedasticity and independence of the residuals. All the statistical analysis was performed using R statistical software (R Core Team, 2015).

Results

Water chemistry and climate of the studied springs

We found a relatively large gradient in water chemistry and climatological features of the surveyed springs. Mean annual temperature (MAT) ranged from 7.3 to 15.7ºC, mean annual precipitation (MAP) from 555 to 1180 mm year^{-1}, pH from 5.7 to 8.8, conductivity from 19 to 2160 µS cm^{-1} and altitude from 100 to 1570 m a.s.l. The springs of the Littoral mountain range are located at lower altitudes, being on average, 328 m above sea level (a.s.l.) and therefore, experienced the highest temperatures (13.9ºC on average) (Table 1). Springs from Lluçanès, Montseny-Guilleries and Garrotxa are found at intermediate altitudes, between 550 and 850 m a.s.l., and recorded temperatures between 11 and 12.5ºC, although some of Montseny springs experienced relatively high temperatures (up to 15.7ºC). Annual precipitation in La Garrotxa is higher than in any other of the studied regions (1049 mm), followed by Montseny-Guilleries, whereas Lluçanès and the Littoral mountain range are drier (Table 1).

In general, pH of the springs surveyed was slightly alkaline. However, pH in Garrotxa's springs is higher than those from the other regions studied (Table 1). Conductivity, though, is much more variable than pH. Lluçanès' springs are, on average, those with highest conductivity (1059 µS·cm^{-1}), whereas springs from the Littoral mountain range and Garrotxa are found in the mid-range (around 700 µS·cm^{-1}), and those from Montseny-Guilleries have the lowest conductivity values of all (286 µS·cm^{-1}) (Table 1).

Relationships between species presence and environmental variables

The sPLS model included conductivity, altitude, spring, summer and autumn temperature, winter, spring, summer and autumn precipitation, and temperature and precipitation seasonality as important predictors of bryophyte species assemblages in springs (Figure 2). The first variate explained 51.1% of the variability in the predictors and 12.9% of the variability of the bryophyte assemblages. As shown by Figure S4, no single species or spring was driving the results of our sPLS model. This variate was positively related to seasonal precipitation, altitude, and temperature seasonality and negatively related to conductivity, seasonal temperatures and precipitation seasonality (Figure S5). The second variate explained 28.1% of the variability in the predictors and 11.0% of the variability of the bryophyte assemblages. This variate was positively related mainly to summer precipitation and altitude and negatively related mainly to seasonal temperatures and precipitation seasonality.

Species were clustered into two big clusters (1 and 2) with two smaller clusters each (a and b, Figure 2). The first cluster of species (1a), formed by *Eucladium verticillatum, Pohlia melanodon, Didymodon tophaceus* and *Pellia endiviifolia,* was found to be related to warm and especially dry climate and high water conductivity. Their presence was also negatively related to altitude of the springs and temperature seasonality and, less strongly, positively related to precipitation seasonality. *Pallustriella commutata* and *Cratoneuron filicinum,* which formed cluster 1b, were found to be associated with dry and cold climate with low temperature and precipitation seasonality, but with rainy summers and located at high altitude. *Pallustriella commutata* was also positively related to high water conductivity, while no relationship was found with *C. filicinum*.

Kindbergia praelonga, Oxyrrhynchium speciosum and *Conocephalum conicum* formed cluster 2a and were more likely to be found in springs that have a warm and relatively dry climate in spring, and especially in summer, and high water conductivity (except *C. conicum*). The species of this cluster were also found in springs of low altitude and low seasonal variability of temperature and precipitation (low continentality). The last cluster of species (2b) was formed by *Plagiomnium undulatum*, *Brachythecium rivulare, Bryum pseudotriquetrum,* and *Platyhypnidium riparioides*. These species were the group of mosses related to the springs with the coldest and wettest climate, highest temperature seasonality but lowest precipitation seasonality, and highest altitude and having also low water conductivity. *Plagiomnium undulatum*, however, was not so strongly related to cold climate.

Of all studied species, *P. endiviifolia and C. conicum* were the species with the lower correlations (Figure 2). Table 3 shows the average values per species for the environmental variables included in the sPLS. Significant differences appear amongst species for all of the variables, which further corroborates results from the sPLS. This indicates that ecological niche separation occurs within the environmental variables considered.

Discussion

Our results show that macroclimate, altitude and water conductivity can explain variability in bryophyte assemblages in semi-natural and man-made springs. The distribution of some of the most abundant bryophyte species in our springs is clearly separated into different ecological niches, defined by the environmental variables we considered. In particular, our findings suggest that models predicting species distributions could be improved by including micro- and mesoscale environmental variables such as water conductivity and altitude in addition to macroscale variables such as climate. However, other environmental variables considered in previous studies (Longton *et al.*, 1983; Cattaneo & Fortin, 2000; Tessler *et al.*, 2014; Corbera *et al.*, 2015), such as main ions (Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, SO_4^{2-}, Cl^{-}, PO_4^{3-}), pollutants such as nitrate (Vanderpoorten & Klein, 1999), or type of microhabitat and its heterogeneity (Suren, 1996) could help to differentiate the ecological niche of bryophyte species even further. Despite that, the present study clearly shows several general trends in bryophyte assemblages in springs.

Determinants of bryophyte assemblages

The role of precipitation on bryophyte assemblages is evident in previous studies (Eldridge & Tozer, 1997; Gignac, 2001; Callaghan & Ashton, 2008a) although some other papers highlight that the number of rainy days per year is sometimes more important than the total amount of precipitation (Ratcliffe, 1968; Callaghan & Ashton, 2008b) because the time that the bryophytes are dry is more determinant than the total amount of water they receive (Pentecost & Zhang, 2006; Proctor *et al.*, 2007). However, this fact may not be so relevant in our study, because most of the sampled springs provide water almost constantly throughout the year. We found that *B. rivulare, P. riparioides, B. pseudotriquetrum,* which are species associated with streams and believed not to tolerate desiccation, are those with the highest affinity for high precipitation (Figure 2, Table 2). However, other species with supposedly equally high affinity for humidity like *P. endiviifolia*, *P. commutata, C. filicinum* and *E. verticillatum* (Table 2), do not show such a strong positive association with precipitation, but even show the opposite (Figure 2).

Kapfer *et al*. (2012) suggested that the length of time water resides in the aquifers of zones with lower precipitation is longer than in zones with higher precipitation (). A longer time in contact with the rock causes the water to dissolve more salts and increase its conductivity. This could explain the presence of known calcicolous species, associated with high conductivity water, in springs with low precipitation (Corbera *et al.*, 2015) (Figure 2). Despite the fact that bryophytes are able to tolerate wide ranges of temperature, we found different average seasonal temperatures and temperature seasonality (which relates to continentality) per species. This effect of temperature on bryophyte assemblages may also be mediated through its effect on the water availability of the spring. Almost all of our springs had forested areas nearby and which typically consume large quantities of subsoil water, especially under high temperatures (Fernández-Martínez *et al.*, 2014), thus reducing water runoff. Also, continentality increases temperature extremes throughout the year, which can certainly be an important factor favouring certain species versus others, depending on their temperature tolerance ranges.

Some studies have shown a great polarization in the distribution of bryophytes in relation to calcareous and non-calcareous lithology (Callaghan & Ashton, 2008a; Virtanen *et al.*, 2009) because of big differences in the cellular exchange capacity of Ca^{2+} (Bates, 1982).

In our study, species with affinity for calcareous lithology, like *D. tophaceus* and *E. verticillatum* (Table 2), have been found in springs with high water conductivity (on average, 972 and 886 $\mu S \cdot cm^{-1}$ respectively) while calcifugous species like *B. rivulare* (Longton *et al.*, 1983) have been found in springs with low water conductivity (292 $\mu S \cdot cm^{-1}$). However, in contrast to other studies carried out with bryophyte communities from peat lands and in the headwaters of rivers (Gignac, 1992; Tessler *et al.*, 2014), we did not find any significant relationship between water pH and bryophyte assemblages (Figure 2). This fact may be the result of having analysed only the 13 most frequent bryophyte species that were found in the springs. As these species are more frequent, they are also supposed to be more likely to be generalists and to tolerate wider ranges of water pH. Further work, based on more intensive sampling and focused on less frequent species, will help elucidate how water pH, conductivity and other environmental variables (e.g., climate, topography and ionic composition of water) affect bryophyte assemblages in Mediterranean springs. However, our results indicate that water conductivity could be a very suitable variable to include in species distribution models to improve their predictions on humid or sub-humid bryophytes, given the fact that it is relatively easy to obtain (it could be inferred from lithology) and significantly separates bryophyte species.

Conclusions

Our results clearly demonstrate that bryophyte assemblages in semi-natural springs can be explained using widely available variables such as mean seasonal temperatures and precipitation, temperature and precipitation seasonality (intra-annual variation), altitude, and water conductivity of the springs. Our results also point out the need to include micro- and mesoscale variables, in addition to macroscale variables to help improve the prediction of species distribution models.

Acknowledgements

MFM is funded by a postdoctoral subsidy of the University of Antwerp. We acknowledge the Institució Catalana d'Història Natural (ICHN) and the Institut d'Estudis Catalans (IEC) for funding the project. Also, to all the volunteers of the Delegation of the Serralada Litoral Central, Grup de Naturalistes d'Osona and Lluçanès Viu for helping with the survey of the springs. We also thank all those people who helped us to find the springs.

Taxonomic Additions and Changes: Nil.

References

Ah-Peng, C., Chuah-Petiot, M., Descamps-Julien, B., Bardat, J., Stamenoff, P. & Strasberg, D. 2007. Bryophyte diversity and distribution along an altitudinal gradient on a lava flow in La Réunion. *Diversity and Distributions*, 13: 654–62.

Andrew, N.R. & Rodgerson, L. 2003. Variation in invertebrate – bryophyte community structure at different spatial scales along altitudinal gradients. *Journal of Biogeography*, 30: 731–46.

Arts, G.H.P. 1990. Aquatic bryophyta as indicators of water quality in shallow pools and lakes in The Netherlands. *Annales Botanici Fennici*, 27: 19–32.

Atherton, I.D.M., Bosanquet, S.D.S. & Lawley, M.2010. *Mosses and liverworts of Britain and Ireland: a field guide*. Cardiff: British Bryological Society.

Bates, J.W. 1982. The role of exchangeable calcium in saxicolous calcicole and calcifuge mosses. *New Phytologist*, 90: 239–52.

Belland, R.J. 2005. A multivariate study of moss distributions in relation to environment in the Gulf of St. Lawrence region, Canada. *Canadian Journal of Botany*, 83: 243–63.

Belland, R.J. & Vitt, D.H. 1995. Bryophyte vegetation patterns along environmental gradients in continental bogs. *Ecoscience*, 2: 395–407.

Bruun, H.H., Moen, J., Virtanen, R., Grytnes, J.-A., Oksanen, L. & Angerbjörn, A. 2006. Effects of altitude and topography on species richness of vascular plants , bryophytes and lichens in alpine communities. *Journal of Vegetation Science*, 17: 37–46.

Callaghan, D.A. & Ashton, P.A. 2008a. Bryophyte distribution and environment across an oceanic temperate landscape. *Journal of Bryology*, 30(1): 23–35.

Callaghan, D.A. & Ashton, P.A. 2008b. Knowledge gaps in bryophyte distribution and prediction of species-richness. *Journal of Bryology*, 30: 147–58.

Le Cao, K.-A., Rohart, F., Gonzalez, I., Dejean, S., Gautier, B., Bartolo, F., Monget, P., Coquery, J., Yao, F-Z. & Liquet, B. 2017. mixOmics: Omics Data Integration Project. Available at: R package version 6.1.1. https://CRAN.R-project.org/package=mixOmics

Casas, C. 1958. Aportaciones a la flora briológica de Cataluña. Musgos y hepáticas del Montseny. *Anales del Instituto Botánico Antonio José Cavanilles de Madrid*, 16: 121–226.

Casas, C. 1959. Aportaciones a la flora briológica de Cataluña. Catálogo de las hepáticas y musgos del Montseny. *Anales del Instituto Botánico Antonio José Cavanilles de Madrid*, 17: 21–174.

Casas, C., Brugués, M. & Cros, R.M. 2001. *Flora de Briòfits dels Països Catalans. I. Molses*. Barcelona: Institut d'Estudis Catalans.

Casas, C., Brugués, M. & Cros, R.M. 2004. *Flora de Briòfits dels Països Catalans. II. Hepàtiques i antocerotes*. Barcelona: Institut d'Estudis Catalans.

Cattaneo, A. & Fortin, L. 2000. Moss distribution in streams of the Quebec Laurentian Mountains. *Canadian Journal Of Botany-Revue Canadienne De Botanique*, 78: 748–752.

Ceschin, S., Aleffi, M., Bisceglie, S., Savo, V. & Zuccarello, V. 2012. Aquatic bryophytes as ecological indicators of the water quality status in the Tiber River basin (Italy). *Ecological Indicators*, 14: 74–81.

Corbera, J., Fernández-Martínez, M., Jover, M., Torner, G., Calpe, M., Ciurana, O. & Sabater, F. 2015. Els briòfits de les fonts de la Serralada Litoral Central: composició específica i efecte dels paràmetres ambientals en la seva distribució. *L'Atzavara*, 25: 105–16.

Eldridge, D.J. & Tozer, M.E. 1997. Environmental factors relating to the distribution of terricolous bryophytes and lichens in semi-arid eastern Australia. *Bryologist*, 100: 28–39.

Fernández-Martínez, M., Bagaria, G., Raya, I., Preece, C., Margalef, O. & Domene, X. 2016. Terra Negra: un sòl sobre pissarres al Montnegre. *L'Atzavara*, 26: 67–72.

Fernández-Martínez, M., Vicca, S., Janssens, I.A., Luyssaert, S., Campioli, M., Sardans, J., Estiarte, M. & Peñuelas, J. 2014. Spatial variability and controls over biomass stocks, carbon fluxes and resource-use efficiencies in forest ecosystems. *Trees, Structure and Function*, 28: 597–611.

Gignac, L.D. 1992. Niche structure, resource partitioning , and species interactions of mire bryophytes relative to climatic and ecological gradients in western Canada. *American Bryological and Lichenological Society*, 95: 406–18.

Gignac, L.D. 1994. Peatland species preferences: An overview of our current knowledge base. *Wetlands*, 14: 216–22.

Gignac, D. 2001. New frontiers in bryology and lichenology: bryophytes as indicators of climate change. *Bryologist*, 104: 410–20.

Gignac, L.D. & Vitt, D. 1990. Habitat limitations of Sphagnum along climatic, chemical and physical gradients in mires of western Canada. *Bryologist*, 93: 7–22.

Grau, O., Grytnes, J.A. & Birks, H.J.B. 2007. A comparison of altitudinal species richness patterns of bryophytes with other plant groups in Nepal, Central Himalaya. *Journal of Biogeography*, 34: 1907–15.

Grytnes, J.A., Heegaard, E. & Ihlen, P.G. 2006. Species richness of vascular plants, bryophytes, and lichens along an altitudinal gradient in western Norway. *Acta Oecologica*, 29: 241–46.

Hill, M.O., Bell, N., Bruggeman-Nannenga, M., Brugués, M., Cano, M.J., Enroth, J., Flatberg, K.I., Frahm, J.-P., Gallego, M.T., Garilleti, R., Guerra, J., Hedenäs, L., Holyoak, D.T., Ignatov, M.S., Lara, F., Mazimpaka, V., Muñoz, J., & Garilleti, R. 2006. An annotated checklist of the mosses of Europe and Macaronesia. *Journal of Bryology*, 28: 198–267.

Kapfer, J., Audorff, V., Beierkuhnlein, C. & Hertel, E. 2012. Do bryophytes show a stronger response than vascular plants to interannual changes in spring water quality? *Freshwater Science*, 31: 625–35.

Lê Cao, K.-A., Rossouw, D., Robert-Granié, C. & Besse, P. 2008. A Sparse PLS for Variable Selection when Integrating Omics Data : Statistical Applications in Genetics and Molecular Biology. *Statistical Applications in Genetics and Molecular Biology*, 7: 1–35.

Longton, R.E., Schuster, R.M., Glime, J.M. & Vitt, D.H. 1983. Reproductive Biology: a comparison of bryophyte species diversity and niche structure of montane streams and stream banks. *Canadian Journal of Botany*, 1: 386–462.

Martín-Vide, J. 1992. El clima. In: C. Carreras, ed. *Geografia General dels Països Catalans. Enciclopèdia Catalana*. Barcelona, pp. 1–110.

Nicholson, B.J., Gignac, L.D. & Bayley, S.E. 1996. Peatland distibution along a north-south transect in the Mackenzie River Basin in relation to climatic and environmental gradients. *Vegetatio*, 126: 119–33.

Ninyerola, M., Pons, X., Roure, J.M., Ninyerola, M., Pons, X. & Roure, J.M. 2000. A methodological approach of climatological modelling of air temperature and precipitation through GIS techniques, A methodological approach of climatological modelling of air temperature and precipitation through GIS techniques. *International Journal of Climatology,* 20: 1823, 1823–41,.

Patiño, J., Sólymos, P., Carine, M., Weigelt, P., Kreft, H. & Vanderpoorten, A.

2015. Island floras are not necessarily more species poor than continental ones. *Journal of Biogeography*, 42: 8–10.

Pentecost, A. & Zhang, Z. 2006. Response of bryophytes to exposure and water availability on some European travertines. *Journal of Bryology*, 28(1): 21–6.

Pentecost, A. & Zhaohui, Z. 2002. Bryophytes from some travertine-depositing sites in France and the U.K.: relationships with climate and water chemistry. *Journal of Bryology*, 24: 233–41.

Peñuelas, J. 1983. La qualitat de les aigües del riu Muga: Dades físiques, químiques i biològiques. *Annals de l’Institut d’Estudis Empordanesos*, 16: 33–49.

Peñuelas, J. & Sabater, F. 1987. Distribution of macrophytes in relation to environmental factors in the Ter River, NE Spain. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, 72: 41–58.

Pons, X. 1996. Estimación de la radiación solar a partir de modelos digitales de elevaciones. Propuesta metodológica. In: *Modelos y sistemas de información geográfica,* pp. 87–97.

Proctor, M.C.F., Oliver, M.J., Wood, A.J., Alpert, P., Stark, L.R., Cleavitt, N.L. &Mishler, B.D. 2007. Desiccation-tolerance in bryophytes: a review. *Bryologist*, 110: 595–621.

R Core Team. **2015**. R: A Language and Environment for Statistical Computing, version 3.2.3. Available at: https://cran.r-project.org.

Ratcliffe, D.A. 1968. An ecological account of Atlatnic Bryophytes in the British Isles. *New Phytologist*, 67: 365–439.

Sabater, F., Fernández-Martínez, M., Corbera, J., Calpe, M., Torner, G., Cano, O., Corbera, G., Ciurana, O. & Parera, J.M. 2015. Caracterització hidrogeoquímica de les fonts de la Serralada Litoral Central en relació a la litologia i als factors ambientals. *L’Atzavara*, 25: 93–104.

Slack, N.G. 1990. Bryophytes and ecological niche theory. *Botanical Journal of the Linnean Society*, 104: 187–213.

Smith, A.J.E. 1978. *The moss flora of Britain and Ireland*. 1st edn. Cambridge: Cambridge University Press.

Smith, A.J.E. 1990. *The liverworts of Britain and Ireland*. Cambridge: Cambridge University Press.

Spitale, D. 2016. The interaction between elevational gradient and substratum reveals how bryophytes respond to the climate. *Journal of Vegetation Science*, 27: 844–53.

Spitale, D., Petraglia, A. & Tomaselli, M. 2009. Structural equation modelling detects unexpected differences between bryophyte and vascular plant richness along multiple environmental gradients. *Journal of Biogeography*, 36: 745–55.

Strohbach, M., Audorff, V. & Beierkuhnlein, C. 2009. Drivers of plant species composition in siliceous spring ecosystems: groundwater chemistry, catchment traits or spatial factors? *Journal of Limnology*, 68: 375–84.

Suren, A.M. 1996. Bryophyte distribution patterns in relation to macro-, meso-, and micro-scale variables in South Island, New Zealand streams. *New Zealand Journal of Marine and Freshwater Research*, 30: 501–23.

Suren, A.M. & Duncan, M.J. 1999. Rolling stones and mosses: effect of substrate stability on bryophyte communities in streams. *Journal of the North American Benthological Society*, 18: 457–67.

Suren, A.M. & Ormerod, S.J. 1998. Aquatic bryophytes in Himalayan streams: Testing a distribution model in a highly heterogeneous environment. *Freshwater Biology*, 40: 697–716.

Tessler, M., Truhn, K.M., Bliss-Moreau, M. & Wehr, J.D. 2014. Diversity and distribution of stream bryophytes : does pH matter? *Freshwater Science*, 33: 778–87.

Tomaselli, M., Spitale, D. & Petraglia, A. 2011. Phytosociological and ecological study of springs in Trentino (south-eastern Alps, Italy). *Journal of Limnology*, 70: 25–53.

Tyler, T., Bengtsson, F., Dahlberg, C.J., Lönnell, N., Hallingbäck, T. & Reitalu, T. 2018. Determinants of bryophyte species composition and diversity on the Great Alvar of Öland , Sweden. *Journal of Bryology*, 40(1): in press.

Vanderpoorten, A. & Klein, J.P. 1999. Variations of aquatic bryophyte assemblages in the Rhine Rift related to water quality. 2. The waterfalls of the Vosges and the Black Forest. *Journal of Bryology*, 21: 109–15.

Vanderpoorten, A. & Palm, R. 1998. Canonical variables of aquatic bryophyte combinations for predicting water trophic level. *Hydrobiologia*, 386: 85–93.

Vieira, C., Aguiar, F.C. & Ferreira, M.T. 2014. The relevance of bryophytes in the macrophyte-based reference conditions in Portuguese rivers. *Hydrobiologia*, 737: 245–64.

Virtanen, R., Ilmonen, J., Paasivirta, L. & Muotka, T. 2009. Community concordance between bryophyte and insect assemblages in boreal springs: a broad-scale study in isolated habitats. *Freshwater Biology*, 54: 1651–62.

Wang, J., Vanderpoorten, A., Hagborg, A., Goffinet, B., Laenen, B. & Patiño, J. 2017. Evidence for a latitudinal diversity gradient in liverworts and hornworts. *Journal of Biogeography,* 44: 487–8.

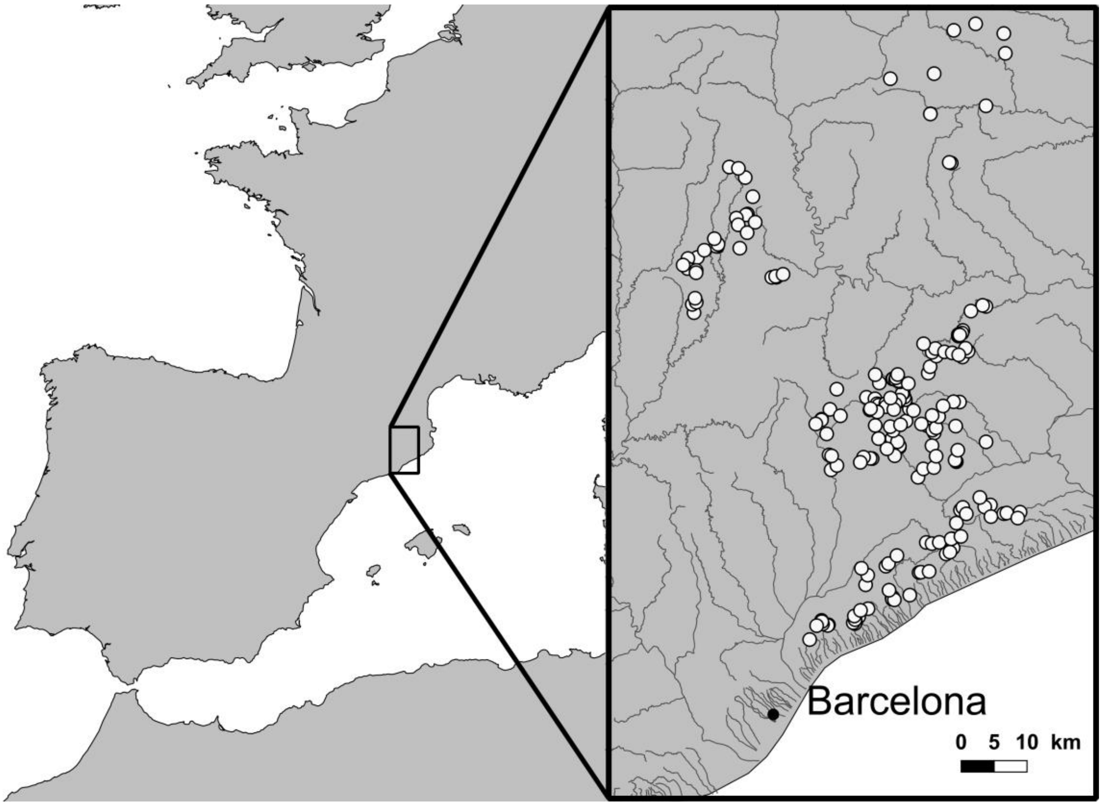
Wolf, J.H.D. 1993. Diversity Patterns and Biomass of Epiphytic Bryophytes and Lichens Along an Altitudinal Gradient in the Northern Andes. *Annals of the Missouri Botanical Garden*, 80: 928–60.

Figure captions

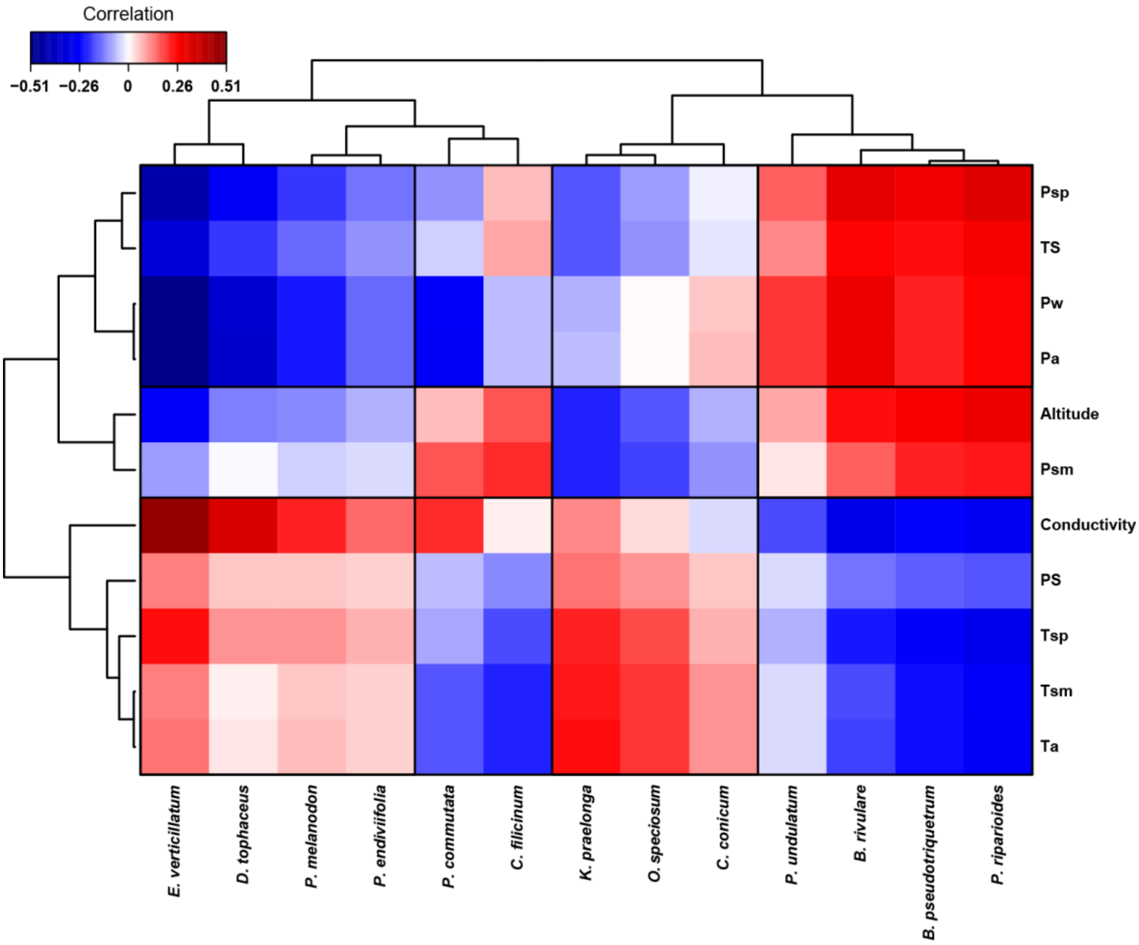
Figure 1. Map showing the location of the surveyed springs (white dots).

Figure 2. Clustered image map of the sPLS model showing the correlations between environmental predictors and presence of bryophyte species. Species and predictors have been clustered according to their similarity forming groups that we split using black lines. Abbreviations: T indicates temperature, P indicates precipitation, and seasons were indicated in lowercase letters as winter (w), spring (sp), summer (sm) and autumn (a). PS and TS indicate seasonality of precipitation and temperature respectively.

Figure 1.



520 **Figure 2**



521

Table 1. Average values for altitude, climate (mean ± standard error of the mean [SE]), water pH and conductivity and bryophyte species richness of the springs and average values per region. Lluçanès is a calcareous region, Montseny and the Central Littoral mountain range have granitic and metamorphic lithology and Garrotxa springs are located over volcanic and calcareous rocks. Acronyms are MAT for mean annual temperature and MAP for mean annual precipitation. Units are degrees Celsius for MAT, mm·year^{-1} for MAP, µS·cm^{-1} for conductivity and metres for altitude.

	Total	**Lluçanès**	**Montseny - Guilleries**	**Central Littoral**	**Garrotxa**
Springs	198	32	101	55	10
MAT	12.01 ± 0.13	11.38 ± 0.12	11.18 ± 0.30	13.90 ± 0.16	12.11 ± 0.52
MAP	863 ± 11	766 ± 22	947 ± 19	728 ± 14	1049 ± 31
Altitude	660 ± 24	703 ± 29	828 ± 53	328 ± 25	640 ± 109
pH	7.17 ± 0.04	7.10 ± 0.09	7.18 ± 0.11	7.09 ± 0.10	7.65 ± 0.13
Conductivity	546 ± 32	1059 ± 100	286 ± 50	701 ± 74	688 ± 186
Sp. richness	2.71 ± 0.12	3.16 ± 0.44	2.75 ± 0.23	2.05 ± 0.21	4.27 ± 1.31

Table 2. List of bryophyte species found in ten or more springs and their habitat preferences according to (Casas, 1958 & 1959; Casas et al., 2001 & 2004; Atherton et al., 2010) and their ecological characterization following Dierßen (2001). Column M/L indicates whether species are mosses (M) or liverworts (L). Column N indicates the number of springs in which each species has been found. Substrate: h.a., highly acidophilous; c.a., considerably acidophilous; m.a., moderately acidophilous; s.n., subneutrophylous; b., basophilous. Humidity: h.h., highly hygrophilic; c.h., considerably hygrophilic; m.h., moderately hygrophilic; m., mesophilous; m.x., moderately xerophilous; c.x., considerably xerophilous; h.x., highly xerophilous.

				Substrate					Humidity						
	M/L	**N**	**Preferred habitat**	**h.a.**	**c.a.**	**m.a.**	**s.n.**	**b.**	**h.h.**	**c.h.**	**m.h.**	**m**	**m.x.**	**c.x.**	**h.x.**
Oxyrrhynchium speciosum (Brid.) Warnst.	M	64	Wet forests, peat lands and springs												
Pellia endiviifolia (Dicks.) Dum.	L	57	Edge of streams, shaded forests, peatlands and wet rocks												
Eucladium verticillatum (With.) Bruch & Schimp.	M	50	Edge of streams, wet and shaded locations. Calcareous water												
Cratoneuron filicinum (Hedw.) Spruce	M	44	Mixed habitats: edge of streams, rocks and barks												
Plagiomnium undulatum (Hedw.) T.J.Kop.	M	36	Wet rocks, forests, grasslands. Occasionally in streams												
Platyhypnidium riparioides (Hedw.) Dixon	M	24	Rocks and roots underwater or semi-underwater in streams												
Brachythecium rivulare Schimp.	M	20	Aquatic environment. Soils and rocks in streams												
Didymodon tophaceus (Brid.) Lisa	M	20	Wet rocks, calcareous soils, muddy edges of bogs and streams												
Palustriella commutata (Hedw.) Ochyra	M	19	Wet habitats like springs. Calcareous substrates.												
Conocephalum conicum (L.) Underw.	L	13	Wet rocks and soils, usually in contact with water.												
Bryum pseudotriquetrum (Hedw.) P.Gaertn. *et al.*	M	12	Edge of rivers, streams or lakes												
Pohlia melanodon (Brid.) A.J.Shaw	M	12	Wet and muddy soils. Usually on the edge of streams												
Kindbergia praelonga (Hedw.) Ochyra	M	11	Grasslands, forests, bark and branches and edges of water streams												

Table 3. Species average (± standard error of the mean) values for environmental variables that were selected in the sPLS for explaining variability in species distribution. Letters (a, b, c, d, e, f) indicate groups according to the post-hoc Tukey HSD test for multiple comparisons. All ANOVA tables were statistically significant at the <0.001 level. Units were metres for altitude, mm year^{-1} for precipitation variables and Celsius degrees for temperature variables and (µS·cm^{-1}) for conductivity. Precipitation seasonality (PS) and temperature seasonality (TS) were unit less. Abbreviations: T indicates temperature, P indicates precipitation, and seasons were indicated in lowercase letters as winter (w), spring (sp), summer (sm) and autumn (a).

Species	**Tsp**		**Tsm**		**Ta**	
Bryum pseudotriquetrum	6.98 ± 0.58	a	16.1 ± 0.6	a	10.2 ± 0.5	a
Brachythecium rivulare	7.50 ± 0.45	a	16.6 ± 0.5	a	10.6 ± 0.4	ab
Platyhypnidium riparioides	7.72 ± 0.41	ab	16.7 ± 0.4	ab	10.7 ± 0.4	abc
Plagiomnium undulatum	9.45 ± 0.32	bc	18.5 ± 0.3	bc	12.2 ± 0.3	cd
Cratoneuron filicinum	9.58 ± 0.30	cd	18.8 ± 0.3	c	12.3 ± 0.3	d
Oxyrrhynchium speciosum	10.15 ± 0.25	cdc	19.2 ± 0.3	cd	13.0 ± 0.2	def
Palustriella commutata	9.88 ± 0.46	cdc	19.4 ± 0.5	cd	12.5 ± 0.4	bcde
Pellia endiviifolia	10.45 ± 0.27	cdc	19.6 ± 0.3	cd	13.2 ± 0.2	def
Conocephalum conicum	11.03 ± 0.56	cdc	20.0 ± 0.6	cd	13.6 ± 0.50	def
Pohlia melanodon	10.88 ± 0.58	cdc	20.1 ± 0.6	cd	13.6 ± 0.5	def
Eucladium verticillatum	11.06 ± 0.28	c	20.2 ± 0.3	d	13.7 ± 0.3	ef
Didymodon tophaceus	10.91 ± 0.45	cdc	20.3 ± 0.5	cd	13.5 ± 0.4	def
Kindbergia praelonga	11.84 ± 0.61	dc	20.5 ± 0.6	cd	14.8 ± 0.6	f

	Pw		**Psm**		**Pa**	
Kindbergia praelonga	159.2 ± 11.8	abc	150.9 ± 15.7	a	252.5 ± 10.7	abcd
Oxyrrhynchium speciosum	182.1 ± 4.9	bcd	175.0 ± 6.5	ab	263.1 ± 4.4	bc
Pohlia melanodon	150.8 ± 11.3	ab	183.2 ± 15.0	abc	233.6 ± 10.2	ab
Conocephalum conicum	196.5 ± 10.9	bcde	188.5 ± 14.4	abcd	267.8 ± 9.8	abcde
Plagiomnium undulatum	201.4 ± 6.3	cde	194.3 ± 8.3	abc	278.0 ± 5.7	cde
Brachythecium rivulare	220.0 ± 8.8	e	196.7 ± 11.6	abcd	293.7 ± 7.9	de
Eucladium verticillatum	146.3 ± 5.5	a	199.2 ± 7.4	abc	234.9 ± 5.0	a
Pellia endiviifolia	177.5 ± 5.2	bc	200.6 ± 6.9	abc	260.4 ± 4.7	bc
Didymodon tophaceus	138.6 ± 8.8	a	210.0 ± 11.6	abcd	226.6 ± 7.9	a
Platyhypnidium riparioides	219.2 ± 8.0	e	216.4 ± 10.6	bcd	295.6 ± 7.2	e
Bryum pseudotriquetrum	221.1 ± 11.3	de	218.9 ± 15.0	abcd	303.3 ± 10.2	e
Cratoneuron filicinum	191.3 ± 5.9	bcde	221.7 ± 7.8	cd	269.5 ± 5.3	bcde
Palustriella commutata	155.7 ± 9.0	ab	248.0 ± 11.9	d	244.7 ± 8.1	ab

Table 3. Continuation.

Species	Psp		PS		TS	
Kindbergia praelonga	18.10 ± 1.01	a	0.25 ± 0.012	cd	0.40 ± 0.03	a
Pohlia melanodon	19.88 ± 0.96	abc	0.24 ± 0.011	abcd	0.48 ± 0.02	abc
Eucladium verticillatum	20.03 ± 0.47	ab	0.25 ± 0.006	d	0.48 ± 0.01	ab
Didymodon tophaceus	20.03 ± 0.75	ab	0.25 ± 0.009	d	0.50 ± 0.02	abc
Oxyrrhynchium speciosum	21.24 ± 0.42	abc	0.23 ± 0.005	bcd	0.50 ± 0.01	bc
Pellia endiviifolia	22.06 ± 0.44	bcd	0.22 ± 0.005	abcd	0.50 ± 0.01	bc
Conocephalum conicum	22.45 ± 0.93	abcdc	0.21 ± 0.011	abcd	0.48 ± 0.02	abc
Palustriella commutata	22.63 ± 0.77	bcdc	0.24 ± 0.009	abcd	0.54 ± 0.02	bcde
Plagiomnium undulatum	23.41 ± 0.53	cdc	0.21 ± 0.006	abc	0.53 ± 0.01	bcd
Cratoneuron filicinum	23.96 ± 0.50	dc	0.20 ± 0.006	a	0.54 ± 0.01	cd
Brachythecium rivulare	24.89 ± 0.75	dc	0.20 ± 0.009	ab	0.61 ± 0.02	de
Platyhypnidium riparioides	25.36 ± 0.68	c	0.20 ± 0.008	ab	0.60 ± 0.02	de
Bryum pseudotriquetrum	25.84 ± 0.96	c	0.20 ± 0.011	abc	0.64 ± 0.02	e

Species	Conductivity		Altitude	
Platyhypnidium riparioides	227.9 ± 82.4	a	893.5 ± 54.3	cd
Brachythecium rivulare	291.9 ± 90.3	ab	890.9 ± 59.4	cd
Plagiomnium undulatum	300.0 ± 64.7	a	670.8 ± 42.6	abc
Bryum pseudotriquetrum	300.7 ± 116.6	ab	1077.6 ± 76.7	d
Conocephalum conicum	429.5 ± 112.0	ab	448.7 ± 3.7	a
Oxyrrhynchium speciosum	505.1 ± 50.5	ab	551.7 ± 33.2	a
Cratoneuron filicinum	560.3 ± 60.9	ab	755.5 ± 40.1	bc
Kindbergia praelonga	582.3 ± 121.8	abc	380.5 ± 80.1	a
Pellia endiviifolia	611.9 ± 53.5	b	589.6 ± 35.2	ab
Palustriella commutata	717.9 ± 92.7	bc	669.2 ± 61.0	abc
Eucladium verticillatum	885.5 ± 57.1	c	516.7 ± 37.6	a
Didymodon tophaceus	971.9 ± 90.3	c	555.2 ± 59.4	ab
Pohlia melanodon	1122.3 ± 116.6	c	558.6 ± 76.7	ab

Supplementary information

Figure S1: Picture of a spring surveyed. The yellow line indicates the sampling zone, coinciding with the area under the influence of water.

Figure S2: Graph showing species richness as a function of the area sampled in a subset of 41 sampled springs. Units of sample area are m^{-2}. No significant correlation between species number and area was found.

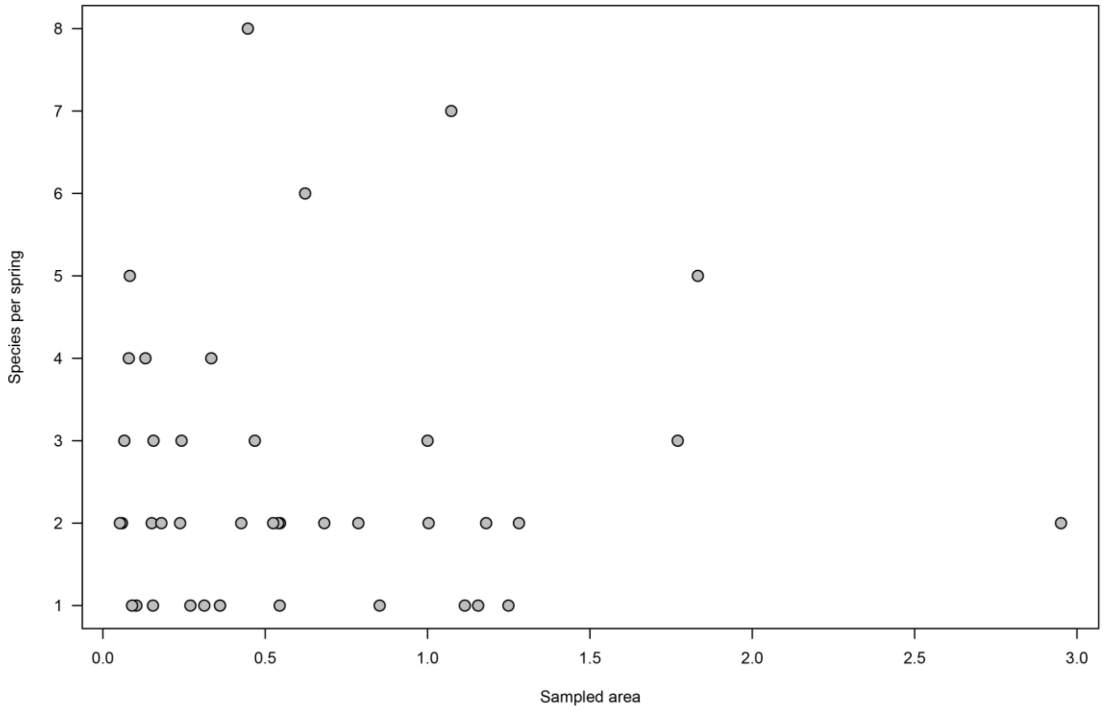


Figure S3. Histogram showing species richness of the studied springs.

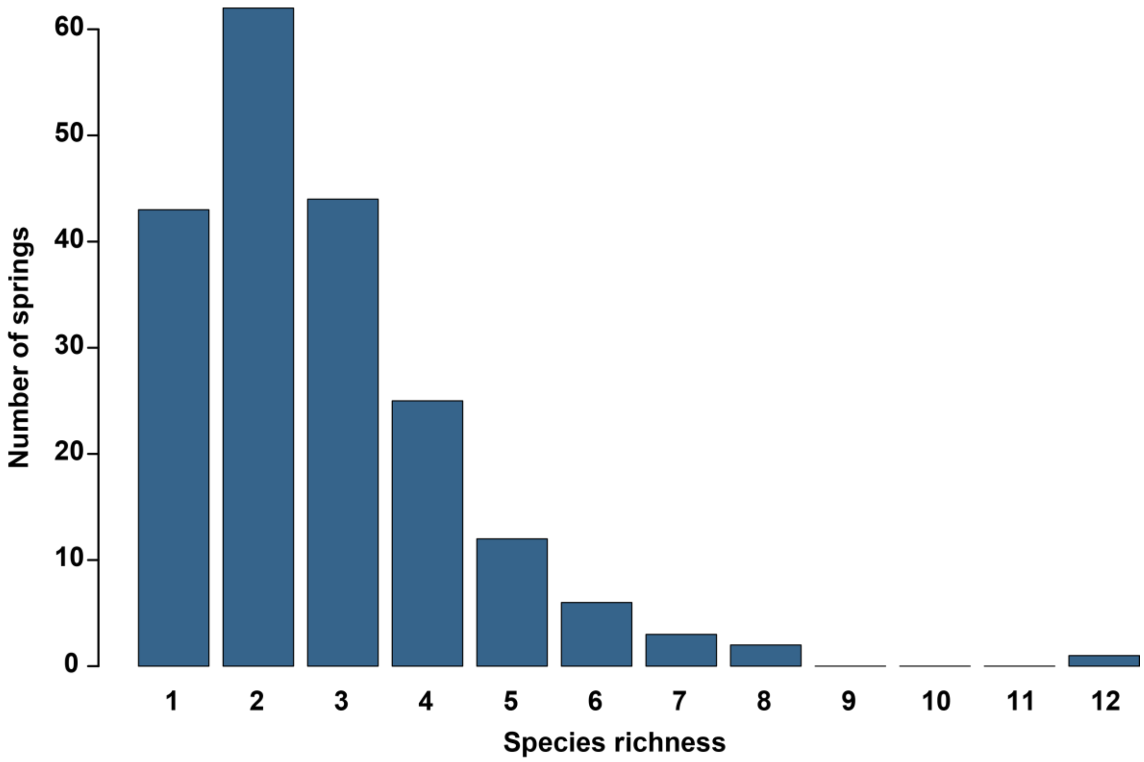


Figure S4: Site scores of the sPLS relating environmental variables (predictors) and bryophyte presence (species). None of the variates were driven by single sites or species.

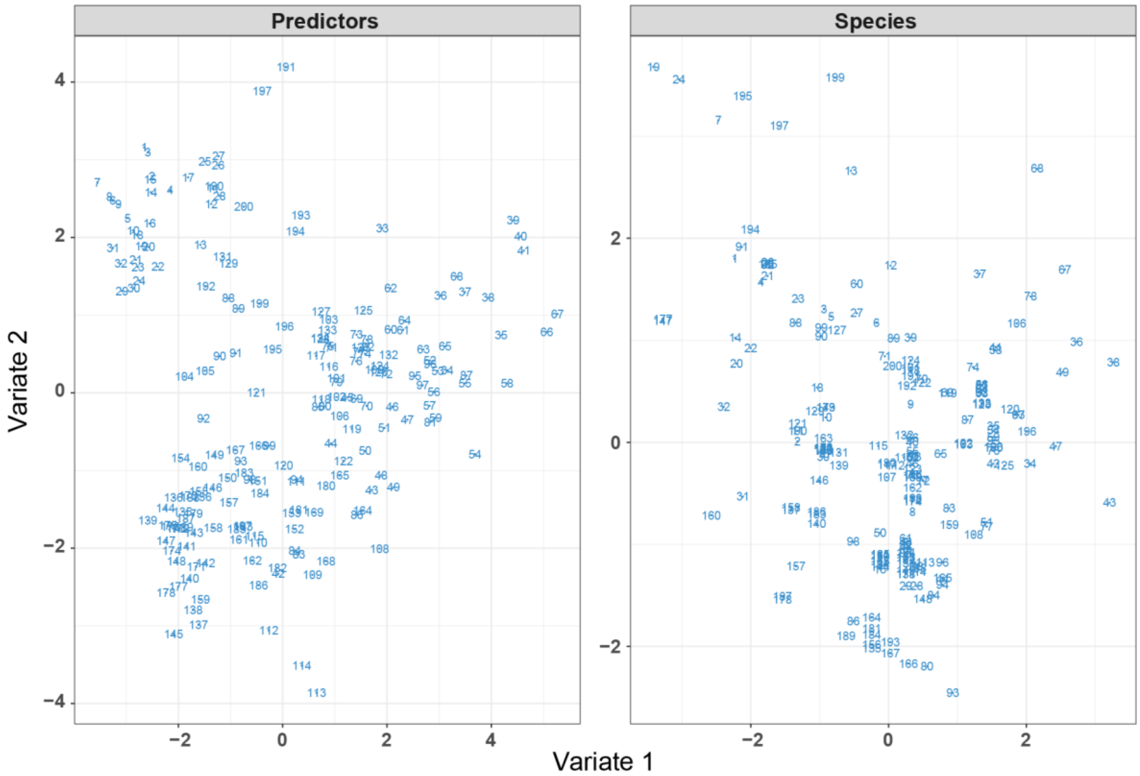


Figure S5: Correlation circle plot showing the results of the sPLS model relating environmental variables (predictors – in blue) and bryophyte presence (species – in green). Prec: precipitation, Temp: temperature.