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Reference:

Fernandez-Martinez Marcos, Preece Catherine, Corbera Jordi, Cano Oriol, Garcia-Porta Joan, Bogdziewicz Michał, Sardans Jordi, Janssens Ivan, Sabater Francesc, Peñuelas Josep.- Nutrients control reproductive traits of hygrophytic bryophytes
Freshwater biology - ISSN 0046-5070 - 66:7(2021), p. 1436-1446
Full text (Publisher's DOI): <https://doi.org/10.1111/FWB.13729>
To cite this reference: <https://hdl.handle.net/10067/1784980151162165141>

Nutrients control reproductive traits of hygrophytic bryophytes

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Running title: Nutrients control reproductive bryophyte traits

Key words: bryophyte, sporophyte, phosphorus, reproduction, springs

Abstract

1. Nutrient economy and plant nutrient concentrations have been suggested as important selective pressures of reproductive traits in vascular plants. However, evidence supporting that the reproduction of bryophytes and their reproductive traits have also been conditioned by nutrient availability is lacking.
2. We here analysed the nutrient (N, P, K, and Fe) concentration of 35 aquatic and semi-aquatic bryophytes and the chemistry of the spring water in which they lived to determine whether bryophyte nutrient concentrations were correlated with the frequency of sexual reproduction, main reproductive mode (sexual vs asexual), dioicy, and spore diameter, while controlling for climate and nutrient concentrations in the spring water.
3. Water nutrient concentrations, except for K, had a small but positive influence on bryophyte nutrient concentrations. P-rich and K-poor species produced sporophytes more often and were more likely to reproduce sexually and be monoicous. Spore diameter was smaller in P- and Fe-rich mosses but larger in N-rich mosses living in humid climates. P-rich species produced thus more and smaller spores and were therefore able to propagate more extensively.
4. Overall, our results suggest that nutrient economy, especially P economy, has played an important role in shaping the reproductive traits of bryophytes.

Introduction

Reproduction is one of the most important features of the life cycle of any organism. Sexual reproduction allows the reshuffling of previously acquired mutations which helps to maximize the genetic and phenotypic variation of the descendants, being the basis for the evolution of all species. Sexual reproduction has been extensively investigated in vascular plants because of the strong repercussions that fruit or seed production have on humans (such as agriculture and food security) and ecosystems (for example, plant dispersal or animal population dynamics). The evolution of reproductive traits, such as the interannually variable reproduction of wild plants (Fernández-Martínez *et al.*, 2019; Herrera *et al.*, 1998) or the evolution of crop yield from wild to domesticated plants (Abbo, Lev-Yadun & Gopher, 2010; Preece *et al.*, 2017), has also been the focus of multiple investigations. However, the study of the reproductive traits of bryophytes has received much less attention, even though a good understanding of how they work may help us better understand the evolutionary constraints of reproductive traits in plants. Bryophytes are also much simpler than vascular plants and highly sensitive to environmental conditions (Porley & Hodgetts, 2005). These features make them extremely interesting subjects of study for testing ecological and evolutionary hypotheses.

The elemental composition of an organism is a strong determinant of how it functions and responds to its environment (Sternner & Elser, 2002; Güsewell, 2004; Sardans & Peñuelas, 2015; Peñuelas *et al.*, 2019, 2020). Fruits and seeds are especially dependent on plant nutrient reserves, because they are enriched in nutrients compared to vegetative tissues (Reekie & Bazzaz, 1987). Consequently, a relatively large set of studies have reported an important role of plant nutrient concentrations in determining the frequency and strength of the reproductive efforts of wild plants. Most of these studies have

concluded that differences in the availabilities of N and P are responsible for differences in reproductive efforts amongst stems, individuals, populations, and species (Han *et al.*, 2013; Fujita *et al.*, 2014; Miyazaki *et al.*, 2014; Fernández-Martínez *et al.*, 2017, 2019b) and that nutrient concentrations (Camarero *et al.*, 2010; Sala *et al.*, 2012) and growth (Sánchez-Humanes, Sork & Espelta, 2011; Vergotti *et al.*, 2019) of vegetative tissues may decrease after reproduction. In algae, instead, the relationship between nutrient availability and reproduction seems to be a bit more complicated. While some species seem to require no limitation of nutrients to engage in sexual reproduction, other species seem to reproduce only under low concentrations of N or P (Agrawal, 2012).

In bryophytes, a few studies have suggested that growth and sexual reproduction (i.e. sporophyte production) are affected by the availability of nutrients, including N, P, K, and Fe (Machlis, 1962; Selkirk, 1979; Chopra & Rahbar, 1982), and that sporophyte production drastically reduces the rate of growth of gametophytes (Ehrlén, Bisang & Hedenäs, 2000; Bisang & Ehrlén, 2002; Rydgren & Økland, 2003). Ecophysiological features of bryophytes and vascular plants are thus more similar than those relating to algae, at least in their response to sexual reproduction and nutrient availability. However, to date, whether differences in bryophyte nutrient (N, P, K, and Fe) concentration amongst species are associated with differences in their reproductive traits, remains unknown.

To fill in this gap, we here collected 253 samples from 35 aquatic and semi-aquatic (hygrophytic) bryophyte species inhabiting springs to determine whether bryophyte nutrient concentrations were associated with their reproductive traits. Springs are important hotspots of biodiversity (Cantonati, Gerecke & Bertuzzi, 2006; Cantonati *et al.*, 2012, 2020), especially in the Mediterranean and semi-arid regions, because they behave like small islands in which water is continuously available (Bes *et al.*, 2018;

Fernández-Martínez *et al.*, 2019), providing the ecological requirements of many taxa that would disappear if the springs would be disturbed, polluted or dried out (Heino *et al.*, 2005; Fernández-Martínez *et al.*, 2020a). One of these groups is aquatic and semi-aquatic bryophytes that, additionally, can act as engineering species (Cantonati *et al.*, 2012) in those environments (e.g., tufa formation). However, the bryophyte species found in spring ecosystems are also found in other semi-aquatic, aquatic or water-dominated ecosystems such as rivers, ponds and fens, for which any conclusion drawn with spring bryophytes can be directly transferred to many other freshwater ecosystems. Additionally, the study system spring-aquatic bryophyte is very convenient to study how environmental nutrient availability (e.g., water chemistry) affects bryophyte nutrient concentrations (Fernández-Martínez *et al.*, 2021).

So the main objective of this study was to test whether bryophyte nutrient concentrations were related to their frequency of sporophyte production. We also determined whether the main reproductive mode (sexual or asexual), dioicy (separate female and male organisms), and spore diameter were correlated with differences in nutrient concentrations of the gametophyte amongst bryophyte species. Given the paramount role that foliar nutrient concentrations play in shaping the reproductive behaviour of vascular plants, we hypothesised that bryophyte N, P, K, and Fe concentrations would also determine bryophyte reproductive traits. Moreover, we hypothesised that the availability of these nutrients in the water from the springs would affect both bryophyte nutrient concentrations and frequency of sexual reproduction. Our results will help us to further understand the role of nutrients in the evolution of reproductive traits of bryophytes and other plants.

Methods

Field sampling and laboratory analyses

We collected 253 bryophyte samples from 35 aquatic and semi-aquatic species (hygrophytic bryophytes (Dierssen, 2001), 29 mosses and six liverworts, **Table S1**) from 164 springs distributed across Catalonia (northeastern Iberian Peninsula, **Figure S1**) along a large gradient of hydrochemical and climatic conditions (Fernández-Martínez et al., 2019). Our study area has a Mediterranean climate, ranging from humid to sub-humid, but with large differences in mean annual temperature (from 4.2 to 15.7 °C) and mean annual precipitation (from 567.4 to 1202.4 mm y⁻¹).

All springs surveyed continuously drained untreated water from aquifers, so the chemical features of the water were constant within a spring over time (Fernández-Martínez *et al.*, 2019a). The bryophytes were in constant contact with water (e.g., by water spray, flooding or dripping) because the water flows continuously throughout the year. Occasional interruptions in water flow occurred only in winter when the water froze or in summer due to intense drought. All springs had a similar morphology, including a wet, rocky wall, one spout (sometimes more than one) from which the water emerges continuously, a sink, and a channel to drain the water. Most of the springs were small human constructions for collecting water from aquifers, but water was not interrupted by the presence of a tap (**Figure S2**). Water discharged varied amongst springs, ranging from dripping over the wall (<1 ml s⁻¹) to more than 2500 ml s⁻¹, with a median discharge of 36 ml s⁻¹.

We measured the pH and electric conductivity of the spring water using a combined pH and conductivity meter. Filtered water samples (at 0.45 µm) were stored frozen in the laboratory at -20 °C until the moment of the analyses of main nutrients: nitrate (NO₃⁻) and K⁺ by ion chromatography, phosphate (PO₄³⁻) by colorimetry following the method of Murphy and Riley (1962), and Fe by inductively coupled plasma-mass spectrometry. The detection limit for K⁺ was 0.1 mg L⁻¹, for NO₃⁻ was 0.8 mg L⁻¹, and for PO₄³⁻ and

Fe was $2 \mu\text{g L}^{-1}$. NH_4^+ was also analysed by ion chromatography, but all springs presented values below the limit of detection ($<0.05 \text{ mg L}^{-1}$). We refer to Fernández-Martínez *et al.* (2019b) for more details of the chemical analyses. We obtained the geographical coordinates of the springs using a GPS device. The coordinates allowed us to extract mean monthly temperature and precipitation from the Climatic Atlas of Catalonia (Pons [1996], Ninyerola *et al.*, [2000], available at <http://www.opengis.uab.cat/acdc/index.htm>). We calculated mean annual temperature as the average of the monthly mean temperatures. We also calculated annual water availability for each spring as the annual sum of monthly precipitation minus evapotranspiration. Evapotranspiration was calculated following Hargreaves' equation (Hargreaves, 1994) and using the irradiance estimated by the latitude of each spring. Hence, the influence of evapotranspiration on the total water budget of each spring depended on the temperature, the latitude and the amount of rainfall at the spring. The calculations were performed with the “spei” R package (Beguería & Vicente-serrano, 2012).

We collected a sample of bryophytes within the spray zone at each spring, in direct contact with the water or submerged. Bryophytes were collected at the same moment that we sampled the water from the springs. Sampling seasons took place during spring, summer and autumn and from 2013 to 2019. Despite the possibility that seasonal differences in the elemental composition of bryophytes may occur, recent literature indicates that these organisms present species-specific elemental compositions (Fernández-Martínez *et al.*, 2021), thus allowing a reliable comparison of elements and traits amongst species. Bryophytes were identified using three identification keys (Casas *et al.*, 2001, 2004 and Smith, 1990) following the nomenclature of Hill *et al.*, (2006). Samples were dried and stored after species identification. Before analysing the

elemental composition of the bryophytes, we first submerged them in a solution of acetic acid at pH 2.7 to remove any CaCO_3 incrustation. The bryophytes were then rinsed with distilled water, dried at 60 °C for 48 h, and ground to a powder using liquid nitrogen and a mortar. Bryophyte N concentration was determined by using a Flash EA1112 and TC/EA coupled to a stable isotope mass spectrometer Delta C through a ConFlo III interface (ThermoFinnigan). Bryophyte P, K, and Fe concentrations were determined by inductively coupled plasma mass/optical emission spectrophotometry (ELAN 600 and Optima 8300 respectively, Perkin Elmer) after the samples were digested overnight at 90 °C with nitric acid and hydrogen peroxide at a 2:1 proportion. All analyses were carried out by the technical staff at the Scientific and Technical Centers of the University of Barcelona.

Due to the difficulty of directly assessing reproductive traits for the 35 species studied here, we used four reproductive traits extracted from the literature (Hill *et al.*, 2007) to determine whether bryophyte elemental composition was associated with bryophyte reproduction: sporophyte frequency (on a semiquantitative scale from 1 to 4 [rare=1, occasional=2, frequent=3, abundant=4]), main reproductive mode (sexual or asexual), dioicy (0 monoicous, 1 dioicous), and mean spore diameter (average of the smallest and largest diameters). Data for sporophyte frequency and reproductive modes from (Hill *et al.*, 2007) were adjusted to match observations in our study area following (Cros, 1985) and as previously used in Fernández-Martínez *et al.*, (2019).

Statistical analyses

We first tested whether bryophyte species inhabiting springs with higher water nutrient (NO_3^- , PO_4^{3-} , K^+ , and total Fe) concentrations also had higher nutrient (N, P, K, and Fe) bryophyte concentrations and in turn had higher frequencies of sporophyte production.

We conducted a phylogenetic path analysis using the R package *phylopath* (Hardenberg & Gonzalez-Voyer, 2013; van der Bijl, 2018), where water nutrient concentrations were set to affect bryophyte nutrient concentrations, which were set to affect sporophyte frequency. We also added a phylogenetic path from bryophyte N concentration to P concentration (this path received more support than the path in the opposite direction based on Akaike's information criterion). We performed this analysis twice: using all bryophytes (mosses + liverworts, N=35) and using only mosses (N=29). We removed *Lophocolea bidentata* from the analysis of all bryophytes (therefore N=34) because of an extremely high PO_4^{3-} concentration in the water of the spring where it was found (see **Table S1**). All variables were log-transformed for these analyses.

We further explored the relationship of bryophyte elemental composition with sporophyte frequency and spore diameter using phylogenetic linear models using the *phylolm* function (using a lambda model to compute the error structure) in the *phylolm* (Tung Ho & Ané, 2014) R package. We fitted our response variable in these models as a function of bryophyte N, P, K, and Fe concentration, the N×P interaction, water conductivity, mean annual temperature, and annual water availability (precipitation - potential evapotranspiration). We included the interaction between N×P because it has been largely used as a measure of P limitation (Güsewell, 2004). We included water conductivity and climate, because they have been shown to affect bryophyte traits in these habitats (Bes *et al.*, 2018; Fernández-Martínez *et al.*, 2019; Fernández-Martínez *et al.*, 2020a). We selected models based on the best subsets using the *dredge* function in the *MuMin* R package (Barton, 2018), allowing the inclusion of a maximum of only five predictors to avoid overfitting our models (N=35 bryophyte species). We repeated this analysis using only moss species (N=29) to identify biases when also including liverworts. All variables were log-transformed, and all model residuals were normally

distributed. We similarly tested whether the predictors were correlated with the reproductive mode of bryophytes (0 mainly asexual, 1 mainly sexual) and whether they were monoicous (0) or dioicous (1) using a phylogenetically informed binomial model using the *phyloglm* function in the *phylolm* R package (Tung Ho & Ané, 2014).

We used the phylogenetic tree of bryophytes developed by Fernández-Martínez *et al.* (2019a) in all our analyses. Statistical analyses were performed using R statistical software (R Core Team, 2018). We used the *visreg* R package (Breheny & Burchett, 2015) for visualising the model results using partial-residual plots and response curves.

Results

Relationships between water nutrient concentrations, bryophyte concentrations, and sporophyte frequency

Our analyses revealed that, through direct effects, sporophyte frequency was higher in bryophyte species with higher P and Fe concentration than species with lower P and Fe concentration (**Figure 1**). Bryophytes with high N and K concentrations had a lower frequency of sporophyte production. The negative effect of bryophyte N concentration on sporophyte frequency, however, was not statistically significant when the positive direct effect between bryophyte N and P concentrations was taken into account (**Table 1**). Water PO_4^{3-} and Fe concentrations were positively correlated with high P and Fe bryophyte concentrations (**Figure 1**). The correlation with Fe concentration, though, was not significant when only mosses were considered. Water NO_3^- concentration tended to be positively correlated with bryophyte N concentration, albeit not significantly. Bryophyte K concentration, in contrast, tended to be negatively correlated with water K^+ concentration, but again not significantly. The direct effect of water nutrient concentrations on bryophyte nutrient concentrations was generally not strong

enough to significantly affect sporophyte frequency (**Table 1**). Nonetheless, moss species in springs with high water PO_4^{3-} concentrations did exhibit significantly higher sporophyte frequencies.

Relationships between bryophyte reproductive traits and nutrient concentrations

Our results for the phylogenetic linear models explaining the variability in sporophyte frequency mainly supported our results from the phylogenetic-path analysis. Bryophytes with high P and low K concentrations produced sporophytes more frequently, explaining 21% of the variance amongst species (**Figure 2a and b, Table 2**). When analysing only mosses, however, we also found a positive effect of Fe and a negative effect of N moss concentrations on sporophyte frequency, and the model explained 37% of the variance (**Figure 2c and d**). Similarly, bryophyte species with low K concentrations were more likely to reproduce sexually. The interaction between N×P and the likelihood of bryophytes reproducing sexually was also negative (**Figure 3, Table 2**), both when including all bryophytes or only mosses in the analysis. Our model indicated that a high N concentration led to a high likelihood of reproducing sexually in P-poor bryophytes, but to a low likelihood in P-rich bryophytes. A high P concentration was similarly correlated only with a high likelihood of sexual reproduction in N-poor bryophytes.

P- and Fe-poor and K-rich bryophytes were significantly typically dioicous. Our results for only mosses, however, indicated only a marginally significant negative effect of P concentration in reducing the likelihood of dioicy. Spore diameter was not correlated with any predictor variable when all bryophytes in our data set were used (**Table 2**). Moss N concentration, however, was positively correlated with spore diameter (**Figure 4**), and moss P and Fe concentrations were negatively correlated with spore diameter. Additionally, species living in springs with higher climate water availability presented

larger spore diameters (**Table 2**). Water availability was the only abiotic factor that statistically significantly correlated with reproductive traits.

Discussion

Effects of abiotic factors on bryophyte nutrient concentrations and reproductive traits

Bryophyte nutrient concentrations, except for K, were generally positively correlated with the water nutrient concentrations. Bryophytes are highly sensitive to environmental changes because of their lack of thick cuticles and true roots; their rhizoids serve mainly as support, although some bryophytes appear to use rhizoids to acquire water and nutrients through providing capillary spaces (Porley & Hodgetts, 2005). These properties probably account for the more flexible elemental concentrations of their tissues than those of vascular plants, although comparative analyses have not yet been performed. Bryophytes also have a large capacity to store elements such as heavy metals, so they are useful for monitoring environmental pollution (Tyler, 1990; Zechmeister, Grodzinska & Szarek-Lukaszewska, 2003). The lack of a positive correlation between water K concentrations and bryophyte K concentrations was not expected, but has been previously reported (Brown & Bates, 1990). These findings indicate that processes other than the availability of K in the environment regulate bryophyte K concentration. Monovalent cations (e.g. Na⁺ and K⁺) are less effectively retained in the cell walls than are divalent ions (e.g. Mg²⁺) (Brown & Bates, 1990). Hygrophytic bryophytes can leak K when desiccated and rehydrated (Brown & Buck, 1979; Deltoro *et al.*, 1998), suggesting that differences in K leaking amongst the species in our study may have been responsible for the differences in bryophyte K concentration.

Bryophyte reproductive traits were linked to their nutrient concentrations, but water chemistry was generally a poor predictor of bryophyte reproductive traits when bryophyte nutrient concentrations were taken into account. This result suggests that these reproductive traits have co-evolved with other adaptations for absorbing and retaining nutrients to maintain different concentrations amongst species. Bryophytes, like vascular plants, must thus have mechanisms to regulate their nutrient concentrations within limits. Nonetheless, mosses living in spring-fed streams with high concentrations of PO_4^{3-} also had high P concentrations (Steinman, 1994), which in turn positively affected the frequency of producing sporophytes. This result indicates that P limitation in the environment can be a main determinant of algae (Grover, 1989), vascular plant (Peñuelas *et al.*, 2013, 2019) and bryophyte ecophysiology.

Additionally, we found that the local climate played a very limited role in determining bryophyte reproductive traits. Only spore diameter of the mosses was positively correlated with water availability; temperature was not correlated with the differences in any of the traits amongst the species. These findings were not unexpected due to the large distributional ranges that bryophytes generally have. Climate may therefore not be an important constraint in the evolution of reproductive traits in hygrophytic bryophytes. Water conductivity, however, was unexpectedly not correlated with any of the reproductive traits when nutrient concentrations were taken into account, even though it is the main environmental constraint of vegetative moss traits and the distribution of bryophytes in springs (Bes *et al.*, 2018; Fernández-Martínez *et al.*, 2019; Fernández-Martínez *et al.*, 2020a). These results thus suggest that species-specific strategies to absorb nutrients are more important than abiotic factors in determining reproductive traits. Nonetheless, other important factors, such as temperature, photoperiod or rain-atmospheric deposition of nutrients, may contribute to explain

reproductive temporal patterns across populations of particular species and reproductive features across bryophyte species. Long time series of sporophyte production in bryophytes, similar to those used to study fruit production in vascular plants (Fernández-Martínez *et al.*, 2017), may provide extremely valuable scientific knowledge regarding the drivers of bryophyte reproduction.

The role of nutrients in bryophyte reproductive traits

Our results clearly indicated that bryophyte nutrient concentrations played a much more important role across species than did other environmental conditions in determining the reproductive traits of the hygrophytic bryophytes. P was the most relevant element controlling bryophyte reproductive traits, albeit the variance explained by elements was still relatively low. P and N concentrations have been demonstrated to be strongly correlated with other functional traits such as photosynthesis, in both vascular plants (Wright *et al.*, 2004; Niinemets, 2015) and bryophytes (Wang *et al.*, 2017). P also plays a paramount role in the production of seeds in vascular plants, increasing reproduction and making it less variable (Fernández-Martínez *et al.*, 2017; Fernández-Martínez *et al.*, 2019; Fujita *et al.*, 2014). Increased mineral nutrition in experiments with bryophytes was found to stimulate gametangial induction and earlier production of sporophytes (Chopra & Rahbar, 1982). Fertilisation with PO_4^{3-} increased gametophyte growth (Machlis, 1962) and induced sporophyte production (Selkirk, 1979). Fe played a similar role as P, increasing sporophyte frequency and the likelihood of monoicy and producing smaller spores. These results again agreed with previous studies focused on bryophytes from different habitats suggesting that Fe favours the onset of the sexually reproductive phase (Chopra & Bhatla, 1983) and that a lack of Fe considerably reduces gametophyte growth (Machlis, 1962).

Constraints imposed by the limitation of P may have played an important role determining bryophyte reproductive traits. A trade-off between growth and reproduction (Sánchez-Humanes *et al.*, 2011; Vergotti *et al.*, 2019) may have been an important selective pressure towards nutrient-conservative reproductive strategies (i.e., highly variable reproduction amongst years) under nutrient limitation, as recently suggested for vascular plants (Fernández-Martínez *et al.*, 2019b, 2020b). These trade-offs between growth and reproduction have also been observed in bryophytes (Ehrlén *et al.*, 2000; Bisang & Ehrlén, 2002; Rydgren & Økland, 2003). There are multiple lines of evidence that indicate that the successful development of the sporophyte is largely dependent on the resource status of the gametophyte (summarised in Stark *et al.*, [2009]). For example the known importance of the transfer of photosynthetic assimilates from gametophyte to sporophyte and the subsequent cost to the gametophyte (Rydgren & Økland, 2003); the positive correlation between gametophyte and sporophyte size (Convey, 1994); trade-offs between vegetative growth and sporophyte maturation (Ehrlén *et al.*, 2000); and density-dependent sporophyte abortion that suggests resource limitation such as is found in seed plants (Stark, Mishler & Mclethie, 2000).

Our comparative analyses across species found that P-rich bryophytes produced spores more frequently and were more likely to reproduce sexually. P-rich bryophytes were also more likely to be monoicous and produce small spores. P-poor bryophytes thus have more resource-conservative reproductive traits than P-rich bryophytes, which we attribute to four potential reasons. *i*) Frequent sporophyte production may jeopardise individual competitiveness due to reduced gametophyte growth compared to individuals that reproduce less often but grow faster and better compete for territory. *ii*) Sexual reproduction may be more beneficial for propagating and creating new populations, allowing genetic recombination and improving adaptability to new habitats. In contrast,

asexual reproduction maintains an already colonised habitat in which the current genotype has already been successful (Longton, 2006). *iii*) Dioicy (having male and female individuals) is likely to be a less resource-dependent strategy than monoicy (individuals are hermaphrodites). This is because trade-offs between growth and reproduction in male and female organs can be shared amongst individuals. Moreover, sexual reproduction is considerably reduced amongst dioicous species due to the spatial separation of male and female gametophytes and their limited range of effective fertilisation (Nath & Asthana, 2001). Finally, *iv*) smaller spores are more likely to travel farther from the source and colonise new habitats compared to larger spores, but their reserves for germination will also be lower and therefore represent a riskier strategy.

Experimental fertilisation with NO_3^- was reported to increase the growth of *Riccia duplex* gametophytes, but delay sexual reproduction (Selkirk, 1979). These effects on sporophyte production, however, were evident only when plants were grown under mineral fertilisation but without NO_3^- or K addition (Selkirk, 1979). Our findings are also consistent with these experimental results; N- and especially K-rich bryophytes produced sporophytes less frequently, but Fe-rich mosses produced sporophytes more frequently. Facilitating water movement and solute transport by controlling transmembrane potentials and osmotic pressure, limiting water loss, and increasing water-use efficiency are amongst the main roles of K in vascular plants (Sardans & Peñuelas, 2015). Plants under water limitation consequently have high foliar K concentrations (Milla *et al.*, 2005; Rivas-Ubach *et al.*, 2012). Low K leakage in bryophytes (associated with high K concentration in our study) has been correlated with a high tolerance of dehydration, which is needed more in drier and less productive environments (Deltoro *et al.*, 1998). Bryophytes with a stronger capacity to tolerate dehydration would thus need to maintain higher K concentrations and be forced to adopt

a more conservative reproductive strategy than bryophytes with a low tolerance of desiccation and high K leakage.

Overall, our results represent the first attempt to comparatively study reproductive traits and nutrient concentrations amongst species of bryophytes and suggest that nutrient scarcity has likely been a main driver of the evolution of reproductive traits throughout bryophytes and, as shown by previous studies, vascular plants (Han *et al.*, 2013; Fujita *et al.*, 2014; Miyazaki *et al.*, 2014; Fernández-Martínez *et al.*, 2017, 2019b). Further studies focusing on the interannual variability and drivers of sporophyte production will help us understand the mechanisms involved, how available nutrients affect sexual reproductive efforts across time, within and across species, and especially the evolutionary constraints of plant reproductive traits.

Acknowledgements

This study was funded by Small Grant SR18/1341 from the British Ecological Society. We acknowledge the Institució Catalana d'Història Natural (ICHN) and the Secció de Ciències Biològiques de l'Institut d'Estudis Catalans (IEC) for additional funding. We also acknowledge all the volunteers and “Teaming” supporters of the Delegation of the Serralada Litoral Central. We thank Dr. Miquel Jover for his help with moss identification and UB-CCiT technicians, Maite Romero and Pilar Rubio. MFM is a postdoctoral fellow of the Research Foundation – Flanders (FWO). IAJ and JP acknowledge support from the European Research Council Synergy grant no. ERC-2013-SyG 610028-IMBALANCE-P.

Author contributions

MFM, CP, and JP planned and designed the research. MFM, CP, JC, OC, and FS conducted the field work. MFM, CP, JC, and OC conducted the laboratory analyses.

MFM analysed the data. All authors contributed substantially to the writing of the manuscript.

Data availability

All data used in this study is available as Supplementary Materials (**Table S1**). The phylogenetic tree can be downloaded from the online materials of (Fernández-Martínez *et al.*, 2019), at <https://doi.org/10.6084/m9.figshare.9209351.v1>.

Conflict of interests

The authors declare no conflict of interests.

References

- Abbo S., Lev-Yadun S. & Gopher A. (2010). Yield stability: an agronomic perspective on the origin of Near Eastern agriculture. *Vegetation History and Archaeobotany* **19**, 143–150. <https://doi.org/10.1007/s00334-009-0233-7>
- Agrawal S.C. (2012). Factors controlling induction of reproduction in algae — review : the text. *Folia Microbiologica* **57**, 387–407. <https://doi.org/10.1007/s12223-012-0147-0>
- Barton K. (2018). MuMIn: Multi-Model Inference. -R package version 1.40.4.
- Beguería S. & Vicente-serrano S.M. (2012). SPEI: Calculation of the Standardised Precipitation-Evapotranspiration Index. R package version 1.1.
- Bes M., Corbera J., Sayol F., Bagaria G., Jover M., Preece C., *et al.* (2018). On the influence of water conductivity, pH and climate on bryophyte assemblages in Catalan semi-natural springs. *Journal of Bryology* **40**, 149–158.

433 <https://doi.org/10.1080/03736687.2018.1446484>

434 van der Bijl W. (2018). phylopath: Easy phylogenetic path analysis in R. *PeerJ* **2018**.
 435 <https://doi.org/10.7717/peerj.4718>

436 Bisang I. & Ehrlén J. (2002). Reproductive effort and cost of sexual reproduction in
 437 female *Dicranum polysetum*. *The Bryologist* **105**, 384–397.
 438 <https://doi.org/10.1639/0007>

439 Breheny P. & Burchett W. (2015). Visualization of regression models using visreg, R
 440 package version 2.2-0

441 Brown D.H. & Bates J.W. (1990). Bryophytes and nutrient cycling. *Botanical Journal*
 442 *of the Linnean Society* **104**, 129–147. [https://doi.org/10.1111/j.1095-](https://doi.org/10.1111/j.1095-8339.1990.tb02215.x)
 443 [8339.1990.tb02215.x](https://doi.org/10.1111/j.1095-8339.1990.tb02215.x)

444 Brown D.H. & Buck G.W. (1979). Desiccation effects and cation distribution in
 445 nryophytes. *New Phytologist* **82**, 115–125. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.1979.tb07565.x)
 446 [8137.1979.tb07565.x](https://doi.org/10.1111/j.1469-8137.1979.tb07565.x)

447 Camarero J.J., Albuixech J., López-Lozano R., Casterad M.A. & Montserrat-Martí G.
 448 (2010). An increase in canopy cover leads to masting in *Quercus ilex*. *Trees* **24**,
 449 909–918. <https://doi.org/10.1007/s00468-010-0462-5>

450 Cantonati M., Füreder L., Gerecke R., Jüttner I. & Cox E.J. (2012). Crenic habitats,
 451 hotspots for freshwater biodiversity conservation: Toward an understanding of
 452 their ecology. *Freshwater Science* **31**, 463–480. <https://doi.org/10.1899/11-111.1>

453 Cantonati M., Gerecke R. & Bertuzzi E. (2006). *Springs of the Alps - Sensitive*
 454 *ecosystems to environmental change: From biodiversity assessments to long-term*
 455 *studies*.

- 456 Cantonati M., Segadelli S., Spitale D., Gabrieli J., Gerecke R., Angeli N., *et al.* (2020).
 457 Geological and hydrochemical prerequisites of unexpectedly high biodiversity in
 458 spring ecosystems at the landscape level. *Science of the Total Environment* **740**,
 459 140157. <https://doi.org/10.1016/j.scitotenv.2020.140157>
- 460 Casas C., Brugués M. & Cros R.M. (2001). *Flora de Briòfits dels Països Catalans. I.*
 461 *Moltes*. Institut d'Estudis Catalans, Barcelona.
- 462 Casas C., Brugués M. & Cros R.M. (2004). *Flora de Briòfits dels Països Catalans. II.*
 463 *Hepàtiques i antocerotes*. Institut d'Estudis Catalans, Barcelona.
- 464 Chopra R.N. & Bhatla S.C. (1983). Regulation of gametangial formation in bryophytes.
 465 *The Botanical Review* **49**, 29–63
- 466 Chopra R.N. & Rahbar K. (1982). Temperature, light and nutritional requirements for
 467 gametangial induction in the moss *Bartramidula bartramioides*. *New Phytologist*
 468 **92**, 251–257. <https://doi.org/10.1111/j.1469-8137.1982.tb03383.x>
- 469 Convey P. (1994). Modelling reproductive effort in sub-and maritime Antarctic mosses.
 470 *Oecologia* **100**, 45–53. <https://doi.org/10.1007/BF00317129>
- 471 Cros R.M. (1985). Flora briològica del Montnegre. In: *Arxius de la Secció de Ciències*,
 472 78. p. 287. Institut d'Estudis Catalans, Barcelona.
- 473 Deltoro V.I., Calatayud A., Gimeno C. & Barreno E. (1998). Water relations,
 474 chlorophyll fluorescence, and membrane permeability during desiccation in
 475 bryophytes from xeric, mesic, and hydric environments. *Canadian Journal of*
 476 *Botany* **76**, 1923–1929. <https://doi.org/10.1139/b98-167>
- 477 Dierssen K. (2001). Distribution, ecological amplitude and phytosociological
 478 characterization of European bryophytes. In: *Bryophytorum Bibliotheca*. (Ed. J.

479 Cramer), pp. 1–289. Berlin and Stuttgart.

480 Ehrlén J., Bisang I. & Hedenäs L. (2000). Costs of sporophyte production in the moss,
 481 *Dicranum polysetum*. *Plant Ecology* **149**, 207–217

482 Fernández-Martínez M., Corbera J., Domene X., Sayol F., Sabater F. & Preece C.
 483 (2020a). Nitrate pollution reduces bryophyte diversity in Mediterranean springs.
 484 *Science of The Total Environment* **705**, 135823.
 485 <https://doi.org/10.1016/j.scitotenv.2019.135823>

486 Fernández-Martínez M., Margalef O., Sayol F., Asensio D., Bagaria G., Corbera J., *et*
 487 *al.* (2019a). Sea spray influences water chemical composition of Mediterranean
 488 semi-natural springs. *Catena* **173**, 414–423.
 489 <https://doi.org/10.1016/j.catena.2018.10.035>

490 Fernández-Martínez M., Pearse I., Sardans J., Sayol F., Koenig W.D., LaMontagne
 491 J.M., *et al.* (2019b). Nutrient scarcity as a selective pressure for mast seeding.
 492 *Nature Plants* **5**, 1222–1228. <https://doi.org/10.1038/s41477-019-0549-y>

493 Fernández-Martínez M., Preece C., Corbera J., Cano O., Garcia-Porta J., Sardans J., *et*
 494 *al.* (2021). Bryophyte C:N:P stoichiometry, biogeochemical niches, and
 495 elementome plasticity driven by environment and coexistence. *Ecology Letters*, in
 496 press. <https://doi.org/10.1111/ele.13752>

497 Fernández-Martínez M., Sardans J., Sayol F., LaMontagne J.M., Bogdziewicz M.,
 498 Collalti A., *et al.* (2020b). Reply to: Nutrient scarcity cannot cause mast seeding.
 499 *Nature Plants* **6**, 763–765. <https://doi.org/10.1038/s41477-020-0703-6>

500 Fernández-Martínez M., Vicca S., Janssens I.A., Espelta J.M. & Peñuelas J. (2017). The
 501 role of nutrients, productivity and climate in determining tree fruit production in

European forests. *New Phytologist* **213**, 669–679.
<https://doi.org/10.1111/nph.14193>

Fernández-Martínez M., Berloso F., Corbera J., Garcia-Porta J., Sayol F., Preece C., *et al.* (2019). Towards a moss sclerophylly continuum: Evolutionary history, water chemistry and climate control traits of hygrophytic mosses. *Functional Ecology* **33**, 2273–2289. <https://doi.org/10.1111/1365-2435.13443>

Fujita Y., Venterink H.O., van Bodegom P.M., Douma J.C., Heil G.W., Hölzel N., *et al.* (2014). Low investment in sexual reproduction threatens plants adapted to phosphorus limitation. *Nature* **505**, 82–6. <https://doi.org/10.1038/nature12733>

Grover J.P. (1989). Phosphorus-dependent growth kinetics of 11 species of freshwater algae. *Limnology and Oceanography* **34**, 341–348.
<https://doi.org/10.4319/lo.1989.34.2.0341>

Güsewell S. (2004). N:P ratios in terrestrial plants: Variation and functional significance. *New Phytologist* **164**, 243–266

Han Q., Kabeya D., Iio A., Inagaki Y. & Kakubari Y. (2013). Nitrogen storage dynamics are affected by masting events in *Fagus crenata*. *Oecologia* **174**, 679–687. <https://doi.org/10.1007/s00442-013-2824-3>

Hardenberg A. von & Gonzalez-Voyer A. (2013). Disentangling evolutionary cause-effect relationships with phylogenetic confirmatory path analysis. *Evolution* **67**, 378–387. <https://doi.org/10.1111/j.1558-5646.2012.01790.x>

Hargreaves G.H. (1994). Defining and using reference evapotranspiration. *Journal of Irrigation and Drainage Engineering* **120**, 1132–1139

Heino J., Virtanen R., Vuori K.M., Saastamoinen J., Ohtonen A. & Muotka T. (2005).

525 Spring bryophytes in forested landscapes: Land use effects on bryophyte species
526 richness, community structure and persistence. *Biological Conservation* **124**, 539–
527 545. <https://doi.org/10.1016/j.biocon.2005.03.004>

528 Herrera C., Jordano P., Guitián J. & Traveset A. (1998). Annual variability in seed
529 production by woody plants and the masting concept: reassessment of principles
530 and relationship to pollination and seed dispersal. *The American Naturalist* **152**,
531 576–594

532 Hill M.O., Bell N., Bruggeman-Nannenga M., Brugués M., Cano M.J., Enroth J., *et al.*
533 (2006). An annotated checklist of the mosses of Europe and Macaronesia. *Journal*
534 *of Bryology* **28**, 198–267. <https://doi.org/10.1179/174328206X119998>

535 Hill M.O., Preston C.D., Bosanquet S.D.S. & Roy D.B. (2007). *BRYOATT: Attributes of*
536 *British and Irish Bryophytes*.

537 Longton R.E. (2006). Reproductive ecology of bryophytes: What does it tell us about
538 the significance of sexual reproduction? *Lindbergia* **31**, 16–23.
539 <https://doi.org/10.2307/20150203>

540 Machlis L. (1962). The Effects of Mineral Salts, Glucose, and Light on the Growth of
541 the Liverwort, *Sphaerocarpos donnellii*. *Physiologia Plantarum* **15**, 354–362.
542 <https://doi.org/10.1111/j.1399-3054.1962.tb08034.x>

543 Milla R., Castro-Díez P., Maestro-Martínez M. & Montserrat-Martí G. (2005).
544 Relationships between phenology and the remobilization of nitrogen, phosphorus
545 and potassium in branches of eight Mediterranean evergreens. *New Phytologist*
546 **168**, 167–178. <https://doi.org/10.1111/j.1469-8137.2005.01477.x>

547 Miyazaki Y., Maruyama Y., Chiba Y., Kobayashi M.J., Joseph B., Shimizu K.K., *et al.*

- (2014). Nitrogen as a key regulator of flowering in *Fagus crenata*: understanding the physiological mechanism of masting by gene expression analysis. *Ecology Letters* **17**, 1299–1309. <https://doi.org/10.1111/ele.12338>
- Murphy J. & Riley J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**, 31–36
- Nath V. & Asthana A.K. (2001). Reproductive biology of bryophytes. *Reproductive Biology of Plants*, 148–174. https://doi.org/10.1007/978-3-642-50133-3_8
- Niinemets Ü. (2015). Is there a species spectrum within the world-wide leaf economics spectrum? Major variations in leaf functional traits in the Mediterranean sclerophyll *Quercus ilex*. *New Phytologist* **205**, 79–96. <https://doi.org/10.1111/nph.13001>
- 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**, 1823–1841. <https://doi.org/10.1002/1097-0088>
- Peñuelas J., Fernández-Martínez M., Ciais P., Jou D., Piao S., Obersteiner M., *et al.* (2019). The bioelements, the elementome, and the biogeochemical niche. *Ecology* **100**, e02652. <https://doi.org/10.1002/ecy.2652>
- Peñuelas J., Janssens I.A., Ciais P., Obersteiner M. & Sardans J. (2020). Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. *Global Change Biology*, gcb.14981. <https://doi.org/10.1111/gcb.14981>

572 Peñuelas J., Poulter B., Sardans J., Ciais P., van der Velde M., Bopp L., *et al.* (2013).
 573 Human-induced nitrogen-phosphorus imbalances alter natural and managed
 574 ecosystems across the globe. *Nature communications* **4**, 2934.
 575 <https://doi.org/10.1038/ncomms3934>
 576 Pons X. (1996). Estimación de la radiación solar a partir de modelos digitales de
 577 elevaciones. Propuesta metodológica. In: *Modelos y sistemas de información*
 578 *geográfica*. pp. 87–97.
 579 Porley R. & Hodgetts N.G. (2005). *Mosses and Liverworts*, 1st edn. HarperCollins
 580 Publishers, London.
 581 Preece C., Livarda A., Christin P.A., Wallace M., Martin G., Charles M., *et al.* (2017).
 582 How did the domestication of Fertile Crescent grain crops increase their yields?
 583 *Functional Ecology* **31**, 387–397. <https://doi.org/10.1111/1365-2435.12760>
 584 R Core Team (2018). R: A Language and Environment for Statistical Computing
 585 Reekie E.G. & Bazzaz F.A. (1987). Reproductive effort in plants. II Does carbon reflect
 586 the allocation of other resources? *American Naturalist* **129**, 897–906
 587 Rivas-Ubach A., Sardans J., Pérez-Trujillo M., Estiarte M. & Peñuelas J. (2012). Strong
 588 relationship between elemental stoichiometry and metabolome in plants.
 589 *Proceedings of the National Academy of Sciences of the United States of America*
 590 **109**, 4181–4186. <https://doi.org/10.1073/pnas.1116092109>
 591 Rydgren K. & Økland R.H. (2003). Short-term costs of sexual reproduction in the
 592 clonal moss *Hylocomium splendens*. *The Bryologist* **106**, 212–220.
 593 [https://doi.org/10.1639/0007-2745\(2003\)106\[0212:scosri\]2.0.co;2](https://doi.org/10.1639/0007-2745(2003)106[0212:scosri]2.0.co;2)
 594 Sala A., Hopping K., McIntire E.J.B., Delzon S. & Crone E.E. (2012). Masting in

- whitebark pine (*Pinus albicaulis*) depletes stored nutrients. *The New phytologist* **196**, 189–99. <https://doi.org/10.1111/j.1469-8137.2012.04257.x>
- Sánchez-Humanes B., Sork V.L. & Espelta J.M. (2011). Trade-offs between vegetative growth and acorn production in *Quercus lobata* during a mast year: the relevance of crop size and hierarchical level within the canopy. *Oecologia* **166**, 101–10. <https://doi.org/10.1007/s00442-010-1819-6>
- Sardans J. & Peñuelas J. (2015). Potassium: a neglected nutrient in global change. *Global Ecology and Biogeography* **24**, 261–275. <https://doi.org/10.1111/geb.12259>
- Selkirk P.M. (1979). Effect of Nutritional Conditions on Sexual Reproduction in *Riccia*. *The Bryologist* **82**, 37–46
- Smith A.J.E. (1978). *The moss flora of Britain and Ireland*, Cambridge. Cambridge.
- Stark L.R., Brinda J.C. & McLetchie D.N. (2009). An Experimental demonstration of the cost of sex and a potential resource limitation on reproduction in the moss *Pterygoneurum* (Pottiaceae). *American Journal of Botany* **96**, 1712–1721. <https://doi.org/10.3732/ajb.0900084>
- Stark L.R., Mishler B.D. & McLetchie D.N. (2000). The cost of realized sexual reproduction: Assessing patterns of reproductive allocation and sporophyte abortion in a desert moss. *American Journal of Botany* **87**, 1599–1608. <https://doi.org/10.2307/2656736>
- Steinman A.D. (1994). The influence of phosphorus enrichment on lotic bryophytes. *Freshwater Biology* **31**, 53–63. <https://doi.org/10.1111/j.1365-2427.1994.tb00838.x>
- Sterner R.W. & Elser J.J. (2002). *Ecological stoichiometry : the biology of elements*

618 *from molecules to the biosphere*. Princeton University Press.

619 Tung Ho L.S. & Ané C. (2014). A linear-time algorithm for gaussian and non-gaussian
620 trait evolution models. *Systematic Biology* **63**, 397–408.

621 <https://doi.org/10.1093/sysbio/syu005>

622 Tyler G. (1990). Bryophytes and heavy metals: a literature review. *Botanical Journal of*
623 *the Linnean Society* **104**, 231–253. <https://doi.org/10.1111/j.1095->

624 [8339.1990.tb02220.x](https://doi.org/10.1111/j.1095-8339.1990.tb02220.x)

625 Vergotti M.J., Fernández-Martínez M., Kefauver S.C., Janssens I.A. & Peñuelas J.

626 (2019). Weather and trade-offs between growth and reproduction regulate fruit

627 production in European forests. *Agricultural and Forest Meteorology* **279**, 107711.

628 <https://doi.org/10.1016/j.agrformet.2019.107711>

629 Wang Z., Liu X., Bader M.Y., Feng D. & Bao W. (2017). The ‘plant economic

630 spectrum’ in bryophytes, a comparative study in subalpine forest. *American*

631 *Journal of Botany* **104**, 261–270. <https://doi.org/10.3732/ajb.1600335>

632 Wright I.J., Reich P.B., Westoby M., Ackerly D.D., Baruch Z., Bongers F., *et al.*

633 (2004). The worldwide leaf economics spectrum. *Nature* **428**, 821–7.

634 <https://doi.org/10.1038/nature02403>

635 Zechmeister H.G., Grodzinska K. & Szarek-Lukaszewska G. (2003). Bryophytes. In:

636 *Bioindicators and biomonitors*. (Eds B.A. Markert, A.M. Breure & H.G.

637 Zechmeister), pp. 329–375. Elsevier Science Ltd.

Figure captions

Figure 1. Standardised direct effects (\pm the standard error of the estimation) of the phylogenetic-path analysis correlating water chemistry (blue boxes), bryophyte elemental composition (green boxes), and sporophyte frequency (SpFr). Blue and red arrows indicate, respectively, positive and negative relationships. See **Table 1** for the total effects. The model including all bryophytes included 34 species, and the model using only mosses used 29 species. Dashed arrows indicate non-significant paths at $P < 0.05$.

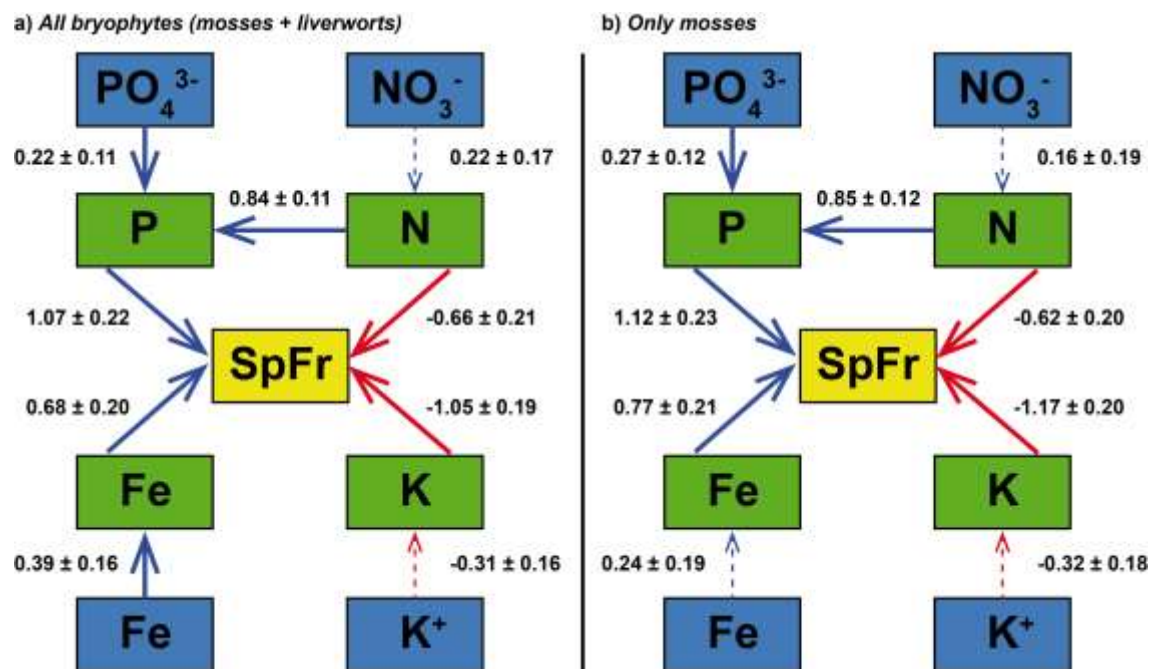


Figure 2. Partial-residual plots of the relationship between bryophyte elemental composition and sporophyte frequency per species; a) and b) mosses and liverworts (N=35), c) and d) only mosses (N=29). Further information on the models is presented in **Methods** and **Table 2**.

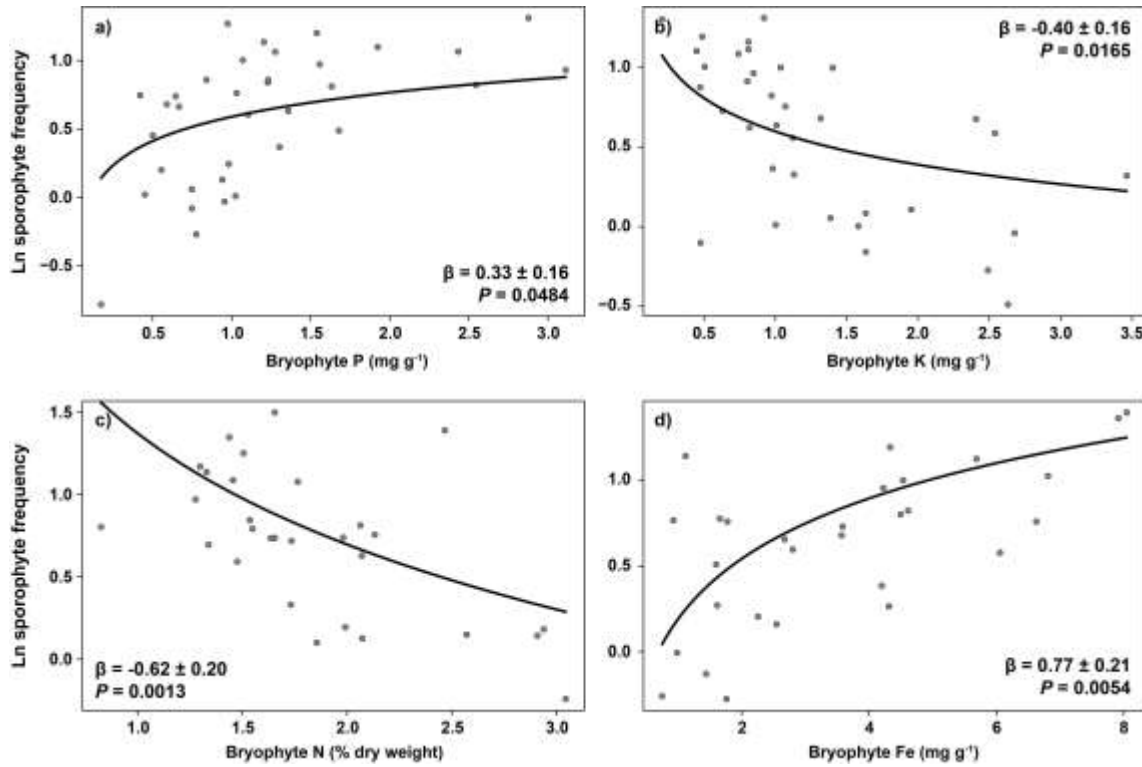


Figure 3. Response plots of the relationship between the interaction of bryophyte N and P concentrations with the likelihood of a bryophyte species showing mainly sexually reproduction. Parameter estimates are presented in **Table 2**. Shaded areas represent the 95% confidence intervals of the curves. N=35.

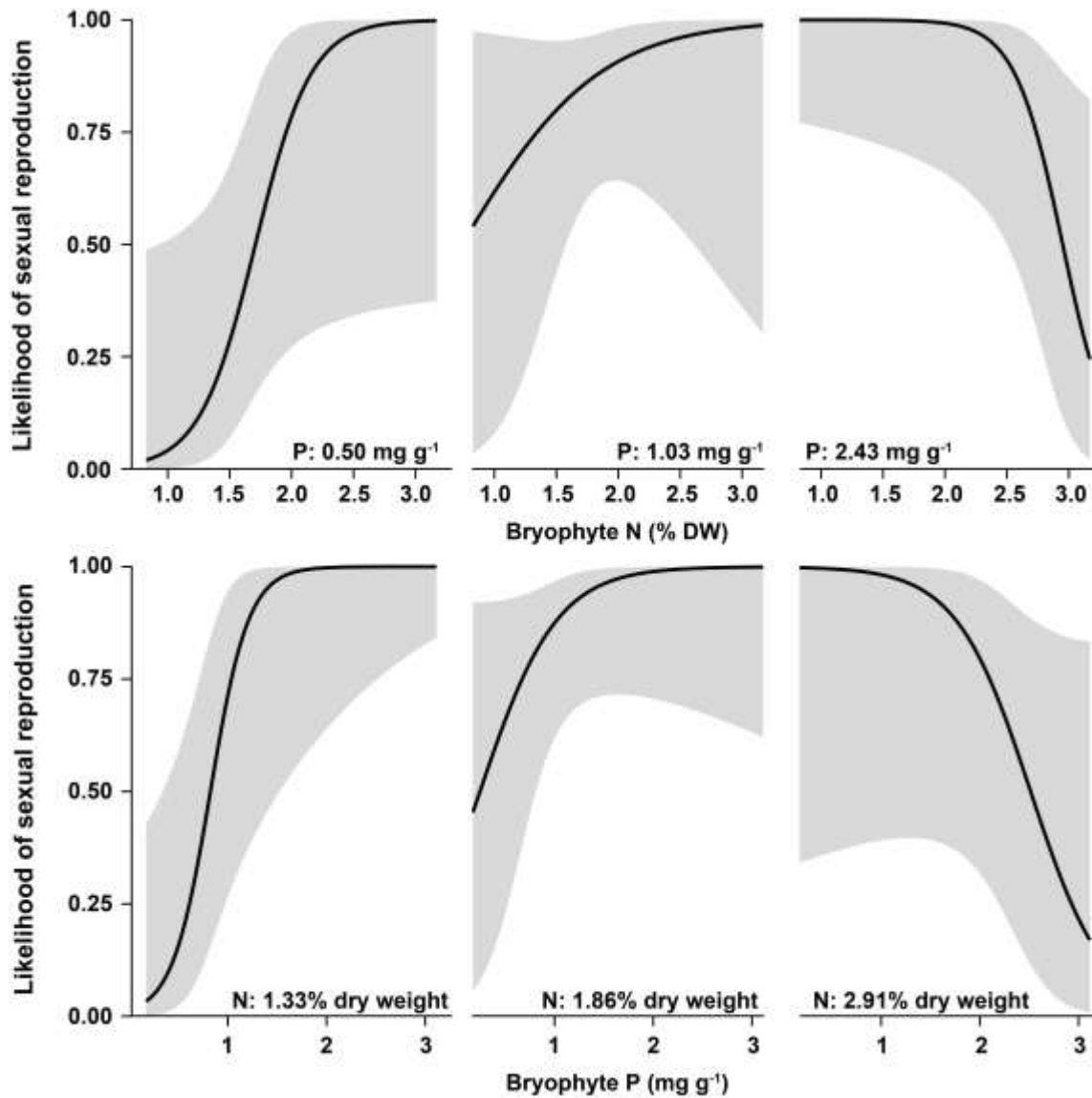


Figure 4 Partial-residual plots of the relationships between spore diameter, moss elemental concentration (a, b and c), and water availability (d, annual precipitation - annual potential evapotranspiration). See **Table 2** for details. β indicates the standardised coefficients \pm standard error. N=29.

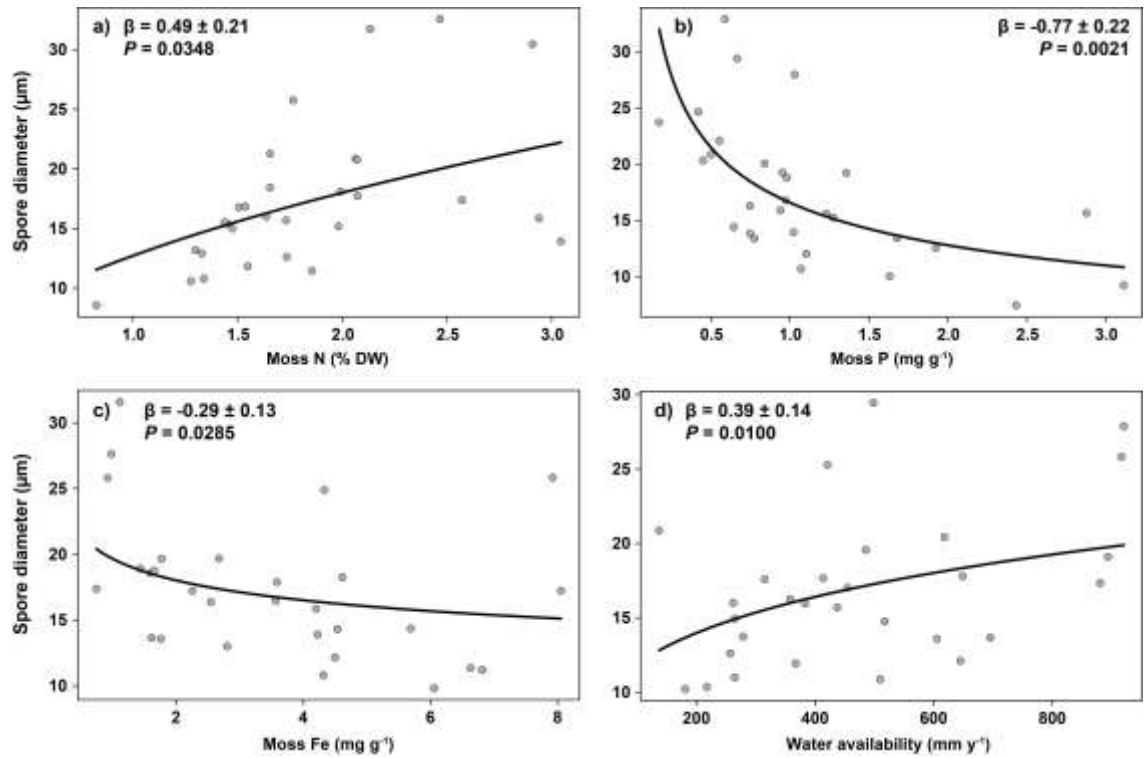


Table 1. Standardised total effects (\pm standard error of the mean) of the phylogenetic-path analysis (**Figure 1**) correlating water NO_3^- , PO_4^{3-} , K^+ , and Fe concentrations (indicated by subscripted “w”), bryophyte nutrient concentration (N, P, K, and Fe), and sporophyte frequency (SpFr). Values indicate the effect of the row variables on the column variables. Bold coefficients indicate significant effects at $P < 0.05$.

	Fe	N	K	P	SpFr
<i>All bryophytes</i>					
<i>(N=35)</i>					
$\text{NO}_3^-_{\text{w}}$		0.22 ± 0.17		0.19 ± 0.15	0.05 ± 0.22
$\text{PO}_4^{3-}_{\text{w}}$				0.22 ± 0.11	0.23 ± 0.13
K_{w}			-0.31 ± 0.17		0.30 ± 0.18
Fe_{w}	0.39 ± 0.16				0.24 ± 0.13
N				0.84 ± 0.11	0.25 ± 0.30
P					1.08 ± 0.22
K					-1.05 ± 0.19
Fe					0.68 ± 0.20
<i>Mosses</i>					
<i>(N=29)</i>					
$\text{NO}_3^-_{\text{w}}$		0.16 ± 0.19		0.13 ± 0.16	0.05 ± 0.26
$\text{PO}_4^{3-}_{\text{w}}$				0.27 ± 0.12	0.30 ± 0.15
K_{w}			-0.33 ± 0.18		0.38 ± 0.22
Fe_{w}	0.24 ± 0.19				0.18 ± 0.15
N				0.85 ± 0.12	0.33 ± 0.31
P					1.12 ± 0.23
K					-1.17 ± 0.20
Fe					0.77 ± 0.21

Table 2. Standardised parameter estimates (β) of the regression models predicting sporophyte frequency, spore diameter, and whether the species reproduce mainly sexually (1) or not (0, binomial model). Lambda (λ) indicates the phylogenetic signal in the models. Phylogenetic correction was negligible when $\lambda = 0$. WA: water availability, annual precipitation - annual potential evapotranspiration.

	All bryophytes		Mosses	
	β	<i>P</i>	β	<i>P</i>
<i>Sporophyte frequency</i>				
N			-0.62 \pm 0.20	0.0013
P	0.33 \pm 0.16	0.0484	1.12 \pm 0.23	<0.0001
K	-0.40 \pm 0.16	0.0165	-1.17 \pm 0.20	<0.0001
Fe			0.77 \pm 0.21	0.0054
λ	0.00		0.91	
R²	0.22		0.38	
<i>Sexual reproduction</i>				
N	0.54 \pm 0.97	0.5780	1.34 \pm 1.15	0.2456
P	1.46 \pm 1.01	0.1502	1.40 \pm 1.07	0.1918
N\timesP	-1.94 \pm 0.67	0.0038	-1.88 \pm 0.77	0.0141
K	-1.28 \pm 0.53	0.0173	-1.70 \pm 0.74	0.0141
λ	0.00		0.00	
R²	0.43		0.44	
<i>Dioicy</i>				
P	-0.89 \pm 0.42	0.0434	-0.96 \pm 0.48	0.0461
K	2.77 \pm 1.31	0.0423		
Fe	-1.59 \pm 0.74	0.0399		
λ	0.00		0.00	
R²	0.37		0.15	
<i>Spore diameter</i>				
WA			0.39 \pm 0.14	0.0100
N			0.49 \pm 0.21	0.0348
P			-0.77 \pm 0.22	0.0021
Fe			-0.29 \pm 0.13	0.0285
λ	0.99		0.86	
R²	0		0.22	

1 **Nutrients control reproductive traits of hygrophytic**
2 **bryophytes**

3 **Authors:** M. Fernández-Martínez^{*1,2}, C. Preece^{3,4}, J. Corbera², O. Cano⁵, J. Garcia-
4 Porta⁶, M. Bogdziewicz^{3,7}, J. Sardans^{3,4}, I.A. Janssens¹, F. Sabater^{2,5}, J. Peñuelas^{3,4}.

5 **Supplementary material**

6 **Figure S1:** Map showing the location of the sampled bryophytes (red dots).

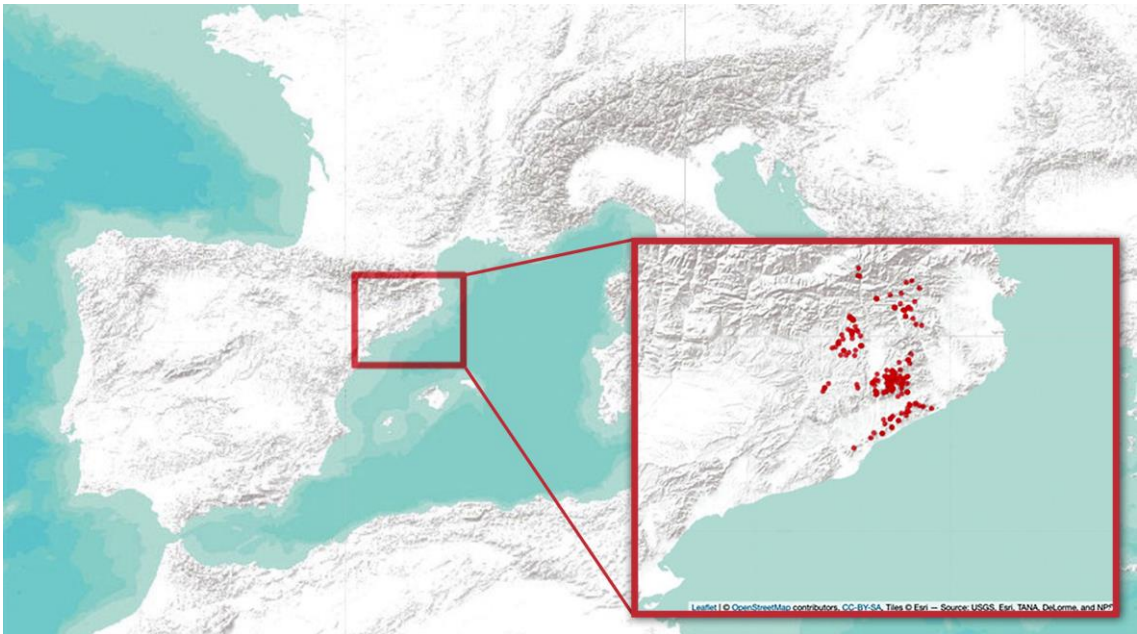


Figure S2: Image showing one of the springs sampled, representative of most of the springs sampled in this study. Only those bryophytes located inside the area directly affected by the water from the spring were sampled.



Table S1: Median values for each species used in the statistical analyses. SpFr, sporophyte frequency (mainly sexual reproduction); SpDi, mean spore diameter; N, bryophyte nitrogen concentration (percentage of dry weight); P, K, and Fe, phosphorus, potassium, and iron concentrations (mg g⁻¹); NO₃⁻ (mg L⁻¹), PO₄³⁻ (μg L⁻¹), K⁺ (mg L⁻¹), and Fe_w (μg L⁻¹), water concentrations of nitrate, phosphate, potassium, and iron; Conductivity, water conductivity (μS cm⁻¹); MAT, mean annual temperature (°C); WA, water availability: annual precipitation - potential annual evapotranspiration (mm y⁻¹); and *n*, number of replicates per species.

Species	SpFr	Sexual	SpDi	N	P	K	Fe	NO ₃ ⁻	PO ₄ ³⁻	K ⁺	Fe _w	Conductivity	MAT	WA	<i>n</i>
<i>Amblystegium serpens</i>	4	1	11.5	3.04	2.43	0.80	6.06	0.01	1.97	1.53	3.69	982	11.4	509.4	1
<i>Anomodon viticulosus</i>	1	0	16.0	1.34	0.78	2.63	6.63	0.02	7.04	0.70	2.48	577	10.3	695.3	1
<i>Apopellia endiviifolia</i>	3	1	75.0	2.39	1.20	0.81	3.52	20.12	5.48	3.06	3.95	588	13.2	407.3	24
<i>Brachythecium rivulare</i>	2	1	18.0	1.86	1.07	0.74	4.31	0.84	15.65	0.38	3.88	88	8.7	644.7	18
<i>Bryum pseudotriquetrum</i>	2	0	18.5	1.33	0.75	1.58	4.54	0.02	10.96	0.16	3.19	33	7.4	880.5	3
<i>Conocephalum conicum</i>	2	1	75.0	1.96	1.56	2.54	2.23	5.64	10.30	1.63	3.16	464	13.0	476.9	21
<i>Cratoneuron filicinum</i>	2	1	13.0	1.98	1.02	1.00	4.23	2.79	7.83	0.90	3.09	283	10.5	517.3	21
<i>Ctenidium molluscum</i>	1	0	14.0	1.30	0.45	1.63	5.69	0.36	15.65	1.50	4.51	712	11.3	279.0	1
<i>Dialytrichia mucronata</i>	2	1	15.0	2.06	0.95	1.63	8.05	0.43	2.40	1.97	14.62	577	11.5	485.4	2
<i>Didymodon tophaceus</i>	3	1	14.0	1.28	0.64	0.45	1.62	20.87	4.90	3.04	4.16	996	11.5	266.6	21
<i>Eucladium verticillatum</i>	1	0	13.0	1.51	0.42	0.49	1.78	7.42	4.67	2.42	3.73	821	12.5	262.2	20
<i>Fissidens crassipes</i>	3	1	23.0	2.47	0.59	0.21	1.12	20.87	2.33	4.63	5.84	820	14.3	137.0	1
<i>Fissidens grandifrons</i>	2	1	21.5	1.77	0.67	1.07	4.33	1.05	7.04	0.95	3.13	348	12.9	421.1	3
<i>Fissidens rivularis</i>	2	1	18.5	1.66	1.36	0.82	0.93	1.10	14.87	0.42	2.96	91	7.5	916.5	1
<i>Fissidens taxifolius</i>	2	1	12.5	2.13	1.03	1.32	7.92	26.07	3.13	1.11	2.93	283	8.1	498.2	1
<i>Fontinalis antipyretica</i>	1	0	15.0	2.91	2.88	0.81	0.98	0.34	18.00	0.19	4.045	31	6.0	920.3	2
<i>Gymnostomum calcareum</i>	1	0	10.0	1.46	0.97	0.92	1.60	42.46	18.79	7.54	3.425	1624	11.4	264.5	2
<i>Leptodictyum riparium</i>	3	1	14.0	2.57	3.11	0.47	0.75	19.50	1.57	2.89	3.225	1130	11.5	257.1	3

<i>Lophocolea bidentata</i>	3	1	17.5	2.38	1.23	0.51	4.59	32.23	420.30	5.57	3.75	260	10.2	644.1	2
<i>Lunularia cruciata</i>	1	0	18.0	2.32	1.30	1.95	5.38	6.73	11.94	0.98	4.96	387	10.5	458.5	4
<i>Oxyrrhynchium speciosum</i>	1	1	18.0	2.07	0.84	0.85	2.67	3.76	4.98	1.32	5.68	357	12.6	314.8	20
<i>Palustriella commutata</i>	1	0	18.0	1.54	0.55	0.98	3.58	1.64	7.04	1.25	3.17	612	12.0	413.6	20
<i>Philonotis caespitosa</i>	1	0	24.0	1.66	0.98	2.68	4.61	0.26	13.31	0.53	3.865	195	8.4	617.9	4
<i>Philonotis fontana</i>	2	1	28.0	2.07	1.92	2.41	4.20	0.08	18.00	0.19	5.3	39	6.1	893.6	1
<i>Plagiomnium undulatum</i>	2	0	28.0	1.99	1.28	1.04	1.44	1.50	7.04	1.00	4.065	338	11.5	454.7	20
<i>Platyhypnidium riparioides</i>	3	1	19.0	1.74	1.10	0.63	2.80	1.96	7.83	1.11	4.09	186	9.5	605.5	20
<i>Pohlia melanodon</i>	2	0	18.0	1.55	0.75	2.49	4.50	27.07	3.13	1.75	4.51	754	11.5	180.9	2
<i>Porella platyphylla</i>	1	0	55.0	3.17	2.55	1.12	3.86	26.07	3.13	1.11	2.93	283	8.1	498.2	1
<i>Rhizomnium punctatum</i>	3	1	37.5	1.48	0.50	1.01	2.26	0.02	9.39	2.16	4.07	296	10.0	649.0	1
<i>Rhynchostegiella teneriffae</i>	3	1	14.5	1.64	1.68	1.13	3.57	6.73	15.94	2.10	4.96	517	13.2	383.2	2
<i>Riccardia chamedryfolia</i>	3	1	18.5	2.68	1.54	1.40	12.93	4.72	19.57	0.68	32.16	142	8.2	491.7	1
<i>Scorpiurium circinatum</i>	1	0	16.0	2.94	1.63	3.46	6.81	0.42	4.98	2.30	29.94	958	14.9	217.5	1
<i>Thamnobryum alopecurum</i>	2	1	11.0	1.44	1.23	0.97	1.66	2.84	10.91	0.88	7.715	388	13.1	357.9	6
<i>Thuidium delicatulum</i>	2	1	18.0	1.73	0.94	1.39	2.55	0.02	1.57	0.50	3.48	706	10.8	437.5	1
<i>Trichostomum crispulum</i>	1	0	17.0	0.83	0.17	0.48	1.76	0.02	6.26	0.65	3.11	589	11.2	367.3	1

19

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21