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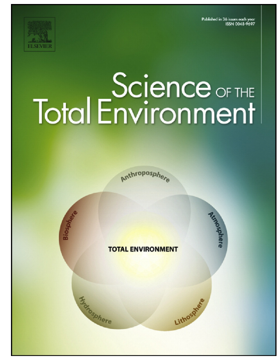

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Nitrate pollution reduces bryophyte diversity in Mediterranean springs

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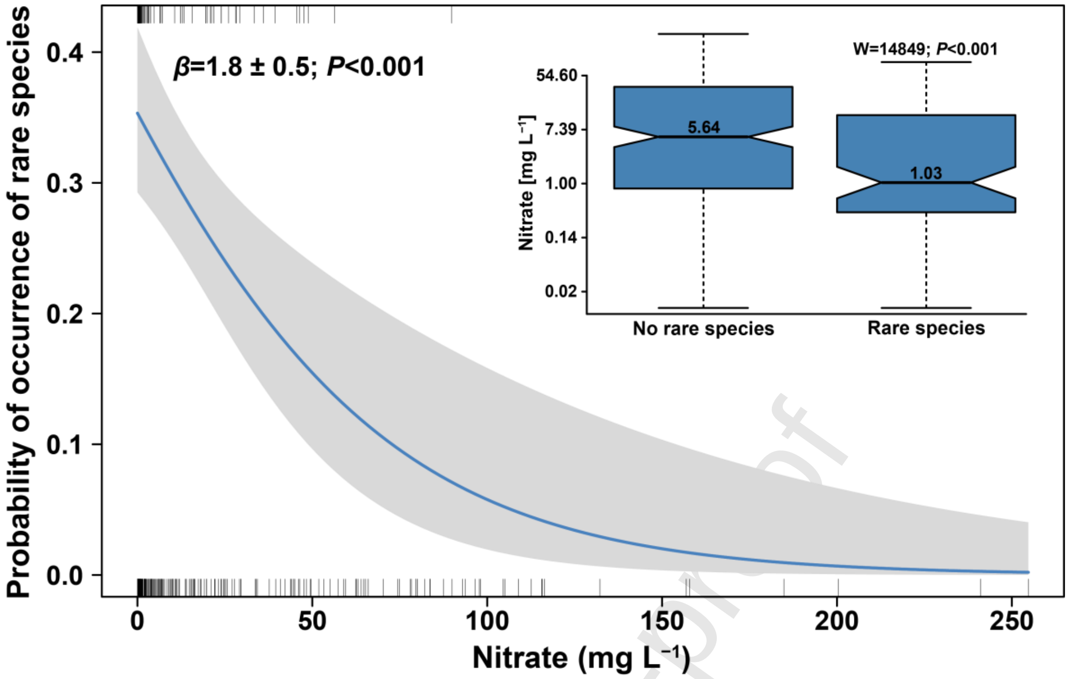
Abstract

Anthropogenic activities and intensive farming are causing nitrate pollution in groundwater bodies. These aquifers are drained by springs which, in the Mediterranean region, act as refugia for preserving biodiversity of species that need continuous water. Some springs are also used for drinking water for wild animals, livestock and humans, so if their water quality is compromised it can become a threat to public health. However, the impact of nitrate pollution on these biotic communities remains unknown. We sampled 338 assemblages of aquatic and semi-aquatic bryophytes (i.e., hygrophytic mosses and liverworts) growing in springs in a gradient of water conductivity, nitrate concentration and climate and distributed across the north-east of the Iberian Peninsula to investigate the impact of nitrate pollution on the diversity of bryophytes and moss functional traits in Mediterranean springs. Based on previous literature suggesting that increased nitrogen load decreases biodiversity in grasslands and freshwater ecosystems, we hypothesised that water nitrate pollution in springs decreases bryophyte diversity at the local and regional scales. Our results indicated that, at the local scale (spring), nitrate pollution reduced the number and the likelihood of finding a rare species in springs. Rare species were found in 4% of the springs with nitrate above 50 mg L^{-1} but in 32% of the springs with nitrate below 50 mg L^{-1}. Moss, liverwort and overall bryophyte diversity was not directly affected by nitrate at the local scale but nitrate consistently decreased diversity of mosses, liverworts and rare bryophyte species at the regional scale. We also found that warmer and drier springs presented fewer bryophyte species. Our results show that the combination of nitrate pollution, increasing temperature and drought could severely threaten bryophyte diversity in Mediterranean springs. Our results indicate that the absence of rare bryophytes could be used as a bioindicator of nitrate pollution in springs.

Graphical abstract



Keywords: Mosses, liverworts, biodiversity loss, intensive farming, groundwater.

1. Introduction

Human activities are altering biogeochemical cycles at the global scale (Peñuelas et al., 2013). Due to extensive use of fertilisers, intensive farming and fossil fuel burning, the nitrogen (N) cycle has been disrupted far beyond the safe planetary operational limits (Steffen et al., 2015). Increased N deposition loads have been shown to entail positive consequences for humans, such as increased terrestrial productivity (Thomas et al., 2010) and reduced ecosystem respiration (Janssens et al., 2010), fostering carbon sequestration in terrestrial ecosystems (De Vries et al., 2009; Fernández-Martínez et al., 2017, 2014) (i.e., helping to mitigate climate change). However, increased N loads have been proven to be generally related to biodiversity loss (Soons et al., 2017). In freshwater ecosystems, increasing N loads can cause large changes through water eutrophication, dramatically changing their biotic communities (Tian et al., 2017). The high solubility of nitrate (NO_3^-) additionally allows N to enter into aquifers, which are very difficult and costly to depurate, entailing a threat to public health if such water is used for water supply for human consumption.

Despite decreases in N deposition across Europe and North America since international efforts were put into reducing NO_x emissions (Fernández-Martínez et al., 2017; Lajtha and Jones, 2013; Menz and Seip, 2004), groundwater pollution by nitrate from intensive farming and other sources is an increasing concern in many countries (Padilla et al., 2018) because of its impact on natural ecosystems and public health. One clear example is Catalonia (north-east Iberian Peninsula), where intensive farming has peaked during the last few decades, causing unprecedented nitrate pollution of their groundwater reserves, increasing their nitrate concentration year after year (García-Galán et al., 2010; Mas-Pla and Menció, 2019; Menció et al., 2011). Some of the springs draining those aquifers currently present concentrations far beyond 400 mg L^{-1} (Menció et al., 2016), even though the safety threshold from the World Health Organisation (WHO, 2011) is set at 50 mg L^{-1}.

Historically, these natural and semi-springs have shaped Mediterranean human societies and their rural activities because they were used for providing water to people and livestock. Furthermore, in the Mediterranean region, natural and semi-natural springs play an important role acting like small islands, where water is continuously available, surrounded by completely different environments where water is completely absent (Bes et al., 2018; Fernández-Martínez et al., 2019a). These spring ecosystems act, therefore, as refugia for animal and plant diversity truly becoming hotspots of biodiversity, sheltering species that would definitely disappear in a short period of time if the spring would dry out (Cantonati et al., 2012, 2006). In spite of their paramount importance for biodiversity conservation, the impact of nitrate pollution on the biotic communities of those springs is largely unknown.

Nitrogen pollution has been related to plant and bryophyte species loss because it can destabilize the competition between species in their communities (Pannek et al., 2015; Sayol et al., 2017; Stevens et al., 2010, 2004; Wamelink et al., 2009). Generally, when there is N pollution, N-tolerant (nitrophilous) species tend to be benefited in terms of productivity with respect to N-intolerant (nitrophobous) species, leading to competitive imbalances that end up simplifying the ecosystems in terms of diversity. These contrasting responses may be related to determined functional traits exhibited by both groups of plants that allow them to take advantage of the excess of N or not. There is also evidence that excess N can be toxic to bryophytes, causing damage to cell membranes and solute leakage, and subsequent reduced growth and even shoot death (Pearce et al., 2003). In terms of ecological monitoring, bryophytes are ideal study species because their lack of thick cuticles and true roots means that they absorb compounds directly through the whole surface of the plant. These characteristics make them very sensitive to environmental changes (Porley and Hodgetts, 2005), changing both their distribution and their functional traits (Fernández-Martínez et al., 2019a). The widespread occurrence of this taxa in natural and semi-natural springs draining aquifers, provides a unique opportunity to investigate the role of nitrate groundwater pollution on spring species diversity.

Here, we sampled the water and the bryophyte assemblages (mosses and liverworts) of 338 semi-natural springs, across a gradient of springs with contrasting water nitrate concentration, water conductivity and climate, in the north-east of the Iberian Peninsula. Our aim was to investigate, at the local and regional scale, how nitrate pollution affects the distribution and species richness of bryophytes in these ecosystems. We hypothesised that nitrate concentration decreases diversity of bryophytes in springs and that this decrease is more evident for rare species because of their potential higher sensitivity. We also investigated whether water nitrate concentration was related to differences in the functional traits (i.e., morphology, physiological, growth forms) of moss communities, suggesting patterns of moss assemblages in water-polluted springs.

2. Methods

2.1 Experimental design, field and laboratory analyses

We surveyed 338 springs located across Catalonia (north eastern Iberian Peninsula, **Figure S1**) capturing a large gradient in climatic and lithological conditions (Fernández-Martínez et al., 2019b). Climate in our springs was mainly Mediterranean (humid and sub-humid), with considerable differences in terms of their mean annual temperature, annual precipitation and their seasonality (Martín-Vide, 1992). The study area comprised a set of regions with contrasting natural (i.e., mountains, forests), agricultural (crops), and residential activities (often with wastewater leaks), leading to a large gradient in groundwater nitrate pollution.

Sampled springs were human-made constructions of reduced dimensions aimed to collect water from aquifers and release it through a spout. All springs continuously drained non-treated water and presented a similar morphology, including a wet rock wall, usually one single spout (sometimes more) from where the water emerges, a small sink to retain water and a small channel to drain the water. Hence, in our springs, bryophytes were in permanent contact with water, with interruptions to water flow only in some springs because of frozen water during winter or intense drought during summer.

In the field, we measured water electric conductivity and pH with a combined pH and conductivity meter for each spring. We also collected a water sample to analyse nitrate (NO_3^-) by ionic chromatography (see (Fernández-Martínez et al., 2019b) for further details). Geographical coordinates of each spring were obtained through a GPS device and used 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 further calculated climate water availability as the monthly precipitation minus evapotranspiration estimated following Hargreaves (1994). We finally calculated annual sums of temperature and water availability per spring. We also recorded whether the springs were located under shade or whether they received direct sunlight.

Bryophytes were sampled by collecting a sample of all species present in the springs that were in direct contact with the water or receiving drops over the splash zone. Given that previous studies reported no species richness – area relationship in these springs (Bes et al., 2018) and the reduced dimensions of most of them (three quarters of springs are less than 1 m^2) we did not standardise our sampling by area. Bryophytes were identified using Smith (1990) and Casas *et al.*, (2001, 2004) identification keys and following the nomenclature proposed by Hill et al., 2006. We found a total of 77 bryophyte species (**Figure S2**).

We additionally used 13 moss functional traits estimated for 30 of our moss species (derived from (Fernández-Martínez et al., 2019a) and (Hill *et al*., 2007)) to test the effect of nitrate on moss functional traits. We used eight morphological traits (leaf length, width, form [leaf length-to-width ratio] and area, moss mass per area [MMA], water absorption capacity [WAC], density and spore diameter) and five life forms (cushions, mat-roughs, turfs, tall turfs and whether they were pleurocarpous or acrocarpous).

2.2 Statistical analyses

We first explored whether water nitrate pollution affects species richness of *i*) bryophytes (moss + liverworts), *ii*) mosses, *iii*) liverworts, and *iv*) rare species of bryophytes (species present in fewer than 8 springs in our database) at the local scale. The threshold to define rare species when found at 8 springs or fewer was chosen to ensure that models predicting the distribution of non-rare species were fitted with enough data points of presence (9 springs or more). In total, 55 of the 77 species were defined as rare species. To do so, we fitted Poisson regressions in which the response variables were species richness per spring of *i* – *iv* and the explanatory variables were nitrate concentration in water, water conductivity, whether the spring was under shade or not, and the interaction between mean annual temperature and annual water availability (annual precipitation – potential annual evapotranspiration, see above). Apart from nitrate, we included these explanatory variables because previous research conducted in these springs showed that they significantly affect the distribution of bryophyte species (Bes et al., 2018; Fernández-Martínez et al., 2019a). Not including them could potentially mask the effect of nitrate on bryophyte species richness and their distribution. These models were fitted using the quasipoisson family to correct for overdispersion.

Similarly, we used a binomial regression model to test the effect of nitrate on the probability of finding any rare species in a determined spring. In that case, the response variable was a binary variable indicating whether the spring had at least one rare species (1) or not (0) and the explanatory variables were the same as described for the Poisson models. We further tested whether springs presenting rare species have statistically different nitrate concentrations than those without rare species using the Wilcoxon signed-rank test. We then tested which bryophyte species (present in more than 8 springs) were more or less tolerant to nitrate pollution while controlling for water conductivity (the main driver of bryophyte distribution in these springs (Bes et al., 2018; Fernández-Martínez et al., 2019a)) using binomial models, where the response variable was the presence (1) or absence (0) of each species per spring and the explanatory variables were water conductivity and nitrate concentration. We then used the estimated standardised coefficients to explore the relationship between the estimated effect of nitrate and water conductivity versus the number of springs in which each species was found. To avoid alpha-inflation (23 models), *P*-values were corrected following Benjamini and Hochberg, (1995).

We further tested the effect of nitrate pollution on bryophyte species richness over multiple springs following a gradient of water nitrate concentration (regional scale). To do so, we first sorted the springs according to their nitrate concentration. We then selected the first 60 springs and performed a bootstrap analysis (repeating 1000 times) over 50 out of the 60 springs to calculate species richness of bryophytes, mosses, liverworts and rare species. We repeated this analysis on a moving window of 60 springs until the last spring was included (N=338). Then, we calculated the Kendall's rank correlation and the Theil-Sen's slope (Ohlson and Kim, 2015) between the average of the bootstrapped species richness and water nitrate concentration in natural logarithmic scale. We repeated the same analyses using water conductivity, mean annual temperature and annual water availability to compare the correlations obtained with nitrate to those with these other explanatory variables.

Finally, we explored the effect of nitrate pollution on moss traits. To do so, we first performed a principal components analyses (PCA), using all 13 moss traits described in the previous section, and extracted the two first axes (PC1 and PC2, **Figure S3**). Then, we used the species scores to estimate a community-weighted score per spring for both axes (based on the presence of moss species). Springs without any of the 30 moss species with traits were excluded from the analyses. We then used linear regressions to test the effect of nitrate concentration, water conductivity, shade, and the interaction between mean annual temperature and annual water availability on PC1 and PC2. Then, we multiplied the correlation between the traits and the axes (in the PCA) with the statistically significant standardised coefficients estimated by the linear models to obtain a pseudo-correlation between the traits and explanatory variables. We finally used a clustered image map to show the results (*cim* function in *MixOmics* (Le Cao et al., 2017) R package).

All analyses were performed using R statistical software (R Core Team, 2018) and model visualisations were extracted using the *visreg* (Breheny and Burchett, 2015) R package.

3. Results

3.1 Effect of nitrate on species richness of bryophytes per spring (local scale)

Our results indicated that species richness of bryophytes (mosses + liverworts), mosses and liverworts per spring were not statistically related to water nitrate concentration at the local scale (**Table 1**). The amount of species per spring of these three groups (bryophytes, mosses and liverworts) presented a statistically significant and positive interaction between mean annual temperature and annual water availability (**Figure 1**) and a higher number of species in springs under shade compared to those receiving direct sunlight (**Table 1**). Instead, the amount of rare species (occurring in fewer than 8 springs) per spring was negatively correlated with water nitrate concentration and positively with annual water availability. Furthermore, the probability of finding at least one rare species in a spring was only significantly and negatively related with increasing water nitrate concentration (**Figure 2**). Consequently, springs without any rare species present had significantly higher nitrate concentration compared to those with at least one rare species. In fact, only two out of 48 springs (4%) with nitrate above 50 mg L^{-1} presented at least one rare species, while 93 out of 290 (32%) with nitrate below 50 mg L^{-1} presented at least one rare species. No rare species were found at all in springs with more than 90 mg L^{-1} of nitrate.

The frequency of occurrence of bryophyte species was not directly related to their correlation with nitrate concentration or water conductivity (**Figure 3**). However, the correlation between species presence and nitrate concentration was generally stronger for negative relationships than for positive ones. It was also found to be negative for more species (13 species plus all rare species together) compared to the number of species showing a positive relationship (9). Nonetheless, only the presence of rare species was significantly predicted by nitrate concentration. A different pattern was shown with water conductivity. A total of six species presented a statistically significant relationship with water conductivity: three of them positive (*Pohlia melanodon*, *Didymodon tophaceus* and *Eucladium verticillatum*) and three of them negative (*Brachythecium rivulare*, *Platyhypnidium riparioides* and *Plagiomnium undulatum*). The presence of rare species was not related to water conductivity.

3.2 Effect of nitrate on bryophyte diversity across springs (regional scale)

Our analyses at the regional scale further supported our analyses at the local scale, but showing a greater effect of nitrate. We found that increasing nitrate concentration was negatively related to bryophyte species richness over groups of 60 springs (**Figure 4** and **Figure 5**). The negative relationship between nitrate and species diversity was of a similar magnitude when analysing overall bryophyte diversity, rare species, mosses or liverworts (**Table 2**). We repeated these analyses using conductivity, mean annual temperature and water availability, instead of nitrate, to further test that the previously observed decrease in bryophyte species richness was due to increasing nitrate pollution and not to other potentially confounding environmental effects. Our results were similar with water conductivity and mean annual temperature, but effect sizes were statistically lower than those found for nitrate (**Table 2,** see confidence intervals of correlation coefficients). Annual water availability, instead, presented a positive correlation with regional bryophyte diversity. Absolute effect sizes were of similar magnitude compared to those of nitrate, except for mosses for which they were statistically significantly lower.

3.3 Effect of nitrate on moss functional traits

Linear models did not find any significant effect of nitrate, water conductivity, mean annual temperature, water availability or shade on the community weighted scores of the first axis of a PCA (explaining 32.0% of the variance in moss traits, **Figure S3**) performed using 13 functional traits and 30 moss species. The second PCA axis (PC2, explaining 24.3% of the variance in moss traits), instead, was negatively related to nitrate concentration (standardised parameter estimates, β=-0.15 ± 0.06, P=0.006), annual water availability (β=-0.26 ± 0.06, P<0.001) and shade (β=-0.11 ± 0.04, P=0.017). Based on these model coefficients and the correlations between the traits and PC2, we found that low density mosses with large leaves and spores, with high water absorption capacity that were more likely to be pleurocarpous and form mats were more likely to be found in springs with high nitrate concentration and high water availability but low water conductivity (**Figure 6**). Conversely, dense mosses with needle-like leaves forming cushions were more likely to be found in springs with low nitrate concentration and annual water availability but high water conductivity.

4. Discussion

Our results confirmed that groundwater nitrate pollution is threatening bryophyte diversity in springs, both at the local and at the regional scale, thus jeopardizing hotspots of biodiversity that should be protected (Bes et al., 2018; Cantonati et al., 2012, 2006). We found that increasing nitrate pollution was the main factor related to losing rare species at the local scale (**Figure 2**) and overall bryophyte diversity at the regional scale (**Figure 4** and **Figure 5**), again potentially because of the disappearance of the less frequent bryophytes from the pool of species. Additionally, the moss communities that benefit from nitrate pollution seem to be characterised by particular functional traits (**Figure 6**), potentially reducing bryophyte functional diversity. Our results also indicated that climate change may threaten bryophyte diversity in Mediterranean springs. Our results show that in springs under a drier climate (i.e., higher temperature and lower annual water availability) bryophyte diversity is lower compared to springs under wetter climate (**Table 1, Table 2, Figure 1**). Given that climate projections for the Mediterranean region point towards increased temperature and reduced precipitation (Alexander et al., 2013), it is expected that spring bryophyte communities, and their associated organisms, will be simplified or partially lost. Therefore, simultaneously increasing nitrate, temperature and drought, could have a synergistic effect on bryophyte species loss in Mediterranean springs. Hence, our study demonstrates that Mediterranean spring ecosystems are threatened by anthropogenic activities, both directly (i.e., groundwater pollution) and indirectly (i.e., climate change). These valuable biodiversity hotspots can act as refugia, increasing the probability of survival of the species. Preserving them should be a top priority in conservation plans of natural areas.

4.1 Mechanisms behind the negative effect of nitrate on bryophyte diversity

The main mechanism by which ecosystem eutrophication has been suggested to reduce biodiversity is through the disruption of the competitive interactions between the species within a community (Hautier et al., 2014, 2009; Soons et al., 2017). In vascular plant communities, plants are often N-limited, so when ecosystem nitrogen availability increases, nitrophilous (often fast-growing) species tend to dominate the community to the detriment of other species unable to benefit from an increase in nitrogen. Bryophytes may not be as N limited as vascular plants, as they require low amounts of nutrients and can take them up directly through the whole surface of the organism

(Porley and Hodgetts, 2005). In fact, bryophytes are known to be highly sensitive to changes in environmental conditions, so different responses to N between species may be more related to the maximum level that they can tolerate rather than the minimum amount they need. They are known to vary in their tolerance to N and the optimum amount, for maximum growth (Salemaa et al., 2008), and there are numerous examples of terrestrial bryophytes being damaged by direct exposure to N (in the form of nitrate and also ammonium), leading to cell membrane damage, shoot death and reductions in bryophyte cover (Carroll et al., 2000; Pearce et al., 2003; Potter et al., 1995).

Thus, for bryophytes in springs, when N increases, different scenarios can occur: *i*) nitrophobous species are lost because of N intolerance, *ii*) the less frequent species are lost first, as other species out-compete them, or *iii*) a combination of both, as rare species may also be nitrophobous. Our results support more the second, and potentially third, mechanism, given the fact that the main effect of nitrate pollution we detected was related to a decrease in rare species (**Figure 2**). Nitrophilous vascular plants often present similar traits, such as larger leaves, lower leaf dry matter content and higher specific leaf area (Shipley et al., 2017). Similarly, we found that, once the main effect of water conductivity was accounted for (Fernández-Martínez et al., 2019a), moss species that benefited from increased nitrate concentration were those with bigger leaves, that absorb more water and are lighter (**Figure 6**). These traits may be related to better moss competitive ability in contrast to dense, small mosses with needle-like leaves which may grow at lower rates. Hence, lighter and bigger mosses may be able to overgrow other less productive species impeding their development. However, species dominance, in bryophytes is usually more related to an efficient retention and a conservative use of captured resources than to a higher competitive ability (Grime et al., 1990). The nature of interactions between bryophytes therefore remain elusive.

Notwithstanding the role of competition between bryophytes themselves, nitrophilous vascular plants may also be favoured in nitrate-rich springs (Hautier et al., 2014), entailing an additional difficulty in terms of survival of small bryophytes (e.g., because of competition for space and light) (Porley and Hodgetts, 2005). Such an outcome, of negative correlations in biomass production between bryophytes and vascular plants under N addition, has been shown in different systems including grasslands (Hejcman et al., 2010; Virtanen et al., 2017) and nutrient-poor calcareous fens (Bergamini and Pauli, 2001).

4.2 Interaction between nitrate and water conductivity

As nitrate pollution in our springs occurs mainly because of manure and mineral fertilizer application into agricultural fields, nitrate pollution can also have additional impacts because of a joint effect with other factors, such as increased water conductivity (Fernández-Martínez et al., 2019b; Menció et al., 2016). Increased water conductivity has been suggested to act as an important environmental filter in these bryophyte communities (Bes et al., 2018; Fernández-Martínez et al., 2019a) because of the particular adaptations required to withstand hard water (e.g., osmotic stress, calcium carbonate crusts). Our results further support these hypotheses, as we found that different moss species present contrasting preferences for water conductivity (**Figure 3**). Nitrate pollution may thus not only remove rare species but also change bryophyte assemblages because of the substitution of bryophytes with preference for low conductivity water (e.g., *Brachythecium rivulare*, *Platyhypnidium riparioides* and *Plagiomnium undulatum*) to bryophytes preferring hard waters (e.g., *Pohlia melanodon*, *Didymodon tophaceus* and *Eucladium verticillatum*). Hence, the joint increase in nitrate and water conductivity may certainly have a large impact on moss communities because of alteration of natural conditions.

5. Conclusions

Our results clearly indicate that nitrate pollution is threatening bryophyte diversity in Mediterranean springs, which are hotspots of biodiversity acting as refugia. Nitrate pollution is especially related to the decrease of rare bryophytes, which can potentially reduce diversity at the local and regional scale if water pollution occurs over a broad area. Impacts may be especially large under warmer and drier environmental conditions produced by climate change. Hence, simultaneously increasing nitrate, temperature and drought may pose a serious threat to the conservation of bryophyte diversity in Mediterranean springs. Strong policies are needed to correct this damage and avoid further diversity loss.

Author contributions

MFM and CP planned and designed the research. MFM, JC, XD, Fe. S, Fr. S, and CP conducted fieldwork. MFM, JC, and CP conducted laboratory analyses. MFM analysed the data. All authors contributed substantially to the writing of the manuscript.

Conflict of interests

The authors declare no conflict of interests.

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Figure captions

Figure 1: Response surface showing bryophyte species richness per spring as a function of climate water availability (annual precipitation - annual evapotranspiration) and mean annual temperature (MAT). Model parameter estimates are shown in **Table 1**.

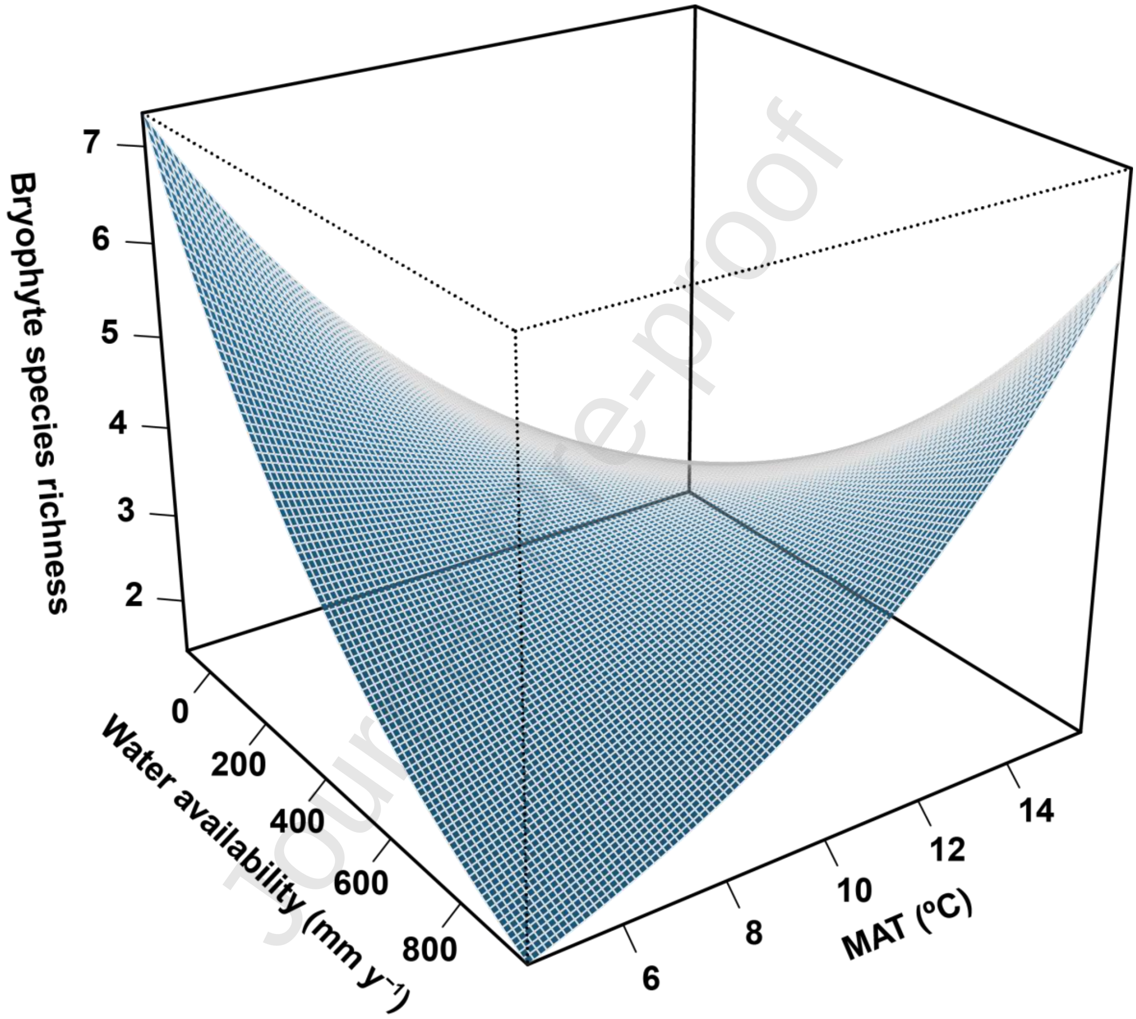

Figure 2. Estimated probability of finding a rare bryophyte species (fewer than 8 occurrences in our database) as a function of water nitrate concentration. The standardised coefficient (*β*) and its statistical significance (*P*), derived from a binomial model, are also shown. The inset illustrates the difference in water nitrate concentration of springs not presenting rare bryophytes versus springs presenting rare bryophytes using a box-plot graph. Differences between groups were tested using the Wilcoxon signed-rank test. Values inside the boxplots show the median values of nitrate of each group.

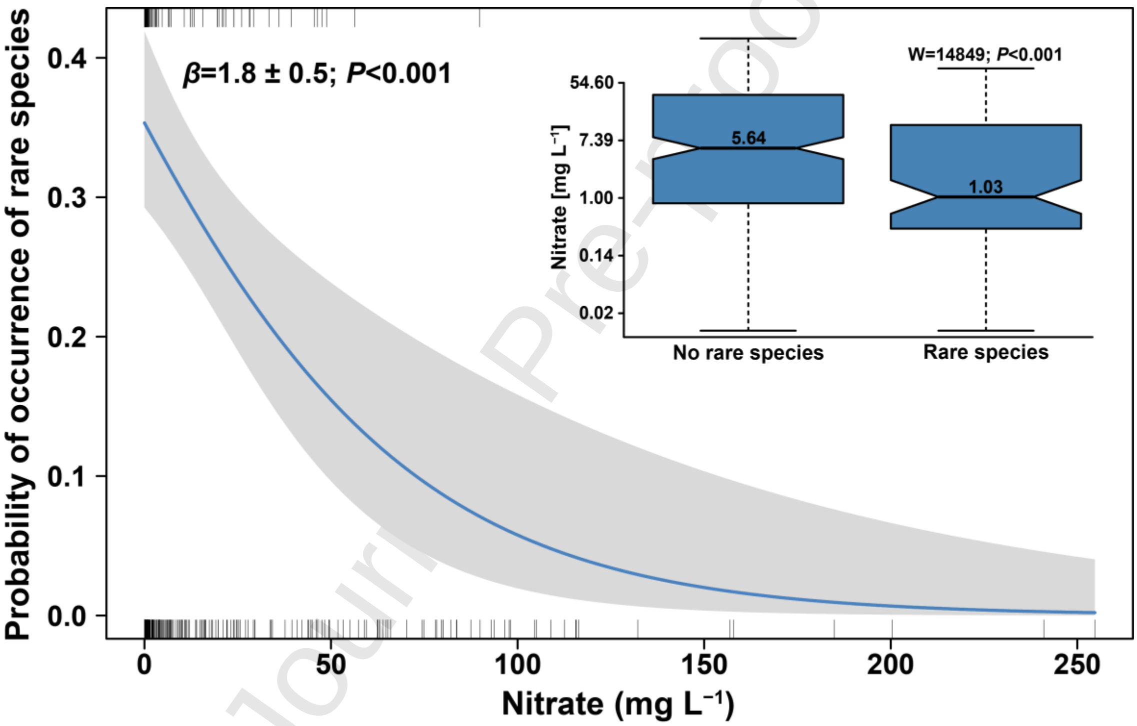

Figure 3. Standardised nitrate (a) and conductivity (b) effects, estimated using binomial regression models predicting bryophyte species occurrence as a function of nitrate concentration and water conductivity. Parameter estimates are plotted against the absolute frequency of occurrence of the bryophyte species used (species occurring in 8 or more springs). Rare species (fewer than 8 records) were all grouped together (triangle). Statistically significant coefficients (*P* < 0.05), after correcting for multiple comparisons (N=23) following (Benjamini and Hochberg, 1995), are denoted with a black point and the name of the bryophyte species.

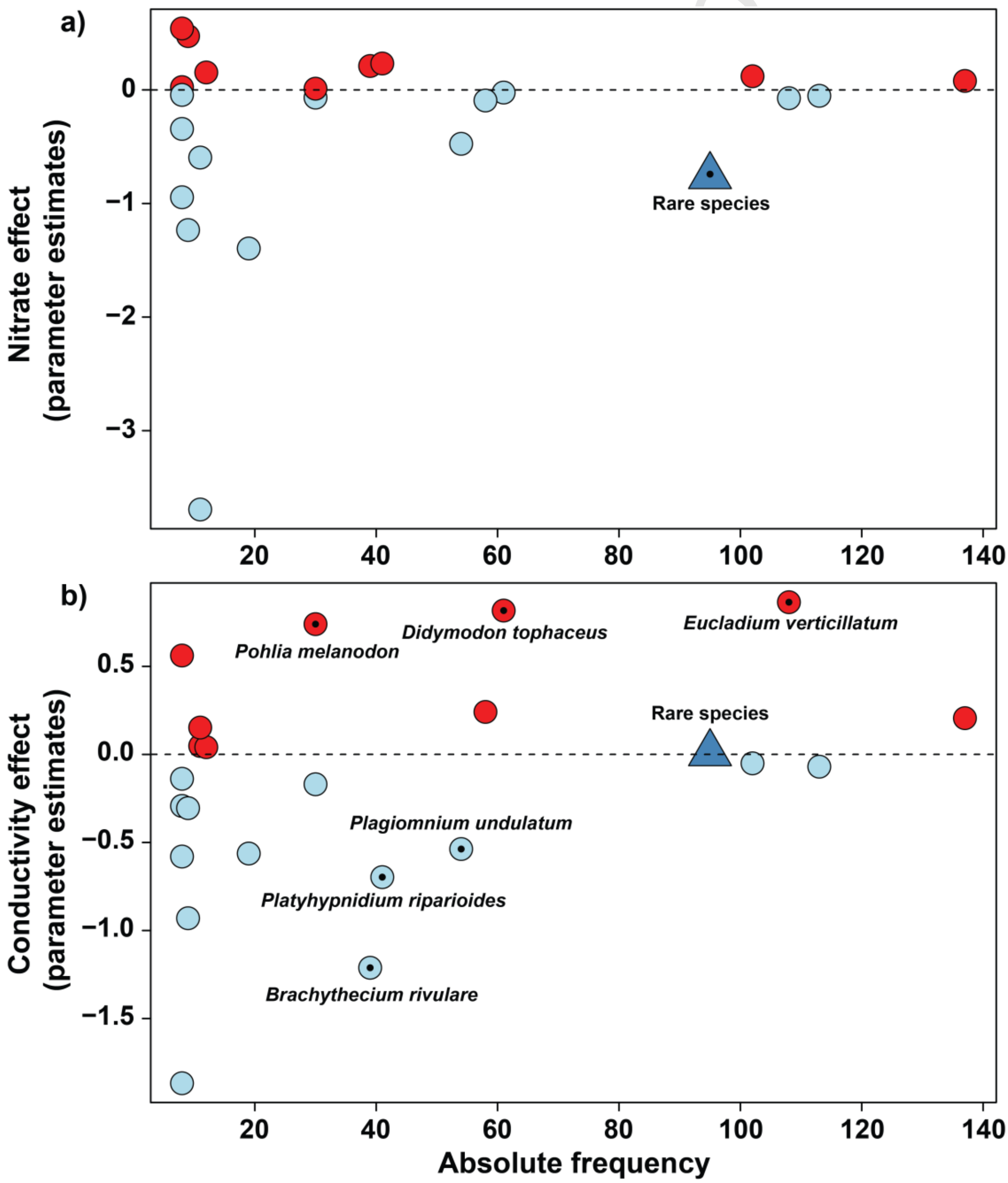

Figure 4: Relationships and Kendall's correlations (τ) between the bootstrapped species richness of bryophytes and rare species (occurring in fewer than 8 springs sampled) over 50 springs on a moving window, going from low to high values of water nitrate concentration. Solid lines indicate the average species richness surrounded by the 95% confidence intervals. Slopes (dashed lines) were calculated using the Theil-Sen's slope estimator. Bootstrapped 95% confidence intervals of *τ* are shown as sub- and superscripts after τ. See **Table 2** for further details.

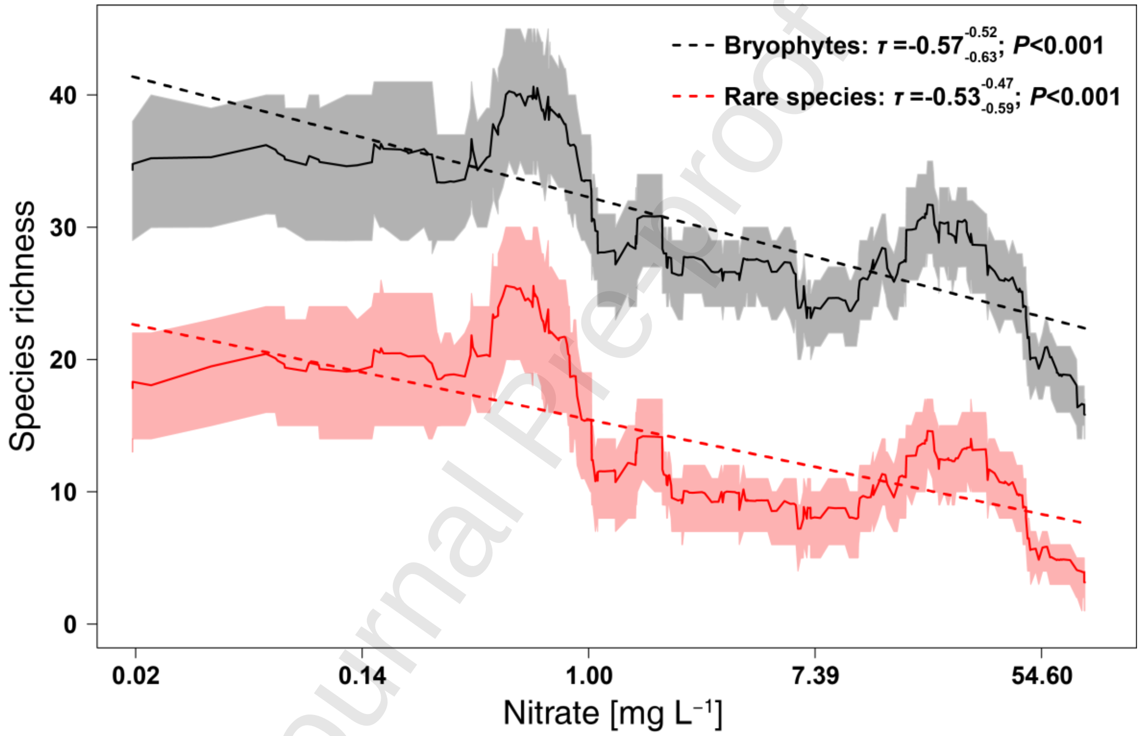

Figure 5. Relationships and Kendall's correlations (τ) between the bootstrapped moss and liverwort species richness over 50 springs on a moving window, going from low to high values of water nitrate concentration. Solid lines indicate the average species richness surrounded by the 95% confidence intervals. Slopes (dashed lines) were calculated using the Theil-Sen's slope estimator. Bootstrapped 95% confidence intervals of τ are shown as sub- and superscripts after τ. See **Table 2** for further details.

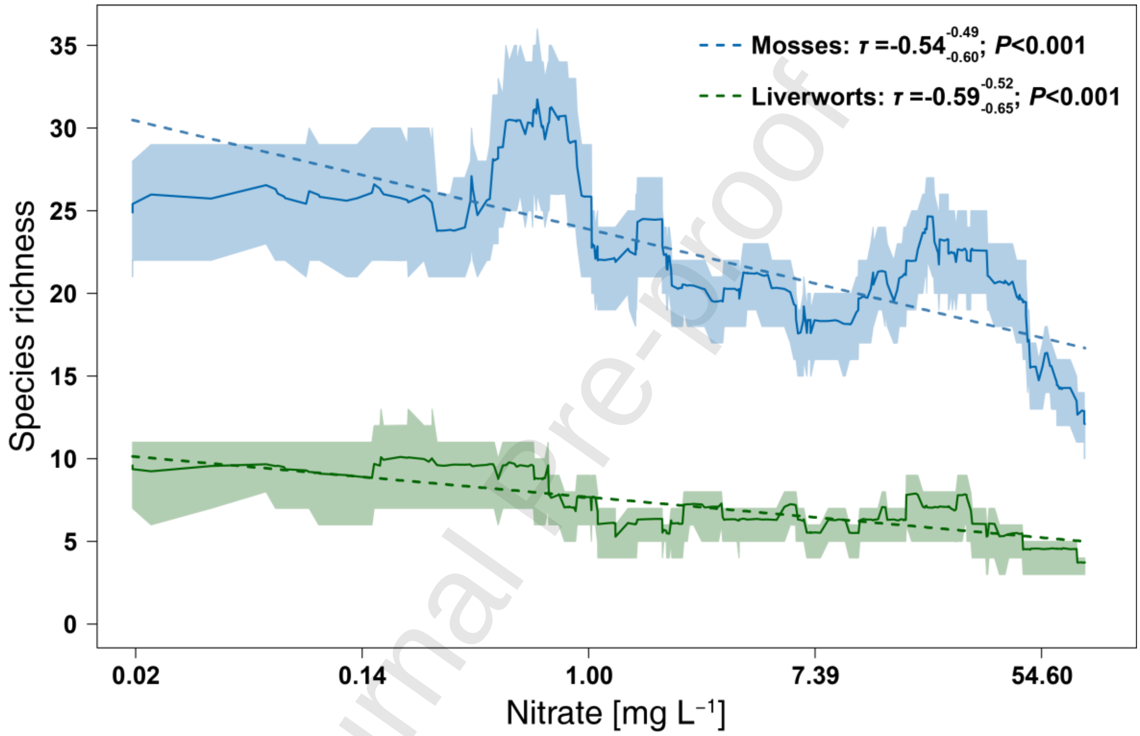

Figure 6. Clustered heatmap showing the effect of water nitrate concentration, water conductivity and climate water availability (annual precipitation - annual evapotranspiration) on 13 moss functional traits. See **Methods** for details on the estimation of correlation coefficients.

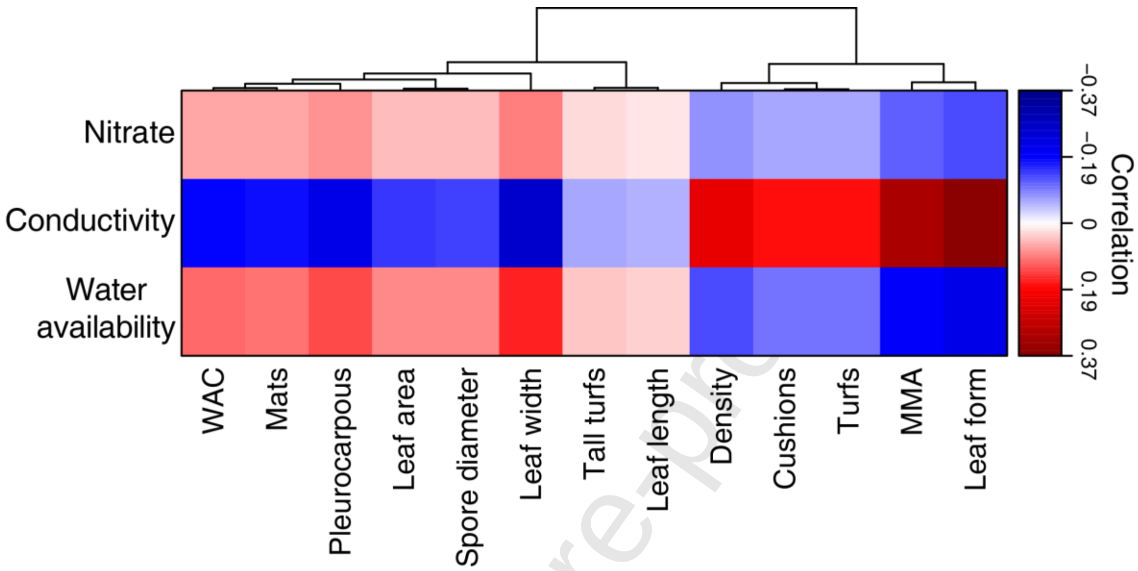

Table 1. Parameter estimates of the Poisson regression models predicting bryophyte, rare species (fewer than 8 occurrences in our database), moss, and liverwort species richness per spring. *Acronyms*: mean annual temperature (MAT), climate water availability (WA: annual precipitation - annual potential evapotranspiration), non-significant effect (n.s.). Significance levels: P<0.001***, P<0.01**, P<0.05*.

	Nitrate	MAT	WA	MAT:WA	Shade
Bryophytes	*n.s.*	-0.129 ± 0.049**	-0.338 ± 0.104**	0.270 ± 0.076***	0.059 ± 0.021**
Rare species	-0.778 ± 0.282**		0.359 ± 0.130**	*n.s.*	*n.s.*
Mosses	*n.s.*	-0.206 ± 0.064**	-0.435 ± 0.129***	0.318 ± 0.095***	*n.s.*
Liverworts	*n.s.*	-0.319 ± 0.238	-1.548 ± 0.578**	1.329 ± 0.418**	0.456 ± 0.135***

Table 2. Kendall's correlations (τ) between the bootstrapped species richness of bryophytes, rare species (occurring in fewer than 8 springs sampled), mosses and liverworts over 50 springs on a moving window, going from low to high values of water nitrate concentration, water conductivity (**Figure 4** and **Figure 5**), MAT and WA. *Acronyms*: mean annual temperature (MAT), annual water availability (WA: annual precipitation - annual potential evapotranspiration).

	Nitrate	**Conductivity**	**MAT**	**WA**
Bryophytes				
Correlation (τ)	-0.57	-0.36	-0.21	0.53
low CI (2.5%)	-0.63	-0.45	-0.28	0.46
high CI (97.5%)	-0.52	-0.26	-0.14	0.60
P	<0.001	<0.001	<0.001	<0.001
Rare species				
Correlation (τ)	-0.53	-0.24	-0.47	0.56
low CI (2.5%)	-0.59	-0.33	-0.52	0.51
high CI (97.5%)	-0.47	-0.14	-0.42	0.60
P	<0.001	<0.001	<0.001	<0.001
Mosses				
Correlation (τ)	-0.54	-0.30	-0.06	0.38
low CI (2.5%)	-0.60	-0.39	-0.13	0.30
high CI (97.5%)	-0.49	-0.21	0.01	0.46
P	<0.001	<0.001	0.122	<0.001
Liverworts				
Correlation (τ)	-0.59	-0.44	-0.26	0.66
low CI (2.5%)	-0.65	-0.51	-0.32	0.61
high CI (97.5%)	-0.52	-0.38	-0.19	0.71
P	<0.001	<0.001	<0.001	<0.001

Nitrate pollution reduces bryophyte diversity in Mediterranean springs

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Highlights

- Intensive farming is increasing groundwater nitrate pollution in many regions

- We studied 338 springs across a gradient of climate, nitrate and water conductivity

- Nitrate pollution was associated with decreased bryophyte diversity in springs

- Rare bryophyte species were not present in highly polluted springs

- Mosses living in springs polluted with nitrate were larger and less dense