Title: Environmental variables driving species composition in Subarctic springs

Short running title: Environmental variation in Subarctic springs

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Abstract

Questions: Which environmental variables are most important in determining plant species composition in Subarctic springs? Do observed patterns differ between typical wetland and general matrix species?

Location: Helocrenic (seepage) springs, Northern Norway

Methods: We sampled 49 helocrenic spring sites, measuring environmental variables (water temperature, water pH, electrical conductivity, discharge volume, geographic position) and recording all species present. We performed a partial canonical correspondence analysis (pCCA) to determine the relative importance of water quality, spatial, and climatic variables for patterns in species composition and to compare the differences in these patterns between wetland generalist and specialist species.

Results: We found that climatic and water quality variables were almost equally important in determining species composition in Subarctic springs, with climatic variables explaining 26.62% of variation in species composition and water quality variables explaining 26.14%. Spatial variables explained the least variation (21.53%). When looking at the variables individually, altitude (10.93%) and mean summer temperature (9.25%) explained the most variation. The trend was the same for matrix species and wetland species, with climatic variables explaining the most variation (matrix: 27.26%; wetland: 24.42%), followed by water quality (matrix: 26.40%; wetland: 24.13%) and spatial variables (matrix: 24.87%; wetland: 16.27%). The main difference between general matrix species and typical wetland species was that the spatial variables explained less variation for wetland species.

Conclusions: The close relationship of species composition (total vegetation as well as separated into wetland and matrix species) with climatic and water quality conditions indicates a sensitivity of Subarctic springs to future climate change. In combination with altitude, which was found to be the most important individual variable, it is likely that the future distribution of spring species tracking climate change will be limited by the occurrence of suitable spring habitats, especially at high altitudes.

Keywords: helocrenic, pCCA, bryophytes, vascular plants, Norway

Introduction

Spring ecosystems are important hotspots of biodiversity (Scarsbrook *et al.*, 2007; Cantonati *et al.*, 2012; Ilmonen *et al.*, 2012; Cantonati *et al.*, 2020). These islet-like systems may be critical for maintaining a high biodiversity because of the specific and stable habitat conditions they provide: high water quality, thermal constancy, and low seasonal variability (Odum, 1971; Hobbie, 1984; Callaghan, 2005; Wrona *et al.*, 2005; Audorff *et al.*, 2011; Cantonati *et al.*, 2012; Glazier, 2012). These factors result in distinct species composition and higher species richness, often including highly specialized organisms, and higher incidence of rare and red-listed species than in surrounding areas with disparate environmental conditions (Rosenzweig, 1995; Cantonati *et al.*, 2009; Gerecke *et al.*, 2011; Tomaselli *et al.*, 2011; Cantonati *et al.*, 2012; Cantonati *et al.*, 2020). Additionally, helocrenic – or seepage – springs are characterized by diffuse outflow and low water current velocity, as well as gentle sloping and rare flooding events, all of which provide a more conducive environment for the establishment of a wide variety of plant species, diatoms, and benthic invertebrates (Rosenzweig, 1995; Cantonati *et al.*, 2009; Gerecke *et al.*, 2011; Ilmonen *et al.*, 2012; Spitale *et al.*, 2012).

The high biodiversity found in springs makes them a critical ecosystem to investigate. Despite that, springs are vastly understudied (Dudgeon *et al.*, 2006; Cantonati *et al.*, 2011; Cantonati *et al.*, 2012). With the exception of a handful of articles in the mid-1900s (e.g. Nordhagen, 1943; Dahl, 1957), scientific investigation of spring vegetation is relatively recent, starting predominantly in the 1990s (Økland and Bendiksen, 1985; Zechmeister and Mucina, 1994; Lindegaard, 1995; van der Kamp, 1995). Studies limited to central Europe have found that the key factors determining spring species composition are pH and altitude (Cantonati *et al.*, 2006; Audorff *et al.*, 2011; Kapfer *et al.*, 2012; Spitale *et al.*, 2012; Schweiger *et al.*, 2015b).

However, these studies leave many questions unanswered. For example, are patterns in the explanation of spring species composition maintained across different regions and climates?

Another key question is whether different groups of spring species respond differently to environmental variables. For example, Kapfer *et al.* (2012) found that bryophytes and vascular plants do not differ in their responses to spring water quality, whereas Horsáková *et al.* (2018) found that the species richness of fen habitat specialists responded to different environmental factors than matrix-derived species (species occupying both fens and surrounding habitats). Therefore, we aim to elucidate whether different or similar environmental variables influence the species composition of general matrix species which are found in spring and wetland habitats as well as other habitats – compared to typical wetland species which are only found in springs and other wetland habitats. It is important to understand specifically which factors are most important for specialist wetland species in order to conserve these species which are found in a limited number of habitats and prone to local extinctions (Horsáková *et al.*, 2018).

This study aims to fill these gaps in our knowledge by investigating the plant species diversity and environmental conditions of helocrenic springs in a previously unstudied region: Northern Norway. Under the extreme environmental conditions of the Subarctic – including long winters, short summers, and generally cold temperatures – species patterns and links to environmental variables may differ from those found in other regions. These patterns are important for understanding and preserving the biodiversity present in these ecosystems. Here, we describe the key characteristics of these Subarctic springs and seek to establish which environmental factors are most important in determining species composition and whether these trends differ between general matrix species and typical wetland species.

Methods

Study area

The studied helocrenic springs are fed by near-surface groundwater originating from forested and alpine catchments within Troms County, Northern Norway, ranging from 69.02 to 69.78 °N and 18.20 to 20.61 °E (Fig. 1). They are characterized by a groundwater-saturated area covering a few square meters to some hundred square meters, seeping out water with constant but rather low discharge rates (≤ 0.5 L/s). Mean water temperature across sites was 6.2 °C, indicating that the groundwater is independent from the influence of more-frequently fluctuating surface air temperatures (Spitale *et al.*, 2012; Schweiger *et al.*, 2015a).

The locations of the spring sites follow a climate gradient from the coast to inland, with altitudes varying from 86 to 852 m above sea level (a.s.l.). The climate of the region is continental Subarctic (Kottek *et al.*, 2006). Temperatures in the study area average 9.0 °C in the summer and -6.3 °C in the winter (Norwegian Meteorological Institute, 2015). Near the coast, winters are relatively mild compared to the inland due to the North Atlantic Current. Annual precipitation in the study sites ranges from 414 to 1385 mm and falls mostly in the form of snow from early November to early April, with a longer snow season at higher altitudes (Norwegian Meteorological Institute, 2015).

The study region is characterized by northern boreal birch forest. The low alpine region is dominated by *Salix* spp., and the middle alpine region is dominated by open mountain heath (Moen, 1999). Treeline occurs at approximately 500-600 m a.s.l (Körner, 1998).

The majority of the study area bedrock is part of the Caledonian nappes, with the exception of some granitic rocks at the Northwestern extent of our study area (Ramberg *et al.*, 2008). On the mainland, the bedrock types in the studied areas include gneiss, granite, slate, quartz, schist, and the occasional strips of calcite marble (Norwegian Geological Survey, 2016).

Data collection

We sampled 49 springs over two summers (10 in 2014, 39 in 2015). Consideration was given to selecting springs with a variety of different plant communities, surrounding ecosystems, bedrock types, and altitudes. We selected spring sites generally along two climate gradients: a temperature gradient, with mean annual temperatures decreasing further inland (with decreasing latitude) and with increasing altitude, and a precipitation gradient, with mean annual precipitation increasing moving west (with decreasing longitude) and with increasing altitude. Table 1 displays descriptive statistics of the environmental variables for each spring, and a complete set of the recorded variables are available in Appendix S1.

At each spring, considering the entire seeping zone (ranging from 1.5-80 m^2), which is demarcated where the spring abruptly transitions to dry ground, we sampled vegetation by listing each species present. For environmental data sampling, we measured water temperature, water pH, electrical conductivity, discharge volume, and spring area. In measuring spring area, we considered the border to be where the seeping zone abruptly transitioned to dry ground. This demarcation was also characterized by an abrupt change in plant community type. We defined the lower border of the spring to be where the spring water started to build a small brooklet with water running downhill perpendicular to the spring seepage area. Water temperature, pH, and conductivity were measured *in situ* with a pH/conductivity-meter (Mettler Toledo, model SevenGo Duo SGD SG23-ELK with InLab Cool glass electrode). A small hole was dug and water allowed to clear before measuring. Discharge was measured in liters per minute by measuring the time it took to fill a 0.5 or 1 L bucket, and then we translated the units to L/s. Altitude and coordinates were taken in the field with a GPS receiver (Garmin model eTrex Vista HCx). Climate data, including mean seasonal temperature and mean total annual precipitation, were downloaded from Norwegian Meteorological Institute maps covering the normal period from 1961 to 1990. For the ten sites visited in 2014, area was not recorded, so this variable was excluded from further analyses. Plot area does not have a significant effect on analysis and classification of fen vegetation as long as plots are 1 m^2 or larger (Peterka *et al.*, 2020). A full list of species present in the springs can be found in Appendix S2. Nomenclature of species follows Lid and Lid (2005) for vascular plants and Damsholt (2002) for liverworts and Smith (2004) for mosses.

Data processing

We used a partial canonical correspondence analysis (pCCA, cca(.) function in the R 'vegan' package) to calculate the amount of variation in species composition that was explained by the environmental variables sorted into three main groups: water quality, which includes hydrophysical (water temperature, electrical conductivity, and discharge), and hydrochemical variables (water pH), spatial (altitude, latitude, and longitude), and climatic (mean annual temperature, mean summer temperature, and total annual precipitation). Overall, we performed three pCCA analyses: the first with all environmental variables individually, the second simplified with four selected variables, and the third for matrix and wetland species with the three main groups of environmental variables. The 'Condition' term was used to partial out the effects of different variable groups on each other. The variables of individual mean seasonal temperature and precipitation were excluded because they correlated highly with the annual means ($r \geq 0.7$, $p < 0.05$). The exception was mean summer temperature, which was included in the analyses because it did not correlate as highly with the mean annual temperature ($r = 0.68$, $p < 0.001$).

We limited the species used in the analysis to those with eight or more presence observations in order to reduce the noise caused by species with few observations. We chose the cut-off at eight observations because moving from seven to eight resulted in a large improvement in the amount of variation that the model explained, while still balancing a desire to keep a greater number of species in the analysis. Reducing the dataset further to nine or ten observations only resulted in small percentage increases in variation explained. The original dataset consisted of 235 species, of which 127 were vascular plants and 105 were bryophytes. We continued with the 54 species that had eight or more observations, consisting of 34 vascular plants and 20 bryophytes.

We tested each of the environmental variables with a logarithmic transformation to ascertain if a more normal distribution could be achieved. This was the case only for water temperature, conductivity, and mean summer temperature, so we continued with these three variables under log transformation and left the remaining variables untransformed. Water pH did not need a transformation because it is already a log-transformed variable. A transformation was not needed for the species data because it was already in binomial (presence/absence) format.

After the initial pCCA, we further narrowed down variables to use in a simplified model in order to explain the maximum amount of species composition variation with fewer variables. All of the environmental variables were tested pairwise, and one was excluded from each pair with a significantly high correlation ($r \geq 0.7$, $p < 0.05$). In most cases, the selected variable from each pair was chosen based on its ability to explain more of the variation in species composition.

In one case, we decided which variable to keep based on ecological significance. Longitude alone explained more variation (6.88%) than mean total annual precipitation (4.93%), but we chose to keep mean total annual precipitation because it was the more relevant variable in order to study the relationship between species composition and climatic conditions. Our final, simplified pCCA model consisted of four environmental variables: altitude, pH, mean annual temperature, and mean total annual precipitation.

Lastly, we performed pCCA separately on species separated into two groups: general matrix species (*n*=26) and typical wetland species (*n*=28). Species were classified based on moisture indicator values, which indicate the soil moisture conditions that a plant species prefers -- low values (1-3) indicate a preference for dry soils, middle values (4-7) indicate a preference for moist soils, and high values (8-12) indicate a preference for wet soils or aquatic conditions (Ellenberg *et al.*, 1992; Hill *et al.*, 1999; Tutin *et al.*, 2001; Hill *et al.*, 2007). Species with a value of 7 or less were considered general matrix species, and species with a value of 8 or greater were considered typical wetland species. This method of classification means that the typical wetland species group will include species with high moisture values that are not actually spring specialists but that prefer water-saturated habitats, such as typical mire species. However, springs often appear as islands within drier habitats, so the occurrence of wet habitat-dependent species is likely to largely depend on the presence of springs for our species sample. All analyses were run using R 3.1.1 statistical software (R Core Team, 2014).

Results

Patterns and drivers of species composition

The total amount of variation explained by all ten variables in the pCCA was 41.76%. The analysis revealed that the climatic variables (mean annual temperature, mean summer temperature, and mean total annual precipitation) are most important in determining species composition: they explained 26.62% of the variation (Fig. 2). The water quality variables (water temperature, water pH, electrical conductivity, and discharge) explained the next largest amount (26.14%), and the spatial variables (altitude, latitude, and longitude) explained the smallest amount (21.53%). There was a large amount of overlap between all three groups (11.58%) and also solely between the groups of water quality and climatic variables (6.84%; Fig 2).

When looking at the effects of individual variables, the most influential variable was altitude, which explained 10.93% of the variation in species composition and 3.28% with the overlapping effects of the other nine variables removed. The second most influential variable was mean summer temperature, which explained 9.25% of the variation and 2.87% with the effects of the other variables removed.

For the simplified pCCA, the first axis corresponded strongly to the altitudinal gradient and the second axis to the pH gradient, with components of mean annual temperature and mean total annual precipitation in both axes (Fig. 3). Individual species differed in their responses to these environmental gradients, with some species strongly associated with one or two variables (e.g. *Saxifraga aizoides* strongly associated with water pH and *Saxifraga cernua* strongly associated with altitude).

Differences between general matrix species and typical wetland species

When separating the species into matrix and wetland species, the importance of the groups of environmental variables remained the same, but to differing extents. For matrix species, climatic variables were found to be the most determinant for species composition (27.26%), followed closely by water quality variables (26.40%) and then spatial variables (24.87%; Fig. 4A). For typical wetland species, the climatic variables remained the most determinant (24.42%), followed again by water quality variables (24.13%) and spatial variables (16.27%; Fig. 4B). However, for the typical wetland species, the amount of variation explained by the spatial variables was smaller than for matrix species.

Discussion

Effects of environmental variables on spring species composition

This study found that climatic variables, as a group, are the most important in determining species composition, followed closely by the water quality variables. However, the difference in our study between climatic and water quality variables was relatively small. The importance of climatic variables in determining species composition is particularly noteworthy as the Subarctic mainland of Norway is projected to experience increased temperatures and precipitation with the progression of climate change (Hassel *et al.*, 2010; Øseth, 2007; Haugen and Iversen, 2008; Førland *et al.*, 2009; Kirtman *et al.*, 2013; Norwegian Meteorological Institute, 2015; CliC/AMAP/IASC, 2016). Most previous studies looked at environmental variables individually, generally focusing on water quality and spatial variables (Hájková *et al.*, 2006; Sekulová *et al.*, 2011; Ilmonen *et al.*, 2012; Kapfer *et al.*, 2012, Spitale *et al.*, 2012). One of the studies that did compare variables in groups, Audorff *et al.* (2011), did not include climatic variables, but similarly found water quality variables to be more determinant than spatial variables.

When looking at individual variables, we found that altitude was the most important environmental variable in determining species composition of the total vegetation studied, followed by summer temperature. This finding is different from several other studies that found pH to be the most important factor (e.g. Audorff *et al.*, 2011; Spitale *et al.*, 2012). These studies also reported altitude as second most important (e.g. Spitale *et al.*, 2012; Schweiger *et al.*, 2015b) or did not include altitude (e.g. Ilmonen *et al.*, 2012). Conductivity may be an important factor, particularly for bryophytes (Kapfer *et al.*, 2012). Low-conductivity springs, like most of those included in our study, often host high biodiversity species assemblages (Cantonati *et al.*, 2009; Cantonati and Lange-Bertalot, 2011). We found that conductivity was only a moderately-important driver of species composition (and it was excluded from most analyses because of its high correlation with pH). Audorff *et al.* (2011) grouped pH with other hydrochemical variables and found that this group was more important than the spatial variables, including altitude. Another study found pH, altitude, and also shading to be the most important factors for bryophytes (Spitale *et al.*, 2012). In contrast, although we did not measure shading, the birch forests surrounding the studied springs in Northern Norway were in general relatively open, and we expect light to be less of a limiting factor. Overall, most studies found altitude and pH to be among the most important factors for mire and spring vegetation (e.g. Hájková *et al.*, 2006; Audorff *et al.*, 2011; Spitale *et al.*, 2012; Schweiger *et al.*, 2015b; Peterka *et al.*, 2017).

One of the few studies to include mean temperature found that it was a significant explanatory variable only for vascular plants in the Western Carpathians, Slovakia (Sekulová *et al.*, 2012). In contrast, in our study, pH ranked behind mean summer temperature, even when the effects of altitude and other variables were removed. This difference may be due to our study sites being colder than most other studied springs. In a warmer region, the mean summer temperature may be high enough that most or all summer days are warm enough for plant growth (e.g. Audorff *et al.*, 2011; Horsák *et al.*, 2018), in contrast to Norway, where the mean summer temperature may be more limiting because some days are too cold for growth (i.e., number of growing degree days with temperatures >5°C is drastically reduced). Thus, summer temperature and the shorter growing season in the Subarctic may become more important.

While studies may disagree over the order of importance of environmental variables, there is more consensus on the reasons for the variables' influences. Altitude is likely critical because of its correlation with temperature (Cantonati *et al.*, 2006). Our data show a strong correlation between altitude and both mean annual temperature and mean summer temperature. The latter is important because the short summer is the primary growing season for plants in the Subarctic, and summer temperature is a major limiting factor for plant growth in the region (Callaghan, 2005). July temperature has been shown to explain 95% of variation in vascular species richness in the Canadian Arctic (Rannie, 1986), thus reinforcing the importance of temperature to Arctic and Subarctic plants during the limited growing season. On the other hand, many studies agree that the importance of pH lies in its link to nutrient availability, both increasing the uptake of nutrients necessary for growth (e.g. nitrogen, phosphate, potassium, magnesium; Beierkuhnlein and Gräsle, 1998; Wheeler and Proctor, 2000; Hájek *et al.*, 2002; Hájková and Hájek, 2008; Strohbach *et al.*, 2009; Audorff *et al.*, 2011; Vicherová *et al.*, 2015) as well as nutrients that can be toxic to bryophytes (e.g. calcium, aluminum, iron; Clymo, 1973; Vicherová *et al.*, 2015; Tyler and Olsson, 2016). This factor may be particularly important to plants in the Subarctic, where nutrient availability is generally low (Callaghan, 2005).

In the Venn diagram, there is a large amount of overlap – about half of the variation explained by each – between the spatial and climatic variables. This is likely to be at least partially a result of the study design following a climatic gradient from the coast to inland. In addition, precipitation correlates with longitude, increasing the overlap between the spatial and climatic variables.

Species-specific relations to the environmental gradients

We found that species differ in their response to the environmental gradients considered in this study. For example, *Saxifraga aizoides, Palustriella falcata* and *Campylium stellatum* were strongly associated with water pH. This finding aligns well with their being calciphilic species (Peterka *et al.*, 2017), and thus strongly influenced by the surrounding soil and water pH. *Saxifraga cernua* is strongly associated with altitude, and this species is typically found at high altitudes or high latitudes in the springs studied. Species like *Solidago virgaurea* and *Salix glauca* are associated with both temperature and precipitation, which indicates that they are largely influenced by the local climate. A few species found exclusively in springs, including *Epilobium alsinifolium*, *Philonotis fontana*, and *Pohlia wahlenbergii* (Fremstad, 1997), were not strongly associated with altitude, temperature, precipitation, or pH. For other spring-exclusive species, temperature (*Philonotis seriata*) or precipitation (*Scapania uliginosa*) was most important. These findings confirm known traits for many species, elucidate the most determinant factors in other species, and highlight a few spring-exclusive species whose presence may be determined by other, unmeasured variables.

Wetland species and matrix species

Climatic variables remained the most important when the species were split into groups of matrix species and wetland species, indicating a sensitivity to changes in climatic conditions. Water quality variables also remained a close second for both groups. These results differ from the findings of Horsáková *et al.* (2018), which identified significant differences between matrix species and specialist fen species; they found that the species composition of matrix derived species was principally driven by waterlogging and pH, whereas geographical location and pH were most important for fen specialists. However, our definition of wetland species differs, including all wetland specialists, whereas Horsáková *et al.* (2018) dealt specifically with fen specialists. In addition, we did not include waterlogging in our study, so perhaps the lack of this variable explains our finding that general matrix species and typical wetland species composition is similarly driven by climatic and water quality factors.

The main difference we found between matrix and wetland species was in how much variation in species composition was explained by the spatial variables: it was less for the wetland species than for the matrix species. This may be because the occurrence of wetland species may depend more upon unmeasured spatial factors that control spring location, such as the underlying geology, topography, land use, and hydrology, rather than altitude, latitude, and longitude, which were studied here.

Conclusion

This study found the species composition of Subarctic springs (both total vegetation and separated into wetland and matrix species) to be almost equally explained by the groups of climatic and water quality variables, closely followed by the spatial variables. As climate change progresses, the Subarctic mainland of Norway is projected to experience increased temperatures and precipitation. The current altitudinal location of a species may affect its ability to shift to higher altitudes as a consequence of climate warming, for example, if it is already located at the highest-altitude spring location or if there are no suitable spring sites located at nearby higher altitudes. Altitude, which was found to be the individual variable most important for species composition, may therefore be a limiting factor for spring species in adapting to climate change. Spatial variables were found to be less important for typical wetland species. This reflects a novel finding and improves our understanding of the factors that influence species composition in Subarctic springs.

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Author contributions:

J.K. conceived of the research idea; J.K. and T.K.M. collected data; J.K., K.H. and T.K.M. identified species; T.K.M. and E.H. performed statistical analyses; T.K.M. and J.K. wrote the paper; all authors commented on the manuscript.

Data availability statement:

All data will be made available on Open Science Framework.

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Tables

Table 1: Descriptive statistics of the variables measured at the springs (n=49) or obtained from Norwegian Meteorological Institute maps.

	Minimum	1st quartile	Median	3rd quartile	Maximum
Water temperature (°C)	2.60	5.36	6.24	7.48	9.40
pH	5.48	6.31	6.66	7.42	8.43
Electrical conductivity (µS cm^{-1})	14	38	54	109	354
Discharge (L s^{-1})	0.005	0.042	0.083	0.133	0.500
Area (m^{2})*	1.5	6	10	24	80
Altitude (m a.s.l.)	86	224	364	457	852
Latitude (°N)	69.02	69.12	69.35	69.51	69.78
Longitude (°E)	18.2	18.59	18.82	19.7	20.61
Total coverage (%)*	40	80	90	96.5	100
Bryophyte coverage (%)*	35	80	85	92.5	100
Herb coverage (%)*	5	15	20	30	65
Shrub and tree coverage (%)*	0	0	0	5.5	45
Mean annual temperature (°C)	-2.25	0.04	0.70	1.34	2.67
Mean spring temperature (°C)	-4.07	-1.93	-0.93	-0.38	0.76
Mean summer temperature (°C)	6.87	7.97	9.48	9.81	11.27
Mean autumn temperature (°C)	-2.01	0.15	0.91	1.48	3.06
Mean winter temperature (°C)	-10.77	-8.30	-6.14	-4.70	-2.58
Mean annual precipitation (mm)	414	732	937	1174	1385
Mean spring precipitation (mm)	59	123	162	192	248
Mean summer precipitation (mm)	109	183	198	236	275
Mean autumn precipitation (mm)	128	230	320	426	511
Mean winter precipitation (mm)	117	207	257	319	389
Species richness	11	19	23	27	50
Vascular plant species richness	5	12	14	18	26
Bryophyte species richness	3	7	8	12	24

* Data only available for 39 spring sites

Figures

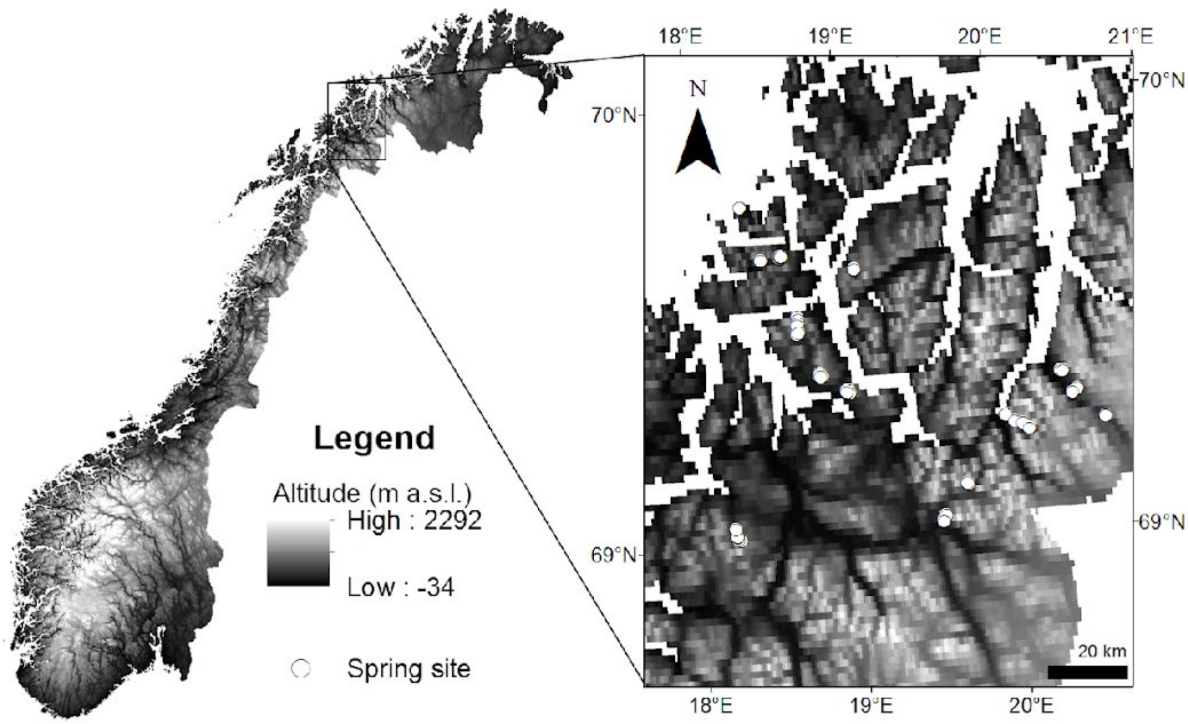


Fig. 1: Map of Norway with spatial distribution of 49 spring sites in the study area.

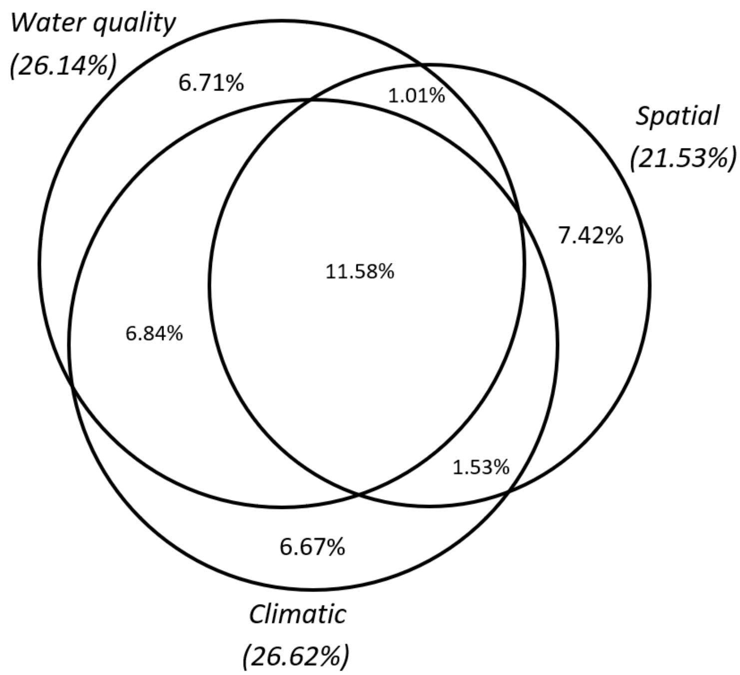


Fig. 2: Venn diagram showing the amount of variation in species composition that is explained by three groups of environmental variables, based on partial canonical correspondence analysis (pCCA). The water quality variables (n=4) include water temperature, water pH, electrical conductivity, and discharge; the spatial variables (n=3) include altitude, latitude, and longitude; and the climatic variables (n=3) include mean annual temperature, mean summer temperature, and mean total annual precipitation. Water temperature, electrical conductivity, and mean summer temperature were log-transformed for the analysis. (pH is already a log-transformed variable.) The total amount of variation explained by all examined variables (n=10) is 41.76%. The size of circles and overlapping portions are approximate.

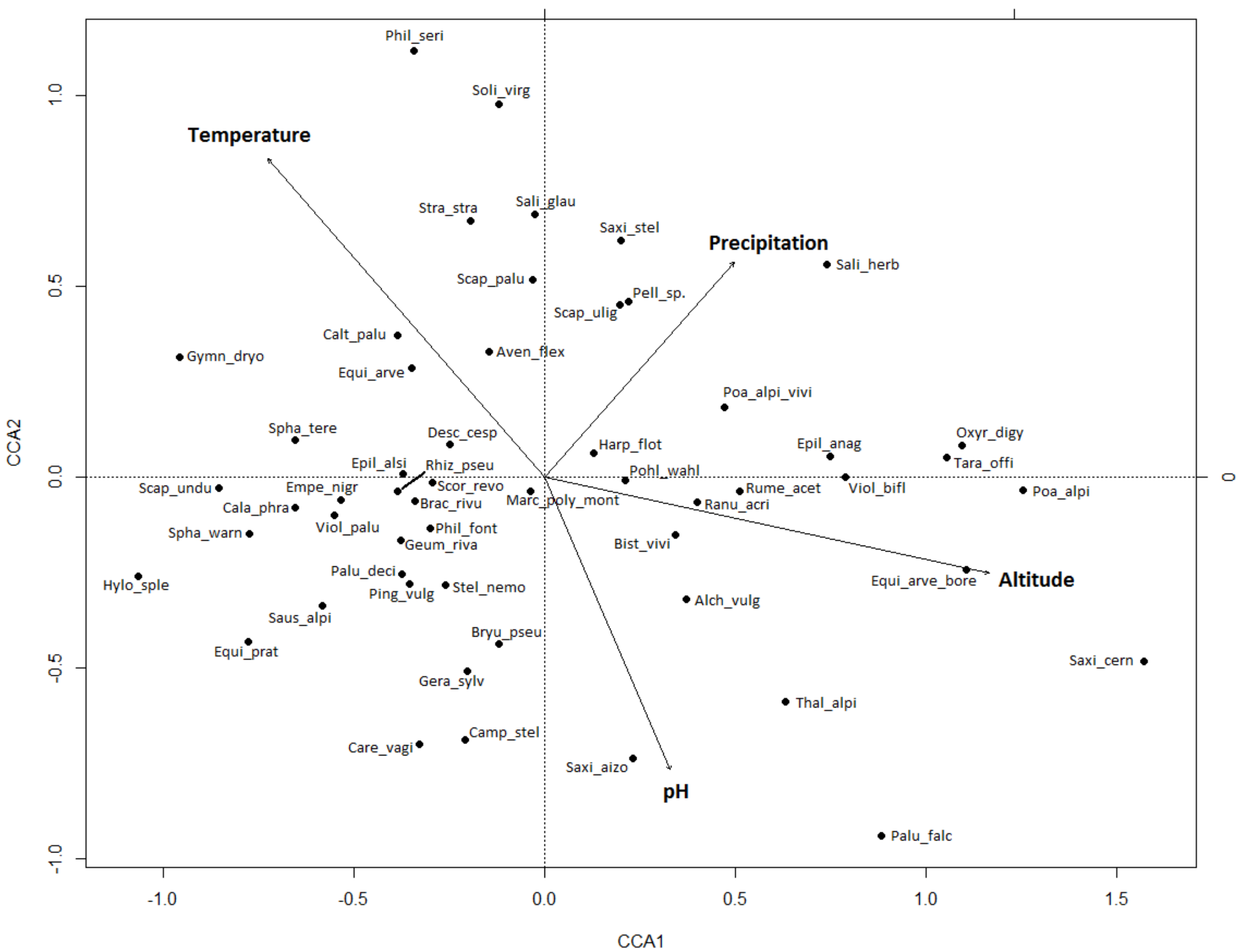
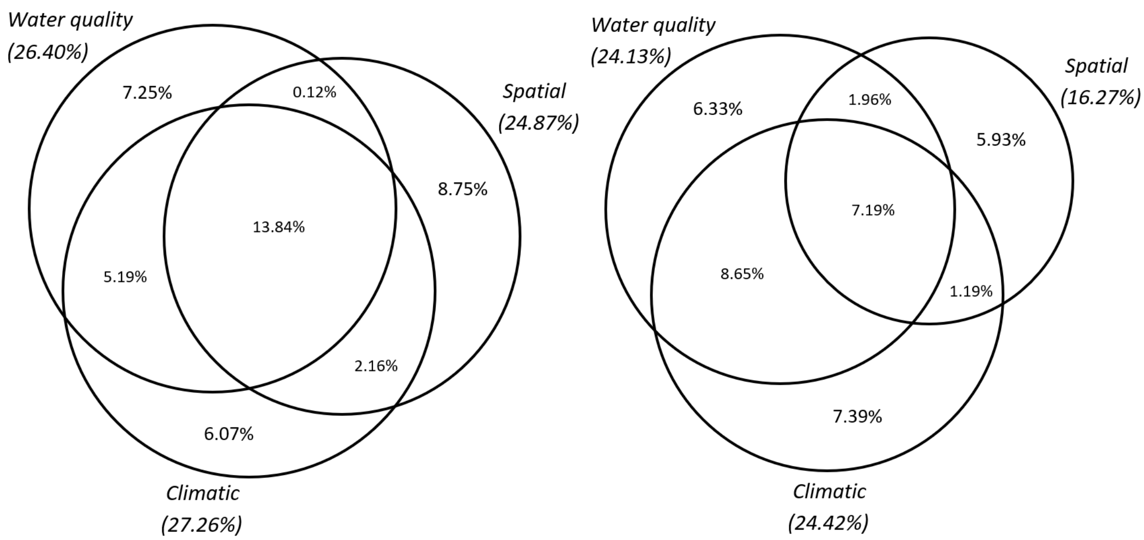


Fig. 3: Partial canonical correspondence analysis, with four key environmental variables and all species with eight or more observations displayed on a biplot of the first two axes. The Temperature label represents mean annual temperature and Precipitation represents mean total annual precipitation. The species name abbreviations are listed in Appendix S2.



A B

Fig. 4: Venn diagram showing the amount of variation in species composition that is explained by three groups of environmental variables, based on partial canonical correspondence analysis (pCCA) with species divided into two groups – wetland generalists (A) and wetland specialists (B). The water quality variables (*n*=4) include water temperature, water pH, electrical conductivity, and discharge; the spatial variables (*n*=3) include altitude, latitude, and longitude; and the climatic variables (*n*=3) include mean annual temperature, mean summer temperature, and mean total annual precipitation. Water temperature, electrical conductivity, and mean summer temperature were log-transformed for the analysis. (pH is already a log-transformed variable.) The total amount of variation explained by all examined variables (n=10) is 43.38% for wetland generalists and 38.64% for wetland specialists. The size of circles and overlapping portions are approximate.

Supporting Information

Appendix S1. Environmental variable data for all spring sites

Appendix S2. Full species list (with figure abbreviations)