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Vegetation ecology of springs: ecological, spatial and temporal patterns

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Vegetation ecology of springs: ecological, spatial and temporal patterns

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Tales sunt aquae, quales terrae, per quas fluunt.

Plinius der Ältere

Contents

Summary

Acidification is a phenomenon, which affected the forested catchments of the northern hemisphere severely over recent decades. Acidic depositions depleted the buffering capacities of soil and groundwater, what lead to an impairment of forests, headwaters, and lakes. Even though the depositions were reduced considerably since the early 1990s, the recovery of catchments was found to occur time-delayed. The grade of recovery was found to vary significantly between regions.

Biomonitoring is an appropriate tool to detect spatial and temporal patterns of ecosystem alterations, such as acidification and recovery. However, to know the interrelationships between organisms and their environment is an indispensable precondition for the identification of indicator species. The complexity of ecosystems and ecological processes hampers this quest oftentimes. Springs provide a natural setting that minimises such constraints. Compared to other habitat types, external factors are less relevant, which makes it easier to relate changes in species abundances to changes in their environment. Studying this species-environment relationship, here the response of plant species to the acidification of the spring waters was of particular interest.

In a survey of five regions in Central Europe - taking spatial, hydrophysical as well as hydrochemical parameters of the springs into account - it was clearly shown that the species composition of springs is essentially determined by the spring water chemistry, and more precisely by the gradient of acidity and nutrient availability. This connection was reflected by spatial patterns within and between the regions. These patterns provide useful ecological information about spring water quality and in return about the acidity status of their forested catchments.

Including catchment traits - like bedrock, climatic parameters, and forest vegetation - in the analyses, these emerged to be relevant for the species composition of springs, but less than the spring water chemistry. A path analysis showed that the catchments affect the vegetation of springs not directly, but indirectly via the determination of spring water quality. Hence, the catchments are a part of the functional chain, which is driven by the atmospheric depositions.

The pH-value was found to represent the gradient of acidity and nutrient availability best. It can serve as a proxy measure that can be related to species occurrence and to species dynamics respectively, aiming to identify indicator species for assessing the status and alterations of spring water quality.

With the aim to delineate niche optima and amplitudes, which in return can serve as indicator values, the realised niches of spring-inhabiting species were modelled with respect to pH. The niche attributes were found to be a matter of sampling scale. Larger plot sizes (grain) weakened the species-environment relationship, what consequently resulted in broader niche amplitudes. In contrast, the grain did not influence the species' pH optima.

Monitoring approaches that target to assess processes in time, such as acidification and recovery, are dependent on the response time of indicator species to changes in their environment. Investigating an interval of four consecutive years, inter-annual variability of the species composition could not be attributed to changes in the acidity of the spring waters. Looking at single species, bryophytes did not show a higher sensitivity to the inter-annual variability of the environment than vascular plants. Actually, only a minority of all species featured abundance changes which were significantly correlated to variations in spring water acidity. Our results suggest that the species inertia retards the vegetation dynamics of forest springs. A delayed or long-term integrating response of potential indicator species must be considered when evaluating their indicator suitability. In conclusion, the biomonitoring of spring water acidification or recovery is expedient only for longer time intervals.

In a nutshell, the vegetation of springs is closely related to the hydrochemical traits of the spring waters, in particular to a gradient of acidity and nutrient availability. Individual species as well as whole plant communities are suitable indicators which allow for the monitoring of the acidity status of forested catchments. The results of this study contribute to a better understanding of the species-environment-relationships, and in return to an improvement of indicator systems.

Zusammenfassung

Bewaldete Einzugsgebiete der nördlichen Hemisphäre wurden in den vergangenen Jahrzehnten stark vom Phänomen der Versauerung beeinflusst. Saure Depositionen haben die Pufferkapazitäten des Bodens und des Grundwassers erschöpft, was zur Schädigung von Wäldern, Bächen und Seen führte. Auch wenn die Depositionen seit Anfang der 1990er Jahre deutlich reduziert wurden, so tritt eine Erholung erst zeitverzögert ein. Das Ausmaß dieser Erholung ist regional deutlich verschieden.

Bioindikation ist ein geeignetes Werkzeug, um räumliche und zeitliche Muster ökosystemarer Veränderungen – wie Versauerung und Erholung – zu erfassen. Unabdingbare Voraussetzung für die Identifikation von Indikatorarten ist es, die Zusammenhänge zwischen Organismen und ihrer Umwelt zu kennen. Die Komplexität der Ökosysteme und der in ihnen stattfindenden Prozesse erschwert dieses Ansinnen oftmals. In Quellen bietet sich eine Ausgangssituation, unter der solche Einschränkungen minimiert sind. Verglichen mit anderen Biotoptypen sind externe Einflussfaktoren weniger relevant. Dies vereinfacht es, Änderungen der Artmächtigkeit zu Umweltänderungen in Beziehung zu setzen. Bei der Untersuchung der Arten-Umwelt-Beziehung ist hier die Reaktion von Pflanzenarten auf die Versauerung der Quellwässer von besonderem Interesse.

Bei einer Bestandsaufnahme in fünf mitteleuropäischen Naturräumen wurden räumliche, hydrophysikalische sowie hydrochemische Eigenschaften von Quellen erfasst. Hierbei wurde klar gezeigt, dass die Artenzusammensetzung von der Quellwasserchemie bedingt wird, genau gesagt von einem Säure- und Nähstoffverfügbarkeitsgradienten. Dieser Konnex wurde von räumlichen Mustern widergespiegelt, und zwar sowohl innerhalb einzelner Landschaften als auch beim Vergleich zwischen diesen. Diese Muster bieten uns ökologische Informationen zur Qualität der Quellwässer und damit auch zum Säurestatus ihrer bewaldeten Einzugsgebiete.

Werden Eigenschaften der Einzugsgebiete – wie Grundgestein, Klima und Waldbestockung – in die Analysen einbezogen, so erwiesen sich diese als relevant für die Artenzusammensetzung der Quellen, waren jedoch von geringerer Bedeutung als die Quellwasserchemie. Mittels einer Pfadanalyse konnte gezeigt werden, dass Einzugsgebiete die Vegetation der Quellen nicht direkt beeinflussen, sondern indirekt über die Beeinflussung der Quellwasserqualität. Somit sind die Einzugsgebiete ein Teil der Wirkungskette, die von den atmosphärischen Depositionen angetrieben wird.

Der Säure- und Nähstoffverfügbarkeitsgradient wurde vom pH-Wert am besten repräsentiert. Stellvertretend kann dieser in Beziehung zum Vorkommen und zur Dynamik der Arten

gesetzt werden, um Indikatorarten für den Zustand und die Veränderung der Quellwasserqualität zu identifizieren.

Die realisierten Nischen quellbewohnender Arten in Bezug auf den pH-Wert der Quellwässer wurden modelliert, um Optima und Amplituden dieser Arten abzuleiten. Diese wiederum können als Indikatorwerte dienen. Jedoch wurde eine Abhängigkeit der Nischeneigenschaften von der räumlichen Erfassungsskala festgestellt. Größere Aufnahmeflächen führten zur Berechnung von weiteren Amplituden, da der Bezug des Artauftretens zu den gemessenen Umweltvariablen dort schwächer war. Im Gegensatz dazu beeinflusste die Größe der Aufnahmefläche die Lage der pH-Optima nicht.

Die Herangehensweise, dynamische Prozesse – wie Versauerung und Erholung - mittels Indikationsverfahren zu erfassen ist abhängig von der Geschwindigkeit mit der Indikatorarten auf Veränderungen ihrer Umwelt reagieren. Bei der Untersuchung von vier aufeinander folgenden Jahren konnte die interannuelle Variabilität der Artenzusammensetzung nicht den Veränderungen des Säuregehaltes der Quellwässer zugeschrieben werden. Bei der Betrachtung einzelner Arten erwiesen sich Moose nicht als empfindlicher gegenüber interannuellen Umweltveränderungen als Gefäßpflanzen. Überhaupt zeigte nur eine Minderheit aller Arten eine signifikante Korrelation zwischen Änderungen ihrer Artmächtigkeits und denen des Säuregehaltes der Quellwässer. Unsere Ergebnisse legen nahe, dass die Vegetationsdynamik von Waldquellen von der Trägheit der Arten verlangsamt wird. Eine verzögerte oder über lange Zeiträume integrierende Reaktion potenzieller Indikatorarten muss bei der Beurteilung ihrer Indikatoreignung berücksichtigt werden. Daraus ist zu schließen, dass die Bioindikation der Versauerung und der Erholung von Quellwässern nur für längere Zeiträume angebracht ist.

Kurz zusammengefasst besteht zwischen der Quellvegetation und den hydrochemischen Eigenschaften der Quellwässer ein enger Zusammenhang, insbesondere hinsichtlich des Säure- und Nährstoffverfügbarkeitsgradienten. Einzelne Arten wie auch ganze Pflanzengesellschaften eignen sich als Indikatoren zum Monitoring des Säurezustands bewaldeter Einzugsgebiete. Die Ergebnisse dieser Studie tragen zu einem besseren Verständnis der Arten-Umwelt-Beziehung bei und dadurch schließlich auch zur Optimierung von Indikatorsystemen.

Introduction

This thesis addresses the species-environment relationship of springs, more specifically, the response of plant species to the acidification of the spring waters. To know about the ecological, the spatial and the temporal context is an essential prerequisite for the identification of indicator species and the assessment of their indicative power.

Background

The following chapter outlines the framework in which the investigations for this thesis are embedded. The habitat traits of springs and their environmental background are depicted shortly, with the aim to set the scene for developing the hypotheses. More detailed information, which is relevant for the respective research objectives, is included in the original manuscripts.

Acidification and recovery

The damage and decline of forest vegetation as well as the alteration of soils, aquifers, headwaters, and lakes were the eye-catching symptoms of acidification, which occurred in the last decades of the 20th century. Forested catchments of the northern hemisphere were affected severely by this phenomenon. The impairments were caused by loads of acidifying compounds, which were considerably increased compared to historical periods (Reuss et al. 1987; Asman et al. 1988; Steinberg & Wright 1994; Kopácek et al. 2001; Bouwman et al. 2002). Though, acidic depositions alone cannot explain the ecological decline of terrestrial and aquatic habitats and the spatial patterns of these. Landscape traits, such as the surface roughness of forest vegetation (e.g. deciduous or coniferous forest) (Williard et al. 2005) as well as altitude, slope and aspect of the catchment (Palmer et al. 2004; Rantakari et al. 2004), and climatic conditions may alter the local deposition load of a specific catchment. The bedrock and the soils in the catchment predetermine its buffering capacity and therefore exert their influence on ecosystem conditions (Probst et al. 1999; Williard et al. 2005). Catchments on siliceous bedrock, like investigated in this thesis, are in particular affected by acidification.

Indeed, since the early 1990s the depositions of various acidifying compounds (e.g. sulphuric acid) were reduced considerably in Central Europe. Thus, the recovery of catchments and limnological systems seems possible (Hultberg et al. 1998; Kopácek et al. 1998), but can be considerably delayed by biogeochemical processes within the catchments (Alewell et al. 2001). Regional patterns are encountered (Stoddard et al. 1999). Anyhow, atmospheric nitrogen loads continue to be extremely high. Accordingly, acidification remains a relevant environmental issue.

Springs and their catchments

Spring waters are characterised basically by the biogeochemical and hydrological processes in their catchment area (Beierkuhnlein & Durka 1993). The 'small catchment approach' (e.g. Moldan & Černý 1994; Church 1997; Likens & Bormann 1999) applies to springs. It is a well established research approach to meet the challenge of functional complexity of ecological processes that can hardly be measured completely in ecosystems. The catchments are considered as 'black-boxes', the headwaters - or in our case springs – serve as proxies, which represent the output of ecosystems in a spatially concentrated manner. In low dynamic forested catchments, springs reflect long-term ecological processes and the biogeochemical background of the landscape. In the context of catchment ecology acidification can be seen as unwanted, large-scale experiment (Schulze & Ulrich 1991).

Due to the dense bedrock of Central European siliceous mountain ranges groundwater flow is located close to the surface (interflow). Under such conditions, springs are generally formed as helocrenic springs (sensu Thienemann 1924). These feature a saturated area of a few to some hundred square meters in size, where the groundwater seeps out continuously. Climate causes minor seasonal fluctuations, which are reflected in a certain variability of discharge. The size of the catchment area modulates this connection. However, spring water temperatures are equilibrated by the permanent energy supply of the groundwater.

Springs – an outstanding habitat type

Springs are a peculiar habitat type which differs remarkably from others. Odum (1971) summarized this when he wrote: "*Springs are the aquatic ecologist's natural constant temperature laboratory. Because of the relative constancy of the chemical composition, velocity of water, and temperature, in comparison with lakes, rivers, marine environments, and terrestrial communities, springs hold a position of importance as study areas that is out of proportion to their size and number*".

Springs represent the interface between aquifers and lotic waters. Indeed, there are numerous attributes which account them for being an interesting subject to study species-environment relationships.

Their constancy of hydrophysical and hydrochemical parameters sets them apart from other habitats (Odum 1971; Ellenberg 1996). In particular, when they are situated in forested catchments, they can be considered to be ecosystems with low dynamics that reflect the biogeochemical background of the landscape and long-term ecological processes. This enables species to adapt to habitat conditions over a relatively long period and to develop a steady state in community composition. In spite of this constancy in time, helocrenic springs (sensu Thienemann 1924) with a relatively large seeping area show a certain spatial variability at the landscape scale (Beierkuhnlein 1994; Durka 1994; Hájková et al. 2004).

The area of seeping spring water is clearly differentiated from the neighbouring habitats. This well-defined boundary is an effect of frost heaving of substrate and vegetation during freeze-thawing cycles in winter and makes it easy to assess whether species are dwelling in the spring itself or the neighbouring terrestrial habitats. The surrounding habitats are characterised by disparate conditions. Even the adjacent brooks clearly differ in the thermic regime. Direct spatial connectivity between springs is negligible. Therefore springs feature a considerable ecological isolation. They resemble islets with specific habitat conditions that do not refer to the surrounding matrix.

Water flow in springs is unidirectional and more or less continuous. Organisms living in the springs are strongly related to the amount and the characteristics of the groundwater, other site conditions are less meaningful. Thus, they are adapted to its relative environmental constancy (Thienemann 1924; Odum 1971). A high proportion of crenobiotic and crenophilic species is stenoecious (Ferrington 1998; Zollhöfer et al. 2000; Cantonati et al. 2006).

As a result of the permanent water saturation and the small extent of spring habitats, land-use options for springs are negligible, and therefore human interest in them is low. Springs in forested areas represent a notable exception to the predominantly anthropogenic ecosystems of Europe. Warncke (1980) refers to them as pristine islands in an ocean of cultivated land. Thus, in springs natural drivers still dominate ecosystem functioning, at least regarding physical factors and land-use. In case an anthropogenic impact on a single spring was encountered anyhow, this site was excluded from the analyses.

However, often the complexity of ecosystems and ecological processes handicaps the separation between cause and effect. Combining the above-mentioned points - the constancy of natural factors, the ecological discreteness, the habitat specialisation of species, and the absence of human land-use - spring ecosystems provide a natural setting that minimises such constraints. Neighbour effects are low and negligible. The landscape context which is influencing vegetation succession in other habitat types (Prach & Řehounková 2006) plays an inferior role in springs. Hence, we expect a higher relevance of internal factors, which makes it easier to relate changes in species abundances to changes in their environment.

Consequences for biomonitoring

Even if springs account only for a small proportion of land surface, they appear numerously on the forested mountain ranges of Central Europe. This fact is a prerequisite for monitoring purposes at the landscape scale. Data from comparable and almost standardised records are provided with a high spatial resolution (Beierkuhnlein & Durka 1993). As mentioned above, spring water quality reflects biogeochemical processes in its catchments. Springs act as a point-shaped outlet of complex, black-box-like ecosystems.

The particular site conditions of springs cause crenobiotic and crenophilic species to be stenoecious in many instances (Ferrington 1998; Zollhöfer et al. 2000). Indeed, species with narrow ecological amplitudes feature a low capacity to resist alterations of their environment. It can be expected that changes in spring water quality will affect first and foremost the stenoecious species (Ferrington 1998; Zollhöfer et al. 2000; Cantonati et al. 2006). Therefore, changing conditions will be responded to by species and their communities (Glazier 1991; Zollhöfer et al. 2000; Cantonati et al. 2006). Especially plants are supposed to be suitable indicator organisms, because they are immobile and consequently not able to avoid unfavourable conditions. However, the capacity of resilience and the inertia of spring-inhabiting species are widely unknown. Up to now, the response time of species and taxonomic groups to changes in the hydrochemical regime has not been investigated.

The preconditions of exact localisation and balanced physical conditions in combination with organisms that are highly sensitive to environmental signals characterise springs and the species dwelling in them to be appropriate indicators for the monitoring of the biogeochemical background of landscapes as well as for the detection of ecological trends.

Research objectives and hypotheses

The purpose of **manuscript 1** is to fathom the driving factors of the species composition in the investigated springs, and therefore to provide a basis for the subsequent investigations. Water flow in springs is unidirectional and more or less continuous, hence spring-inhabiting organisms are strongly depending on the quantity and quality of the outpouring groundwater. Regarding Central Europe, spring water chemistry was reported to be the main determinant of the plant species composition, in particular the gradient of acidity and nutrient availability (Beierkuhnlein 1994; Durka 1994; Hájek et al. 2002; Hájková et al. 2008). This coincides with various studies in springs and fens of the northern hemisphere (Vitt & Chee 1990; Wheeler & Proctor 2000; Bragazza & Gerdol 2002; Nekola 2004; Tahvanainen 2004), but some of these studies included several mire types or did not specify the investigated habitat precisely. Indeed, the habitat characteristics differ substantially with increasing distance from the source point of groundwater. This aboveground flow path length has not been accounted for (or at least not been reported) in the above-mentioned studies. To avoid restrictions by aboveground water flow (i.e. an altered water chemistry by outgassing of CO_2, buffering processes, and lateral impacts), we confined ourselves solely to these areas of helocrenic springs (sensu Thienemann 1924), where groundwater is continuously seeping to the surface (fens and mires with larger spatial extension were avoided, just like rheocrenic springs where the physical conditions of spring brooks are dominating right from the source point). There we recorded the vegetation, taking spatial, hydrophysical and hydrochemical variables into account. This way springs on five siliceous mountain ranges in Central Europe were surveyed. The environmental data were related to the vegetation data in a two-step approach, by means of a partial canonical correspondence analysis (pCCA), with the goal of specifying the species-environment relationship of springs.

Spring waters are known to reflect the biogeochemical properties of their catchments (Beierkuhnlein & Durka 1993), the ‘small catchment approach’ (e.g. Moldan & Černý 1994; Church 1997; Likens & Bormann 1999) applies also to the investigated springs. Spring water acidity is not only dependent on the acidic depositions, but also on the buffering processes in the associated catchments. Therefore it stands to reason, that also the vegetation of springs is influenced by catchment traits, such as relief, bedrock, soil, climatic parameters, and forest vegetation. **Manuscript 2** intends to decipher the magnitude and the paths of influence that catchments exert on springs and their vegetation. It was hypothesized that the plant species composition mainly reflects hydrochemical conditions of the spring water itself. To test this hypothesis, the influence of spring water chemical properties and catchment traits on the vegetation of springs was investigated, using non-metric multidimensional scaling (NMDS), Mantel tests, and path analyses. In addition the datasets were tested for spatial dependencies, applying the same methods.

Having explored the driving factors of the species composition of springs in the first two manuscripts, one can go for more detailed investigations of ecological, spatial, and temporal patterns, which might affect the identification of indicator species and the assessment of their suitability for certain ecological issues.

The realised niche of a species was defined by Hutchinson (1978) as its amplitude of tolerance in natural communities in the presence of competitors. Indeed, each species responds to its environment at a range of different scales (Levin 1992). Therefore the modelling of species-habitat relationships is dependent on the appropriate sampling of the spatial variation of the environmental parameters on which the models are based (Gillison & Brewer 1985; Cushman & McGarigal 2004). Consequently, the problem of the appropriate sampling design complicates the derivation of those niches from empirical data. However, the appraisal of ecological phenomena from only one scale of observation may hamper the detection of the driving factors of ecosystem behaviour (Cushman & McGarigal 2004), and may even bias the detected relation of niche mechanisms (species sorting along environmental gradients) versus neutral mechanisms (species determined spatially by dispersal limitations) (Bell 2005). In **manuscript 3** we delineated species' realised niches with respect to spring water pH, using generalized additive models. Niche attributes (which in return can serve as indicator values) were calculated for two spatial scales - an entire-spring scale as well as a subplot-based within-spring scale - with the aim to depict effects of spatial scale (grain, i.e. plot size) on niche position (optimum) and niche width (amplitude). Our hypothesis was that the probabilities of species occurrence with respect to pH, and therefore the species' pH optima and pH amplitudes, are biased by the sampling scale.

Of course, environmental characteristics of springs are also a matter of processes in time (e.g. acidification or climate change). There, changing conditions are likely to be responded to by species and their assemblages (Glazier 1991; Zollhöfer et al. 2000; Cantonati et al. 2006). Considering the fact that springs are assumed to be balanced habitats regarding hydrophysical and hydrochemical factors (Odum 1971; Ellenberg 1996; Mc Cabe 1998; Zollhöfer et al. 2000), extraordinary events such as the hot and dry summer in 2003 with a reduced and locally absent groundwater discharge may greatly contribute to vegetation response. However, the inertia of spring communities as well as their capacity of resilience is unknown. The responses of spring communities to the inter-annual variability of their environment have not yet been investigated and neither has the response time of species and communities to environmental change. **Manuscript 4** addressed the short-term vegetation dynamics, and studied them over a period of four consecutive years. We hypothesised a reflection of the inter-annual variability of environmental parameters, in particular the acidity and nutrient availability status, by the abundances of springs-inhabiting plant species. For calculating the turnover of species composition, we used the Bray-Curtis dissimilarity index. Additionally, we related inter-annual changes of species abundances to those of environmental factors, in order to explore the driving factors behind inter-annual vegetation dynamics.

Indeed, shifts in species abundance do not occur instantaneously after an alteration of their environment, rather they follow time-delayed. The discussion on the response time of organisms and their assemblages to environmental changes is currently mainly going on in the context of climate change (e.g. Menéndez et al. 2006; Borrvall & Ebenman 2008) and conservation issues (e.g. Tilman et al. 1994; Helm et al. 2006). Doubtless, it is also relevant for the suitability of indicator species (Milner et al. 2006; Paltto et al. 2006). However, regarding indicator systems, a deferred response can have desirable as well as unwanted effects. It goes without saying, that an immediate response of indicator organisms is essential for an early warning system. In contrast, a response which is integrating over a longer time-interval can eliminate noise (e.g. due to short-term fluctuations) from the general information.

Groups of organisms differ in their dependence and response to environmental change. Bryophytes absorb water and therein dissolved nutrients and contaminants directly by their surface, this way they can be directly affected (Brown 1984; Bates 2000). Bryophytes are expected to respond rapidly to a changing environment also because of their mostly short life cycles. Therefore Frahm & Klaus (2001) as well as Bates et al. (2005) presume bryophytes to be more sensitive indicators for environmental variations than vascular plants. Concerning these facts, we hypothesise bryophytes to show a stronger correlated response to the inter-annual variability of the environment than vascular plants. We compared both taxonomic groups in **manuscript 5**, regarding the response of species abundances to inter-annual-changes in spring water pH-values.

Synopsis

Outline of the manuscripts

Manuscript 1 provides an attempt to detect the driving factors of the species composition of springs. In a spatial approach the vegetation of springs on five siliceous mountain ranges in Central Europe was surveyed, taking spatial, hydrophysical and hydrochemical parameters into account. The hydrological factors, in particular a gradient of acidity and nutrient availability, turned out to be the most important drivers of the plant community composition of springs. This ecological pattern was clearly depicted in the spatial patterns of the spring water characteristics as well as in the spatial patterns of species composition.

When discussing the reasons for these spatial patterns, it became clear that a concentration on the spring sites themselves might be insufficient. As acidifying processes are closely related to the acidic depositions and the buffering processes in the catchments, it was indispensable to include catchment traits in the analyses. Accordingly, the purpose of **manuscript 2** was to unravel the magnitude and the paths of influence that catchments exert on springs and their vegetation. We found the catchment traits - like bedrock, climatic parameters, and forest vegetation - to be relevant for the species composition of springs. Though, the catchments affect the vegetation of springs not directly, but indirectly via the determination of spring water quality.

Both above-mentioned manuscripts document the connection of the species composition of springs to the hydrochemical characteristics of the spring water, especially to the gradient of acidity and nutrient availability. The pH-value was found to represent this gradient best. Therefore it can serve as a proxy measure that can be related to species occurrence, aiming to identify indicator species for assessing the status and alterations of spring water quality. Hence, the following papers deal with the definition of indicator species and the examination of their suitability for certain issues in space and time.

Manuscript 3 investigates the realised niches of spring-inhabiting species with the aim to delineate niche attributes, which in return can serve as indicator values. We modelled the probability of plant species occurrence with respect to pH, using generalized additive models. To compare the niche attributes, these were derived separately for two spatial scales. Whereas the sampling scale did not systematically influence the species' pH optima, we found that a smaller plot size (grain) narrowed their pH amplitudes significantly. Consequently, the delineation of niche characteristics is a matter of the spatial sampling scale.

Acidification and recovery are processes in time. Monitoring approaches that target to assess these processes therefore are dependent on the response time of indicator species to changes in their environment. This topic is addressed by the last two manuscripts. **Manuscript 4** analyses the species response to inter-annual variability in hydrological parameters over a period of four consecutive years. In order to decipher the driving factors behind inter-annual vegetation dynamics we related inter-annual changes of species abundances to those of environmental factors. Unlike expected, the inter-annual variability of the vegetation of springs could not be attributed to changes in the acidity of the spring waters.

This result coincides with the findings of **manuscript 5**, where the indicator suitability of bryophytes and vascular plants with respect to inter-annual-changes in spring water pH-values were compared. Bryophytes did not show a higher sensitivity to the inter-annual variability of the environment than vascular plants. Actually, only the minority of all species showed abundance changes which were significantly correlated to variations in spring water acidity. We presume that the vegetation dynamics of forest springs is subject to species inertia (delayed response, persistence patterns) and can only be affected by a modified acidity status in the long run.

Summarising conclusions and emerging research challenges

Driving factors of species composition

Combining the results of the **manuscripts 1 and 2**, it was clearly shown that the species composition of springs is essentially determined by the spring water chemistry, and more precisely by the gradient of acidity and nutrient availability. This gradient was reflected mainly by the pH-value. Acidic spring waters featured higher concentrations of the toxic minerals Al, Cd, and Mn, contrary circumneutral spring waters were characterised by higher concentrations of the nutrients Mg and Ca. Spatial factors, first and foremost the altitude, were related closely to the hydrochemical factors, but have been found to be less meaningful than those. Catchment traits (bedrock, climatic parameters, and forest vegetation) executed an influence on the vegetation of springs indirectly, hence the catchment is a part of the functional chain. This chain is driven by the atmospheric depositions. Their impact was most effective in catchments on siliceous bedrock with a poor buffering capacity. The acidification of ground and surface waters reflects this circumstance.

Springs are pristine, but abundant habitats where disturbance and human land-use are rare, in particular if they are located in forested catchments. They are known to be low dynamic ecosystems (Odum 1971; Ellenberg 1996). This allows species to adapt to habitat conditions over a relatively long time and to develop a steady state in community composition. This natural setting provides a sound basis for the biomonitoring of the acidity status of spring waters. We could show that spatial patterns in the vegetation of springs emerge on the landscape scale. These patterns provide valuable ecological information about spring water quality and in return about the acidity status of their forested catchments.

Even though in Central Europe the acidic depositions were reduced considerably since the early 1990s, the recovery of catchments seems to be delayed (Alewell et al. 2001). The question arises, if and when a recovery from acidification can be observed in the springs. Our dataset provides the fundament for repeated measurements, targeting at a long-term monitoring.

Attributes of species' realised niches

The pH-value - as a proxy of the acidity and nutrient availability gradient - was also a strong predictor for the occurrence of individual species, like shown in **manuscript 3**. When modelling the probability of plant species occurrence with respect to pH, significant response curves were yielded for the majority of species. These response curves represent the species' realised niches. The optima and the amplitudes of the latter can be computed. In so doing, we found that the delineation of niche attributes is a matter of sampling scale, in particular for the species' ecological amplitudes. Larger plot sizes (grain) weakened the species-environment relationship, what consequently resulted in broader niche amplitudes. In contrast, the grain did not systematically influence the species' pH optima.

Our findings have profound implications for numerous ecological applications, such as niche-based distribution models (e.g. Guisan & Zimmermann 2000; Guisan & Thuiller 2005). It has been reported that the predictive capacity of such models is limited by the environmental range over which the models are fitted (Thuiller et al. 2004) and by the transferability over regions (Randin et al. 2006; Hájková et al. 2008). We suppose that also the choice of the sampling scale affects the calculation of thresholds and therefore the predicted results.

The resulting niche optima with respect to the spring water acidity were not affected by the sampling scale, they can serve as empirically-grounded indicator values. We will strive to compare these with expert-based indicator values (Ellenberg et al. 1992), but also with such derived by other empirical methods (e.g. multivariate ordination methods), regarding their ability to predict spring water pH-values by species occurrences.

The constancy of hydrological factors in springs brings about a high proportion of stenoecious species to be encountered there (Ferrington 1998; Zollhöfer et al. 2000;

Cantonati et al. 2006). It still has to be tested, if the scale-dependence of the niche-delineation proves true for species with narrower ecological amplitudes.

Species response to environmental change

Because the species occurrence was clearly connected to the spring water acidity when taking single points in time into account (static approach), it was to expect that acidification and recovery processes will evoke changes in species abundances (dynamic approach). Indeed, we found an inter-annual variability of the vegetation, but could not verify this as a species response to inter-annual changes in the investigated hydrological factors (**manuscript 4**). Our results suggest that the species inertia (delayed response, persistence patterns) retards the vegetation dynamics of forest springs.

However, groups of organisms differ in their response to environmental change. This aspect is addressed by **manuscript 5**, where bryophyte species were opposed to vascular plant species with regard to their indicator sensitivity for spring water quality. Bryophytes do not have a rooting system and take up nutrients by the plant surface. They are directly exposed to the medium whose conditions are to be indicated. Hence they are proposed to be more sensitive indicators than vascular plants (Frahm & Klaus 2001; Bates et al. 2005). But, we could not confirm this assumption. Independent of their membership in one of the two taxonomic groups, only a minority of species featured abundance changes which were significantly correlated to pH variations. Thus we conclude that the biomonitoring of spring water acidification or recovery is feasible only for longer time intervals.

In general, a delayed or long-term integrating response of potential indicator species must be considered in the evaluation of their indicator suitability. The bio-indication of ecological processes (e.g. acidification, climate change) is reasonable only when the response times of indicator species are adequate to the velocity of the observed process. However, we had to recognise that the time-lag between environmental change and species response is widely unknown.

Estimating such response times for individual species or even for entire plant communities clearly emerged as an upcoming research challenge. Presently, this issue is discussed especially in the context of climate change (e.g. Menéndez et al. 2006; Borrvall & Ebenman 2008). Indeed, it is also of great importance for conservation issues (Tilman et al. 1994; Helm et al. 2006) and the suitability of indicator species (Milner et al. 2006; Paltto et al. 2006). Our dataset provides a basis for long-term investigations, like Walther (2007) recommends for the monitoring of environmental changes. The methods used in the manuscripts 4 and 5 can be applied to longer time-intervals analogously.

Summing up the above-mentioned points, this study has shown that the vegetation of springs is closely related to the hydrochemical traits of the spring waters, in particular to a gradient of acidity and nutrient availability. Individual species as well as whole plant communities can indicate the acidity status of forested catchments in space and time. With the results from all our approaches, a better understanding of the species-environment-relationships and in return an improvement of indicator systems can be expected. Deficits in the monitoring approaches have been identified, but – as springs provide a natural setting that facilitates to unravel complex ecosystem interrelationships - we suppose them to be suitable research subjects to meet these challenges.

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List of manuscripts and specification of own contribution

„Writing“ means the actual formulation of sentences and paragraphs. Comments and inputs from discussions with the co-authors are covered under „discussion“. Proof-reading and grammar editing before submission was done by professionals.

Manuscript 1

Authors	Volker Audorff, Jutta Kapfer & Carl Beierkuhnlein	
Title	**The role of hydrological and spatial factors for the vegetation of Central European springs**	
Status	in preparation	
Journal	Ecohydrology	
Contributions		
> Audorff	75 %	idea, data collection, data analysis and figures, writing, discussion, editing, corresponding author
> Kapfer	15 %	data collection, data analysis and figures
> Beierkuhnlein	10 %	idea, discussion, editing

Manuscript 2

Authors	Michael Strohbach, Volker Audorff & Carl Beierkuhnlein	
Title	**Drivers of species composition in siliceous spring ecosystems: groundwater chemistry, catchment traits or spatial factors?**	
Status	submitted (03.01.2009)	
Journal	Journal of Limnology	
Contributions		
> Strohbach	45 %	data collection, data analysis and figures, writing, discussion
> Audorff	45 %	idea, data collection, data analysis and figures, writing, discussion, editing, corresponding author
> Beierkuhnlein	10 %	idea, discussion, editing

Manuscript 3

Authors	Christian Zang, Volker Audorff & Carl Beierkuhnlein	
Title	**Is the delineation of niche attributes a matter of spatial scale?**	
Status	submitted (02.01.2009)	
Journal	Journal of Biogeography	
Contributions		
> Zang	45 %	idea, data collection, data analysis and figures, writing, discussion
> Audorff	45 %	idea, data collection, data analysis and figures, writing, discussion, editing, corresponding author
> Beierkuhnlein	10 %	idea, discussion, editing

Manuscript 4

Authors	Volker Audorff, Jutta Kapfer & Carl Beierkuhnlein	
Title	**Inter-annual vegetation dynamics in forest springs**	
Status	submitted (22.12.2008)	
Journal	Freshwater Biology	
Contributions		
> Audorff	60 %	idea, data collection, data analysis and figures, writing, discussion, editing, corresponding author
> Kapfer	30 %	data collection, data analysis and figures, writing, discussion
> Beierkuhnlein	10 %	idea, discussion, editing

Manuscript 5

Authors	Jutta Kapfer, Volker Audorff, Eduard Hertel & Carl Beierkuhnlein	
Title	**Are bryophytes better indicators for inter-annual changes in spring water quality than vascular plants?**	
Status	submitted (01.01.2009)	
Journal	Ecological Indicators	
Contributions		
> Kapfer	45 %	data collection, data analysis and figures, writing, discussion
> Audorff	45 %	idea, data collection, data analysis and figures, writing, discussion, editing, corresponding author
> Hertel	5 %	data collection, discussion
> Beierkuhnlein	5 %	idea, discussion, editing

Manuscript 1

The role of hydrological and spatial factors for the vegetation of Central European springs

Volker AUDORFF, Jutta KAPFER & Carl BEIERKUHNLEIN

Ecohydrology, in preparation

Ecohydrology, in preparation

The role of hydrological and spatial factors for the vegetation of Central European springs

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Abstract

Understanding the driving factors of species occurrence is a prerequisite for estimating the indicator suitability of spring-inhabiting plants with respect to the processes of acidification and recovery. We analysed 18 environmental variables in a two-step approach, regarding their ability to explain the species composition of 222 springs on siliceous mountain ranges in central Germany and the north west of the Czech Republic (49.9° – 50.8° N, 10.6° – 12.8° E). First, we compared the three subsets of spatial, hydrophysical and hydrochemical parameters by means of a partial canonical correspondence analysis (pCCA). Subsequently that subset with the best explanatory power was examined in detail, using a canonical correspondence analysis (CCA).

Hydrochemical factors turned out to play the fundamental role in determining the plant community composition of the investigated springs. Spatial factors, in particular the altitude, were correlated with the hydrochemical factors, but were less meaningful than those. Hydrophysical factors played only a marginal role. More precisely, the species occurrence was mainly driven by a gradient of acidity and nutrient availability. This gradient was primarily represented by the pH-value. High Al, Cd, and Mn concentrations characterised acidic spring waters, high Ca and Mg concentrations were encountered in circumneutral spring waters.

Springs are known to be low dynamic ecosystems, allowing species to adapt to habitat conditions and to develop a steady state in community composition. Additionally, springs are abundant, but pristine habitats where disturbance and human land-use are rare. This natural setting provides a sound basis for a biomonitoring approach. Indeed we could show that spatial patterns in the vegetation of springs emerge and provide valuable ecological information on the water quality of springs and in return on the acidity status of their forested catchments.

Keywords

acidification, biomonitoring, hydrochemistry, partial canonical correspondence analysis pCCA, species composition, spring water, vegetation ecology

Introduction

Forested catchments of the northern hemisphere were affected severely by acidification during the last decades and loads of acidifying compounds were considerably increased compared to historical periods (Reuss et al. 1987; Asman et al. 1988; Steinberg & Wright 1994; Kopácek et al. 2001; Bouwman et al. 2002). Its symptoms were the damage and the decline of forest vegetation as well as the alteration of soils, aquifers and headwaters. Indeed, in Central Europe acidic depositions were reduced considerably since the early 1990s. Nevertheless, acidification continues to be a relevant environmental issue.

Though, the recovery of catchments and limnological systems seems possible (Hultberg et al. 1998; Kopácek et al. 1998) but can be considerably delayed by biogeochemical processes within the catchments (Alewell et al. 2001). Regional patterns occur (Stoddard et al. 1999). Obviously, the impairment and recovery of ecosystems does not respond directly to the recent status of acidic depositions alone. The surface roughness of forest vegetation (e.g. deciduous or coniferous forest) (Williard et al. 2005) as well as altitude, slope and aspect of the catchment (Palmer et al. 2004; Rantakari et al. 2004) may alter the local deposition load of a specific catchment. After all, the buffering capacity of the soils in the catchment exerts its influence on ecosystem conditions (Probst et al. 1999; Williard et al. 2005).

Springs represent the interface between aquifers and lotic waters. They are particularly characterised by their constancy regarding hydrochemical and hydrophysical factors (Odum 1971; Ellenberg 1996). But, there are further attributes which account them for being an interesting subject to study species-environment relationships.

Spring water reflects biogeochemical and hydrological processes of the catchment area (Beierkuhnlein & Durka 1993). That way springs represent the output of ecosystems in a spatially concentrated manner. Due to their functional complexity ecological processes can hardly be measured completely in ecosystems. In our approach, forest ecosystem catchments are regarded as it were black boxes. The 'small catchment approach' (e.g. Moldan & Černý 1994; Church 1997; Likens & Bormann 1999) applies to springs. In low dynamic forested catchments, springs reflect long-term ecological processes and the biogeochemical background of the landscape.

In Central European siliceous mountains groundwater flows mainly close to the surface (interflow). Here, springs are generally formed as helocrenic springs (sensu Thienemann 1924). These feature a saturated area of a few to some hundred square meters in size, where the water seeps out continuously from the ground. Climate and the size of the catchment area cause minor seasonal fluctuations, which are reflected in a certain variability of discharge. However, water temperatures in springs are equilibrated by the permanent energy supply of the groundwater.

As water flow in springs is unidirectional and more or less continuous, the spring-inhabiting organisms are strongly depending on the quantity and quality of the oozing out groundwater. Other site conditions such as the mesoclimate of the forest ecosystem itself are less important (Strohbach et al. submitted). It is well known that springs provide a habitat for specialised organisms which are adapted to the relative environmental constancy (Thienemann 1924; Odum 1971). Most crenobiotic and crenophilic species are stenoecious (Ferrington 1998; Zollhöfer et al. 2000; Cantonati et al. 2006). Hence, the species composition of springs clearly differs from the adjacent ecosystems where the short-term and seasonal variability of the environment is more pronounced.

Spring water chemistry, in particular the gradient of acidity and nutrient availability, is discussed as the major driving factor for the plant species composition of forest springs (Beierkuhnlein 1994; Hájek et al. 2002; Hájková et al. 2008). This coincides with various studies in springs and fens of the northern hemisphere (Vitt & Chee 1990; Wheeler & Proctor 2000; Bragazza & Gerdol 2002; Nekola 2004; Tahvanainen 2004). However, the type of habitat is often not precisely defined or restrained. Only at sources with small spatial extension or at point sources of groundwater the influence of the terrestrial environment becomes negligible. Spatially extended fens are more exposed to insolation and wind. Night-time cooling during winter and snow cover on frozen topsoil is also more important there.

The sampling distance from the source point or in other words the aboveground flow path length has to be accounted for. Indeed, already short distances from the emerging spring itself can alter water chemistry significantly, via outgassing of CO_2, buffering processes and lateral impacts. Water quality is modified when percolating over several meters through compact vegetation. The larger the habitat is (e.g. fens, mires) and the less clearly defined the location of the water sample is, the more comparisons between investigations are hampered by these facts.

In forested areas of Central Europe, springs are more or less the last representatives of pristine habitats. Due to their small extent and to the permanent water saturation of the soil there are almost no land use options and human concern is low. This is why in these habitats natural drivers still dominate ecosystem functioning, at least regarding physical factors and land use. Therefore they are a remarkable exception within the anthropogenic landscapes of Central Europe. Albeit springs capture only a minor portion of the surface, they appear frequently in forested mountain ranges of Central Europe. Their abundance makes them suitable subjects for monitoring purposes at the landscape scale. It offers a high spatial resolution of comparable and almost standardised records (Beierkuhnlein & Durka 1993).

Helocrenic springs (sensu Thienemann 1924), where groundwater slowly seeps out to the surface at a non-point source, are surrounded by habitats with disparate conditions. They resemble islets with specific habitat conditions that do not refer to the surrounding matrix. As the ecological particularity and first of all the thermic constancy gets lost in the mountain brook, springs feature a considerable ecological isolation. Direct spatial connectivity is negligible. Together with the natural constancy of hydrological factors this natural setting permits to relate species occurrences and environmental conditions. This is the basis to indicate upcoming environmental changes.

Summing up these points, springs and the species dwelling in them are supposed to be powerful indicators for the biogeochemical background of landscapes. Exact localisation and balanced physical conditions in combination with organisms that are highly sensitive to environmental change are requested preconditions for the biomonitoring of long-term ecological trends. When environmental conditions change, responses by spring-inhabiting species and their assemblages are likely to occur (Glazier 1991; Zollhöfer et al. 2000). It can be expected that changes in spring water quality will affect first and foremost the stenoecious crenobiotic and crenophilic species (Ferrington 1998; Zollhöfer et al. 2000; Cantonati et al. 2006). Individually, non-mobile groups of organisms - such as plants - are especially suited as indicators.

As our aim was to fathom the indicative power of springs regarding the processes of acidification and recovery, we studied the plant species composition of springs and its relation to hydrophysical, hydrochemical and spatial variables across a large area of predominantly forested landscapes. Spatial gradients (and temporal trends) can be detected.

As the deposition of various acidifying compounds (e.g. sulphuric acid) has been reduced drastically, the regeneration of Central European forest ecosystems is a realistic option.

However, atmospheric nitrogen loads are still extremely high. Future comparisons will allow identifying the rate and inertia of recovery separating direct catchment response and the indirect effects on spring communities. Interactions between pollution pressure on ecosystems and the ongoing and expected climatic changes will be a novel concern. Our study delivers an excellent setting to tackle such questions.

Material and Methods

Study area and study sites

We investigated springs in the five neighbouring mountain ranges Thüringer Wald, Thüringer Schiefergebirge, Frankenwald, Fichtelgebirge and Erzgebirge. These landscapes are located in central Germany and the north west of the Czech Republic (fig. 1). They exhibit altitudes between 250 and 1240 m a.s.l.. The bedrock material consists of silicates throughout and is relatively homogeneous in each of the 5 regions. Climatic conditions are on the transition from oceanic to continental. Detailed landscape characteristics are given in table 1. The study areas are predominantly covered by forests and were exposed to severe acid depositions culminating in the 1970s and 1980s. These loads resulted also in the acidification of ground-, spring-, and headwaters. Mountain lakes and ponds were affected as well.

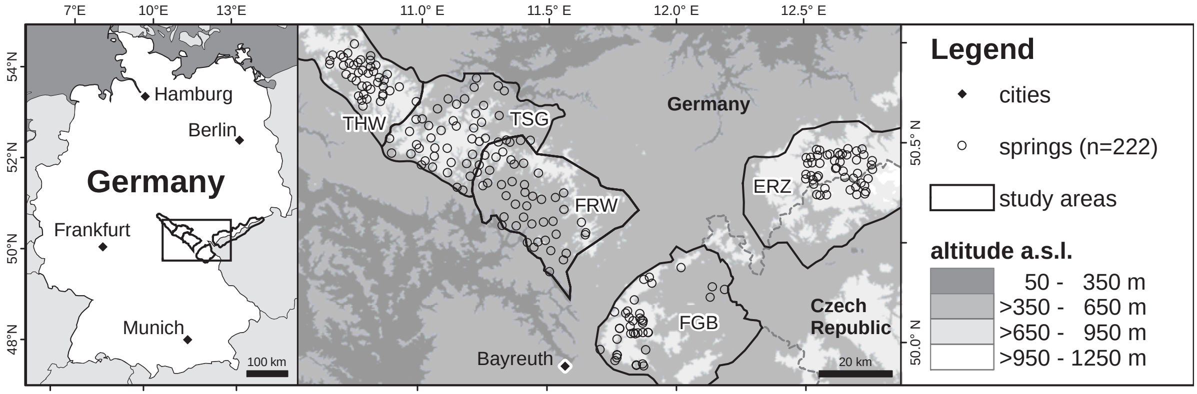

Figure 1: Spatial setting of the investigated springs (n = 222) and monitored landscapes in central Germany and the north west of the Czech Republic. Abbreviations denote the five regions: THW = Thüringer Wald, TSG = Thüringer Schiefergebirge, FRW = Frankenwald, FGB = Fichtelgebirge and ERZ = Erzgebirge.

Table 1: Landscape traits of the five studied regions.

region	Thüringer Wald	Thüringer Schiefergebirge	Franken-wald	Fichtel-gebirge	Erz-gebirge	all 5 regions
landscape trait						
altitude a.s.l. [m]	300 - 980	250 - 870	350 - 790	450 - 1050	400 - 1240	250 - 1240
predominant bedrock	porphyry, conglomerate	schist, quarzite	schist	granite, phyllite	granite, gneiss phyllite	siliceous
precipitation [mm a^{-1}]	800 - 1200	550 - 1200	600 - 1000	600 - 1350	700 - 1200	550 - 1350
air temperature [°C]	4 - 7	4 - 7	5 - 7	4 - 6	3 - 6	3 - 7
dominating tree species	*Picea abies*, (*Fagus sylv.*)	*Picea abies*, (*Fagus sylv.*)	*Picea abies*, (*Fagus sylv.*)	*Picea abies*	*Picea abies*	*Picea abies*, (*Fagus sylv.*)

In our study areas the springs are generally formed as helocrenic springs (sensu Thienemann 1924) with a saturated area of a few to some hundred square meters in size, where water seeps out continuously from the ground. The discharge rates are usually low (< 2.0 l s^{-1}), but there are certain seasonal fluctuations, depending on climate and the size of the catchment. The spring water temperatures, and consequently the surface-near air temperatures as well, are equilibrated in these springs by the permanent energy supply of the groundwater.

Initially we investigated 251 springs, but 29 of them had to be omitted due to insufficient discharge (< 0.02 l s^{-1}) and therefore missing values in the hydrological dataset. Hence 222 springs remain for analysis. All these and their catchments are located in forests, which are mainly dominated by Norway spruce (*Picea abies*), partly also by beech (*Fagus sylvatica*). The altitude of the spring sites ranges from 270 to 925 m a.s.l., the pH-value of the siliceous, nutrient-poor spring waters is acidic to neutral.

Sampling of environmental and vegetation data

In vegetation relevés we estimated the abundance/dominance score of each vascular plant, moss and liverwort species growing in the entire, well-differentiated seeping area. This was done either in July or in August by means of the method of Braun-Blanquet (1964), but using a slightly modified scale (see table 2). Nomenclature follows Frahm & Frey (2004) for bryophytes and Oberdorfer (2001) for vascular plants.

Environmental data were collected in three categories, *hydrophysical* parameters (n=2), *hydrochemical* parameters (n=13) and *spatial* parameters (n=3). Spring water was sampled in late September and October, always at the uppermost point of the seeping area zone with above ground flowing water. There a small pit was impressed and - before measurements were conducted - the spring water was allowed to clarify. In situ we measured the *hydrophysical* parameters discharge and electrical conductivity as well as the *hydrochemical* parameter pH-value. The *hydrochemical* parameter acid neutralising capacity (ANC) was quantified in vitro, via titration with 0.1 M HCl to pH 4.3. Spring water was analysed for the *hydrochemical* compounds NH_4^+, NO_3^-, SO_4^{2-}, PO_4^- and elements Ca, Mg, K, Mn, Al, Cd, Na. The *spatial* parameters latitude, longitude and altitude were derived from topographic maps. Space, of course, is not a driver of ecological relations, but it can be used as a representative for spatial factors and processes (Leduc et al. 1992).

Table 2: Modified Braun-Blanquet's abundance/dominance scores and the code replacement used for statistical analysis.

Br.-Bl. score	Cover [%]	Code replacement
r	0 - 0.01	0.1
+	0.01 - 0.2	0.5
1	0.2 - 5	2.5
2a	5 - 15	10.0
2b	15 - 25	20.0
3	25 - 50	37.5
4	50 - 75	62.5
5	75 - 100	87.5

We are summarising data of 5 regions as a whole. All these have been surveyed using the same sampling protocol and analytical methods. But, the investigations were conducted in different years (1993: Thüringer Schiefergebirge; 1997: Thüringer Wald and Erzgebirge; 2003: Frankenwald and Fichtelgebirge). However, as our main focus is to identify the driving forces of species composition, we suppose that this inconsistency is not crucial here.

Statistical analyses

Initially, to get an overview over the hydrological characteristics of the spring waters, all environmental data were examined by a linear regression analysis, using Pearson's coefficient as the correlation measure.

Aiming to disclose the determinant factors of species composition in springs, a canonical analysis of vegetation and environmental data was carried out. Beforehand, vegetation data were reduced by removing species which were found in less than 10 springs. Braun-Blanquet's abundance/dominance scores of the remaining species were converted into percentage cover (for code replacement see table 2). Then, cover data were square-root transformed to down-weight dominant and to up-weight rare species, like Legendre & Gallagher (2001) recommend for comparing communities to environmental data.

Environmental data were not transformed.

To unveil the driving forces behind plant community composition, we chose a two-step approach. First, we calculated a partial canonical correspondence analysis (pCCA) to identify the proportion of total variance which is explained by each of the three environmental parameter subsets *hydrophysics*, *hydrochemistry* and *space* purely as well as in combination. pCCA is a variance partitioning procedure which allows testing for effects of a subset of explanatory variables, while partialling out effects of an other subset of explanatory variables. For the subsequent detailed analysis (canonical correspondence analysis CCA) we decided to retain merely the subset which was correlated best with community data in the pCCA. That way the canonical analysis benefits, since the autocorrelations in the environmental dataset are confined by a pre-selection of important variables. All analyses were computed using *R* (R Development Core Team 2008). CCA and pCCA were performed using the *R* package *vegan* (Oksanen et al. 2008).

Results

In all five regions spring waters are acidic to circumneutral, they can be characterised as nutrient-poor throughout. Their pH-values ranged from 3.76 to 7.80, their electrical conductivity from 27.0 to 719.0 µS cm^{-1}. Regarding several hydrophysical and hydrochemical parameters differences between the regions were detectable. The mean values and the ranges of the measured hydrological factors are summarised in table 3, separately for each of the regions as well as conjointly for all of them. The interrelationships between the individual environmental parameters are depicted in table 4.

According to the wide variety of environmental conditions, the 222 investigated springs accommodated 284 species in total, with an average of 28 species per spring (minimum 6, maximum 55; cf. table 3). 110 species occurred in at least 10 springs and fulfilled therefore the criterion to be included in the further analyses.

Table 3: **Characteristics of the 222 springs. Aside from the analysed spatial (n = 3), hydrophysical (n = 2) and hydrochemical parameters (n = 13), also the size of the seeping area, water temperature, and number of species are depicted. Values < LOQ are considered as zero values.**

region		**Thüringer Wald**	**Thüringer Schiefergebirge**	**Franken-wald**	**Fichtel-gebirge**	**Erz-gebirge**	**all 5 regions**
no. of sites		41	47	44	41	49	222
	unit	**mean ± sd** min - max	**mean ± sd** min - max	**mean ± sd** min - max	**mean ± sd** min - max	**mean ± sd** min - max	**mean ± sd** min - max
spatial parameters							
longitude	[°]	10.6 - 11.0	10.8 - 11.4	11.2 - 11.7	11.7 - 12.2	12.5 - 12.8	10.6 - 12.8
latitude	[°]	50.5 - 50.8	50.3 - 50.7	50.1 - 50.6	49.9 - 50.2	50.3 - 50.5	49.9 - 50.8
altitude a.s.l.	[m]	**712 ± 80** 535 - 830	**609 ± 122** 270 - 810	**564 ± 83** 410 - 730	**712 ± 107** 500 - 910	**796 ± 82** 625 - 925	**679 ± 127** 270 - 925
hydrophysical parameters							
discharge	[l s^{-1}]	**0.20 ± 0.19** 0.02 - 1.00	**0.06 ± 0.10** 0.01 - 0.50	**0.09 ± 0.13** 0.02 - 0.50	**0.16 ± 0.22** 0.02 - 1.00	**0.34 ± 0.26** 0.05 - 1.00	**0.17 ± 0.22** 0.01 - 1.00
electrical conductivity	[µS cm^{-1}]	**124.1 ± 76.9** 44.3 - 442.9	**133.5 ± 77.9** 33.5 - 387.0	**136.9 ± 109.0** 57.0 - 719.0	**73.3 ± 46.8** 27.0 - 223.0	**90.7 ± 26.2** 38.4 - 184.7	**111.9 ± 76.3** 27.0 - 719.0
hydrochemical parameters							
pH		**6.20 ± 0.61** 5.04 - 7.23	**5.85 ± 0.80** 4.50 - 7.80	**6.33 ± 0.87** 4.65 - 7.50	**5.54 ± 0.56** 3.76 - 6.69	**4.85 ± 0.29** 4.44 - 5.53	**5.73 ± 0.85** 3.76 - 7.80
ANC	[mmol l^{-1}]	**0.312 ± 0.274** 0.050 - 1.270	**0.301 ± 0.458** 0.040 - 2.150	**0.428 ± 0.482** 0.035 - 1.980	**0.132 ± 0.093** 0.000 - 0.530	**0.107 ± 0.047** 0.040 - 0.230	**0.254 ± 0.345** 0.000 - 2.150
NH_4	[mg l^{-1}]	**0.005 ± 0.020** 0.000 - 0.095	**0.018 ± 0.014** 0.004 - 0.072	**0.010 ± 0.025** 0.000 - 0.127	**0.015 ± 0.047** 0.000 - 0.240	**0.000 ± 0.000** 0.000 - 0.000	**0.010 ± 0.026** 0.000 - 0.240
NO_3	[mg l^{-1}]	**7.03 ± 2.41** 2.86 - 12.00	**5.69 ± 6.11** 0.00 - 36.49	**6.92 ± 7.80** 0.37 - 45.90	**4.16 ± 2.67** 0.00 - 10.00	**5.69 ± 2.03** 2.08 - 11.00	**5.90 ± 4.89** 0.00 - 45.90
SO_4	[mg l^{-1}]	**21.63 ± 6.43** 5.84 - 36.60	**26.42 ± 16.44** 6.05 - 88.46	**21.50 ± 6.47** 11.40 - 43.70	**18.95 ± 19.16** 2.57 - 92.50	**28.64 ± 8.79** 5.13 - 48.00	**23.67 ± 12.96** 2.57 - 92.50
PO_4	[mg l^{-1}]	**0.094 ± 0.198** 0.000 - 0.590	**0.039 ± 0.061** 0.003 - 0.408	**0.000 ± 0.000** 0.000 - 0.000	**0.000 ± 0.000** 0.000 - 0.000	**0.034 ± 0.122** 0.000 - 0.634	**0.033 ± 0.111** 0.000 - 0.634
Ca	[mg l^{-1}]	**11.99 ± 5.84** 4.44 - 26.20	**8.53 ± 9.12** 0.40 - 55.80	**11.20 ± 9.02** 2.50 - 44.80	**5.11 ± 5.13** 1.11 - 25.90	**7.16 ± 3.06** 2.04 - 15.80	**8.76 ± 7.23** 0.40 - 55.80
Mg	[mg l^{-1}]	**1.97 ± 0.94** 0.41 - 6.22	**2.68 ± 1.61** 0.50 - 8.00	**4.44 ± 2.23** 1.69 - 12.30	**1.90 ± 1.60** 0.28 - 6.80	**1.68 ± 0.71** 0.75 - 3.87	**2.53 ± 1.81** 0.28 - 12.30
K	[mg l^{-1}]	**3.075 ± 2.106** 0.000 - 10.500	**0.651 ± 0.486** 0.140 - 2.570	**0.954 ± 0.388** 0.297 - 1.910	**1.215 ± 0.650** 0.386 - 3.230	**1.308 ± 0.429** 0.560 - 2.130	**1.408 ± 1.298** 0.000 - 10.500
Mn	[µg l^{-1}]	**1.51 ± 9.68** 0.00 - 62.00	**29.66 ± 46.00** 0.10 - 214.00	**68.85 ± 174.64** 0.00 - 900.00	**43.17 ± 61.07** 0.00 - 230.00	**130.16 ± 124.35** 0.00 - 560.00	**56.91 ± 111.38** 0.00 - 900.00
Al	[µg l^{-1}]	**30.6 ± 51.7** 0.0 - 332.0	**257.7 ± 493.7** 2.1 - 1914.0	**116.0 ± 231.6** 1.4 - 843.0	**338.8 ± 544.7** 2.7 - 2080.0	**1021.7 ± 939.3** 0.0 - 4050.0	**371.3 ± 662.0** 0.0 - 4050.0
Cd	[µg l^{-1}]	**0.065 ± 0.179** 0.000 - 1.100	**0.096 ± 0.123** 0.000 - 0.540	**0.122 ± 0.258** 0.000 - 1.620	**0.186 ± 0.229** 0.000 - 1.140	**0.503 ± 0.252** 0.000 - 1.280	**0.202 ± 0.269** 0.000 - 1.620
Na	[mg l^{-1}]	**5.58 ± 8.39** 1.04 - 37.50	**3.12 ± 3.45** 0.30 - 17.80	**7.56 ± 13.33** 1.37 - 80.70	**4.20 ± 3.49** 1.66 - 14.40	**3.44 ± 2.44** 1.42 - 18.80	**4.73 ± 7.48** 0.30 - 80.70
further parameters (not included in analyses)							
seeping area	[m^2]	**25 ± 17** 6 - 90	**45 ± 40** 8 - 200	**94 ± 96** 7 - 489	**52 ± 81** 1 - 387	**18 ± 8** 6 - 48	**46 ± 64** 1 - 489
water temp.	[°C]	**7.4 ± 1.3** 5.6 - 11.3	***n.a.*** *n.a.*	**6.8 ± 1.4** 3.6 - 9.0	**5.6 ± 1.5** 1.0 - 8.6	**6.7 ± 0.7** 5.5 - 9.2	**(*6.6 ± 1.4*)** (*1.0 – 11.3*)
no. of species		**29 ± 7** 17 - 44	**28 ± 11** 6 - 51	**37 ± 10** 10 - 54	**25 ± 11** 11 - 55	**23 ± 6** 15 - 38	**28 ± 10** 6 - 55

Table 4: **Pearson linear correlation of environmental parameters (r values). Significance levels are marked as follows: $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), $p \geq 0.05$ (n.s.). Abbreviations denote: long = longitude, lat = latitude, alt = altitude, disch = discharge, cond = electrical conductivity.**

	spatial		**hydrophysical**		**hydrochemical**													
	lat	**alt**	**disch**	**cond**	**pH**	**ANC**	**NH_4**	**NO_3**	**SO_4**	**PO_4**	**Ca**	**Mg**	**K**	**Mn**	**Al**	**Cd**	**Na**	
long	**-0.437** ***	**0.389** ***	**0.326** ***	**-0.213** **	**-0.547** ***	**-0.252** ***	-0.117 n.s.	-0.097 n.s.	**0.164** *	**-0.154** *	**-0.209** **	**-0.163** *	**-0.230** ***	**0.354** ***	**0.486** ***	**0.575** ***	-0.076 n.s.	**long**
lat		-0.042 n.s.	0.053 n.s.	**0.271** ***	0.122 n.s.	0.118 n.s.	-0.081 n.s.	**0.196** **	**0.204** **	**0.248** ***	**0.264** ***	0.009 n.s.	**0.338** ***	-0.022 n.s.	-0.059 n.s.	-0.043 n.s.	0.057 n.s.	**lat**
alt			**0.303** ***	**-0.338** ***	**-0.645** ***	**-0.414** ***	-0.051 n.s.	-0.129 n.s.	**-0.323** ***	0.065 n.s.	**-0.374** ***	**-0.599** ***	0.108 n.s.	**0.272** ***	**0.461** ***	**0.427** ***	-0.073 n.s.	**alt**
disch				**-0.150** *	**-0.227** ***	**-0.132** *	**-0.194** **	0.025 n.s.	-0.080 n.s.	**0.167** *	-0.114 n.s.	**-0.226** ***	0.123 n.s.	0.041 n.s.	0.123 n.s.	**0.206** **	-0.015 n.s.	**disch**
cond					**0.241** ***	**0.385** ***	**0.174** **	**0.224** ***	**0.394** ***	0.041 n.s.	**0.703** ***	**0.653** ***	**0.242** ***	**0.215** **	-0.095 n.s.	**0.191** **	**0.787** ***	**cond**
pH						**0.715** ***	-0.022 n.s.	0.091 n.s.	0.019 n.s.	0.075 n.s.	**0.519** ***	**0.456** ***	-0.018 n.s.	**-0.544** ***	**-0.641** ***	**-0.642** ***	-0.042 n.s.	**pH**
ANC							0.009 n.s.	0.042 n.s.	0.110 n.s.	0.104 n.s.	**0.764** ***	**0.413** ***	-0.131 n.s.	**-0.257** ***	**-0.286** ***	**-0.312** ***	-0.042 n.s.	**ANC**
NH_4								-0.054 n.s.	**0.152** *	-0.050 n.s.	0.024 n.s.	**0.136** *	-0.075 n.s.	0.102 n.s.	0.033 n.s.	0.098 n.s.	0.100 n.s.	**NH_4**
NO_3									**0.163** *	0.065 n.s.	**0.269** ***	**0.152** *	**0.197** **	-0.090 n.s.	-0.017 n.s.	-0.020 n.s.	0.030 n.s.	**NO_3**
SO_4										0.025 n.s.	**0.435** ***	**0.330** ***	0.078 n.s.	0.098 n.s.	0.121 n.s.	**0.197** **	0.034 n.s.	**SO_4**
PO_4											0.105 n.s.	-0.122 n.s.	**0.151** *	-0.054 n.s.	0.008 n.s.	-0.043 n.s.	-0.058 n.s.	**PO_4**
Ca												**0.465** ***	**0.158** *	-0.101 n.s.	**-0.214** **	-0.074 n.s.	**0.279** ***	**Ca**
Mg													0.002 n.s.	0.006 n.s.	**-0.307** ***	-0.073 n.s.	**0.468** ***	**Mg**
K														-0.060 n.s.	-0.096 n.s.	0.121 n.s.	**0.314** ***	**K**
Mn															**0.659** ***	**0.708** ***	**0.357** ***	**Mn**
Al																**0.707** ***	-0.038 n.s.	**Al**
Cd																	**0.326** ***	**Cd**

The pCCA revealed the hydrochemical parameters to be the most important category for the determination of the species composition (figure 2). 16.66 % of the total variance could be explained by the hydrochemical variables, 9.53 % purely by these. The spatial and hydrophysical variables were less relevant, explaining purely 3.44 % and 1.04 %, respectively. 7.75 % of the total variance was a matter of the different combinations between the three environmental categories. Conjointly the three categories of spatial, hydrophysical and hydrochemical parameters explain 21.76 % of the total variance, 78.24 % remain unexplained. In conclusion, the results of the pCCA suggest a concentration on the examination of the hydrochemical parameters, when searching for the driving factors of the species composition of springs.

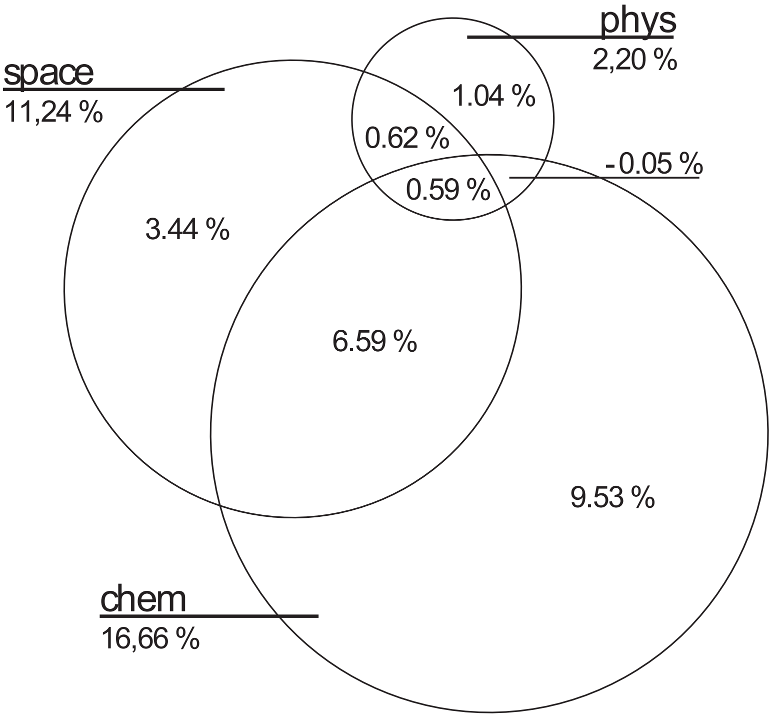

Figure 2: Proportion of total variance explained by the three groups *space* (n = 3 spatial parameters), *phys* (n = 2 hydrophysical parameters) and *chem* (n = 13 hydrochemical parameters), calculated by partial canonical correspondence analysis (pCCA). Conjointly 21.76 % of the total variance is explained. The size of circles and segments is approximated.

When enlightening the driving factors of species composition in detail (by means of a CCA with respect to only the 13 hydrochemical variables), a gradient of acidity and nutrient availability turned out to be the most effective factor (figure 3a). The first axis of CCA is loaded highest by the pH-value. High values in ANC and high Mg and Ca concentrations are loading the first axis of CCA positively as well. These are marking circumneutral waters. In the opposite direction, acidic spring waters are characterised by high concentrations of Al, Cd, and Mn, all three factors load the CCA first axis negatively. This acidic end of the main gradient is widely characterised by the occurrence of peat-building mosses (*Polytrichum commune* and several species of *Sphagnum*) and clonal grasses (*Calamagrostis villosa*, *Deschampsia flexuosa*). Under neutral conditions, herbaceous species are predominant (figure 3b, table 5).

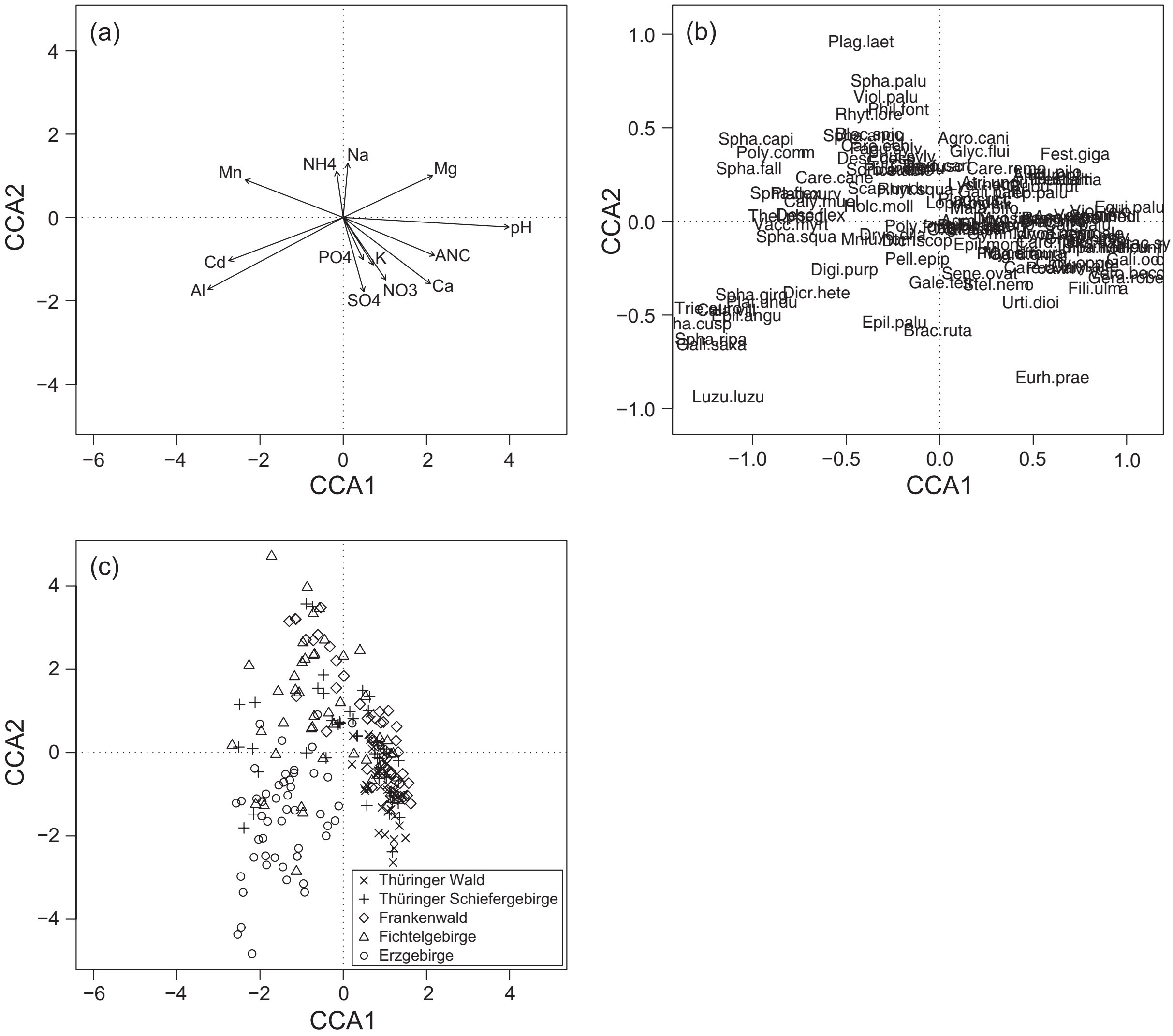

Figure 3: Ordination diagrams for the canonical correspondence analysis (CCA), showing (a) 13 hydrochemical variables, (b) 110 species, and (c) 222 sites. 16.66 % of total variance is explained by 13 hydrochemical parameters.

Table 5: **CCA axis scores for those of the 110 species, which are ranked within the lowest 20 and highest 20 CCA first axis scores. Additionally, their frequency and their occurrence in the five regions are given. Abbreviations denote the regions: THW = Thüringer Wald, TSG = Thüringer Schiefergebirge, FRW = Frankenwald, FGB = Fichtelgebirge and ERZ = Erzgebirge.**

Species	**CCA 1**	**CCA 2**	**Freq. sites**	**Freq. regions**	**THW**	**TSG**	**FRW**	**FGB**	**ERZ**
Sphagnum cuspidatum	-1.332	-0.554	10	2	.	.	+	.	+
Trientalis europaea	-1.251	-0.463	31	4	+	+	.	+	+
Galium saxatile	-1.245	-0.654	44	4	+	+	+	.	+
Sphagnum riparium	-1.240	-0.637	36	3	.	.	+	+	+
Calamagrostis villosa	-1.156	-0.472	93	4	+	+	.	+	+
Luzula luzuloides	-1.144	-0.934	12	3	+	+	.	.	+
Epilobium angustifolium	-1.047	-0.511	32	4	.	+	+	+	+
Sphagnum fallax	-1.036	0.277	65	4	.	+	+	+	+
Sphagnum girgensohnii	-1.020	-0.399	53	***5***	+	+	+	+	+
Sphagnum capillifolium	-1.001	0.435	12	4	.	+	+	+	+
Plagiothecium undulatum	-0.961	-0.428	60	***5***	+	+	+	+	+
Polytrichum commune	-0.878	0.360	90	***5***	+	+	+	+	+
Sphagnum flexuosum	-0.843	0.144	10	3	+	.	+	.	+
Thelypteris phegopteris	-0.835	0.023	49	***5***	+	+	+	+	+
Vaccinium myrtillus	-0.811	-0.025	87	4	.	+	+	+	+
Sphagnum squarrosum	-0.788	-0.087	68	***5***	+	+	+	+	+
Plagiothecium curvifolium	-0.730	0.141	10	3	+	+	.	.	+
Deschampsia flexuosa	-0.710	0.037	82	***5***	+	+	+	+	+
Dicranella heteromalla	-0.670	-0.380	16	***5***	+	+	+	+	+
Calypogeia muelleriana	-0.649	0.100	64	***5***	+	+	+	+	+
…									
…									
Festuca gigantea	0.708	0.352	10	4	+	.	+	+	+
Chrysosplenium oppositifolium	0.711	-0.227	118	***5***	+	+	+	+	+
Caltha palustris	0.725	-0.024	12	3	.	.	+	+	+
Chaerophyllum hirsutum	0.735	-0.086	50	***5***	+	+	+	+	+
Chrysosplenium alternifolium	0.764	-0.246	63	***5***	+	+	+	+	+
Lamium galeobdolon	0.775	-0.070	31	4	+	+	+	+	.
Impatiens noli-tangere	0.806	-0.145	70	***5***	+	+	+	+	+
Veronica montana	0.808	0.033	33	4	+	+	+	+	.
Cirsium palustre	0.821	-0.119	21	4	+	+	+	+	.
Chiloscyphus polyanthos	0.831	-0.085	42	4	+	+	+	+	.
Filipendula ulmaria	0.844	-0.356	19	4	+	+	+	+	.
Viola reichenbachiana	0.845	0.056	12	3	+	.	+	+	.
Stellaria media	0.869	0.029	11	2	.	.	+	+	.
Plagiomnium undulatum	0.890	-0.131	76	4	+	+	+	+	.
Geranium robertianum	0.979	-0.298	24	4	+	+	+	+	.
Veronica beccabunga	0.982	-0.273	10	3	+	+	+	.	.
Equisetum palustre	1.002	0.068	10	2	.	.	+	+	.
Melica uniflora	1.032	-0.136	12	3	+	+	+	.	.
Galium odoratum	1.062	-0.200	11	2	+	.	+	.	.
Stachys sylvatica	1.099	-0.121	17	3	.	+	+	+	.

Reckoning the sites, the main gradient found by the CCA is represented also by the five regions (figures 3c and 4, table 6). Whereas positive CCA first axis scores mark circumneutral conditions in the Thüringer Wald throughout, in the Erzgebirge acidic conditions are prevailing, the latter indicated by the lowest mean CCA first axis scores. In the three central regions, the Thüringer Schiefergebirge, the Frankenwald and the Fichtelgebirge, a wider range of CCA first axis scores was encountered. There, acidic spring waters are confined mainly to the highest altitudes (figures 4 and 5).

Table 6: CCA axis scores for the 222 sites, given as mean values and ranges for the five regions and for the entirety of investigated springs.

region	Thüringer Wald	Thüringer Schiefergebirge	Franken-wald	Fichtel-gebirge	Erz-gebirge	all 5 regions
	mean ± sd min - max	**mean ± sd** min - max	**mean ± sd** min - max	**mean ± sd** min - max	**mean ± sd** min - max	**mean ± sd** min - max
CCA axis scores						
CCA 1	**0.954 ± 0.328** 0.211 - 1.498	**0.091 ± 1.181** -2.509 - 1.355	**0.559 ± 0.868** -1.297 - 1.624	**-0.663 ± 0.959** -2.669 - 1.203	**-1.444 ± 0.701** -2.576 - 0.215	**-0.135 ± 1.220** -2.669 - 1.624
CCA 2	**-0.870 ± 0.820** -2.641 - 0.945	**0.120 ± 1.220** -2.381 - 3.571	**0.606 ± 1.397** -1.276 - 3.483	**1.022 ± 1.592** -2.852 - 4.713	**-1.588 ± 1.292** -4.829 - 0.910	**-0.177 ± 1.603** -4.829 - 4.713

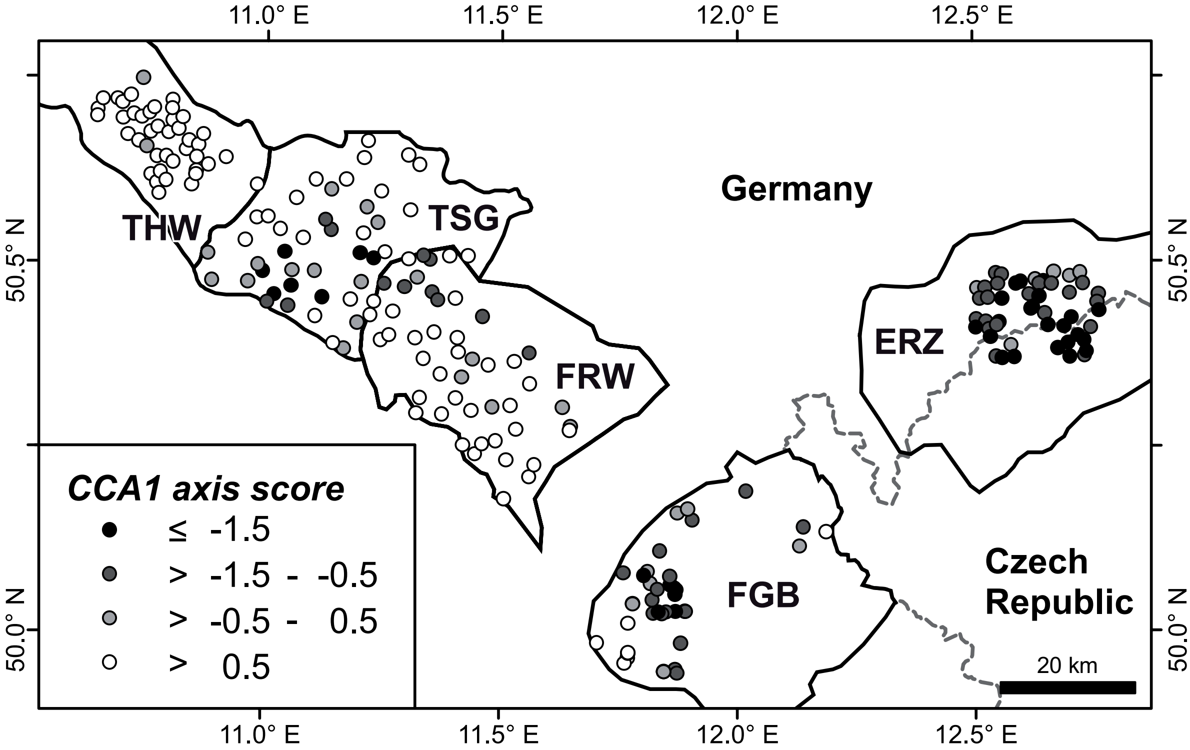

Figure 4: Map of CCA first axis scores. Abbreviations denote the five regions: THW = Thüringer Wald, TSG = Thüringer Schiefergebirge, FRW = Frankenwald, FGB = Fichtelgebirge and ERZ = Erzgebirge.

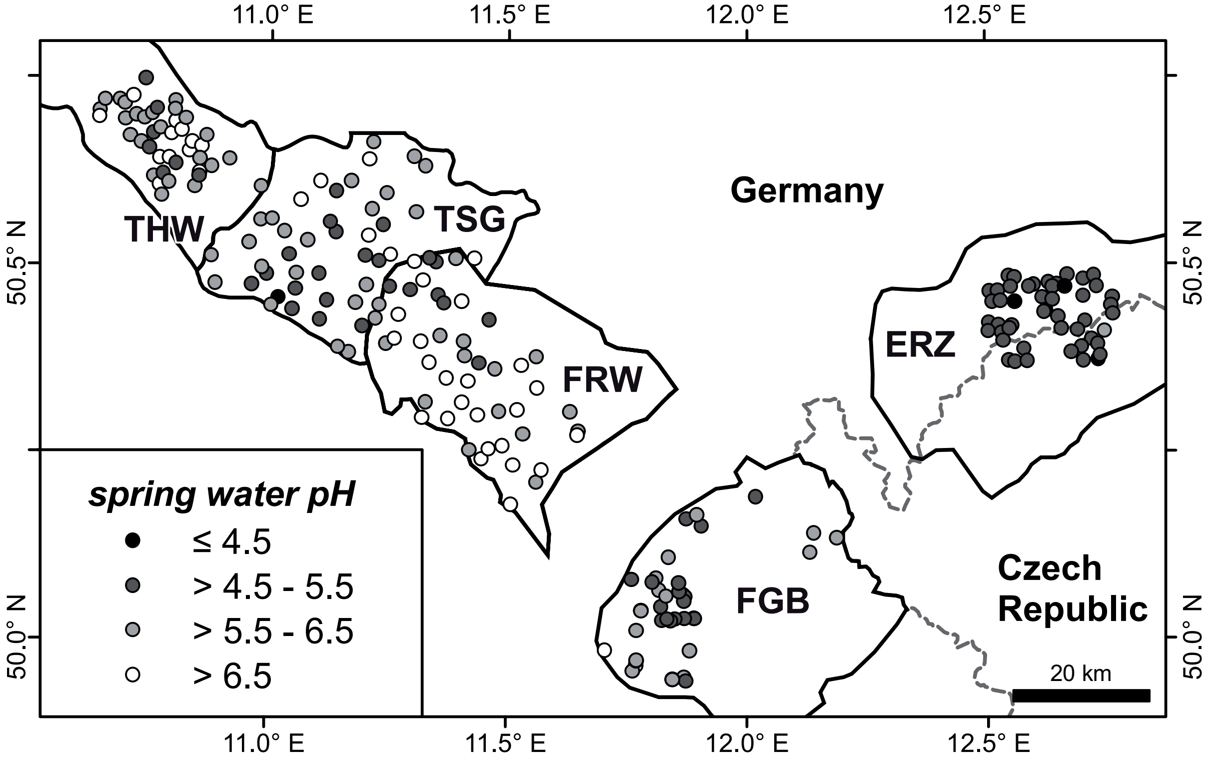

Figure 5: Map of spring water pH-values. Abbreviations denote the five regions: THW = Thüringer Wald, TSG = Thüringer Schiefergebirge, FRW = Frankenwald, FGB = Fichtelgebirge and ERZ = Erzgebirge.

Discussion

Spring water characteristics and spatial relations

The five investigated regions showed remarkable differences in the properties of their spring waters (table 3), in particular regarding the hydrochemical parameters and to a lesser extent for their hydrophysical parameters. Even if the basic principles are connatural - siliceous bedrock, surface-near aquifer, helocrenic form of the springs, forested catchments, and climatic traits - spatial patterns of hydrochemical characteristics were found. Overall, a longitudinal gradient was detected (table 4, figure 5).

The acidity increased from west to east, most prominently marked by a significant decline of the pH-values and the ANC. Our data suggested that this is an effect of the altitude (figure 6), which is also significantly related to the longitude (table 4): the eastern regions Fichtelgebirge and Erzgebirge are more elevated. However, the geochemical traits of the regions might also contribute to the longitudinal acidity gradient. In spite of the fact that the bedrock is siliceous all-around, the buffering capacities differ between the types of rock (cf. table 1) and soils, and therefore between the regions. This has been discussed also for other regions (Probst et al. 1999; Williard et al. 2005). Hence, a concluding interpretation regarding the stress situation of the catchments is not possible, when taking only the spring waters into account. Further catchment traits influencing acidifying processes - such as bedrock, climatic parameters, and forest vegetation - have to be included in the analyses.

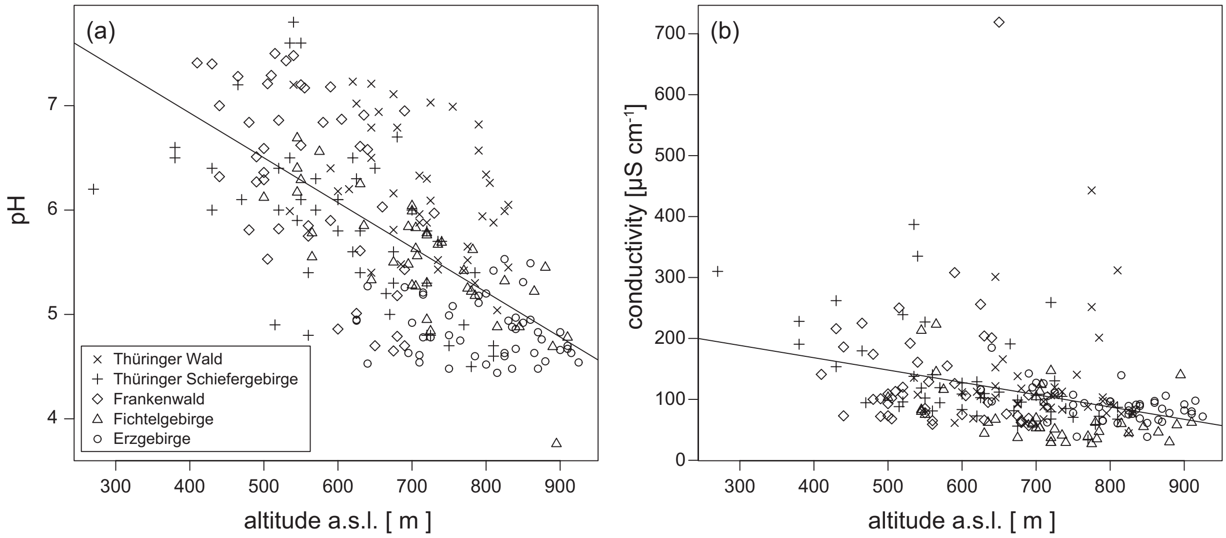

Figure 6: Altitudinal gradients of pH-value and electrical conductivity, fitted by a Pearson linear correlation. (a) pH-value ($r^2 = 0.416$, $p < 0.001$), and (b) electrical conductivity ($r^2 = 0.114$, $p < 0.001$).

Within each of the five regions differences between the individual springs with the respect to the acidity gradient appeared. The springs in the Thüringer Wald (all pH-values above 5.00) and in the Erzgebirge (all pH-values below 5.55) covered narrower ranges of acidity. In contrast, the variability within single regions was most pronounced in the three central regions, the Thüringer Schiefergebirge, the Frankenwald and the Fichtelgebirge, where a broader range of pH-values was encountered. There, highly acidic springs were substantially linked to higher altitudes. As historical mining activities may also blur catchment traits and hydrochemical properties of headwaters (Peiffer et al. 1997) such areas were avoided. However, the slope and the orientation of the catchment (Palmer et al. 2004; Rantakari et al. 2004) as well as the surface roughness of vegetation (e.g. deciduous or coniferous forest; Williard et al. 2005) are able to alter the deposition load of the catchment and therefore the acidity of spring waters. Those factors were not included in our investigation.

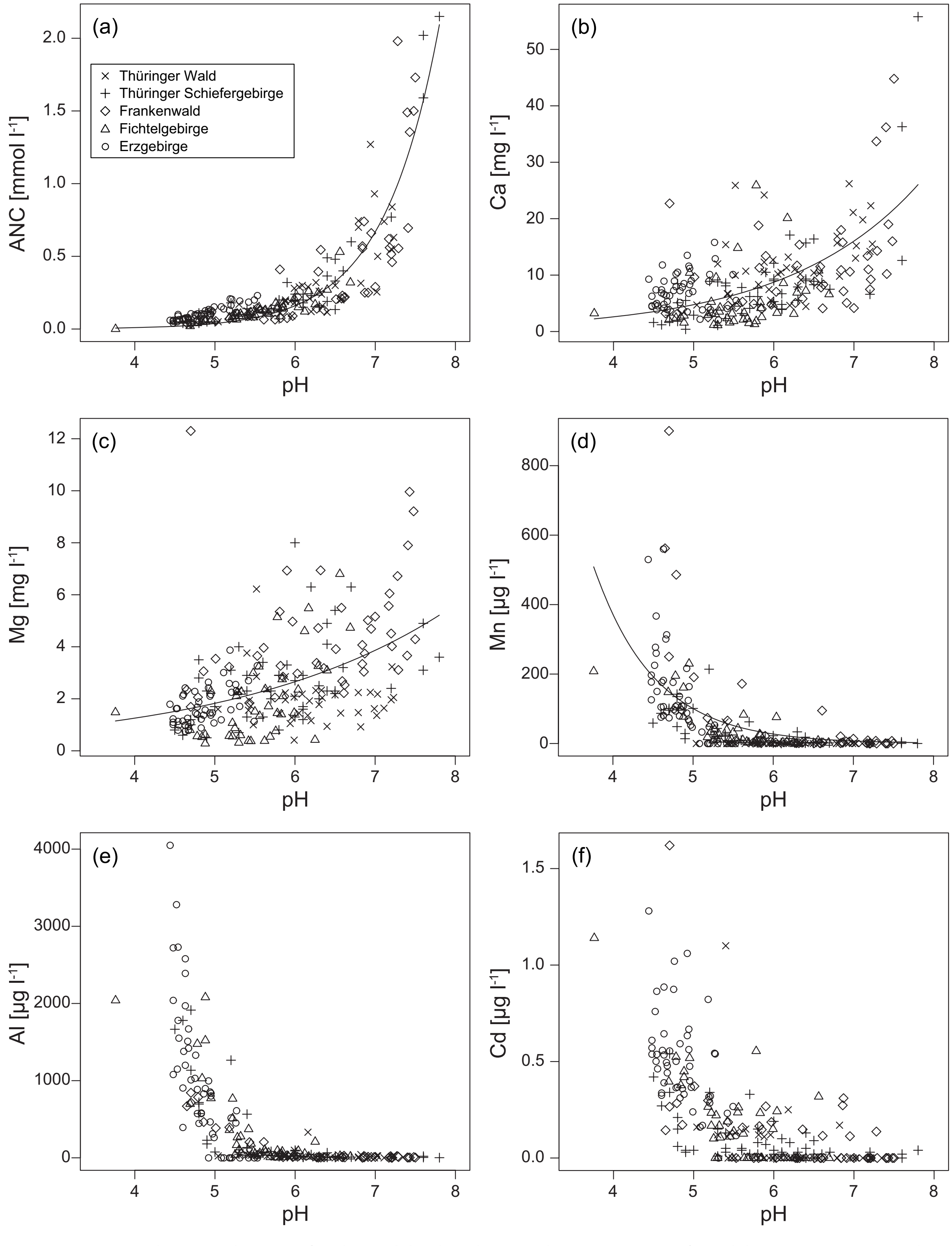

Figure 7: Acidity gradients of selected hydrochemical parameters, fitted by an exponential regression. (a) Acid neutralising capacity ($r^2 = 0.8071$, $p < 0.0001$), (b) Calcium ($r^2 = 0.3535$, $p < 0.0001$), (c) Magnesium ($r^2 = 2171$, $p < 0.0001$), (d) Manganese ($r^2 = 0.3907$, $p < 0.0001$), (e) Aluminium (not significant), and (f) Cadmium (not significant).

In order to appraise the processes of acidification and recovery in springs and their catchments, or just the acidity status of them, it is not sufficient to take just the pH-values into account. Even to quantitate the acidifying compounds $NO_3^-$and SO_4^{2-} itself in the spring waters is less meaningful (cf. table 4). Indeed, the concentrations of elements, which are involved in the buffering systems in soil and groundwaters can hint at the ability of ecosystems to counterbalance acidic depositions. Acidified spring waters are characterised by high concentrations of Al, Cd and Mn. Circumneutral waters, which are a matter of carbonate and silicate buffering, incorporate high concentrations of Ca and Mg (table 4, figure 7). These hydrochemical interrelationships apply to between-region comparisons as well as to within-region comparisons.

As a consequence of the physically dense bedrock in the siliceous mountain ranges of Central Europe, groundwater flow mainly drains close to the surface as so called interflow. This fact is affirmed by the high abundance of springs, as well as by their chemism and their discharge performance. There, spring water quality is very closely linked to the status and processes of the catchments. Thus, springs can act as suitable indicator systems for the qualitative situation of their forested catchments in this area (Beierkuhnlein & Durka 1993). However, to quantify export budgets would require to delineate the catchments exactly and to measure amount and quality of spring water discharge continuously (e.g. Lischeid et al. 1998), what is certainly not feasible for the biomonitoring at the landscape scale.

Driving factors of the vegetation of springs

The hydrochemical subset of the environmental parameters was found to determine the plant community composition principally. Hydrophysical and spatial parameters were less important. A relatively high proportion of variance in the pCCA could be explained by combinations of two or of all three environmental subsets. This is plausible as environmental parameters belonging to different subsets were shown to be correlated. In particular the altitudinal dependencies of the pH-value and of the element concentrations of Al, Ca, and Mg (which are involved in buffering processes) connect the spatial subset to the hydrochemical subset of the environmental parameters.

When concentrating on the hydrochemical parameters, the CCA revealed an acidity gradient as main driving factor of the species composition of springs. But actually, the acidity does not influence the occurrence of species in a direct way. It rather affects the availability of nutrients such as Ca and Mg and the presence of potentially toxic elements such as Al, Cd, and Mn. During buffering processes Al, Cd, and Mn are released at pH-values < 4.2. The nutrients Mg and Ca are mainly released at pH-values between 6.2 and 8.6. This indicates that the species occurrence and therefore the plant community composition are responding indirectly to acidity. However, the pH-value can serve as powerful proxy to represent this gradient. Our findings are in accordance with studies that found the vegetation composition of springs and spring fens to be fundamentally determined by water chemistry, first and foremost by a gradient of acidity and nutrient availability (Beierkuhnlein and Gräsle 1998; Wheeler & Proctor 2000; Hájek et al. 2002; Hájková et al. 2008).

Apart from this gradient also other environmental factors might influence the species composition. Beierkuhnlein & Gräsle (1998) detected that light supply, which in turn reflects the shade provided by the surrounding forest stands, modulates the vegetation composition of springs, albeit of minor importance than acidity. Spatial factors, such as the extent of the spring habitats or the distance between them, can affect seed dispersal and consequently species occurrence and establishment in the island-like spring habitats. However, in our study these restrictions are less notable, because the helocrenic habitats were selected following clear criteria. Fens and mires with larger spatial extension were avoided just like point source rheocrenic springs where the physical conditions of spring brooks were prevalent right from the beginning.

Conclusion

For helocrenic springs in Central European mountain ranges the hydrochemical factors have been found to be the fundamental drivers of plant community composition. Spatial factors, first and foremost the altitude, are related closely to the hydrochemical factors, but have been found to be less important. Hydrophysical factors play a marginal role. In particular, species occurrence is affected by the gradient of acidity and nutrient availability. This gradient is characterised by higher Ca and Mg concentrations in circumneutral spring waters, whereas Al, Cd, and Mn are found higher concentrated in acidic spring waters. The pH-value represents this gradient of acidity as a proxy measure.

In general, springs can be considered to be low dynamic ecosystems, particularly if they are located in forested catchments. This allows organisms to adapt to habitat conditions over a relatively long time and to develop a steady state in community composition. On the other hand, external factors like disturbances or human land-use are negligible. This provides a good basis for the biomonitoring of the acidity status of spring waters. Spatial patterns in the vegetation of springs emerge on the landscape scale and provide valuable ecological information about spring water quality and in return about the acidity status of their forested catchments.

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Manuscript 2

Drivers of species composition in siliceous spring ecosystems: groundwater chemistry, catchment traits or spatial factors?

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Drivers of species composition in siliceous spring ecosystems: groundwater chemistry, catchment traits or spatial factors?

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ABSTRACT

Spring water reflects the hydrochemistry of the aquifer in the associated catchments. On dense siliceous bedrock, the near-surface groundwater flow is expected to be closely related to the biogeochemical processes of forest ecosystems, where the impact of land use is also low. We hypothesize that the plant species composition of springs mainly reflects hydrochemical conditions. Therefore, springs may serve as indicator systems for biogeochemical processes in complex forest ecosystems.

To test this hypothesis, we investigate the influence of spring water chemical properties, catchment traits, and spatial position on plant species composition for 73 springs in forested catchments in central Germany, using non-metric multidimensional scaling (NMDS), Mantel tests, and path analyses.

Partial Mantel tests and path analyses reveal that vegetation is more greatly influenced by spring water chemistry than by catchment traits. Consequently, the catchment's influence on vegetation is effective in an indirect way, via spring water. When considering spatial aspects (in particular altitude) in addition, the explanatory power of catchment traits for spring water properties is reduced almost to zero. As vegetation shows the highest correlation with the acidity gradient, we assume that altitude acts as a sum parameter that incorporates various acidifying processes in the catchment. These processes are particularly related to altitude – through bedrock, climatic parameters and forest vegetation. The species composition of undisturbed springs is very sensitive in reflecting such conditions and may serve as an integrative tool for detecting complex ecological processes.

KEYWORDS

acidification, catchment biogeochemistry, landscape traits, partial Mantel test, path analysis, spring water

1. INTRODUCTION

Springs are a habitat type that is remarkably different from other habitat types. Odum (1971) summarized this fact when he wrote: "*Springs are the aquatic ecologist's natural constant temperature laboratory. Because of the relative constancy of the chemical composition, velocity of water, and temperature, in comparison with lakes, rivers, marine environments, and terrestrial communities, springs hold a position of importance as study areas that is out of proportion to their size and number*".

Most ecosystems of temperate Central Europe are strongly influenced, controlled or even determined by direct human impact such as intensive land use and frequent disturbances related to mobility and urbanization. Communities with low turnover rates that are dominated by natural drivers of ecosystem functioning are rarely found.

The subject of this study, springs in forest areas, represents an exception to the predominantly anthropogenic ecosystems of Europe. Land use options are negligible as a consequence of the permanently saturated moisture regime. In addition, human interest is low due to the small extent of spring sites. Warncke (1980) refers to them as islands in an ocean of cultivated land. In forested catchments in particular, they can be regarded as low dynamic semi-terrestrial ecosystems that reflect long-term ecological processes and the biogeochemical background of landscape compartments. On dense siliceous bedrock, water is transported mainly as interflow close to the surface in Pleistocene layers of solifluction. In these landscapes, springs generally develop as helocrenic springs – a saturated mire of small extent (with a mean of approx. 50 to 100 m^2) where water seeps out more or less continuously from the ground. Discharge rates are usually low (< 2.0 $l\ s^{-1}$). Depending on the climate and the size of the catchment area, seasonal fluctuations are reflected by a certain variability of discharge. Water temperatures, and therefore also surface-near air temperatures, are equilibrated in these springs by the permanent energy supply by groundwater. Due to their ecological constancy (as addressed by Odum 1971), springs provide a habitat for stenoecious species that cannot be found in the surrounding ecosystems. Despite their small size Heino et al. (2005) regard them as “key biotopes” because springs enhance the biodiversity of the terrestrial landscape.

The following aspects of Central European siliceous, helocrenic forest springs, make them an interesting subject of research: (1) They are small in size but the water quantity and quality reflects hydrological and biogeochemical processes of a much larger catchment area (Beierkuhnlein & Durka 1993). Springs act as a point-shaped outlet of complex, black-box-like ecosystems. (2) The area of seeping spring water is clearly differentiated from the surrounding habitats by surface morphology. This well-defined boundary is a consequence of frost heaving of substrate and vegetation during freeze-thawing cycles in winter and makes it easy to assess whether species are dwelling in the spring itself or the neighbouring terrestrial habitats. Their clear delimitation renders springs appropriate tools to measure biogeochemical fluxes. (3) Organisms living in the springs are strongly related to the quality and quantity of outpouring groundwater. Thus, they are adapted to relative environmental constancy (Thienemann 1924; Odum 1971), which qualifies them as bio-indicators of overall and mean environmental properties (Cantonati et al. 2006). (4) In spite of the fact that springs make up only a small proportion of the land surface, they are rather abundant in the vast forested mountain ranges of Central Europe. This high frequency is a prerequisite for investigations at the landscape scale because it provides data with a high spatial resolution (Beierkuhnlein & Durka 1993; Beierkuhnlein 1996).

Spring water reflects the properties of the percolated volume that makes up their catchment and of the processes that occur there, or as Church (1997) puts it: “*Where water goes in a forested catchment, how long it lingers, what materials and organisms it encounters on its journey - all interact to determine its character*”. The *“small catchment approach”* (e.g. Moldan & Černý 1994; Church 1997; Likens & Bormann 1999) applies to siliceous springs.

Over recent decades acid deposition has been a severe environmental problem in forested catchments of the northern hemisphere, accompanied by damage to and the decline of forest vegetation as well as by the alteration of soils, ground-, spring- and headwaters. Even though acid depositions in Central Europe have gone down considerably since the early 1990s, the recovery of catchments seems to be delayed (Alewell et al. 2001). Acidification remains an important environmental issue. The link between acid depositions and ecosystem damage is modified by the buffering capacity of the soils in the catchment (Probst et al. 1999; Williard et al. 2005), but also dependent on factors that alter the deposition load of the catchment like the surface roughness of vegetation (e.g. deciduous or coniferous forest) (Williard et al. 2005) or the altitude, slope and orientation of the catchment (Palmer et al.

2004; Rantakari et al. 2004).

In contrast with other communities, the species composition of springs and fens is congeneric over wide areas of the northern hemisphere. Species occurrence and composition are assumed to be controlled substantially by the gradients of nutrient occurrence and nutrient availability (i.e. acidity) (Vitt & Chee 1990; Hájek et al. 2002; Nekola 2004; Tahvanainen 2004). In the springs of Central Europe, the water pH-value has been shown to be the most important explanatory variable (Beierkuhnlein & Gräsle 1998; Hájková et al. 2008). Hence, crenobiotic plant species have been proposed as indicators for monitoring acidification (Beierkuhnlein 1996). In order to take a closer look at the indicative power of the vegetation of springs, we studied how the three compartments - catchment properties, spring water chemistry and vegetation - are interrelated. We hypothesize that plant species composition mainly reflects hydrochemical conditions. Additionally we tested the datasets for spatial dependencies. The objective of this study was to reveal paths and the magnitude of the impact of the catchment's traits on plant species composition in springs.

2. METHODS

2.1 Sites and study area

We investigated springs and their catchments, located in the Frankenwald and Fichtelgebirge mountain ranges, in the center of Germany (49.9° - 50.6° N, 11.2° - 12.2° E; see Fig. 1). These landscapes cover an area of roughly 900 km^2 each, ranging in altitude from 350 to 1050 m a.s.l. Bedrock material consists of palaeozoic silicate rocks - schist, phyllite, greywacke, gneiss and granite. Long-term mean annual air temperatures vary between 5 and 7 °C with annual precipitation between 750 and 1200 mm. All investigated springs (n = 73) and their catchments are located in state-owned forests that are dominated by Norway spruce (*Picea abies* (L.) H. Karst.). The altitude of the spring sites ranges from 388 to 909 m a.s.l., and their catchment area varies between 800 and 200 000 m^2. The pH-value of the nutrient-poor spring water is acidic to neutral (annual mean between 3.86 and 7.33), and electrical conductivity ranges from 26.9 to 892.1 µS cm^{-1} (annual mean).

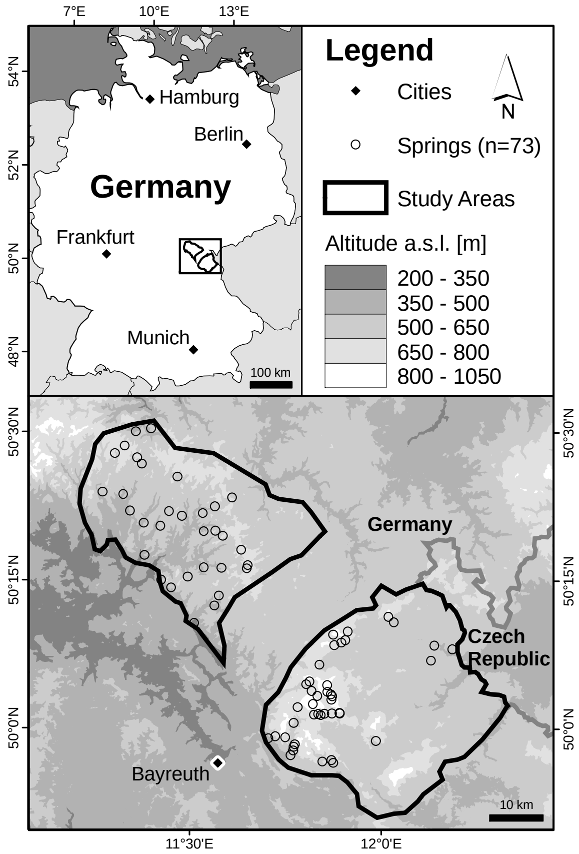

Fig. 1. **Location of the 73 springs investigated in Central Germany.**

2.2 Data collection

The abundance/dominance score of each vascular plant, moss and liverwort species growing in the well-differentiated saturated area of the helocrenic spring was estimated once in the summer of 2003. In total 220 plant species were recorded with an average of 31 species per spring (minimum 10, maximum 69).

Spring water was sampled 10 times from September 2003 through to August 2004 at regular intervals, and always at the uppermost point of the saturated area with aboveground flowing water. *Electrical conductivity*, *pH-value* and *acid neutralization capacity* were measured in situ. For every recording water samples were taken and analyzed for the dissolved concentrations of *DOC*, NO_3^-, SO_4^{2-}, *Ca, Mg, K, Fe, Mg, Al, Zn, Cu, Ni, Cd, Na, Cl, dissolved N, dissolved S* and *Si.* We included the annual mean, maximum and minimum of all measured parameters. As it can be expected that the extreme values of e.g. pH-value or Al^{3+} might not be tolerated by some species, the extreme values could be more important for the formation of plant communities in springs than the average values. The extreme values and the mean values led to 63 variables derived from 21 spring water parameters.

A digital elevation model (DEM) of the area was created by interpolation of the vectorized contour lines of the digital topographic map (1 : 25 000) using the ESRI *ArcGIS 3D Analyst* and the *Nearest Neighbor* algorithm. Based on the DEM, the catchment for each given pour point was calculated (using ESRI *ArcGIS Spatial Analyst 8.2*). At the landscape scale springs appear as a point. To cope with spatial inexactness in location and extension, a buffer was applied around each pour point. In order to delineate the catchments for all springs in one step, an estimation of the best buffer radius had to be made. For a number of buffer sizes, the catchment was delineated to calculate the catchment areas. The latter, multiplied with the average winter precipitation (December to February, season with the least evapotranspiration), resulted in a parameter that equates to the *potential winter spring water discharge*. Finally, a buffer with a 12 m radius was selected, for which the correlation between measured winter spring water discharge and potential winter spring water discharge was highest.

Subsequently landscape traits were sampled using GIS (ESRI *ArcGiS 8.2*) by intersecting different information layers with the catchment areas. Additionally the tree cover above the spring was included in this set of data to represent light availability. For practical reasons and to make a clear distinction from the water chemistry (obtained from a point measurement in the spring), this information based on the catchment area (cf. Tab. 1) will simply be referred to as catchment data in the following. The 35 highly collinear climatic parameters that had been included were condensed, using a principal components analysis. The first three principal components, representing 55, 10 and 7 % of the total variance of the climate parameters, were extracted and used as surrogate climatic parameters. The first principal component showed a high positive loading of all temperature parameters, and a negative loading of precipitation and number of foggy days. The radiation budget in the spring and summer displayed a negative loading and wind speed displayed a positive loading on the second principal component, while the main factor that loads the third principal component is radiation in October. The principal components analysis was calculated using the function *prcomp* from the *stats* package in *R* (R Development Core Team 2007). In total the catchment data set consists of 52 parameters, which are presented in Tab. 1.

Tab. 1. **An overview of data sources, parameters, data types and units of catchment data.**

Data	Source	Parameter description	Data type and unit	Number
Forest management maps scale 1 : 10 000	Bavarian State Forest Administration	deciduous trees in forest stand	proportion of catchment area covered by deciduous trees [%]	1
		4 stand age classes	proportion of catchment area covered by class [%]	4
		6 usage classes	proportion of catchment area covered by class [%]	6
Forest fertilization maps scale 1 : 10 000	Bavarian State Forest Administration	liming location and year (3 periods: years 1980-1988; 1989-1996; 1997-2003)	proportion of catchment area limed in time span [%]	3
Forest habitat / soil maps scale 1 : 10 000	Bavarian State Forest Administration	5 substrate classes	proportion of catchment area covered by class [%]	5
		7 fertility classes	proportion of catchment area covered by class [%]	7
		8 water accessibility classes	proportion of catchment area covered by class [%]	8
Climate maps (years 1961–1990) resolution 400 m x 400 m	Bavarian Climate Research Council	first 3 principal components of 35 climate parameters (temperature, precipitation, solar radiation, wind, fog and phenology) that counts for the majority of the catchment	artificial parameters: principal components (axis scores) [unitless]	3
Geological maps scale 1 : 25 000	Bavarian Environment Agency	53 geological units (classified into 6 groups resembling mineral content)	proportion of catchment area covered by group [%]	6
Topographic maps scale 1 : 25 000	Bavarian Office for Surveying and Geographic Information	flow length to spring (from contour lines via DEM)	flow length [m]	1
		hill slope (from contour lines via DEM)	hill slope [degree]	1
		orientation (from contour lines via DEM)	proportion of catchment area covered by direction class (N, E, S, W) [%]	4
		road in < 100 m vicinity of catchment	presence / absence [unitless]	1
		catchment size (from DEM)	area [m^2]	1
Shading	own vegetation relevès	tree cover	proportion of spring area covered by trees [%]	1
			Total	**52**

2.3 Data transformation

Vegetation data were homogenized by removing the abundances of all tree species (only juvenile trees were found, which can't survive in the saturated areas and are therefore not representative) and by omitting the abundances of species, which were found only in one single spring. Braun-Blanquet's abundance/dominance scores of the 126 remaining species were converted into a percentage cover. To down-weight species with high abundances, cover data were square-root transformed. Legendre & Gallagher (2001) recommend up-weighting rare species when comparing communities to environmental data. Therefore the vegetation data was additionally submitted to *wisconsin double standardization* – whereby species are standardized to maximum cover and samples to equal totals.

Water chemistry parameters were homogenized in order to resemble the pH-value. At first the element and compound concentrations below the Limit of Quantitation (LOQ) were substituted by $0.5 \cdot \mathrm{LOQ}$. Then the common logarithm was calculated for all parameters except for the *pH-value* which is the negative common logarithm of the hydronium concentration, but also excluding the *acid neutralization capacity* which includes zero values. Instead, a square root transformation was chosen for the latter parameter.

2.4 Data analysis

The sampling process resulted in three different sets of data. Fig. 2 shows the presumed model of the relationships between the variables. To compare the data, Mantel tests were carried out (Mantel 1967). These enable community data and their environment to be compared (Legendre & Legendre 1998). Clarke & Ainsworth (1993) proposed iterative Mantel tests to detect the subset of environmental parameters, which is best correlated with community data. The dissimilarity matrix of the plant community is correlated with the matrices that are obtained from all of the possible subsets of the environmental data and the subset with the best correlation is identified.

Unfortunately, this method is still limited by computational power, with *n* parameters leading to $2^{(n-1)}$ possibilities and 63 water chemistry parameters leading to $4.6 \cdot 10^{18}$ possibilities. Therefore a pre-selection of variables was carried out with the help of non-metric multidimensional scaling (NMDS). A two dimensional NMDS of the community data was calculated by means of the function *metaMDS* that is incorporated in the statistical package *vegan* in *R* (Oksanen et al. 2007).

The NMDS is based on a similarity matrix calculated from the vegetation data using the Bray-Curtis index. This similarity matrix will simply be referred to as ***vegetation matrix*** below. The environmental vectors with the maximum correlation with corresponding environmental variables were fitted on the NMDS ordination by the function *envfit* from the same package. These vectors indicate the direction of the ordination in which the variable changes most rapidly. On pre-selection only those variables with a significant correlation ($p < 0.1$) were accepted for further analysis, with significance being calculated in *envfit* using 1000 permutations. Out of the minimum, maximum and mean of each hydrochemical parameter only the one with the highest correlation was considered. In total 15 hydrochemical parameters (Tab. 2) and 20 catchment parameters were preselected (Tab. 3).

Tab. 2. Preselected chemical parameters of the spring water. Preselection was carried out using the significance ($p < 0.1$) of environmental vectors fitted to an NMDS ordination of the vegetation data. Those 3 parameters that constitute the *spring water matrix* are printed in bold characters.

Parameter	r^2	p
pH $_{avg}$	**0.719**	**< 0.001**
Al $_{avg}$	**0.663**	**< 0.001**
Mg $_{avg}$	**0.599**	**< 0.001**
Mn $_{min}$	0.535	< 0.001
ANC $_{avg}$	0.511	< 0.001
Ca $_{max}$	0.481	< 0.001
Cd $_{avg}$	0.312	< 0.001
DOC $_{avg}$	0.199	< 0.001
Zn $_{min}$	0.162	0.003
$NO_{3\ min}$	0.148	0.005
$SO_{4\ min}$	0.131	0.003
Fe $_{min}$	0.115	0.008
Ni $_{avg}$	0.111	0.009
Na $_{max}$	0.090	0.038
Cl $_{max}$	0.088	0.037

Based on these preselected variables iterative Mantel tests were calculated using the function *bioenv*, that is also included in the statistical package *vegan* in *R* (Oksanen et al. 2007), with the vegetation matrix, Euclidian distance matrices of the spring water and catchment data and Pearson's coefficient as a correlation measure. Finally, partial Mantel tests were calculated using the statistical package *ecodist* in *R* (Goslee & Urban 2007) and path analyses were carried out. Partial Mantel tests enable the correlation of two dissimilarity matrices while controlling for the effect of others.

Path analysis works in a comparable way: path coefficients, which resemble standard partial regression coefficients, are calculated. Both methods give an insight into (statistical) relationships between collinear variables, or in our case collinear distance matrices. The application of path analyses on Mantel regression coefficients rather than regression coefficients was carried out according to Nantel & Neumann (1992).

Tab. 3. **Preselected catchment parameters. Preselection was carried out using the significance ($p < 0.1$) of environmental vectors fitted to an NMDS ordination of the vegetation data. The 9 parameters that constitute the catchment matrix are printed in bold characters.**

Parameter	r^2	p
Climate PRC1	**0.650**	**< 0.001**
Bedrock 6 (granite)	**0.404**	**< 0.001**
Soil type 2 (loamy sand)	**0.331**	**< 0.001**
Soil type 3 (loam)	0.295	< 0.001
Bedrock 2 (slate)	**0.271**	**< 0.001**
Soil water 1 (dry)	**0.171**	**0.001**
Soil water 8 (wet)	0.158	0.003
Soil water 5 (moist)	0.150	0.003
Cover of deciduous trees	**0.140**	**0.006**
Soil fert. 6 (soil on slope)	0.140	0.005
Soil type 1 (sand)	0.137	0.006
Bedrock 5 (phyllite)	0.135	0.005
Tree cover	**0.129**	**0.006**
Avg. slope of catchment	**0.123**	**0.011**
Catchment facing west	0.113	0.013
Soil fert. 4 (rich in humus)	0.111	0.004
Soil fert. 2 (poor)	0.097	0.036
Climate PRC2	0.094	0.037
Eastward orientation	**0.091**	**0.037**
Soil type 5 (bog and fen)	0.086	0.046

The results of the partial Mantel test pointed to an unmeasured parameter connected to catchment traits and spring water. That is why spatial parameters were included in the analysis. Space itself is not directly linked to ecological processes, but it can be used to represent factors and processes that are modified in space (Leduc et al. 1992). Horizontal and vertical geographical positions were analyzed respectively as additional parameters in the described Mantel tests, partial Mantel tests and path analyses (using Euclidian distance as the distance measure).

3. RESULTS

3.1 Interrelation of catchment, spring water and species composition

The highest correlation between the vegetation matrix and the catchment traits was found by the Mantel tests for a Euclidian distance matrix calculated using 9 parameters: *1st principal component of the climate parameters, proportional area of deciduous trees,* two *bedrock* parameters *(weak metamorphous bedrock* and *granite),* two *soil* parameters *(loamy sand* and *dry soil), tree cover, slope and eastward orientation*. This distance matrix will be referred to as the ***catchment matrix*** below.

The analogously highest correlation with the vegetation matrix was found for the Euclidian distance matrix containing the three spring water parameters: *average pH-value*, *average Mg* and *average Al*. This distance matrix will be referred to as ***spring water matrix*** in the following.

The results of the Mantel tests between the vegetation matrix, spring water matrix and catchment matrix are shown in Tab. 4. The vegetation matrix was both significantly correlated ($p < 0.001$) with the spring water matrix ($r_M = 0.54$) and the catchment matrix ($r_M = 0.47$). The spring water matrix and the catchment matrix were also correlated ($r_M = 0.41$, all $p < 0.001$). This shows that the spring water and the catchments' characteristics could explain similar quantities of the variation in the vegetation data, but as they are also correlated with each other, no conclusion about their combined explanatory power could be drawn.

Partial Mantel tests decreased all correlations. This indicates that to a certain degree the correlation between the vegetation matrix and the catchment matrix as well as the correlation between the vegetation matrix and the spring water matrix was due to a spurious correlation. There was however no explanation for the strong decline in the correlation between the spring water matrix and the catchment matrix when controlling for the effect of the vegetation matrix ($r_M = 0.41$ to $r_{pM} = 0.21$). It is likely that an unmeasured parameter connects the two.

Tab. 4. **Results (Pearson correlation) of Mantel tests (r_M , above diagonal) and partial Mantel tests (r_{pM} , below diagonal) between vegetation (VEG), spring water chemistry (H2O) and catchment traits (CATCH). All correlations are significant ($p < 0.001$).**

	VEG	H2O	CATCH
	Mantel test r_M =		
VEG		0.54	0.47
H2O	0.43		0.41
CATCH	0.32	0.21	
	partial Mantel test r_{pM} =		

The path analysis for the model shown in Fig. 2, with the catchment influencing both vegetation (path p_1) and water chemistry (path p_2) and the water chemistry influencing vegetation (path p_3), fortify the result of the partial Mantel tests. The resulting path coefficients were $p_1 = 0.30$, $p_2 = 0.41$ and $p_3 = 0.41$ (cf. Fig. 2). The total correlation between the catchment matrix and the vegetation matrix was $r_{tot} = 0.47$. 36 % of this correlation was indirect, which means that the catchment attributes influence the vegetation by altering the spring water. The vegetation of the springs was more greatly influenced by the water chemistry than by the catchment parameters ($p_3 > p_1$). 23 % of the correlation between the vegetation matrix and the spring water matrix was due to a spurious correlation. 64 % of the variation in the vegetation matrix was not explained by the model.

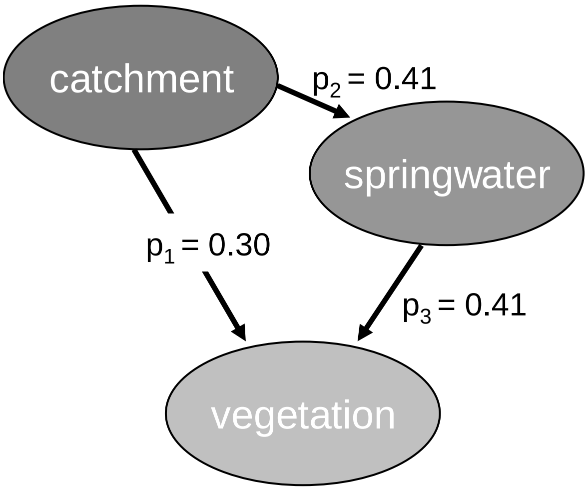

Fig. 2. **Presumed relationships between the vegetation of springs, the spring water and the catchment. The path coefficients were calculated in a path analysis between the distance matrices of the three parameter sets.**

3.2 Influence of geographical position

The correlation between the vegetation matrix and the horizontal distance was weak ($r_M = 0.14$) and it ceased ($r_{pM} = -0.02$) when it was controlled for the catchment and spring water effects with partial Mantel tests (Tab. 5). Other correlations with the horizontal distance were also weak in the Mantel tests and were weakened even further in the partial Mantel tests. The partial correlation between vegetation, spring water and catchment matrices remained - albeit lower - similar to the correlation without horizontal distance. Hence, horizontal geographical distance contained either no or little information on missing spatial variables.

Tab. 5. **Results (Pearson correlation) of Mantel tests (r_M, above diagonal) and partial Mantel tests (r_{pM}, below diagonal) between vegetation (VEG), spring water chemistry (H2O), catchment traits (CATCH) and horizontal geographical distance (hDIST). All correlations are significant ($p < 0.001$).**

	VEG	H2O	CATCH	hDIST
		Mantel test r_M =		
VEG		0.54	0.47	0.14
H2O	0.43		0.41	0.22
CATCH	0.32	0.19		0.22
hDIST	-0.02	0.15	0.14	
		partial Mantel test r_{pM} =		

Tab. 6. **Results (Pearson correlation) of Mantel tests (r_M, above diagonal) and partial Mantel tests (r_{pM}, below diagonal) between vegetation (VEG), spring water chemistry (H2O), catchment traits (CATCH) and vertical geographical distance (vDIST). All correlations are significant ($p < 0.001$).**

	VEG	H2O	CATCH	vDIST
		Mantel test r_M =		
VEG		0.54	0.49	0.50
H2O	0.32		0.41	0.62
CATCH	0.26	0.05		0.50
vDIST	0.14	0.45	0.30	
		partial Mantel test r_{pM} =		

In contrast, there were strong correlations between vegetation ($r_M = 0.50$), spring water ($r_M = 0.62$) and catchment ($r_M = 0.50$) matrices and the vertical geographical distance (Tab. 6). When controlled for the effects of the other variables, the correlation between the vertical distance and the water chemistry remained high ($r_{pM} = 0.45$,) while the partial Mantel correlation between the catchment parameters and the water chemistry decreased from $r_M = 0.41$ to $r_{pM} = 0.05$. Obviously, the vertical geographical distance, or intelligibly the altitude of the spring, represented unmeasured parameters that have a strong influence particularly on water chemistry, while the correlation between the catchment matrix and the spring water matrix was to a large extent a spurious correlation.

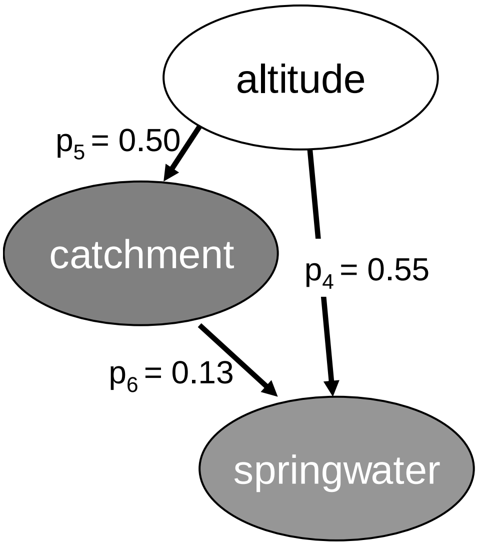

Fig. 3. **Presumed relationships between the altitude, the catchment and the spring water. Altitude is used as a representative for spatial factors and processes. The path coefficients were calculated in a path analysis between the distance matrices of the three parameter sets.**

The strong influence of the altitude on spring water chemistry was confirmed by a path analysis for the model sketched in Fig. 3, where the vertical geographical distance had an influence on the spring water (path p_4) and on the catchment (path p_5) and the catchment also had an influence on the spring water (path p_6). The respective path coefficients are $p_4 = 0.55$, $p_5 = 0.50$ and $p_6 = 0.13$. The total correlation between the vertical distance and the spring water matrix was $r_{tot} = 0.62$. Only 11 % of this correlation was indirect via the catchment matrix. 68 % of the correlation between the vegetation matrix and the spring water matrix was due to a spurious correlation. 61 % of the variation in the vegetation matrix could not be explained by the model.

4. DISCUSSION

4.1 Spring water as a determinant of species composition

Mantel tests (Tab. 4) and path analysis (Fig. 2) revealed that plant community composition was best explained by the hydrochemical parameters of the spring waters, and first and foremost by the average values of *pH*, *Al* and *Mg*, which determine the distance matrix with the highest correlation. Within the preselected hydrochemical parameters, all those, which were found to be highly significant by the NMDS ($p < 0.001$; see Tab. 2), were closely related to the acidity gradient. This confirms results of previous studies (Beierkuhnlein 1996; Hájková et al. 2008). High concentrations of *Al*, accompanied by *Mn*, *Cd* and a low *acid neutralizing capacity*, mark the acidic end of this gradient (lowest annual mean pH-value was 3.86), whereas concentrations of *Mg*, accompanied by *Ca*, are linked to near-neutral spring waters (highest annual mean pH-value was 7.33). Acidity, expressed in particular by the *pH-value*, is influenced by the acidic depositions and the buffering processes in soils and the groundwater of the catchments. Low pH-values correspond to high deposition rates and a low buffering capacity. The latter is the case in the investigation area particularly on granite. Toxic Al^{3+} is released at pH-values < 4.2. The nutrients *Mg* and *Ca* are mainly released from the soil at pH-values between 6.2 and 8.6.

It was expected, that out of the minimum, mean and maximum concentrations (over 10 samples from one year) the maximum values of the phytotoxic metals *Al*, *Mn* and *Cd* would be crucial, as well as on the other hand the minimum values of the nutrients *Mg* and *Ca* would be limiting. But surprisingly, in most cases the average values were found to be more significant. This suggests that (1) vegetation is quite resilient and responds to the year-round hydrochemical characteristics of the spring water, rather than to short-term extreme conditions; and (2) that plant community composition as a whole is not restricted by the captured acidity range margins of the investigated springs.

4.2 The role of the catchment

Catchment traits play a role in explaining the patterns of our data, but their interpretation is difficult. They did not affect vegetation composition directly, but indirectly via spring water chemistry (Fig. 2). Out of nine catchment parameters that constituted the distance matrix with the highest correlation four are related to the micro- and meso-climate: *1st principal component of the climate parameters, slope, eastward orientation* and *tree cover*. *Slope and tree cover* as well as the *proportional area of deciduous trees* can be attributed to light availability as more light reaches spring sites on steeper slopes and in open areas.

Springs located under deciduous trees are subject to a strong seasonality of light availability. Trophic conditions are reflected in the *bedrock* and *soil* parameters. Finally, most parameters incorporated in the catchment matrix can be construed in terms of acidity. *Bedrock* and *soil* parameters reflect the buffering capacity of the catchment. The *slope* is connected to the residence time of water flowing through the catchment, having an effect on the time available for buffering processes. The factors *1st principal component of the climate parameters, slope, eastward orientation* and *proportional area of deciduous trees* determine the deposition of acidifying compounds. Summing up, catchment traits characterize a complex, interconnected system that is difficult to unravel with field studies.

Surprisingly, the explanatory power of the delineated catchment parameters for the spring water chemistry almost disappeared, when taking spatial relations into account. The vertical geographical distance, solely calculated from the altitude a.s.l., could explain the spring water matrix better (Fig. 3, Tab. 6) than the entirety of catchment parameters. In contrast, integrating horizontal geographical distance into the calculations as a wild card for non-measured landscape characteristics revealed little evidence of the missing variables.

There are four potential ways to interpret the results, differing in their evidentiary value - which will be discussed in the following. (**1**) There is no relationship between catchment parameters and the water chemistry that drives the vegetation of springs. (**2**) There is a relationship between catchment parameters and the water chemistry that drives the vegetation of springs, but the parameters that were derived did not cover them. (**3**) There is a relationship between catchment parameters and the water chemistry that drives the vegetation of springs, but they all depend on the altitude. Therefore altitude might represent biogeochemical processes in the catchment, acting as a sum parameter. (**4**) There is a relationship between catchment parameters and the water chemistry that drives the vegetation of springs, but acidification has superimposed most differences between the catchments.

Point (**1**) is highly unrealistic considering all the facts we know about the interrelations between catchment traits and headwater chemistry, both on the global and regional scale (e.g. Reuss et al. 1987; Beierkuhnlein & Durka 1993; Moldan & Černý 1994; Church 1997; Likens & Bormann 1999; Probst et al. 1999; Matzner 2004; Palmer et al. 2004; Björkvald et al. 2008). Furthermore, the results of our Mantel and partial Mantel tests (Tabs 4, 5 and 6) give clear evidence that catchment traits and spring water chemistry are interrelated.

Therefore, point (**2**) seems to be somewhat more realistic, especially if one takes into account possible errors, such as spatial uncertainties of the spring locations themselves and of the DEM, a difference between the aboveground delimitation and the belowground boundaries of the catchment and the fact that primarily almost all catchment data were not collected for our research objective (cf. Tab. 1). However, connections between catchment traits and spring water chemistry have been proven several times (Probst et al. 1999; Nebe & Abiy 2002). Nebe & Abiy related patterns in spring water chemistry to altitude, which leads us to point (**3**), as most relevant catchment traits investigated in our study are correlated with the altitude. The bedrock material found in the highest localities is usually granite, which influences soil formation and buffering capacity. Hence, bedrock material is directly related to the soil type. Furthermore, soil depth declines with increasing altitude. Likewise, climatic parameters are highly predetermined by altitude. In contrast to the air temperature the total amount of precipitation increases with altitude. Furthermore, the amount of precipitation falling as fog and snow is modified by altitude.

We assume that altitudinal differences in the air temperatures do not alter the vegetation growing in the saturated area of the springs, as crenobiotic species benefit from balanced spring water temperatures (Odum 1971; Ellenberg 1996). However, climatic conditions also account for the forest type in the catchments, and this includes both natural conditions as well as forestry. With altitude, the stand density becomes lower, whereas the proportion of conifers (mainly *Picea abies* (L.) H. Karst.) increases at the expense of deciduous trees (mainly *Fagus sylvatica* L.). Conifer litter is known to contribute to the acidification of soils and groundwater. As a matter of course, horizontal patterns of bedrock, climate (caused by slope and orientaion) and forest types occur. As our results show, these cannot be clearly attributed to the horizontal geographical position or to catchment traits, because they are superimposed by altitudinal issues.

Due to the fact that bedrock, climate parameters and forest vegetation – meaning all three main compartments influenced by altitude – are linked to the process of acidification, we presume that acidic depositions are likely to be the “unmeasured parameter” that connects catchment traits with spring water and makes up the correlation with altitude. This underpins point (**4**). The acidification of soils is a natural process in the studied area but it has been accelerated by anthropogenic emissions since the industrial revolution. Unfortunately, there is neither knowledge on spring water chemistry nor on the plant community for the area that dates back any longer than 20 years. Findings for the Vosges Mountains by Probst et al. (1999) have shown that the pH-value and the alkalinity of springs has declined since the 60s. Springs in poorly buffered catchments (siliceous sandstone and conglomerate) particularly started to become acidified in the 1970s while springs in catchments with a higher buffering capacity (clay enriched sandstone) became acidified in the 1980s and 1990s. Similar trends can be expected for our study area. To verify the impact of acidification on spring water and vegetation, it would be necessary to include the factor *depositions* in our analyses, but deposition data are only available for three locations in our study area and therefore there is a lack of spatially-detailed, catchment-specific information.

5. CONCLUSIONS

Our study supports the hypothesis that the plant species composition of the water saturated area of forest springs in siliceous landscapes is essentially determined by spring water chemistry, and more precisely by an acidity gradient that is reflected mainly by *pH-value*, *Al-* and *Mg*-concentrations. Catchment traits execute a minor influence on the vegetation of springs, but they are part of the functional chain. This chain is driven by the atmospheric depositions that had a strong impact on Central European forested catchments over recent decades and were most effective on siliceous bedrock with a poor buffering capacity. The acidification of ground and surface waters reflects this circumstance. Since the vegetation of springs represents the acidity status of the seeping groundwater, we propose using the plant species composition as an indicator system for spring water acidity. Indeed the spatial patterns that emerge from spring vegetation can provide valuable ecological information for both hydrologists and foresters.

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Manuscript 3

Is the delineation of niche attributes a matter of spatial scale?

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Is the delineation of niche attributes a matter of spatial scale?

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ABSTRACT

Aim: To determine the effects of different spatial sampling scales on the delineated characteristics of species' realised niches.

Location: 222 Helocrenic springs in siliceous mountain ranges in central Germany and the north west of the Czech Republic (49.9° – 50.8° N, 10.6° – 12.8° E).

Methods: The probability of bryophyte and vascular plant species occurrence with respect to pH was modelled, using generalized additive models (GAM). To compare species optima and amplitudes, these were derived separately for two spatial scales, by pursuing an entire-spring and a subplot-based within-spring approach.

Results: Species occurrence is driven by an acidity gradient, which is directly related to nutrient availability. The pH value best explains species composition, regardless of the scale of observation. The derivation of niche characteristics is not impartial to the sampling scale. Although the sampling scale did not systematically influence the species' pH optima, we found that a smaller plot size (grain) narrowed their pH amplitudes significantly.

Main conclusions: The delineation of attributes of species' realised niches is a matter of sampling scale, in particular for ecological amplitudes. This affects ecological applications, such as niche-based distribution models. Hence, the predictive capacity of such models are limited by the sampling scale (i.e. grain) that is chosen.

KEYWORDS

ecological amplitudes, generalized additive models GAM, niche modelling, response optima, realised niches, scale-dependence, species response curves, springs

INTRODUCTION

Modelling species-habitat-relationships based on measured environmental parameters is determined by the appropriate sampling of the spatial variation of those parameters (Gillison & Brewer, 1985; Cushman & McGarigal, 2004). The realised niche of a species is understood as its amplitude of tolerance in natural communities in the presence of competitors (Hutchinson, 1978). Deriving those niches from empirical data is complicated by the problem of an appropriate sampling design. This is due to the fact that each species responds to the environment at a range of different scales (Levin, 1992). Consequently, drawing conclusions about phenomena from only one scale of observation may not only lead to a misinterpretation of the driving factors for system behaviour (Cushman & McGarigal, 2004), but may even bias the detection of niche mechanisms (species sorting along habitat gradients) versus neutral mechanisms (species determined spatially by dispersal limitations) (Bell, 2005). At small spatial scales, assumed habitat factors are homogeneous; at this scale neutral dynamics emerging from dispersal limitation are likely to be observed. On the other hand, medium scales depict habitat patches; here niche dynamics are more likely to be observed. However, when habitats are heterogeneous at a small spatial scale, it is more likely that a combination of both neutral and niche mechanisms is observed (Gilbert & Lechowicz, 2004; Karst et al., 2005; Dalberg Poulsen et al., 2006; Girdler & Connor Barrie, 2008).

In this study we delineate species' realised niches in spring ecosystems at two spatial scales, with a view to detecting the scale effects on niche position and niche width. We assume springs to be interesting research subjects for studying the niches of species for several reasons. (1) Their constancy of hydrophysical and hydrochemical parameters sets them apart from other habitats (Odum, 1971; Ellenberg, 1996). In particular, when they are situated in forested catchments, they can be considered to be ecosystems with low dynamics that reflect the biogeochemical background of the landscape and long-term ecological processes. This enables species to adapt to habitat conditions over a relatively long period and to develop a steady state in community composition, expressed by their realised niche. In spite of this constancy in time, helocrenic springs (sensu Thienemann, 1924) with a relatively large seeping area show certain spatial, within-site variability (Illies & Botosaneanu, 1963; Hájková et al., 2004; Cantonati et al., 2006). (2) Spring-inhabiting organisms largely depend on groundwater characteristics, with other site conditions being less meaningful (Strohbach et al., submitted). Slowly seeping spring water clearly differentiates springs from their surrounding habitats. Such clear boundaries help to assess whether species are rooting in the spring itself or in the surrounding terrestrial habitats. (3) As a result of the small extent of spring habitats and the fact that these are permanently soggy, land use options for springs are negligible, and therefore human interest in them is low. Consequently, springs are a noteworthy exception to the predominantly anthropogenic ecosystems of Europe. They can be regarded as pristine islands surrounded by cultivated land. (4) Although springs only account for a small proportion of land surface, they are abundant in forested mountain ranges of Central Europe. Their frequent appearance qualifies them for investigations at the landscape level (Beierkuhnlein & Durka, 1993). (5) Spring water chemistry and in particular its acidity and nutrient availability gradient, is the major determining factor for the species composition of springs in Central Europe (Hájek et al., 2002; Hájková et al., 2008; Strohbach et al., submitted). This is consistent with other studies on springs and fens of the northern hemisphere (Bragazza & Gerdol, 2002; Nekola, 2004; Tahvanainen, 2004). The acidity range in our study covers acidic as well as circumneutral spring waters. Hence we expect to find species with diverse niche positions (pH optimum) and niche widths (pH amplitude).

Our goal is to determine the effects of different spatial sampling scales in spring ecosystems on the niche characteristics that were modelled. We expect the characteristic patterns of the interrelation between hydrological parameters and vegetation to differ between the within-spring and the entire-spring scales. Hence our hypothesis is that the probabilities of species occurrence with respect to pH, the species' pH optima and pH amplitudes are biased by the sampling scale, indicated by a stronger relationship between species occurrence and environmental factors on the larger scale (coarser grain) compared to a smaller scale (finer grain).

MATERIALS AND METHODS

Sites and the study area

Springs, located in 5 lower mountain ranges in central Germany and the north west of the Czech Republic (Thüringer Wald, Thüringer Schiefergebirge, Frankenwald, Fichtelgebirge and Erzgebirge; latitude 49.9° to 50.8° N; longitude 10.6° to 12.8° E; cf. Fig. 1) were the subject of our investigations. Throughout the study area bedrock material consists of silicates - porphyry, schist, phyllite, granite and gneiss. Climate conditions border between oceanic and continental with mean air temperatures between 4 and 7 °C and annual precipitation between 750 and 1350 mm. The mountain ranges were affected by acid depositions, which peaked in the 1970s and 1980s, leading to the acidification of soil, ground-, spring- and headwaters.

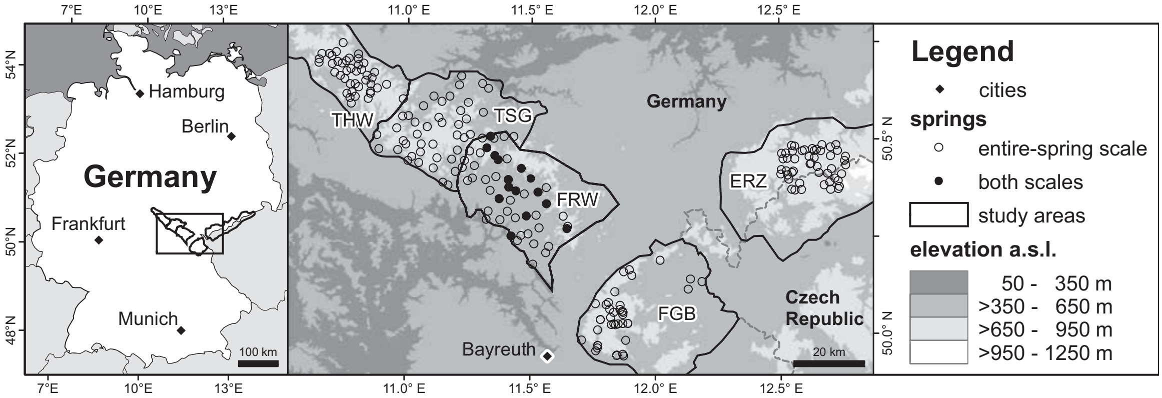

Figure 1 Location of the springs investigated in central Germany and the north west of the Czech Republic. 222 springs were investigated at the entire-spring scale, of which 15 also at the within-spring-scale. Abbreviations denote the regions: THW Thüringer Wald, TSG Thüringer Schiefergebirge, FRW Frankenwald, FGB Fichtelgebirge and ERZ Erzgebirge.

All of the springs that were investigated were located at an elevation between 270 and 925 m a.s.l. These springs along with their catchments are situated in forests, which are mainly dominated by Norway spruce (*Picea abies* (L.) H. Karst.), partly also by beech (*Fagus sylvatica* L.). The electrical conductivity of the siliceous, nutrient-poor spring waters ranges from 27 to 719 $\mu S\ cm^{-1}$, with a pH that is acidic to circumneutral (between 3.76 and 7.80; both autumn values).

Data collection

Springs may appear as a point when viewed on the landscape scale but they actually cover an area. In the studied regions they are generally formed as helocrenic springs (sensu Thienemann, 1924) with a saturated area covering up to 500 m^2 in size, where groundwater is seeping to the surface. To consider this fact and to compare species' performance at different scales we collected data in two ways, (1) for the entire spring and (2) on the within-spring scale by means of a raster-based subplot approach.

Of initially 251 springs that were investigated, 29 had to be omitted due to insufficient discharge (< 0.02 l s^{-1}) and therefore missing values in the hydrological dataset. Hence 222 springs remained for the analysis, and in all of these springs vegetation (vascular plants, mosses and liverworts) and hydrological data (discharge, electrical conductivity, pH, acid neutralizing capacity ANC and concentrations of NH_4, NO_3, SO_4, PO_4, Ca, Mg, K, Mn, Al, Cd, Na) were surveyed.

A subset of 15 springs, all in the Frankenwald region (for map see Fig. 1), was additionally sampled at a within-spring scale, using an equidistant hexagonal sampling grid with circular subplots. Subplot size was 0.25 m^2. The distance between subplot centres was 1.56 m in all directions, resulting in a minimum distance of 1.0 m from subplot boundary to subplot boundary (Fig. 2). Subplots were spread over the entire seeping area of the springs, leading to a number of 21 to 89 subplots per spring, and a total of 706 subplots. However a sufficient amount of water for measuring the below-mentioned parameters was only available in 407 of those. Consequently, the 299 subplots that had almost dried up had to be excluded from further analysis.

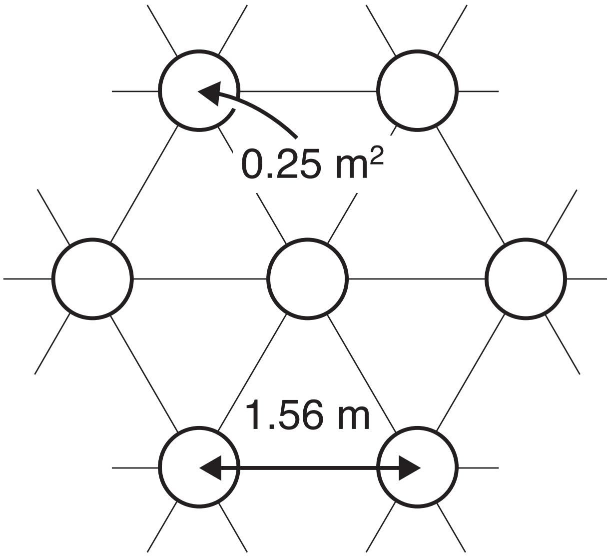

Figure 2 Sampling design for the within-spring approach. Subplots are spread over the whole seeping area of the helocrenic spring.

Spring water was sampled from mid-September to mid-October (years corresponding to vegetation records, see below), always in a dry period after at least one week without intense precipitation, to avoid short-term influences by rain events. For the entire-spring approach the in-situ measurements as well as water sampling for analyses in the laboratory were conducted at the uppermost point of the seeping area with water flowing above ground. There a small pit was formed and before taking measurements we waited until the spring water had clarified.

As not all subplots in the within-spring approach featured water flowing above ground, we used short piezometers (Ø 3.0 cm, slitted from 0.0 to 12.0 cm in depth) at the subplot centre to gain hydrological data from the rhizosphere. After installation we waited at least three days before starting to measure the water level, pH value, electrical conductivity, and water temperature in the piezometers. Prior to measurement all piezometers were emptied using a syringe. Subsequently we allowed the piezometers to be refilled with fresh water from the surrounding area to prevent any bias from hydrochemical alteration due to a possible retention time. The seepage waters of the subplots were characterised by an electrical conductivity ranging from 36 to 1118 µS cm^{-1} and a pH value between 4.20 and 7.19. The 15 springs selected for the within-spring approach showed an electrical conductivity ranging from 59 to 718 µS cm^{-1} and a pH range from 4.65 to 7.21 on the entire-spring scale.

For the entire-spring approach **plant species occurrence** was originally determined by means of the Braun-Blanquet method (1964). This way the abundance/dominance score of each vascular plant, moss and liverwort species growing in the clearly differentiated seeping area was estimated (nomenclature follows Frahm & Frey, 2004 for bryophytes, and Oberdorfer, 2001 for vascular plants). This was always carried out in either July or August, but in varying years (1993, 1997 and 2003) for different regions. We assume that this time inconsistency is not crucial, as our aim was only to find general rules for species occurrence. Vegetation data was transformed into binary data (presence/absence). In total 284 species were found in the 222 springs investigated with an average of 28.3 species per spring (minimum 6, maximum 55). Species with a frequency of less than 5 % were omitted. 100 species fulfilled this 5 % criterion and remained for further analyses.

In the within-spring approach vegetation data were sampled from August to September 2005 by directly detecting the plant species' presence/absence for each circular subplot. 119 species were found in total, with an average of 8.0 species per subplot (minimum 1, maximum 21). On the within-spring scale 41 species fulfilled the 5 % frequency criterion.

Data analysis

First, to reveal the primary driving factors of species composition and therefore also of single species occurrence, we carried out a non-metric multidimensional scaling (NMDS). Beforehand vegetation data for the entire-spring scale were square-root transformed and additionally submitted to Wisconsin double standardisation to give a higher weighting to rare species. Environmental covariables were also square-root transformed, except for the pH value, which is already a logarithmic measurement. The NMDS was only calculated for the entire-spring dataset (222 springs, 100 species), as the overall variation is expected to be greater there and more environmental parameters were available.

For all species with a frequency > 5 % at both scales of observation we calculated species response curves with respect to the pH using generalized additive models (GAM; Hastie & Tibshirani, 1990) with a binomial error distribution and a logit link function. For the predictor we used smoothing splines with the number of degrees of freedom selected by generalized cross validation (Gu & Wahba, 1991), but limited to a maximum number of three. The cross validation process is an improvement compared to testing some integer alternatives of degrees of freedom (Oksanen & Minchin, 2002). We computed the response optimum of each species as the pH value where the maximum predicted probability of occurrence was achieved. The species' pH amplitudes were calculated as the part of the curve where the calculated probability of occurrence exceeds the species prevalence, i.e. the proportion of observed occurrences in the respective data set (cf. Peppler-Lisbach, 2008). For a comparison of the pH optima and amplitudes between scales only those 24 species were taken into account that yielded a significant model ($p < 0.05$) at both scales of observation. We used paired Wilcoxon signed rank tests to compare the entirety pH optima and pH amplitudes, respectively. For single species, the significance of the differences between scales was calculated by bootstrapping (1000 permutations) the aberrations of the two respective optima or amplitudes.

To learn about the potential species pool, we computed the potential number of species along the pH gradient as the sum of the species whose pH amplitudes overlap at each given pH value (rounded to the nearest 0.01 and smoothed with a cubic spline). By treating the real species numbers of the springs and subplots in the same way, we also calculated a real species pool for both scales. In all cases only those 24 species with a significant model ($p < 0.05$) at both observational scales were considered.

All calculations were performed using R 2.8.0 (R Development Core Team, 2008). NMDS was calculated by means of the function metaMDS implemented in the package vegan 1.15-0 (Oksanen et al., 2008), generalized additive models were fitted using the package mgcv 1.4-1 (Wood, 2008).

RESULTS

Driving factors of species occurrence

NMDS clearly revealed acidity and nutrient availability as principal driving factors for species occurrence for the entire-spring approach (Fig. 3). This gradient was made up by the factors pH ($r^2 = 0.60$), Ca (0.29), Mg (0.24), ANC (0.20), electrical conductivity (0.15) and NO_3 (0.06) at the neutral end, whereas high concentrations of Al ($r^2 = 0.55$), Mn (0.44), and Cd (0.43) marked its acidic end. All of these mentioned correlations with the NMDS axis 1 were highly significant ($p < 0.001$; p-values based on 1000 permutations). As the pH value turned out to be a main actuating variable we confined ourselves to this parameter as a proxy, when calculating species response curves in the following.

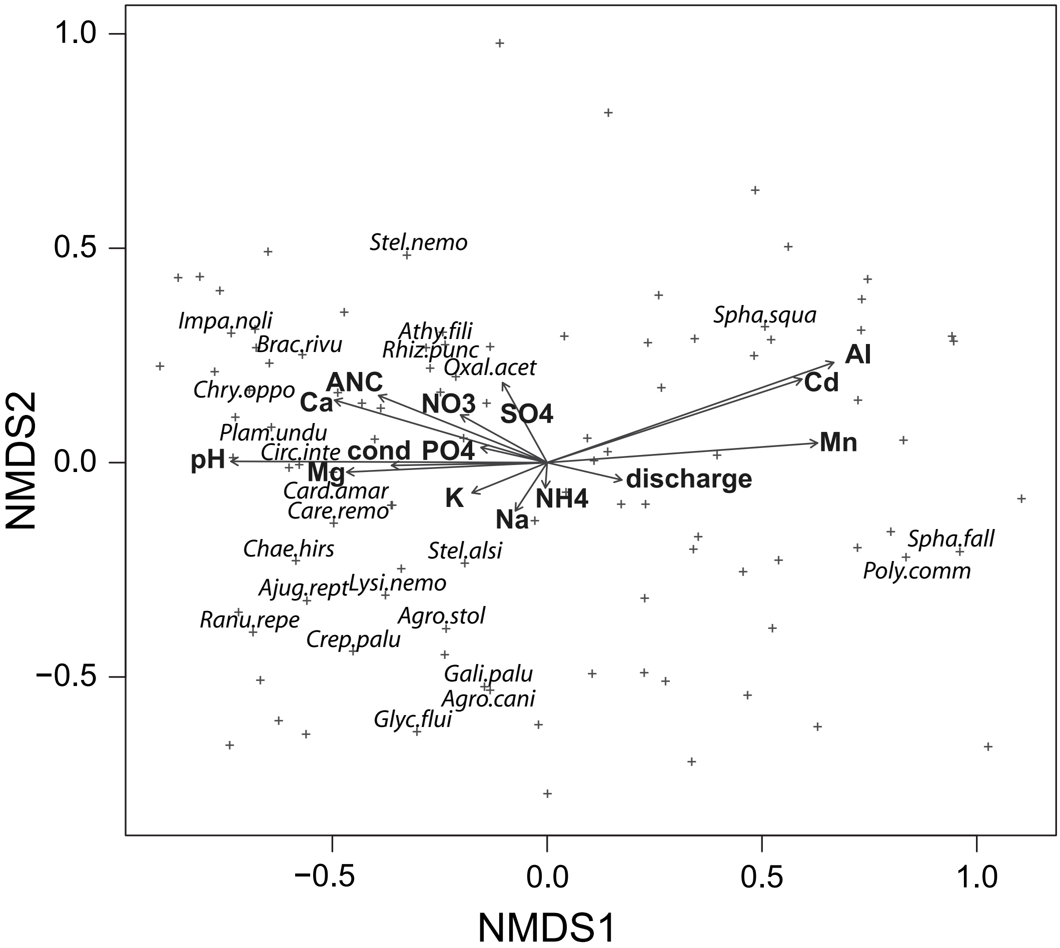

Figure 3 NMDS ordination of the entire-spring approach (222 springs). 100 species that fulfil the 5 % frequency criterion are included in the analysis (crosses), only those 24 species with a significant GAM fitting at both observational scales ($p < 0.05$) are labelled with abbreviations (for full species names, see table 1). Environmental variables are considered as covariables.

Species response curves

39 species occurred with a frequency greater than 5 % at both scales. The probability of occurrence was able to be predicted by generalized additive models (GAM) with respect to the pH for 28 of these species on the entire-spring scale and for 33 species on the within-spring scale (model significance $p < 0.05$). For the remaining species no significant response of occurrence with regards to the pH could be observed. An overlap of 24 species with significant models on both scales was obtained (Table 1; Fig. 4). 20 of those species shared a maximum probability of occurrence in neutral conditions (pH > 5.5) at both scales, and three of those species in acidic conditions (pH < 5.5). Only *Agrostis stolonifera* showed a different performance for each scale. Generally, the probability of occurrence at the within-spring scale was lower than for the entire-spring scale over the entire pH range (14 species) or at least for wide parts of the pH range (9 species). Only *Sphagnum fallax* was more likely to be observed on the within-spring scale for the most part of the pH range.

Table 1 Model characteristics for 24 species with a significant GAM fitting for both scales ($p < 0.05$), sorted by their pH optima for the entire-spring scale. Besides model performance (d.f., r^2, p), species frequency, maximum probability of occurrence, pH optimum and pH amplitude are given for both scales. The significance of differences in pH optima and amplitudes between both scales is also specified (n.s. = not significant: $p > 0.05$).

	entire-spring scale							within-spring scale							between scales	
species	**freq. (%)**	**d.f.**	**r^2**	**p**	**max. p.o.**	**pH opt.**	**pH amp.**	**freq. (%)**	**d.f.**	**r^2**	**p**	**max. p.o.**	**pH opt.**	**pH amp.**	**p Δ opt.**	**p Δ amp.**
Polytrichum commune	40.5	1.00	0.227	< 0.001	0.918	3.76	1.85	18.6	1.88	0.257	< 0.001	0.655	4.20	1.35	0.014	0.005
Sphagnum fallax	29.3	1.22	0.224	< 0.001	0.877	3.76	1.76	31.6	2.19	0.332	< 0.001	0.850	4.20	1.53	0.018	n.s.
Sphagnum squarrosum	30.6	1.62	0.079	< 0.001	0.554	3.76	2.00	11.0	1.25	0.049	< 0.001	0.310	4.20	1.43	n.s.	n.s.
Stellaria alsine	53.6	2.31	0.122	< 0.001	0.715	6.14	1.82	28.4	2.93	0.139	< 0.001	0.537	7.19	1.92	n.s.	n.s.
Agrostis stolonifera	52.3	2.07	0.044	0.013	0.625	6.16	1.92	18.9	2.80	0.075	< 0.001	0.584	4.20	1.88	0.002	n.s.
Agrostis canina	42.3	2.42	0.142	< 0.001	0.613	6.22	2.19	10.5	2.56	0.081	< 0.001	0.267	5.81	1.18	n.s.	0.002
Galium palustre	41.9	2.27	0.117	< 0.001	0.607	6.31	1.90	10.0	2.70	0.058	< 0.001	0.199	6.35	1.08	n.s.	n.s.
Lysimachia nemorum	37.4	2.18	0.110	< 0.001	0.554	6.41	2.14	23.3	1.35	0.039	< 0.001	0.392	7.19	1.35	n.s.	0.048
Glyceria fluitans	25.7	2.12	0.087	< 0.001	0.414	6.48	2.22	21.3	1.00	0.024	0.002	0.356	7.19	1.29	n.s.	n.s.
Ranunculus repens	36.5	2.54	0.301	< 0.001	0.689	6.48	2.24	26.0	2.87	0.159	< 0.001	0.709	7.19	1.64	n.s.	0.026
Cardamine amara	47.3	2.39	0.280	< 0.001	0.744	6.59	2.34	39.5	2.21	0.411	< 0.001	0.837	7.19	1.33	n.s.	< 0.001
Crepis paludosa	38.7	2.27	0.246	< 0.001	0.674	6.60	2.24	14.2	2.21	0.058	< 0.001	0.237	6.21	1.32	n.s.	0.001
Ajuga reptans	20.7	2.09	0.113	< 0.001	0.391	6.66	2.15	7.6	2.36	0.167	< 0.001	0.596	7.19	0.69	n.s.	n.s.
Carex remota	18.5	1.97	0.063	0.003	0.310	6.74	2.19	17.4	2.04	0.079	< 0.001	0.540	7.19	0.93	n.s.	n.s.
Athyrium filix-femina	73.9	2.69	0.152	< 0.001	0.940	6.77	2.25	22.3	1.27	0.014	0.026	0.312	7.19	1.39	n.s.	n.s.
Plagiomnium undulatum	34.2	2.19	0.401	< 0.001	0.777	7.13	2.04	15.9	2.32	0.123	< 0.001	0.320	6.33	1.23	0.008	< 0.001
Chaerophyllum hirsutum	22.5	1.73	0.156	< 0.001	0.625	7.80	1.97	8.8	2.84	0.061	< 0.001	0.257	5.61	1.16	< 0.001	0.009
Oxalis acetosella	72.5	1.72	0.055	0.004	0.838	7.80	2.39	24.8	2.55	0.069	< 0.001	0.399	5.95	1.30	0.042	0.011
Rhizomnium punctatum	63.1	1.00	0.222	< 0.001	0.980	7.80	2.21	11.5	2.14	0.058	< 0.001	0.199	6.29	1.39	< 0.001	0.002
Circaea intermedia	33.3	1.89	0.241	< 0.001	0.776	7.80	2.06	7.8	2.04	0.026	0.015	0.125	6.30	1.58	0.023	0.011
Stellaria nemorum	30.6	1.63	0.047	0.005	0.472	7.80	2.17	15.4	2.11	0.151	< 0.001	0.347	6.76	1.18	n.s.	0.012
Brachythecium rivulare	59.9	2.01	0.418	< 0.001	0.955	7.80	2.32	37.7	2.87	0.223	< 0.001	0.721	7.19	1.72	n.s.	< 0.001
Chrysosplenium oppositif.	53.2	2.01	0.488	< 0.001	0.964	7.80	2.23	46.8	2.62	0.386	< 0.001	0.796	7.19	1.56	n.s.	< 0.001
Impatiens noli-tangere	31.5	2.08	0.294	< 0.001	0.764	7.80	2.04	19.1	2.87	0.079	< 0.001	0.533	7.19	1.76	n.s.	0.029

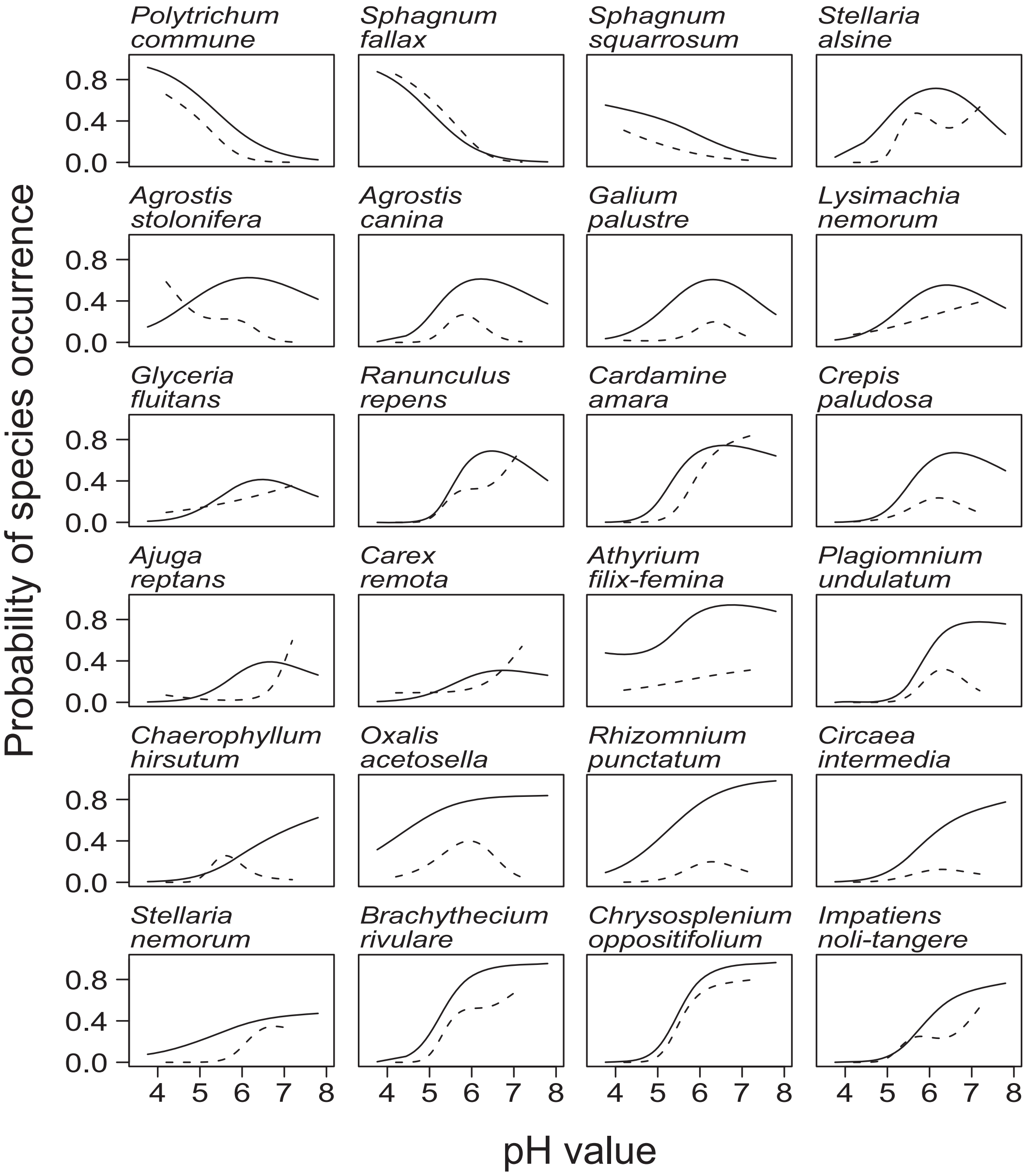

Figure 4 Species response curves for 24 species with a significant GAM fitting ($p < 0.05$). Solid lines represent the entire-spring scale, dashed lines the within-spring scale.

The potential species pool, calculated from the pH amplitudes overlapping at each given pH value, showed comparable species numbers and the same characteristics for both observational scales (Fig. 5). Along the pH gradient the potential number of species was lowest at pH < 5, increased between pH 5 and 6 and reached maximum values at pH > 6. Consisting of a maximum of 21 (entire-spring) and 18 species (within-spring scale) respectively, the dominating proportion of species with a significant GAM fitting contributed to this circumneutral peak of the potential species pool. The real species pool shows the same curve characteristics (Fig. 5), but lower maximum species numbers (14 for the entire-spring and 7 for the within-spring-scale). For the within-spring scale potential species numbers exceed real species numbers throughout the entire pH range. However, the calculation of the species pools for the entire-spring scale resulted in higher real rather than potential species numbers in the acidic section of the pH range, between pH 4.1 and 5.4.

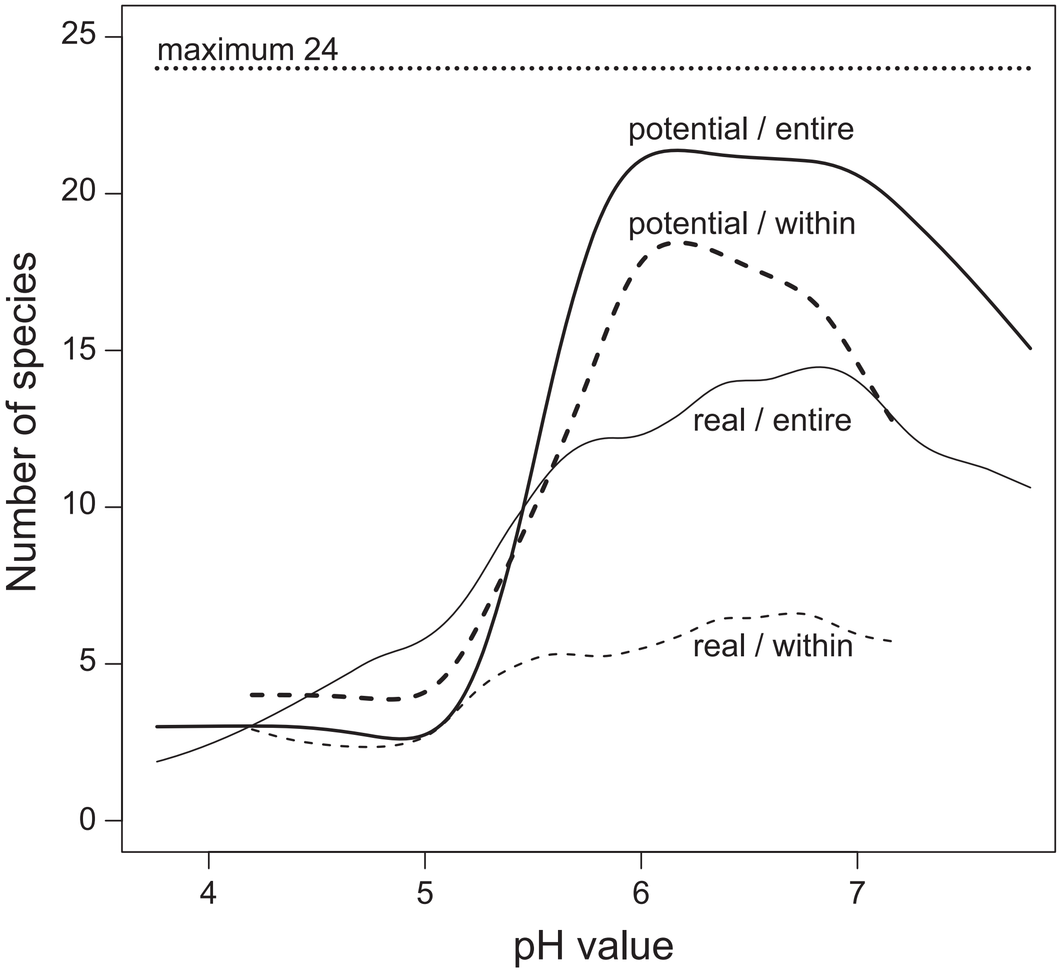

Figure 5 Potential and real pool of species along the pH gradient (rounded to 0.01 pH units and smoothed with a cubic spline). The potential species pool was calculated as the sum of those species whose pH amplitudes overlap at the given pH value. In all cases only the 24 species with a significant GAM fitting ($p < 0.05$) at both scales were considered.

Comparison of scales

On examining the entirety of species, the pH optima did not differ significantly between scales, as shown by the paired Wilcoxon signed rank test ($p = 0.31$; Fig. 6a). Even when looking at discrete species, their majority showed roughly the same optima at both observational scales (Fig. 6b). However, eight species displayed a significant shift in their response optimum ($p < 0.05$; Table 1 and Fig. 6b). Within the latter, *Agrostis stolonifera*, *Plagiomnium undulatum*, *Chaerophyllum hirsutum*, *Oxalis acetosella*, *Rhizomnium punctatum* and *Circaea intermedia* could be assigned to a higher pH optimum for the entire-spring scale. *Polytrichum commune* and *Sphagnum fallax* were the only species that had a significantly higher pH optimum at the within-spring scale.

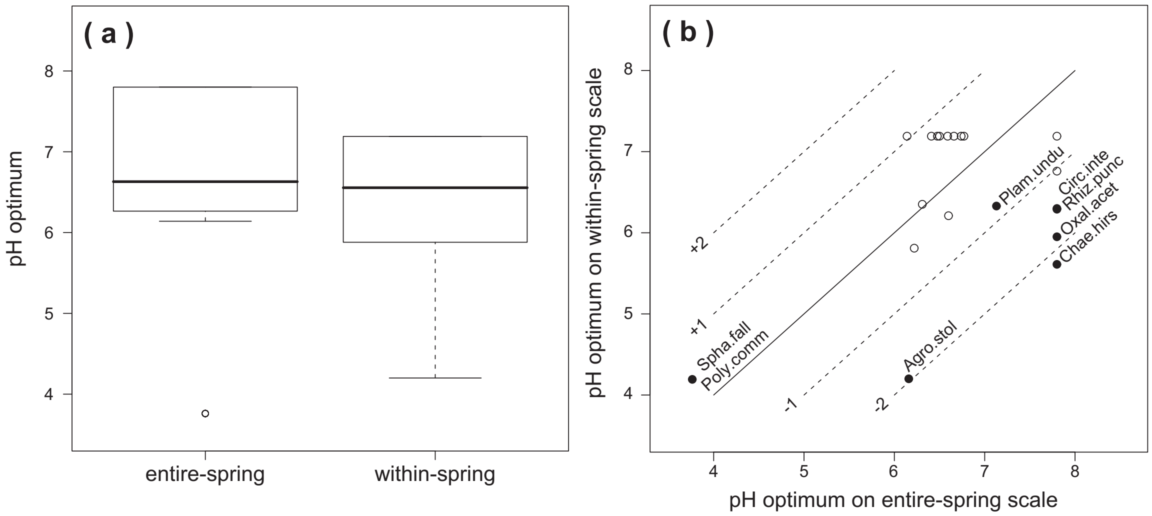

Figure 6 Comparison of pH optima between scales. (a) For the entirety of species, optima do not significantly differ between scales (p = 0.31). (b) Single species with significant differences (p < 0.05) of their optima are marked by full circles and labelled (for full species names, see table 1), non-significant ones by empty circles. The solid line marks the 1:1 ratio; dashed lines represent discrepancies of ±1 and ±2 pH-units.

The optima of only four species was at the centre of the captured pH ranges at both observational scales. For all other species the pH optimum was positioned at the margin of the measured pH range on at least one scale. Each three species at the neutral end (*Brachythecium rivulare*, *Chrysosplenium oppositifolium*, *Impatiens noli-tangere*) and the acidic end of the gradient (*Polytrichum commune*, *Sphagnum fallax* and *Sphagnum squarrosum*) were characterised by the pH optima bound to the range margins of both observational scales.

In contrast to the pH optima, the pH amplitudes of species differed significantly between the scales of observation (p < 0.001; Fig. 7a) when taking all 24 species into account. For individual species, pH amplitudes were generally narrower at the within-spring scale, with *Stellaria alsine* as the only exception (not significant). This shift between observational scales was most pronounced for *Polytrichum commune*, *Agrostis canina*, *Lysimachia nemorum*, *Ranunculus repens*, *Cardamine amara*, *Crepis paludosa*, *Plagiomnium undulatum*, *Chaerophyllum hirsutum*, *Oxalis acetosella*, *Rhizomnium punctatum*, *Circaea intermedia*, *Stellaria nemorum*, *Brachythecium rivulare*, *Chrysosplenium oppositifolium* and *Impatiens noli-*

tangere. These 15 species displayed a significant curtailment of the amplitude between scales ($p < 0.05$; Table 1 and Fig. 7b).

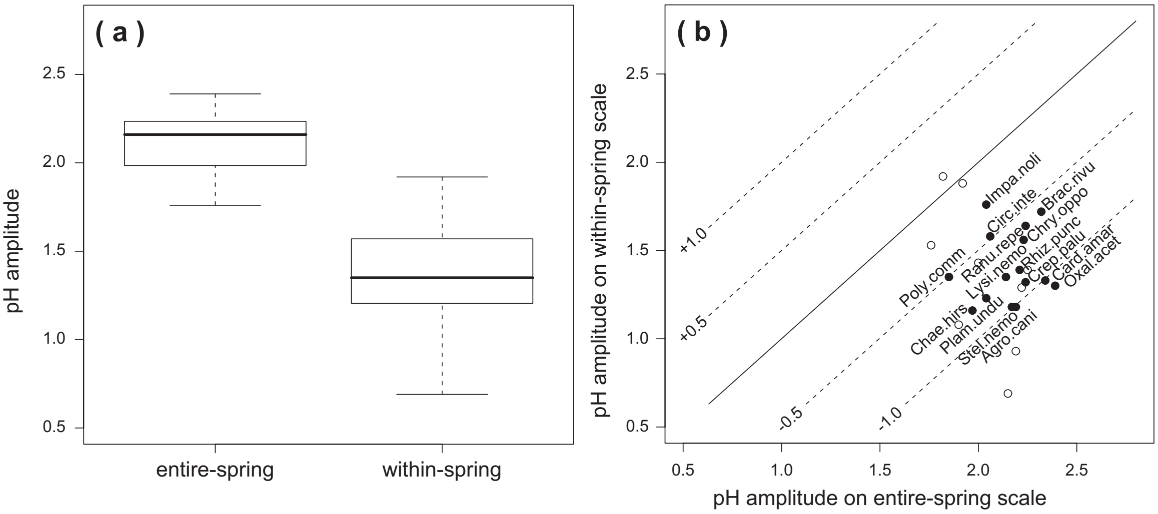

Figure 7 Comparison of pH amplitudes between scales. (a) For the entirety of species amplitudes significantly differ between scales ($p < 0.001$). (b) Single species with significant differences ($p < 0.05$) of their amplitudes are marked by full circles and labelled (for full species names, see table 1), non-significant ones by empty circles. The solid line marks the 1:1 ratio; dashed lines represent discrepancies of ±0.5 and ±1.0 pH-units.

DISCUSSION

Characteristics of species response to pH

Species diversity was shown to be correlated with pH, with the highest species numbers found in circumneutral conditions, regardless of the scale of observation (Fig. 5). This finding coincides with the potential species pools calculated for mires in Bulgaria and in the West Carpathians by Hájková et al. (2008). The threshold between species-poor acidic springs and species-rich circumneutral springs is around pH 5.5. This agrees with Wheeler & Proctor (2000), who proposed a general bimodal division of mire types (including springs) into Ca-poor 'bogs' at pH values < 5 and Ca-rich 'fens' at pH values > 6. In general, both the potential and the real species pool showed the same curve characteristics (Fig. 5). Unlike expected, the potential number of species did not exceed the real species numbers over the entire pH range. We ascribe this partial abnormality in the acidic section of the entire-spring approach to the accentuation, which is a result of the methodical constraint (a maximum of three degrees of freedom) when calculating the species response curves.

On dissecting the entirety of species occurrences by implementing an NMDS (Fig. 3), it emerged that the acidity gradient governs the vegetation patterns by controlling nutrient availability. High concentrations of Ca, Mg and NO_3 characterise circumneutral spring waters, whereas acidic conditions coincided with high concentrations of Al, Mn and Cd. The pH value acts as a leading representative of this acidity and nutrient availability gradient. Our findings coincide with Hájek et al. (2002), Hájková et al. (2008), and Strohbach et al. (submitted), who also found this gradient to be the major determinant for the species composition of springs in central Europe.

For almost all species, the response curves of the entire-spring and the within-spring scale provided a roughly synchronous description of occurrence in relation to spring water pH (Fig. 4). *Agrostis stolonifera* is the exception, but this might be due to the fact that it is difficult to identify species of this genus if not flowering.

Effect of the sampling scale

Despite this clear connection of species occurrence with the acidity gradient, surprisingly only 62% of the considered species have reasonably established niches with respect to pH, which were computed to be significant for both scales (Table 1; Fig. 4). However, when looking at each of the two observational scales separately, the percentage of species yielding a significant model was higher at the within-spring scale (83 %) compared to the entire-spring scale (62 %), both for species with a frequency > 5 % for the respective scale. Only three species (*Lamium galeobdolon*, *Scapania undulata*, *Thuidium tamariscinum*) fulfilling the 5 % frequency criterion at both scales did not yield significant models on the within-spring scale. On the contrary, nine of those species only had significant species response curves at the within-spring scale, but not at the entire-spring scale (*Deschampsia flexuosa*, *Epilopium palustre*, *Holcus mollis*, *Mnium hornum*, *Pellia epiphylla*, *Picea abies*, *Plagiomium affine*, *Sphagnum palustre*, and *Viola palustris*). This ratio suggests that niche effects are of importance at the smaller scale.

The dominance of dispersal limitation as the driving force for species distribution at small scales (Bell, 2005) did not hold true for our dataset. Indeed, several studies denote a concurrence of neutral mechanisms due to dispersal limitation and niche mechanisms at small scales, when the environment exhibits a strong spatial structure (Gilbert & Lechowicz, 2004; Karst et al., 2005; Dalberg Poulsen et al., 2006; Girdler & Connor Barrie, 2008). Our trend towards significantly lower maximum probabilities of occurrence at the within-spring scale (paired Wilcoxon signed rank test; $p < 0.001$) could therefore be explained by an increasing influence of dispersal limitation at the within-spring scale. On the other hand the influence of pH-driven niche mechanisms was still strong enough to yield significant correlations and was indicated by the high number of species response curves that could be modelled significantly for the within-spring scale.

Here it comes into play that springs bear a likeness to islands. They are an aquatic biotope, surrounded by terrestrial habitats, in our case forests, and thus fragmented naturally. Spring water flow is unidirectional and permanent, away from the spring. As the transport of diaspores of mosses, liverworts and vascular plants by groundwater can be excluded, the dispersal vectors to the spring habitats are restricted. Hence springs also feature a limited dispersal at larger scales.

In general, springs are known for their constancy of hydrophysical and hydrochemical parameters (Odum, 1971; Ellenberg, 1996). Nevertheless, helocrenic springs with a relatively large saturated area, where groundwater seeps to the surface, show certain within-site variability (Illies & Botosaneanu, 1963; Hájková et al., 2004; Cantonati et al., 2006). Therefore environmental factors can also influence niche characteristics at the within-spring scale.

Summing up the abovementioned points, dispersal limitations as well as environment-driven niche mechanisms are less scale-dependent in springs than in other habitat types. We assign the synchronicity of the pH optima (Fig. 6a) as well as the similar characteristics of the species response curves (Fig. 4) to this reason. Taking this point into consideration, we have to overturn our hypothesis that species' pH optima depend on the sampling scale. Consequently, the minority of species show significant differences in pH optima, in contrast to pH amplitudes which are significantly different for the majority of species (Table 1; Figs. 6b and 7b). However, some of those differences might be an effect of truncated species response curves at the pH range margins.

The within-spring patterns of the environment and vegetation are only adequately captured by the raster-based subplot approach. Although the entire-spring approach encompasses all species dwelling in the seeping area, the hydrochemical parameters were only measured at one single point. As spring-inhabiting species are often stenoecious (Zollhöfer et al., 2000; Cantonati et al., 2006) they do indeed show a fine-scale habitat selection. However, they are assigned to spatially and therefore also hydrochemically inaccurate environmental data by the entire-spring approach. Consequently, this leads to the calculation of wider pH amplitudes (Fig. 7). The calculation of pH optima is not affected by this inaccuracy, as errors average to the mean value. Hence, plot size (grain) is relevant for the delineation of niche characteristics, but first and foremost for the niche amplitudes and only marginally for the niche optima. With regards to species' pH amplitudes, we can therefore corroborate our hypothesis that this depends on the sampling scale.

We assume that shifts and the curtailment of amplitudes cannot be ascribed to geographical discrepancies, because the within-spring approach uses a centric subset of the springs used for the entire-spring approach (Fig. 1). Moreover, the subset almost covers the whole pH range of the entire-spring dataset.

CONCLUSIONS

Species occurrence in helocrenic springs is closely correlated with an acidity gradient that controls nutrient availability. The pH value represents this gradient as a proxy. Potential species diversity (calculated from the amplitudes of the species response curves) was shown to depend on pH, regardless of the scale of observation. Lowest species numbers are found in acidic conditions and the highest in circumneutral conditions.

Springs are characterised by two special features. On the one hand they are groundwater-dependent aquatic biotopes surrounded by terrestrial habitats and therefore quasi-islands. On the other hand helocrenic springs show certain small-scale within-site variability. Therefore dispersal limitation as well as niche mechanisms are less scale-dependent here than in other habitat types. Hence, we were able to derive significant species response curves to acidity at both observational scales.

We could not confirm the hypothesised dependency of niche characteristics from the sampling scale for the pH optima. In contrast, pH amplitudes were found to be dependent on the scale. Larger plot sizes (grain) weakened the species-environment relationship, what consequently resulted in broader niche amplitudes. Consequently, the delineation of niche characteristics is a matter of sampling scale, in particular for ecological amplitudes.

Numerous ecological applications deal with niche attributes, for instance niche based distribution models (e.g. Guisan & Zimmermann, 2000; Guisan & Thuiller, 2005) that are valuable tools in conservation biology and species management (Randin et al., 2006). Our findings suggest that the predictive capacity of such models is not only limited by the environmental range over which the models are fitted (Thuiller et al., 2004) and the transferability over regions (Randin et al., 2006; Hájková et al., 2008), but also by the choice of sampling scale. Furthermore, the different numbers of significant model fittings between scales imply that even the sheer quantity of indicator species identified for ecological monitoring depends on the scale of observation at which the data was obtained.

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Manuscript 4

Inter-annual vegetation dynamics in forest springs

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Inter-annual vegetation dynamics in forest springs

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Summary

1. Hydrological parameters, herein mainly the acidity and nutrient availability gradient, are known to be major environmental factors that determine plant species composition in forest spring communities. Here we analyse species response to inter-annual variability in hydrological parameters over a period of four consecutive years (2003 to 2006).

2. Annual data for both the vegetation of 57 forest springs (NE-Bavaria, Germany) and corresponding hydrological parameters were recorded. First, we detected determinants of species composition by CCA ordination (static approach), then we analysed the inter-annual turnover of species composition using the Bray-Curtis-dissimilarity index. In order to decipher the driving factors behind inter-annual vegetation dynamics (dynamic approach) we applied multivariate statistics (CCA) to inter-annual change values of environmental and vegetation data.

3. The pH-value clearly emerged as a major driving factor of species composition (static approach). An inter-annual variability of the vegetation of forest springs was indeed found, but its hypothesised sensitivity to changes in the hydrological properties of the spring water could not be proven (dynamic approach).

4. We presume that the vegetation dynamics of forest springs is subject to species inertia (delayed response, persistence patterns) and can only be affected by a modified acidity status in the long run.

Keywords

inertia, resilience, persistence, species response, species turnover

Introduction

The relative constancy of hydro-physical and hydro-chemical parameters in springs sets them ecologically apart from other habitats (Odum, 1971; Ellenberg, 1996). As water flows from them more or less continuously, spring organisms refer strongly to groundwater hydrology. Numerous investigations have shown the vegetation of springs and fens to be fundamentally determined by the gradient of acidity and nutrient availability (Vitt & Chee, 1990; Wheeler & Proctor, 2000; Bragazza & Gerdol, 2002; Hájek et al., 2002; Nekola, 2004; Tahvanainen, 2004; Hájková et al., 2008). Acidity has been documented as a major driver of plant species occurrence and composition in Central European springs (Beierkuhnlein & Gräsle, 1998; Hájková et al., 2008). Therefore, highly specialised crenobiotic plant species can be seen as suitable indicators for the monitoring of groundwater acidification.

Changing ambient conditions in springs (e.g. due to acidification or climate change) are likely to be responded to by species and their assemblages (Glazier, 1991; Zollhöfer et al., 2000). However, in many cases due to the complexity of processes, cause and effect can hardly be separated. The lack of short-term disturbances such as land use and perturbations in combination with the high continuity of the spring environment provides a natural setting that avoids these restrictions and is also localised precisely. Changes in spring water quality are expected to affect the stenoecious crenobiotic and crenophilic species in particular (Ferrington, 1998; Zollhöfer et al., 2000; Cantonati et al., 2006).

With regards to the discussion on global climate change, which is forecasted to entail a higher frequency of extreme weather events, questions arise concerning the potential influence of short-term variability of environmental conditions on vegetation (Gutschick & BassiriRad, 2003). Intense short-term events are expected to cause stronger responses in vegetation compared to gradual shifts in mean values (White & Jentsch, 2001; Scheffer & Carpenter, 2003). Considering the fact that springs are assumed to be balanced habitats with both a constant water supply and water quality (Odum, 1971; Ellenberg, 1996; Mc Cabe, 1998; Zollhöfer et al., 2000), extraordinary events and potential tipping points such as the hot and dry summer in 2003 with reduced and locally absent groundwater discharge may greatly contribute to vegetation response. On the other hand, the capacity of resilience and the inertia of spring communities are unknown. The short-term responses of spring communities to such events have not yet been investigated and neither has the response time of species and communities to environmental change.

We hypothesise a reflection of the inter-annual variability of environmental parameters, in particular the acidity and nutrient availability status, by the abundances of plant species inhabiting the seeping area of forest springs.

Methods

Study area & sites

The investigation areas (Frankenwald and Fichtelgebirge mountain ranges) are located in north-eastern Bavaria, central Germany (49.9° - 50.6° N, 11.2° - 12.2° E). They each cover an area of roughly 900 km^2, with the elevation of the Frankenwald between 350 and 800 m a.s.l. and the Fichtelgebirge between 450 and 1050 m a.s.l. Their petrography can be characterised as relatively homogenous, consisting of silicates - palaeozoic schist, phyllite, greywacke, gneiss and granite. Climatic conditions show mean air temperatures from 4 to 7 °C and annual precipitation from 750 to 1350 mm. Forests of Norway spruce (*Picea abies* (L.) H. Karst.) and to some extent also beech (*Fagus sylvatica* L.) dominate the landscapes. In the 1970s and 1980s the region was exposed to high loads of acid depositions leading to the acidification of soil, ground-, spring- and headwaters.

In our study areas springs generally occur as helocrenic springs (sensu Thienemann 1924), with a saturated area of a few to 500 m^2 in size, where water seeps out slowly. Due to the compact bedrock with a low pore volume, the groundwater flow is concentrated in layers that are close to the surface (interflow). Springs arise frequently on hill slopes, and are characterised by very small catchments (0.1 to 25 ha) and low discharge rates (mostly below 2.0 $l\ s^{-1}$). All 57 springs and catchments in this study are located in forests. The pH-values of those nutrient-poor spring waters are acidic to neutral (pH 4.3 to 7.5). Electrical conductivity ranges from 28 to 1130 $\mu S\ cm^{-1}$ (autumn values from the years 2003 to 2006).

Data acquisition and preparation

Field data was collected over four consecutive years, from 2003 to 2006. The sampling of ***hydrological data*** was carried out each year in autumn (end of September), always during a climatically dry period after at least one week without intensive rainfall, in order to avoid short-term singularities related to rain events. Because of the summer drought in 2003, nine springs had insufficient discharge for sampling. In 2004 two springs, in 2005 and in 2006 one spring respectively could not be sampled for the same reason (cf. Figure 1). Hence the possibilities of combining vegetation with hydrological data were reduced accordingly in single springs and years. Five hydrological parameters were included in this study, namely discharge, water temperature, electrical conductivity and pH-value (all of which were measured in situ) as well as the acid neutralizing capacity (ANC; in vitro). *In situ* measurements and sampling were performed at the uppermost point with above ground flowing water.

Vegetation (mosses, liverworts and vascular plants) was recorded annually during July and August by means of defined and fixed line transects. These were established perpendicular to the direction of discharge at the position with the largest width in the saturated area, and were permanently marked. The length of the transects differed between 1.1 and 24.7 m. Presence-absence data were recorded in sections of 10 cm along the transect line. We chose this method because it has the advantages of being precise, repeatable, efficient, and sensitive to slight alterations in vegetation composition. Plants rooting on micro-sites without contact to the seeping water (e.g. deadwood, stones) were excluded from the data. Nomenclature follows Oberdorfer (2001) for vascular plants and Frahm & Frey (2004) for mosses and liverworts.

As our aim was to detect inter-annual changes of species performance, it was necessary to make the transect lines of differing length comparable in order to detect generality in species response patterns. Consequently, the percentage abundance for each species in each year was calculated as a ratio between the number of line sections with this species present and the total number of sections in the specific line transect. All analyses were based on these relative abundances. Species occurring in less than 5 springs (over an average of four years) were omitted from the dataset. Consequently, the number of species that remained for further analyses was reduced from 165 to 62.

Temporal dynamics of species abundances and environmental parameters could be detected when ***matrices of deviation*** between pairs of consecutive years (interval 1 year; n = 3) as well as between pairs of non-consecutive years (interval 2 or 3 years; n = 3) were used. This subtraction produced changes in abundances of every single species (turnover) and changes in every environmental parameter, both in terms of differences. These values built the basis for further statistical analyses, i.e. correlations between water and vegetation.

Data analysis

With the aim of revealing inter-annual dynamics of species abundances, we compared the corresponding data sets of pairs of (consecutive as well as non-consecutive) years. We used the function *dist.tmp* from the R-package *simba* (Jurasinski, 2007) with Bray-Curtis as the distance measure.

Interrelations between inter-annual changes of species abundances and those of environmental conditions were investigated by ***multivariate ordination***. The R-package *vegan* (Oksanen et al., 2007) was used for ordination, particularly for canonical correspondence analysis (CCA). Species abundance as well as environmental data were log-transformed, except for pH-values, which already represent a negative logarithm. The change values were standardised into a range from 0 to 1. This procedure enabled information to be kept on the direction of abundance changes when comparing pairs of two years' data sets. A canonical correspondence analysis (CCA) was chosen as the ordination method. CCA was calculated in two different ways. First, we analysed data representing single points of time across four years of investigation (***static approach***). In a second step we then focused on values of inter-annual changes of species abundances and of environmental parameters (***dynamic approach***), with the aim of unravelling the driving forces behind inter-annual species dynamics. For the latter we separated pairs of consecutive and pairs of non-consecutive years, to get some idea about the speed of species responses to environmental change.

Results

Inter-annual variability of the environment

Autumn hydrological conditions of spring water were found to be relatively constant over the investigation period. Nevertheless, we detected temporal variations (Figure 1). Spring water temperature increased in years with warmer atmospheric conditions. Spring water temperature was lowest in 2004 (8.3 ± 0.9°C) and exhibited maximum values in 2003 and 2006 (9.1 ± 1.94°C and 9.2 ± 1.7°C). Discharge showed minimal values of 0.14 ± 0.20 l s^{-1} in 2003, but was comparable between the following years (2004: 0.41 ± 0.39 l s^{-1}, 2005: 0.39 ± 0.35 l s^{-1}, 2006: 0.46 ± 0.42 l s^{-1}). The variance of discharge increased from year to year, with a maximum in 2006. The pH-value decreased slightly from 5.91 ± 0.82 in 2003 to 5.54 ± 0.61 in 2006 (Figure 1). Following this trend, acid neutralizing capacity (ANC) was highest in 2003 and continuously decreased until 2006 (2003: 0.27 ± 0.3 mmol l^{-1}, 2004: 0.21 ± 0.27 mmol l^{-1}, 2005: 0.17 ± 0.2 mmol l^{-1}, 2006: 0.15 ± 0.17 mmol l^{-1}). Autumn values of electrical conductivity neither increased nor decreased. Values ranged between 112 µS cm^{-1} and 128 µS cm^{-1}. Values over 300 µS cm^{-1} were measured, but could be identified to be outliers due to a long-distance transport of road salt in the aquifer close to the surface.

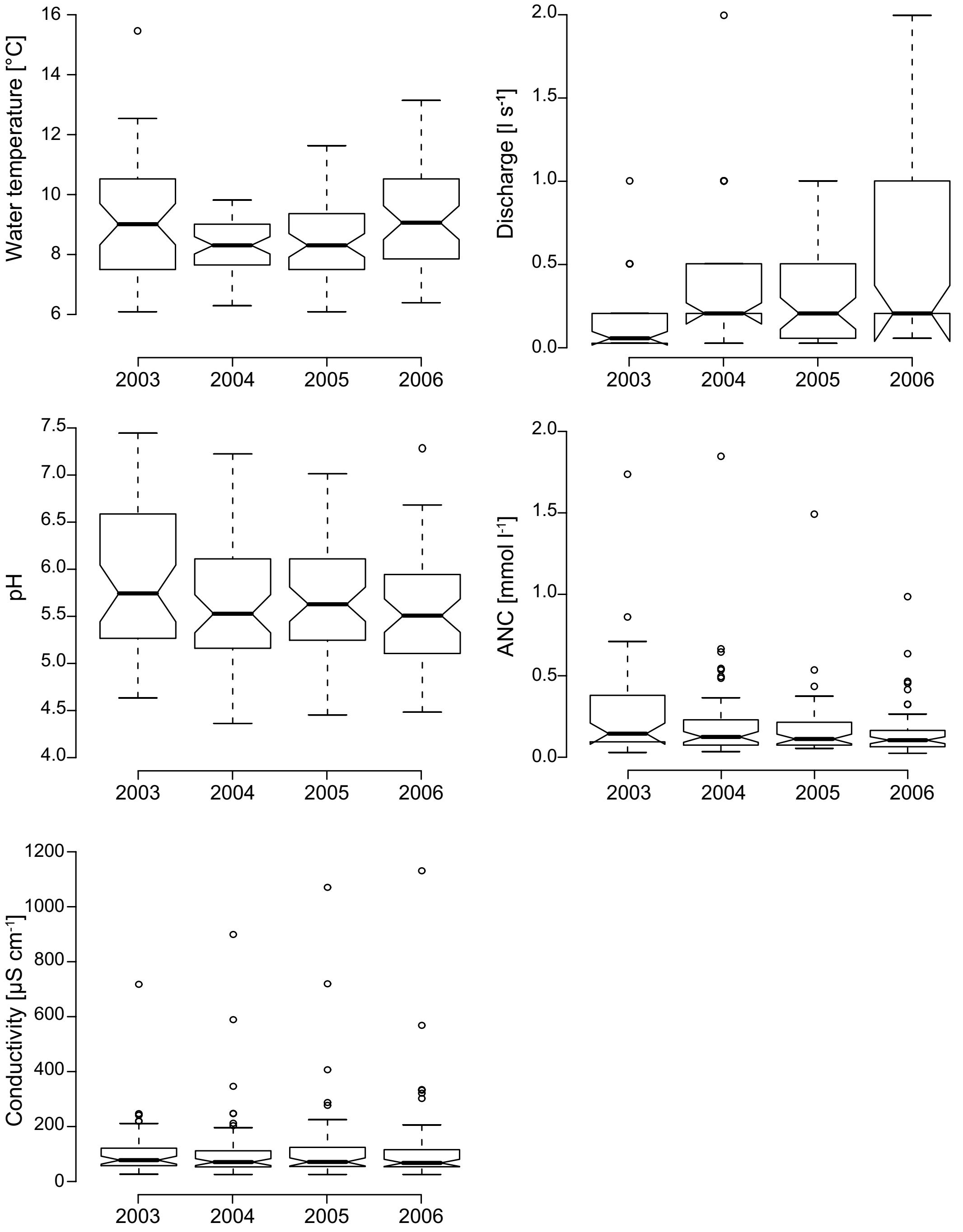

Figure 1: Autumn values of environmental parameters in the four investigation years; Box-Whisker-Plots, thick line = median, box = 50 %, whisker = 90 % of variation, points = outliers, notches are approximations of the 95 % confidence interval of the median. As some of the 57 investigated springs lacked in discharge their quantity was reduced to n_{2003} = 48, n_{2004} = 55, n_{2005} = 56 and n_{2006} = 56.

As expected, the correlation between the pH-value and ANC was significant ($r = 0.71$, $p < 0.001$, Table 1). Spring water temperature was significantly correlated with discharge ($r = -0.38$, $p < 0.001$), but also with the pH-value ($r = 0.33$, $p < 0.001$). However, these parameters are not necessarily linked. Springs in lower elevations might integrate larger catchments that are in addition less affected by acid depositions compared to higher elevations.

Table 1: Pearson linear correlation of environmental parameters (all four investigation years included). Significance levels are marked as follows: $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), $p < 0.1$ (^), $p \geq 0.1$ (n.s.).

	Temperature	**Discharge**	**Conductivity**	**pH**
ANC	0.17 *	-0.09 n.s.	0.07 n.s.	0.71 ***
pH	0.33 ***	-0.22 **	-0.12 ^	
Conductivity	0.26 ***	-0.05 n.s.		
Discharge	-0.38 ***			

Inter-annual variability of vegetation

Inter-annual dissimilarities of species abundances (Bray-Curtis distance) increase with the length of the investigated time interval (Figure 2). Median values are lowest for consecutive years (0.198 to 0.225), intermediate for non-consecutive years with a time interval of two years (0.252 and 0.268), and highest for non-consecutive years with a time interval of three years (0.318). Even though these median values represent a relatively high similarity between years, single springs show remarkable differences (outliers), irrespective of the investigated time interval.

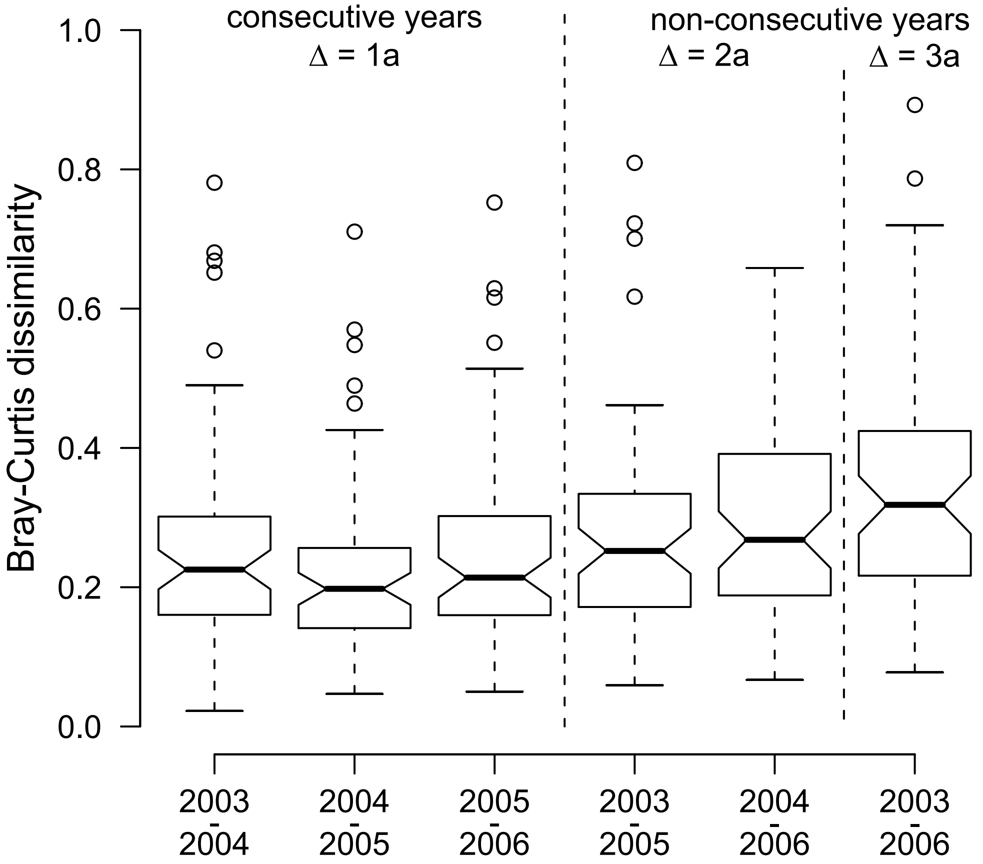

Figure 2: Inter-annual dissimilarities (Bray-Curtis distance) of species abundances (turnover) for pairs of years. Box-Whisker-Plots, thick line = median, box = 50 %, whisker = 90 % of variation, points = outliers, notches are approximations of the 95 % confidence interval of the median.

Driving factors of species composition

To get an idea of the reasons for vegetation dynamics, we first looked for the driving forces behind species composition by analysing the absolute values of species abundances and environmental parameters, implying a ***static approach*** that included data from all four years. Because the pH-value was highly correlated with ANC ($r = 0.71$, $p < 0.001$) and the pH-value explained a larger proportion of the variance in ordination, ANC was omitted from the analysis. As shown in the CCA ordination diagram (Figure 3), the pH-value was highly correlated with the first ordination axis ($r = 0.92$), which explained 24.1 % of the species variation. Therefore the pH-value was the factor, which was found to be most connected with species composition. The second CCA axis, which explained 8.3 % of the species variation, was mainly represented by electrical conductivity. All environmental parameters together (four constrained axes) explained 38.4 % of the species variation.

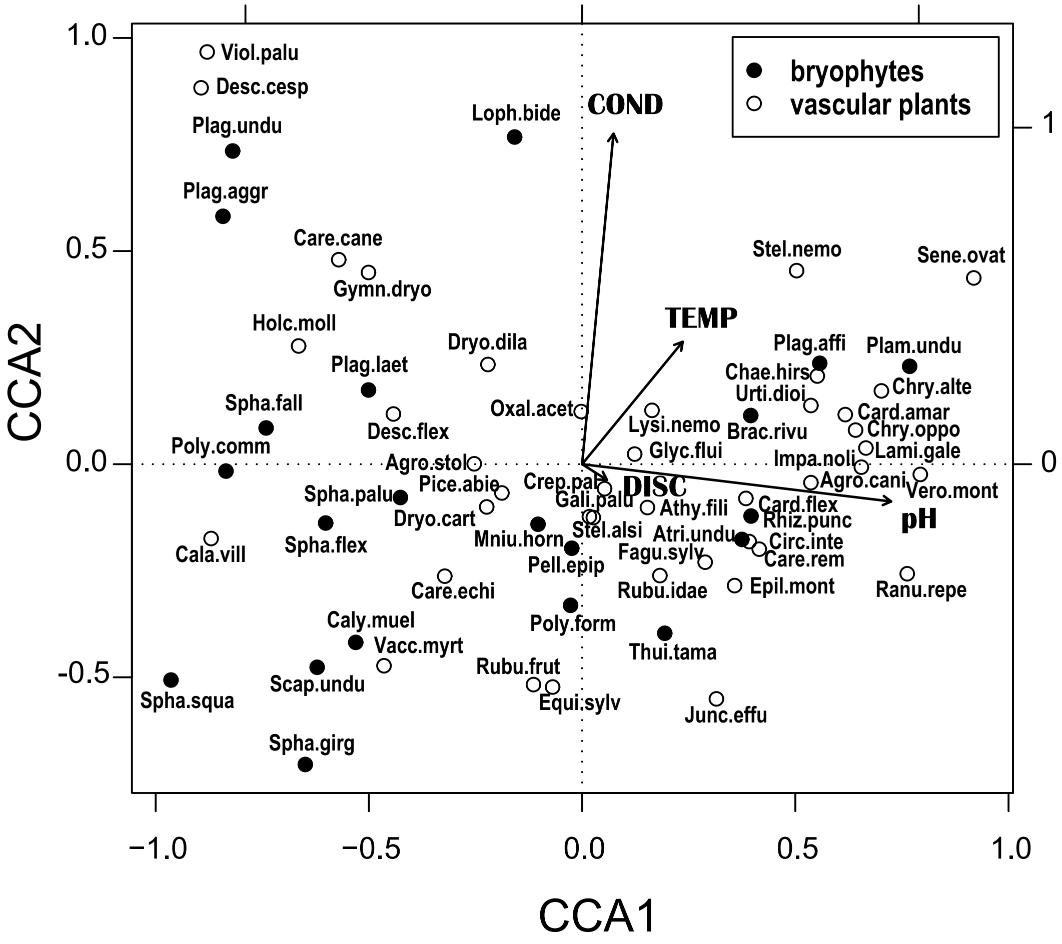

Figure 3: Driving factors of species composition (static approach). CCA is calculated with annual species abundances and the environmental parameters pH, electrical conductivity (COND), spring water temperature (TEMP) and discharge (DISC).

The herbaceous plants *Veronica montana* L., *Impatiens noli-tangere* L., *Ranunculus repens* L. and two *Chrysosplenium* species were closely connected to circumneutral pH-values. In contrast, the mosses *Sphagnum fallax* Klinggr. and *Polytrichum commune* Hedw. as well as the grasses *Calamagrostis villosa* (Chaix) J.F. Gmel. and *Holcus mollis* L. represented acidic conditions. As opposed to the pH, only a few species were linked closely to electrical conductivity.

Driving factors of vegetation dynamics

In contrast to CCA where values represent single points of time (static approach, cf. Figure 3), we additionally calculated a CCA based on the inter-annual turnover in species abundance and in environmental parameters, with the goal of unraveling the driving forces behind inter-annual species dynamics. In this ***dynamic approach*** correlations and ordinations were calculated separately for pairs of consecutive years (interval 1 year) and for pairs of non-consecutive years (interval 2 and 3 years), with a view to gaining some insights into the response time of species to changes in environmental conditions. For pairs of consecutive years the changes in environmental parameters together (4 constrained axes) explained a total of only 3.3 % of the variation of species abundance changes. For pairs of non-consecutive years this value increased to 6.3 %. Consequently, the explanatory power of the four environmental parameters investigated increased with the length of the time interval.

Discussion

Inter-annual environmental changes

Contrary to the assumption of constant environmental conditions in springs (Odum, 1971; Ellenberg, 1996), we found considerable inter-annual variability in the hydro-physical and hydro-chemical properties of spring waters (Figure 1). This was the result that we expected, as Beierkuhnlein & Gräsle (1998) already indicated the seasonal variability in siliceous springs. Spring water pH-values were highest in the extremely hot and dry summer of 2003, which was accompanied by the lowest discharge with some springs actually running dry. As low discharge reflects a low intensity of groundwater fluxes in the aquifer, it can be expected that the residence time in the catchment was longer as a result. A longer residence time in mineral soils, however, is accompanied by geo-chemical reactions and leads to an increased buffering of acidic waters (Kleber et al., 1998).

Lower pH-values and higher discharge rates of the following years (2004 to 2006) were related to higher amounts of summer precipitation. These findings contrast with studies that found a decrease in pH-values along with drought in mires, while the pH-value in springs and fens was found to reach maximum values as a result of aeration caused by turbulent water flow (Sparling, 1966; Tahvanainen & Tuomaala, 2003; Hájková et al., 2006). If the percolated substrate is strongly influenced by acidic humic substances, then longer residence times might actually contribute to increasing acidification. Furthermore, aeration might contribute to hydro-chemical differences when carbonic acid is high in spring water. However, the springs that were investigated in this study neither showed a large discharge capacity nor a large discharge variability.

Springs in our study area can be characterised as perennial, with minor variations of discharge. Nevertheless, in exceptionally dry years such as 2003, aerobic processes in the topsoil can replace anaerobic conditions during dry phases, which occur during precipitation, leading to the recurrence of spring water discharge and saturated phases. Kleber et al. (1998) relate the acid-base status of springs mainly to oxidative and respectively reductive processes in the mineral substrate of the catchment. This is supported by the hydro-geological setting of shallow aquifers and small catchments with high drainages and consequently low residence times in the interflow. The residence time, which is a function of catchment size, slope as well as the amount and form of precipitation, determines the time, which is available for buffering processes. In this way, exceptionally dry conditions can prolong the residence time and consequently enhance the pH-values of spring water. Hence, we conclude that hydrological patterns within springs are subject to climate-induced, short-term oscillations around a certain, more or less constant equilibrium value.

Vegetation's response to inter-annual environmental changes

In the ***static approach*** the ordination clearly emphasised the pH-value as a major driver of species composition (Figure 3), by determining nutrient availability. This confirms several studies from temperate ecosystems in the northern hemisphere that found the vegetation composition of mires, fens and springs to be strongly determined by water chemistry, mainly following a nutrient and acidity gradient (Vitt & Chee, 1990; Wheeler & Proctor, 2000; Bragazza & Gerdol, 2002; Tahvanainen, 2004; Hájková et al., 2008). Hence it is a tentative theory that inter-annual (as well as long-term) changes in species abundances are also driven by changes of spring water acidity. Hájková et al. (2004) assume that even small variations in the water level and nutrient richness may cause a response from spring fen vegetation.

In the ***dynamic approach*** we observed predominantly low dissimilarities between pairs of consecutive years regarding species composition and abundance structure (Bray Curtis distance; Figure 2), in spite of varying environmental conditions. Nevertheless, these inter-annual changes are remarkable, as they contradict the assumption of springs being constant ecosystems. Spring communities are mostly characterised by a few dominant species, making an investigation of the reaction of plant communities to changes in the environment easier. Cantonati et al. (2006) describe springs as stable ecosystems integrating in time and space, and therefore ideal study sites for long-term ecological research. But, species-poor communities (and hence also springs with only a few dominant key species) are assumed to show low resilience in the face of an accelerated frequency of disturbance events (White & Jentsch, 2001). Heino et al. (2005) demonstrated how formerly subordinate spring bryophytes dominated more after disturbance, whereas these species remained rather constant through time in near-pristine springs with low human impact.

Even though the pH-value acts as a leading determinant of species composition in forest springs by affecting nutrient availability, its changes cannot explain species inter-annual variation. Likewise, the other investigated environmental parameters (i.e. water temperature, discharge, conductivity) cannot be given as a reason for inter-annual changes in species abundances, as shown by their marginal explanatory power in the CCA based on inter-annual change values (3.3 % for pairs of consecutive years; 6.3 % for pairs of non-consecutive years). However, the explanatory power of the four environmental parameters almost doubles from consecutive years (with an interval of 1 year) to non-consecutive years (an interval of 2 or 3 years). This finding suggests that species response to environmental changes in spring communities can only be encountered over longer time-scales. The static approach supports this theory, as the respective CCA generates fairly good results. There the environmental parameters explain 38.4 % of species variation. We attribute slow changes in plant species abundance to their inertia (delayed or long-term integrating response) and persistence patterns, and therefore the 'extinction debt' (Tilman et al., 1994) as well as the 'colonisation lag' (Menéndez et al., 2006).

Apart from the acidity and nutrient richness gradient other environmental factors may influence inter-annual shifts in species performance. Beierkuhnlein & Gräsle (1998) detected that spring vegetation depends on light supply, which in turn reflects the shade provided by the surrounding forest stands. Even if Beierkuhnlein & Gräsle (1998) rank the influence of near-surface radiation behind that of the acidity gradient, it is possible that light could modulate plant species abundances and therefore cause inter-annual variations. Hájková et al. (2004) confirmed pH-values and conductivity as major variables that are related to species composition. However, they characterise these parameters as remaining steady compared to other hydrological parameters. They discussed water level variations as the main reason for the short-term variability of spring fen vegetation.

Other hydro-chemical constraints, like toxic substances in the spring water seem to be of little importance for inter-annual variability, since they are relatively constant in forested catchments. Spatial factors, such as the extent of the spring habitats or the distance between them, can affect seed dispersal and consequently species occurrence and establishment in island-like spring habitats. This issue however is also only expected to become crucial in the long run.

Conclusion

Inter-annual variability of the vegetation of forest springs was indeed found, but its hypothesized connection with changes to hydrological properties, in particular the acidity and nutrient availability status, was not verifiable (dynamic approach). However, as ordination methods clearly indicate that the pH-value is a major determinant of species composition (static approach), we suggest that changes in abundances are subject to species inertia and are driven by pH-changes in the long run.

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Manuscript 5

Are bryophytes better indicators for inter-annual changes in spring water quality than vascular plants?

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Abstract

Due to the constancy of thermic and hydric site conditions, springs and spring-inhabiting organisms are considered to serve as sensitive indicator systems for biogeochemical changes within their catchments. However, groups of organisms differ in their dependence and response to environmental change. Species that are directly exposed to a medium whose conditions have to be indicated are expected to be more sensitive than species that take up water and nutrients via the soil. This is why we hypothesise that even partly or temporarily submerged bryophytes that have no rooting system and take up nutrients directly at the plant surface will serve as more sensitive indicators for spring water quality than vascular plants, where nutrients and other compounds are incorporated via the soil and the root.

In this study, we analysed species response to inter-annual changes in spring water quality over a period of four consecutive years, aiming to compare the indicator suitability of bryophytes and vascular plants. For this purpose, we first calculated the inter-annual turnover of species composition for both taxonomic groups separately, using Bray-Curtis-dissimilarity index. Then we related inter-annual change values of species abundances to alterations of spring water pH-value. Unlike expected, bryophytes did not show a stronger correlated response to inter-annual variability of the environment than vascular plants. Actually, only the minority of all species showed abundance changes which were significantly correlated to variations in spring water acidity.

A delayed or long-term integrating response of species to environmental changes restricts the possibilities of indicating inter-annual environmental changes. Therefore, the biomonitoring of acidification or recovery processes in springs is feasible only on longer time-scales. In general, indicator suitability for investigating a status quo (static approach) is different to that for the detection of environmental changes (dynamic approach). The response times of indicator species must be adequate to the velocity of the observed process, when appraising indicator applicability for the monitoring of ecological alterations like acidification and climate change.

Keywords

ecosystem dynamics, indicator suitability, inertia, resilience, response time, species turnover

1. Introduction

The time that species need to respond to alterations of their environment or specific site conditions is an important issue in ecological research. Elementary biogeographical phenomena such as the ‘extinction debt’ (Tilman et al., 1994) and the ‘colonisation lag’ (Menéndez et al., 2006) are caused by a ***delayed response*** of organisms to changes in their environment. But, not only these fundamental incidents of extinction and colonisation are brought about by a lagged response, also alterations in species abundance and therefore in community composition occur time-delayed. Processes which are responsible for this time-lag, like inertia, persistence, and resilience as well as habitat isolation and dispersal limitation are effective on a multitude of temporal and spatial scales.

Currently, the topic of response time of organisms and their communities to environmental changes is discussed predominantly in the context of climate change (e.g., Prentice, 1986; Gutschick and BassiriRad, 2003; Menéndez et al., 2006; Bêche and Resh, 2007; Walther, 2007; Borrvall and Ebenman, 2008), but is also relevant for conservation issues (Tilman et al., 1994; Helm et al., 2006) and the assessment of the suitability of indicator species (Milner et al., 2006; Paltto et al., 2006). However, regarding ***indicator suitability***, a prolonged response time can have unwanted as well as desirable effects. As a matter of course, for an early warning system an immediate response of indicator organisms is indispensable. On the other hand, a lagged response - which is integrating over a longer time-interval - can eliminate short-term fluctuations from the general information. This way, indicator species exhibit an advantage over instrument-based analytical methods, which only represent single moments in time. In order to contribute to this indicator suitability issue, we compare the performance of bryophyte and vascular plant species regarding their response to inter-annual variability in hydrological parameters over a period of four consecutive years.

Bryophytes do not own an efficient rooting system, no conducting tissue, and no epidermal tissue; they absorb water and therein dissolved mineral nutrients and contaminants directly by their surface. Therefore nutrients and toxic substances can affect bryophytes directly (Brown, 1984; Bates, 2000). Some bryophytes perform short life cycles and hence are expected to respond rapidly to a changing environment. Therefore, bryophytes are considered to be more sensitive indicators for environmental variations than vascular plants (Frahm and Klaus, 2001; Bates et al., 2005). Concerning these facts, we hypothesise bryophytes to show a stronger correlated response to the inter-annual variability of the environment than vascular plants.

Springs provide some features which constitute them to be an interesting object for studying species-environment relationships. They make up the interface between the aquifer and lotic waters and are known for their constancy regarding hydrochemical and hydrophysical factors (Odum, 1971; Ellenberg, 1996). Because water flow is unidirectional and more or less continuous, spring organisms refer strongly to ground water hydrology. For the vegetation of springs the gradient of acidity and nutrient availability plays the fundamental role, as several studies have shown (Beierkuhnlein and Gräsle, 1998; Wheeler and Proctor, 2000; Hájek et al., 2002; Hájková et al., 2008; Strohbach et al., submitted). Hence, highly specialised crenobiotic and crenophilic plant species can be seen as suitable indicators for the monitoring of ground water quality (Cantonati et al., 2006), and in particular ground water acidification (Beierkuhnlein, 1996). When environmental conditions are altered (e.g. due to acid depositions or climatic changes), responses by spring-inhabiting species and their assemblages are likely to occur (Glazier, 1991; Zollhöfer et al., 2000). It is expected that such changes in spring water quality will affect particularly the stenoecious crenobiotic and crenophilic species (Ferrington, 1998; Zollhöfer et al., 2000; Cantonati et al., 2006). But, the capacity of resilience and the inertia of spring-inhabiting species are widely unknown. The response time of species and taxonomic groups to alterations of spring water quality has not yet been investigated.

However, in many cases the complexity of ecological processes hampers the separation between cause and effect. Spring ecosystems provide a natural setting that reduces these constraints. Especially helocrenic springs (sensu Thienemann, 1924), where ground water seeps out to the surface at low discharge rates creating a spatially extended but small scaled spring fen, are surrounded by habitats with disparate characteristics. For many species, these sites resemble islands within a matrix of unfavourable conditions. Therefore neighbour effects are low and negligible. In combination with the unidirectional water flow, springs feature a reduced connectivity. Hence, we expect a higher relevance of internal factors, which makes it easier to relate changes in species abundances to changes in their environment. The landscape context, which influences vegetation succession elsewhere (Prach and Řehounková, 2006), plays an inferior role in springs.

The small extent of spring habitats and the permanently high wetness don't allow for land use options, hence human interest is low. Consequently, springs are pristine habitats and therefore a notable exception to the predominantly anthropogenic ecosystems of Europe. Even though springs cover only a small portion of the landscape, they are abundant in forested mountain ranges of Central Europe. This numerous appearance qualifies them for investigations on the landscape scale (Beierkuhnlein and Durka, 1993).

2. Materials and Methods

2.1. Study area and sites

We investigated 57 springs, located in Frankenwald and Fichtelgebirge mountain ranges (NE-Bavaria, central Germany, 49.9° - 50.6° N, 11.2° - 12.2° E, 350 - 1050 m a.s.l.). The petrography of these landscapes is relatively homogenous, it throughout consists of silicates - palaeozoic schist, phyllite, greywacke, gneiss and granite. Long-term mean air temperature ranges from 4 to 7 °C and precipitation from 750 to 1350 mm. Forests, which are mainly dominated by Norway spruce (*Picea abies* (L.) H. Karst.), in parts also by beech (*Fagus sylvatica* L.), are the main constituent of these landscapes. Acidic depositions affected the region in the 1970s and 1980s, leading to an acidification of ground, spring and head waters.

In our study area ground water flow takes place in near-surface layers (interflow), because of the compact bedrock with little pore volume. Springs are generally formed as helocrenic springs (sensu Thienemann, 1924). Here, ground water seeps out slowly from a saturated area of a few to 500 m^2 in size. Springs occur frequently on hill slopes. They feature small catchments (0.1 to 25 ha) as well as low discharge rates (mostly below 2.0 $l\,s^{-1}$). The pH-values of those nutrient-poor spring waters are acidic to neutral (pH 4.3 to 7.5), their electrical conductivity ranges between 28 and 1130 $\mu S\ cm^{-1}$ (autumn values from all investigation years). All investigated springs and their catchments are located in forests.

2.2. Field sampling and data preparation

We collected hydrological and vegetation data over four consecutive years, from 2003 to 2006. ***Hydrological data*** were sampled each year in autumn (end of September), always during a climatically dry period after at least one week without intensive precipitation, in order to avoid short-term anomalies related to rain events. We measured four hydrological parameters in situ, namely discharge, water temperature, electrical conductivity and pH-value. These measurements as well as the water sampling for the titration of the acid neutralising capacity (ANC, in vitro) were conducted at the uppermost point of the spring fen with flowing water at the surface. Because of the summer drought in 2003, nine springs were lacking in discharge ($< 0.02\ \mathrm{l\ s^{-1}}$), consequently sampling was impossible there. In 2004 two springs, in 2005 and in 2006 one spring respectively could not be sampled due to the same fact. Accordingly, the possibilities of combining vegetation with hydrological data were reduced in single springs and years.

By means of defined and fixed line transects we collected ***vegetation data*** annually during July and August. Transects were established perpendicular to the direction of discharge. They are positioned at the place with the largest width in the saturated area, and are permanently marked. Their length differs between 1.1 and 24.7 m. Presence-absence data of bryophytes and vascular plants were recorded in sections of 10 cm along the transect line. This method provides benefits regarding precision, repeatability, efficiency, and sensitivity to slight alterations in vegetation composition. Plants rooting on microsites (e.g. deadwood, stones) without contact to the seeping water were excluded from the dataset. Nomenclature follows Frahm and Frey (2004) for bryophytes and Oberdorfer (2001) for vascular plants.

To detect generality in species response patterns necessitates to make the transect lines of differing length contrastable. Consequently, the percent abundance for each species in each year was calculated as a ratio between the number of line sections with presence of this species and the total number of sections in the specific line transect. All analyses were based on these relative abundances. Species with less than 20 occurrences (in sum of four years) were omitted from the dataset. Thus, the number of species was reduced from 165 initially detected to 62 for data analyses. 21 bryophyte species and 41 vascular plant species remained.

As our aim was to detect the temporal dynamics of species abundances and environmental parameters, we calculated ***matrices of deviation*** between pairs of consecutive years (interval 1 year; $n = 3$) as well as between pairs of non-consecutive years (interval 2 or 3 years; $n = 3$). The separation of these two time scales offers the chance to get an idea of the velocity of species responses. Changes of abundances in every single species and changes in every environmental parameter are both depicted in terms of differences. Further statistical analyses regarding the species dynamics are based on these values.

2.3. Data analysis

In order to detect temporal patterns of species performance, ***dissimilarity*** between pairs of samples was calculated. We compared the corresponding data sets of pairs of years, aiming to reveal inter-annual dynamics of species abundances. Because differences between the dynamics of bryophytes and the dynamics of vascular plants were of particular interest, dissimilarities were calculated separately for these two taxonomic groups. We used the function *dist.tmp* from the R-package *simba* (Jurasinski, 2007) with Bray-Curtis as the distance measure. Due to different species numbers in the two compared taxonomic groups (n = 21 bryophytes, n = 41 tracheophytes) a calculation of significances is not included. To compensate this shortcoming and to allow estimations of the significance of disparities between both groups we display the results as notched boxplots. The notches are approximations of the 95 % confidence interval of median position.

Additionally, we applied a ***correlation analysis*** to compare the inter-annual dynamics of the abundances of single species with the dynamics of environmental factors. Since in previous studies the pH-value was identified as factor, which was best correlated to species composition at a certain point in time (Beierkuhnlein and Gräsle, 1998; Audorff et al., submitted), its role was of particular interest in this dynamic approach. As our aim was to detect species as potential indicators for short-term changes of the acidity status of the spring waters, we used Pearson linear correlation to characterise species in three ways: (1) the direction of correlation (positive or negative) specifies the response type of an indicator, (2) its slope reflects the response intensity, and (3) its significance is an indicator for the response quality.

For both approaches, the dissimilarity as well as the correlation analysis, we separated between pairs of consecutive (interval 1 year; n = 3) and pairs of non-consecutive years (interval 2 or 3 years; n = 3). This was done with the goal of identifying the velocity of species responses to environmental change.

Additionally, we carried out a ***canonical correspondence analysis*** (CCA) including species abundances and environmental data of all four years of investigation (representing single points in time, not differences between years). This was done with the aim of relating the dynamic performance of single species to its position along the main gradient of the species-environment relationship (static performance). CCA was calculated by means of the R-package *vegan* (Oksanen et al., 2007). Beforehand, species abundance as well as environmental data were log-transformed, except for pH-values, which already represent a negative logarithm. Because the pH-value was autocorrelated with ANC and the pH-value explained a larger portion of the variance in ordination, ANC was omitted from the analysis. Subsequently, species' CCA first axis scores (representing static performance) and the results of the abovementioned correlation analysis (representing dynamic performance) were interrelated.

3. Results

For the entirety of springs, autumn hydrological conditions of spring waters were relatively constant over the four year investigation period. Anyhow, we detected inter-annual variations. Looking at individual springs, these inter-annual differences were intensified (Audorff et al. submitted).

Inter-annual dissimilarities of species abundances (Bray-Curtis distance) did not show differences between bryophytes and vascular plants. The according notch sections of the boxplots were overlapping clearly in all pairs of consecutive years (Figure 1, left) as well as in all pairs of non-consecutive years (Figure 1, right). Thus, inter-annual changes in species abundances of both taxonomic groups occurred in a similar way. However, for pairs of consecutive years (interval 1 year) the medians of inter-annual dissimilarities of species abundances (turnover) were higher for bryophytes than for vascular plants, in each of the three cases (Figure 1, left). For pairs of non-consecutive years (interval 2 or 3 years) the comparison of medians resulted in the opposite (Figure 1, right). Bryophytes showed a higher range of variation in most year-to-year comparisons (total box lengths). Comparing the time scales, the medians of dissimilarity between pairs of years increased with the length of the time interval, both for bryophytes and for vascular plants.

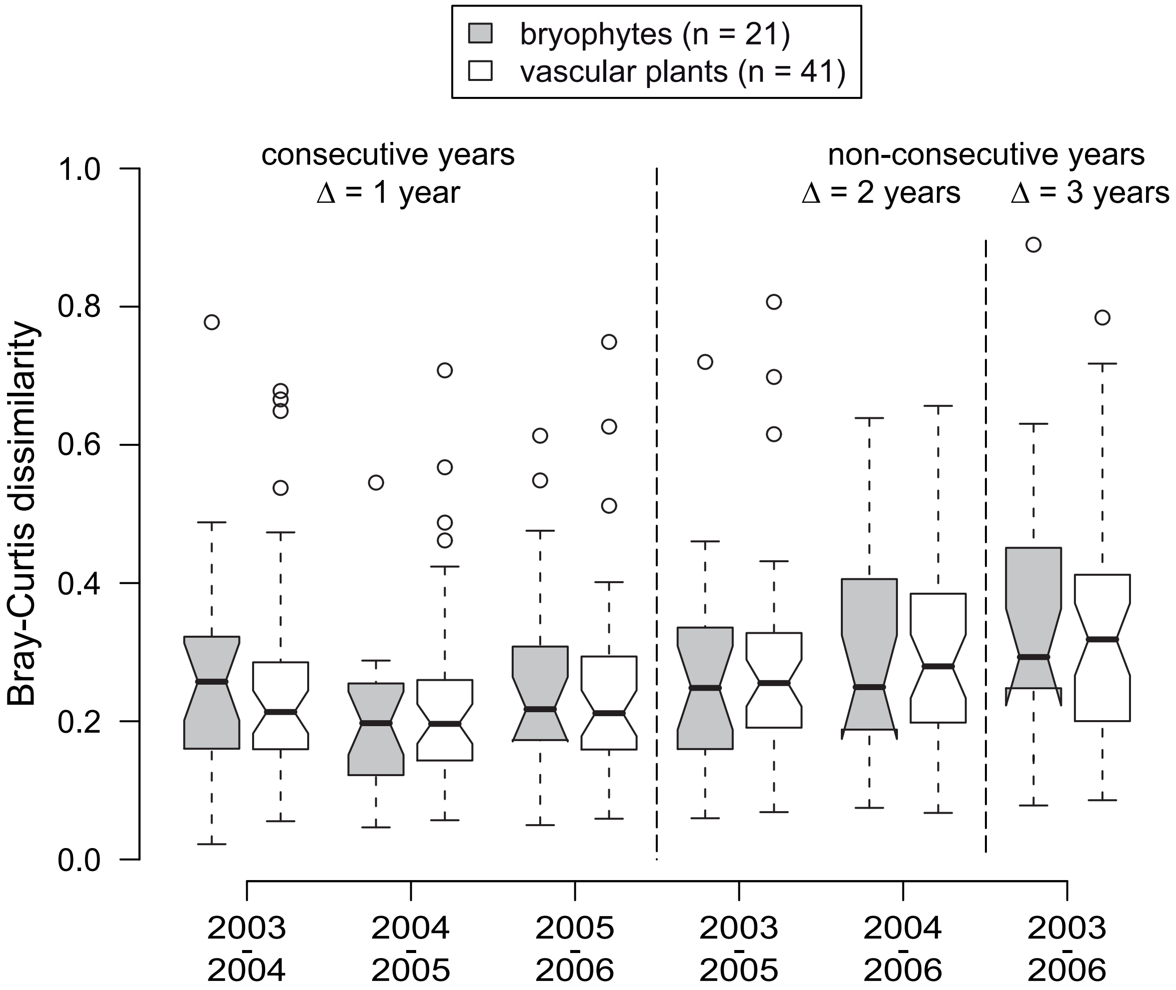

Figure 1: **Inter-annual dissimilarities (Bray-Curtis distance) of species abundances (turnover) of bryophytes and vascular plants for pairs of consecutive (left) and non-consecutive years (right). Box-Whisker-Plots, thick line = median, box = 50 %, whisker = 90 % of variation, points = outliers, notches are approximations of the 95 % confidence interval of median position.**

Concerning the response of single species to inter-annual changes in spring water pH-value, differences between species concerning response type, intensity and quality clearly turned out for both time scales (Figures 2 and 3). However, significant correlations of changes in species abundance with pH alterations were only found for the minority of species (14.5 % for consecutive, 22.6 % for non-consecutive years). Those were predominantly marking positive correlations, meaning an increasing abundance with increasing pH-value. Bryophyte species did not show stronger correlations to short term changes in pH-value than vascular plant species. When comparing both taxonomic groups no differences concerning response type, intensity and quality could be detected.

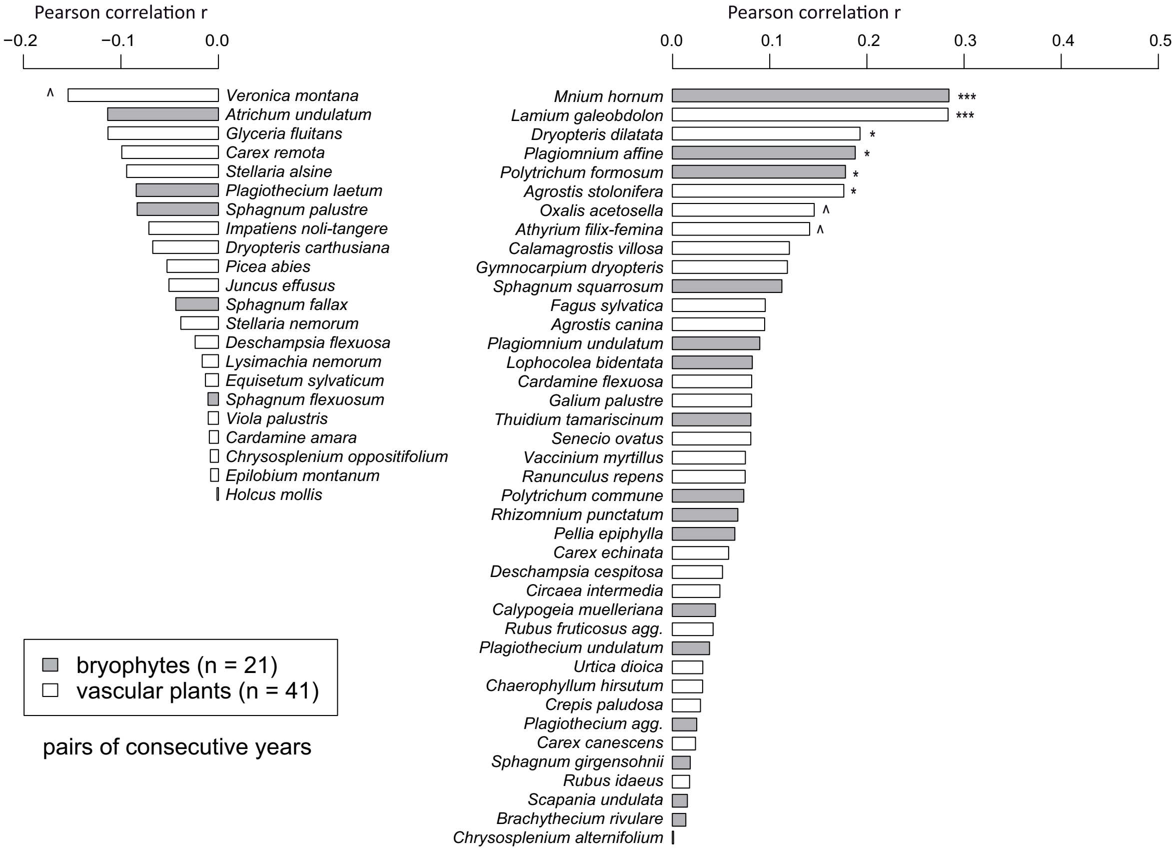

Figure 2: **Pearson linear correlation of inter-annual changes (pairs of consecutive years) in abundances with those in spring water pH. Response type (left side: negative = increasing abundance with acidification, right side: positive = vice versa) and response intensity (length of bars) of bryophyte (n = 21) and vascular plant species (n = 41) are depicted. Response quality is reflected by the significance levels: $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), $p < 0.1$ (^).**

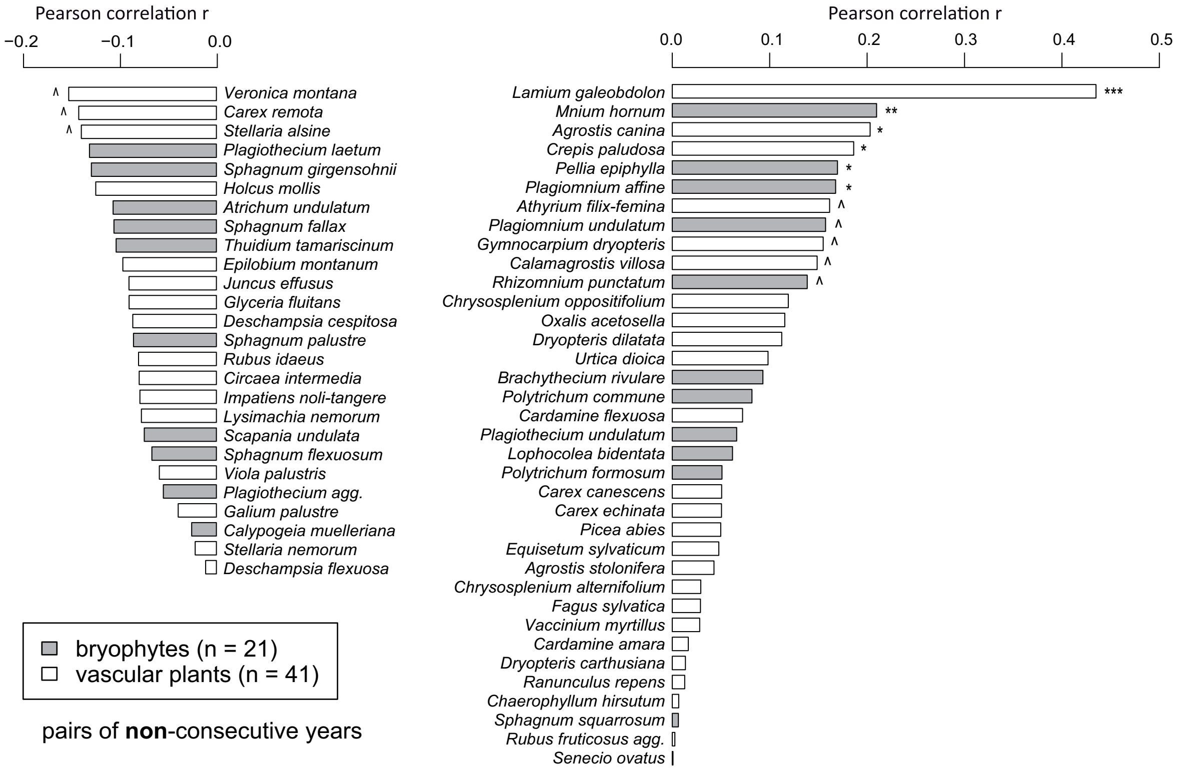

Figure 3: **Pearson linear correlation of inter-annual changes (pairs of non-consecutive years) in abundances with those in spring water pH. Response type (left side: negative = increasing abundance with acidification, right side: positive = vice versa) and response intensity (length of bars) of bryophyte (n = 21) and vascular plant species (n = 41) are depicted. Response quality is reflected by the significance levels: $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), $p < 0.1$ (^).**

In the CCA ordination (Figure 4) the first ordination axis, which explained 24.1 % of the species variation, was highly correlated with the pH-value ($r^2 = 0.85$). Hence the pH-value was found to represent species composition best. The second CCA axis, which explained 8.3 % of the species variation, was mainly connected to electrical conductivity. All environmental parameters together (four constrained axes) explained 38.4 % of the species variation.

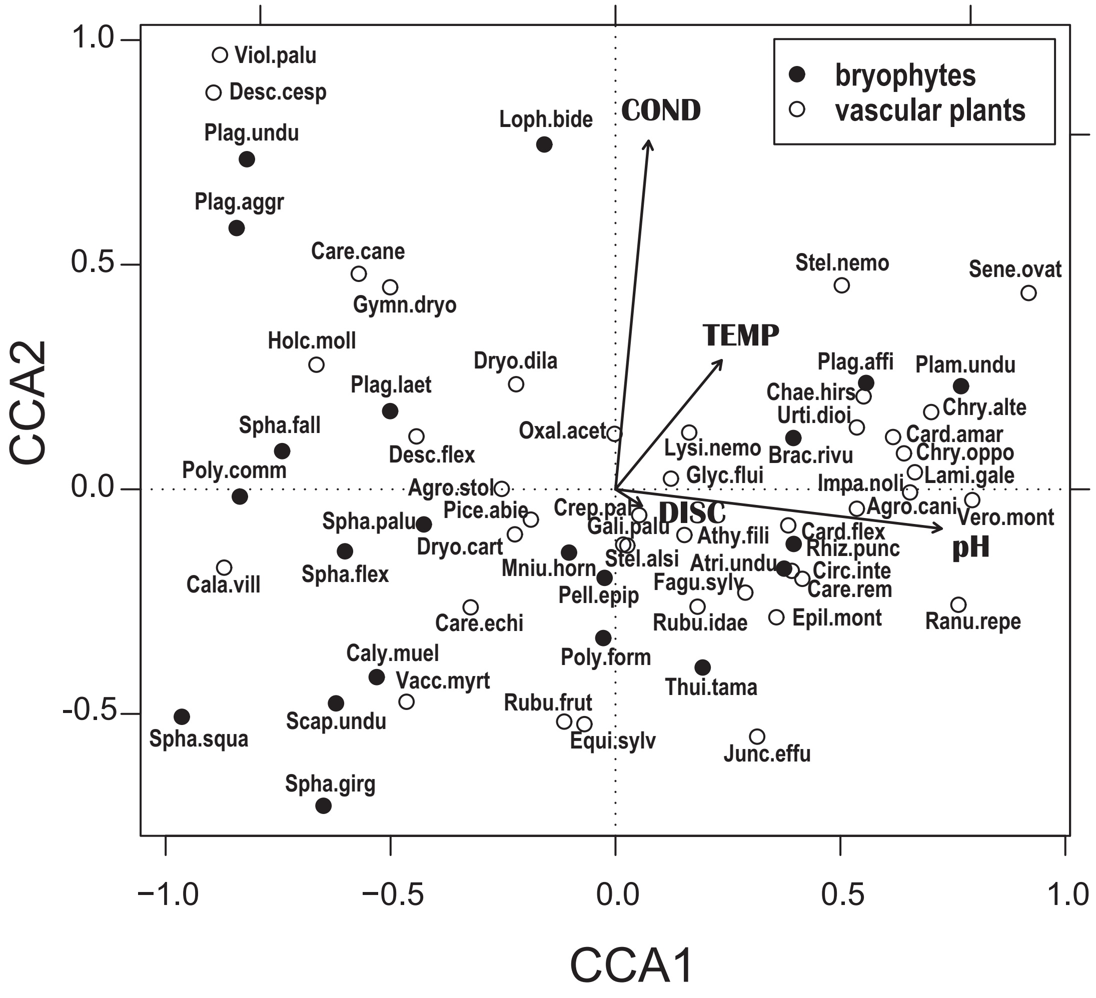

Figure 4: **CCA ordination diagram, including abundances of 62 species (21 bryophytes and 41 vascular plants) and the environmental parameters pH, electrical conductivity (COND), spring water temperature (TEMP) and discharge (DISC).**

Comparing species locations in CCA ordination (static performance) with those in Pearson correlations (dynamic performance) by correlating species' CCA first axis scores (cf. Figure 4) with their Pearson r-values (cf. Figures 2 and 3), no significant relationship appeared (consecutive years: $r = -0.11$, $p = 0.374$; non-consecutive years: $r = 0.18$, $p = 0.154$). Species spanning the first CCA axis, thus marking the extremes of the pH gradient, were arranged widespread in the Pearson correlation ranking (Figures 2 and 3), which represents the species' suitability for indicating pH alterations.

4. Discussion

4.1. Vegetation responds to inter-annual environmental changes

Albeit the Bray-Curtis distances indicated relatively low inter-annual dissimilarities regarding species composition and abundance structure (Figure 1), these inter-annual vegetation changes are noteworthy. After all, they confine Odum's (1971) and Ellenberg's (1996) assumption of springs to be constant ecosystems.

The CCA in our study clearly highlighted the pH-value as main driver of species composition (Figure 4), presumably by regulating nutrient availability. This finding is in accordance with several other studies, which report the gradient of acidity and nutrient availability to be the fundamental determinant of the vegetation of springs (Beierkuhnlein and Gräsle, 1998; Wheeler and Proctor, 2000; Hájek et al., 2002; Hájková et al., 2008; Strohbach et al., submitted). Accordingly, we anticipated that changes in the pH-value would also be the driver of inter-annual changes in species abundances. But, contrary to our expectation, pH changes could explain species inter-annual variation significantly only for the minority of species (Pearson correlation; Figures 2 and 3), independent of the investigated time scale. However, the proportion of species with a significant response to changes in spring water pH was higher for non-consecutive (22.6 %) than for consecutive years (14.5 %). This suggests that species response to environmental changes is time-delayed or integrating over longer time intervals and hence is a matter of longer time-scales.

Comparing the species performance in the CCA ordination (static performance; Figure 4) with those in the Pearson correlations (dynamic performance; Figures2 and 3), no clear patterns showed up. Species spanning the first CCA axis, thus marking the extremes of the pH gradient, were arranged widespread in the Pearson correlation ranking, thus describing species' dynamic response. This holds true for both time scales of consecutive and non-consecutive years. In conclusion, this result has serious implications regarding indicator suitability. Species indicating the acidity status of spring waters are no appropriate indicators for inter-annual alterations of spring water acidity. Whether in return these species are able to signalise long-term trends of acidification or recovery has to be shown by further studies.

We attribute the slow changes in plant species abundance to species' inertia (delayed or long-term integrating response) and persistence patterns. The imbalance between the positive and the negative species response types (Figures 2 and 3) underpinned this assumption, as especially species which mark the acidic end of the gradient (most negative CCA first axis scores in Figure 4) were ambiguous regarding their response type. Those species were as well grasses growing in carpets (*Calamagrostis villosa* (Chaix) J.F. Gmel.) or tussocks

(*Deschampsia cespitosa* (L.) P.B.) as peat-forming mosses (*Polytrichum commune* Hedw., *Sphagnum squarrosum* Crome, *S. fallax* Klinggr. and *S. girgensohnii* Russ.), which are known to have long life cycles and to be rather persistent (Wild et al., 2004; Rydin et al., 2006). Our results also agree with the observational and experimental studies of Kooijman (1992) and Kooijman and Bakker (1995), who show *Sphagnum* mosses in altered conditions to be superior competitors to habitat specialist bryophytes.

4.2. No differences between bryophytes and vascular plants

Unlike expected, bryophytes did not show a stronger correlated response to inter-annual changes in environment than vascular plants. Some recent studies (Bragazza and Gerdol, 2002; Miserere et al., 2003) indeed demonstrate that bryophytes show a more sensitive reaction to acidity-alkalinity gradients than vascular plants (which are mainly determined by the availability of nutrients). Hence, differences in those species groups' responses to altered conditions were to be assumed. Malmer et al. (1994) give an account of a competitive advantage of *Sphagnum* species by their more efficient use of nutrients and therefore the reduction of the nutrient supply for vascular plants. Hájková and Hájek (2004) report that bryophytes and vascular plants respond in an analogous way to the base-richness gradient in spring fens, though vascular plants are restricted also by additional ecological factors like water level variations.

Comparing the Bray-Curtis dissimilarity values between pairs of consecutive and non-consecutive years, we could not find significant differences in the inter-annual variability of species abundances between the two taxonomic groups. In the Pearson correlation ranking we found twice as many vascular plant species as bryophyte species significantly correlated to inter-annual changes in pH-value (6 vs. 3 in consecutive, 9 vs. 5 in non-consecutive years), which was consistent to the ratio of species investigated (41 vs. 21). We could not identify differences concerning response type, response intensity and response quality between the two taxonomic groups.

However, the individual species performance and consequently species composition and species richness are influenced by a multitude of abiotic factors such as spring size, elevation, light supply, water chemistry, and discharge variations (Beierkuhnlein and Gräsle, 1998; Hájková and Hájek, 2003; Hájková et al., 2004; Hájková et al., 2006). But also biotic factors such as above-ground biomass (Gough et al., 1994; Beierkuhnlein and Gräsle, 1998; Bergamini et al., 2001) and other competition-related processes within a single plant community (e.g., Moore and Keddy, 1989; Virtanen et al., 2001; Körner et al., 2007) can affect individual species performance. In our case, changes in the species abundance on the inter-annual time scale could not be attributed to changes in spring water pH-value alone. Aside from other environmental factors, also species' functional traits and strategies which condition species' ability to respond, may act here. Those do not necessarily depend on the taxonomic categories of bryophytes and vascular plants, most likely the response behaviour is species-specific. If differences in the dynamic performance (i.e. response time) between bryophytes and vascular plants occur at all, they are a matter of longer time-intervals.

5. Conclusion

Regarding their dynamic performance, no significant differences were found between the two taxonomic groups of bryophytes and vascular plants, neither by comparing inter-annual dissimilarities of their species composition nor by relating species response to inter-annual changes in environment, i.e. pH-value. Thus we have to rebut our hypothesis: bryophytes did not show a stronger correlated response to inter-annual variability of the environment than vascular plants. Indeed, only the minority of species featured abundance changes which could be correlated significantly to pH variations, independent of its membership in one of the two taxonomic groups. We suggest that species inertia retards changes in species abundances. Nonetheless, as CCA ordination clearly revealed the pH-value as major determinant of species composition (static performance), we expect pH-alterations to be effective in the longer run. Thus we conclude that the biomonitoring of spring water acidification or recovery requires longer time-scales.

Our findings have profound consequences for biomonitoring issues. The attribution of a single species to be a suitable indicator differs between the investigation of a status quo (static approach) and the detection of environmental changes (dynamic approach). A delayed or long-term integrating response of a potential indicator species must be considered in the evaluation of its indicator suitability. A monitoring of ecological processes (e.g. acidification, climate change) by means of indicator species is meaningful only when the velocity of their response is adequate to the velocity of the observed process. Admittedly, it's all very well to say that, because finally we have to recognise that the time-lag between environmental change and species response is widely unknown.

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List of publications

* Publications which are part of this thesis are marked with an asterisk.

Peer-reviewed Journals

* **Audorff V.**; Kapfer J. & Beierkuhnlein C. (in prep.): The role of hydrological and spatial factors for the vegetation of Central European springs. Ecohydrology.

* Strohbach M.; **Audorff V.** & Beierkuhnlein C. (submitted): Drivers of species composition in siliceous spring ecosystems: groundwater chemistry, catchment traits or spatial factors? Journal of Limnology.

* Zang C.; **Audorff V.** & Beierkuhnlein C. (submitted): Is the delineation of niche attributes a matter of spatial scale? Journal of Biogeography.

* **Audorff V.**; Kapfer J. & Beierkuhnlein C. (submitted): Inter-annual vegetation dynamics in forest springs. Freshwater Biology.

* Kapfer J.; **Audorff V.**; Hertel E. & Beierkuhnlein C. (submitted): Are bryophytes better indicators for inter-annual changes in spring water quality than vascular plants? Ecological Indicators.

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