Chapter 9

Hydromorphology and Biodiversity in Headwaters — An Eco-Faunistic Substrate Preference Assessment in Forest Springs of the German Subdued Mountains

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Additional information is available at the end of the chapter

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1. Introduction

Springs are autochthonous inland freshwater ecosystems, which occur where groundwater reaches the surface [1-2]. From a limnological point of view springs are divided into two subtypes: the springhead (eucrenal) and the springbrook (hypocrenal), because of a differentiation in their species composition caused by differences of structural and environmental parameters [3]. That is only part of the reality for the hypocrenal when springs connected with flowing surface waters and be integrated into the upper part of a stream system (headwater). Regarding the common limnological spring types based on hydromorphological properties (rheocrene spring: fast flowing or falling water occurrence; helocrene spring: diffuse or laminar flowing water occurrence; limnocrene spring: water occurrence in a still water pool), springs can also occur in still surface waters without run-off [4-5]. This is of importance for the understanding and interpretation of species presence and biodiversity of springs, because depending on the spring type it is a lotic or a lentic aquatic ecosystem with an appropriate flow velocity as a hydromorphological factor (lotic: 0.1 to 1 m/s; lentic: 0.001 to 0.01 m/s) [6]. Furthermore, it should be emphasized that springs are ecotones with boundary or transition areas between different habitats [7]. The species composition is influenced by interacting with other different species communities and can be characterized as taxa rich regarding the whole habitat (crenon) [8]. Beside typically aquatic spring species (crenocenosis) other aquatic fauna elements occur from groundwater (stygobionts) and related surface waters (brook/river biota or still-water biota). Also semi-aquatic and terrestrial fauna are an integrated part in spring ecotones with specific transition zones as fauna elements (semi-aquatic: Fauna hygropetrica, Fauna liminaria; terrestrial: hydrophilic terrestrial fauna) [9-10]. Springs in the German subdued mountains are commonly cold stenothermic habitats, which means the mean annual
water temperature is about the local mean air temperature (8-12°C) without higher annual amplitudes for the springhead (2°C) and moderate low annual amplitudes for the springbrook (5°C). This abiotic peculiarity of a more or less isotherm setting means that in spring ecosystems relatively constant environmental conditions are proclaimed. However, there are other important key factors or filters, especially geochemical parameters (e.g. pH value, nutrient content) that influence the occurrence and distribution of species in springs. In this case, the spatial dimension or scale is taken into consideration. The spring area size is usually small (a few square meters), but structures and functions of the spring ecosystem are an integral component of the landscape and manifold linked with other landscape elements. Based on the concept that a water body is strongly influenced by landform and land use within the surrounding catchment at multiple scales, the term springscape illustrates the relationship and spatial embedding of ecological structures and functions regarding biodiversity. Most ecological studies of spring species and communities focus on the distribution within the entire spring area as the habitat, e.g. to characterize the strength of binding to the spring habitat (stenotypy). Undisturbed forest springs of the mid-latitudes in Europe have a predominantly mosaic hydromorphological structure that suggests a potential differentiation of the colonization of substrates as microhabitats. It follows that the eucrenal itself is not a discrete spatial entity at the micro scale, because it is made of different substrate types that build heterogeneous mosaic-like structures or patches. It is possible to subdivide the spring level at the nano scale, because invertebrates and other organisms inhabit the substrata. However, the fauna-microhabitat-relationship of springheads (eucrenal) has not been studied sufficiently, so this research wants to fill that gap to quantitatively describe and qualitatively assess substrate preferences of invertebrates in springheads as an ecotone.

2. The ecohydrological importance of substrates as microhabitats in springheads — State of research and open questions

Substrate is a complex variable of the physical environment and itself a basic material usually to build out heterogeneous patches in aquatic ecosystems. Substrate is an important ecohydrological component that influences the occurrence, adaptation, survival and reproduction of the springhead fauna. Catchment properties like land use pattern, parent rock material of soil genesis, slope position and slope inclination as well as hydrological structures like spring type (flow regime), surface roughness, vegetation / forest structures and soil texture determine substrate types and their composition. There are inorganic or mineral and organic substrate types with separate corresponding nomenclature and classification. Table 1 show a classification based on size categories of mineral particles. Organic substrate types vary greatly in size and a systematic classification by size class does not seem very practical. However, a consistent and therefore comparable nomenclature in freshwater ecology is helpful for interpreting structures and functions of microhabitats. Here, especially for the river bed assessment within the implementation of the European Water Framework Directive a standardized designation for organic substrates as organic microhabitats exists and provides the basis for adaptation to the conditions of springheads (Table 2).
Table 1. Nomenclature and classification of inorganic / mineral substrate types in springheads. See [1] and [33].

Structures and functions of substrates in running waters with a distinct hydrological flow regime are very well investigated [13, 18-19] and a special discipline has evolved: The river bottom ecology [20]. The research results from fluvial ecosystems cannot be transferred automatically, because of the special environmental conditions of springheads (e.g. cold stenothermic, oligotrophic, mostly low flow velocity) and their small sized ecotone characteristics. However, the bottom substrate in fluvial ecosystems like brooks and rivers is often one of the most significant factors affecting the species composition of the benthic fauna in the substratum [21-26]. Some studies consider the mapping of substrate types in springs for a hydromorphological based water typology [27-29], but without a given classification scheme and a method instruction for the assessment of substrate type coverage within a field survey. Referring to an estimation procedure by [30] a first combining example for a coverage classification and a description for an ecological assessment procedure for springheads and springbrooks is given by [31]. There are five classes based on the aggregation of levels of

Figure 1. Influence of hydromorphological properties on the springhead fauna. Modified after [32].
coverage: 0 – absent; 1 – low level (10-20 % coverage); 2 – medium level (30-40 % coverage); 3 – strong level (50-60 % coverage) and 4 – continuous level (> 70 % coverage). The mapping of the substrate as a potential microhabitat, combined with a simultaneous integrated field sampling of the invertebrate fauna based on the coverage of substrate types as a water type specific method has so far been developed only for brooks (rhithral) and rivers (potamal), e.g. [33-34]. Studies on the role of substratum for species richness in springs are executed, but with different levels of detail of the research question with regard to the substrate preference of species. A study in the limestone Alps of Austria shows that different substrate types emphasized the differences of species composition and abundance in springs on carbonate substrata [35]. The microhabitat preferences of benthic invertebrates and especially for Oligochaeta has been studied and illustrated by the example of karst springs in the Krakow-Czestochowa Upland in Southern Poland [17]. Here, the substrate type was found to be the main discriminatory factor with regard to the fauna density. Orthocladiinae (subfamily of non-biting midges), Cyprididae (ostracods), Turbellaria (planarians) and only one Oligochaeta (Nais communis) were more abundant in coarse mineral substratum whereas Chironominae (non-biting midges), Limnephilidae (northern caddisflies), Bythinellinae (prosobranch spring snails) and most Oligochaeta (subclass of earthworms) were more numerous in fine mineral substratum. Another outcome of this research is, that from a higher substrate heterogeneity results a higher biodiversity. In a study with the aim to find environment variables, which represent the species composition of fauna in certain assemblages of Danish springs, the result is that higher substrate heterogeneity increases the biodiversity especially in helocrene springheads [36]. The correlation between substrate type diversity and richness in species are also confirmed by investigations in Canadian springs [37] and in springs of the USA [38] for North America. Even [39] can also show a clear relationship between the species composition of insects in springs of the Sacra catchment (Adamello Brenta Regional Park, Italy) and the grain size of mineral substrate. The occurrence of certain substrate types determines fauna assemblages as a key factor beside physical-chemical parameters in a study in perennial limestone springs in Northwest Switzerland [40]. In a different geological setting of perennial siliceous sandstone springs in the Nationalpark Pfälzerwald (Southwest Germany) also the

| Organic Substrate          | Description                                                                 |
|----------------------------|-----------------------------------------------------------------------------|
| Emergent macrophytes       | Spring-fed herbaceous macrophytes                                           |
| Submerged macrophytes      | Subaquous herbaceous macrophytes (partly above the water or completely under water) |
| Moss cushions              | Contiguous patches or layers of mosses                                      |
| Fine roots                 | Floated living fine roots of the riparian area                               |
| Xylal                      | Dead wood, non-living tree trunks, branches and/or roots                     |
| CPOM                       | Course particular organic material (e.g. leaf litter)                        |
| Coniferous litter          | Only needle litter of coniferous trees or shrubs                             |
| FPOM                       | Fine particular organic material                                             |
| Algae                      | Filamentous algae, algal tufts                                              |

Table 2. Nomenclature of organic substrate types in springheads. See [10] and [33].

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substratum leads to main separations in the species composition of aquatic invertebrates [41]. For alpine limestone springs in the Schütt catchment in Kärnten, Austria the role of habitat structure on the community composition was studied with a particular focus on the spring-dwelling animals colonizing the aquatic and the adjacent aquatic-terrestrial transition zones [42]. Here, microhabitat composition and the concomitance of lotic and lentic areas in the springheads furthered a high species diversity and abundance without an influence of the altitude of the investigated springs. In certain dominant microhabitats taxa specific substrate preferences were detected. Ephemeroptera (mayflies) prefer micro- and mesolithal, the caddisfly *Crunoeia irrorata*, however found mainly in CPOM. The study shows also a certain distribution of taxa according to the different spring ecotone zones, at which crenobionte species mainly occur in semi-aquatic areas (Fauna hygropetrica and Fauna liminaria) and only a few crenobionte taxa exclusively in the aquatic environment. This finding underlines the importance to investigate springheads as an ecotone and to include all transition zones from aquatic to terrestrial areas within the methodological concept of eucrenal studies (Figure 2).

For diatoms, only the grain size of mineral substrates has an influence on the colonization of certain species, because a significant correlation with different microhabitats in springs cannot be determined [43]. There are also studies that achieve no or an unclear relationship between substrate occurrence and species diversity in the results. [44] deduce a mixture of substrate specific microhabitat types and general spring types from empirical field data: Mineral dominated springbrooks, helocrene springs, moss cushions, limnocrene springs. For all these...
subtypes of springs specific inhabited taxa of Crustaceans (Crustacea) and insects are found, with the exception of helocrene springs. A statistically significant correlation between these habitat types and species diversity cannot be described. For the latter result, it should be noted that it is not useful to aggregate data of fauna assemblages at different spatial scales (e.g. cumulate microhabitat and habitat scale) to run statistical analysis to differentiate fauna communities, because the hierarchical levels of spatial scales must be considered [15, 45]. By using multivariate statistical ordination methods to analyze fauna composition in the eucrenal of springs in Northwest Switzerland (Swiss Plateau and the Jura Mountains) on the habitat scale the most important identification criteria are the spring type, substrate and discharge intensity [46]. A further regional specific faunistic relevant differentiation of spring types is possible on the basis of the criteria substrate (microhabitat scale). Especially for the structural separation into fine and coarse mineral substrates and particularly specific organic substrates such as CPOM and emergent macrophytes a faunistic relevance is detectable. Even in studies of karst springs in the Wye catchment in the Peak District National Park (Derbyshire) in England no dominant relationship between the occurrence of different microhabitats and the species composition of invertebrates was found [47-48]. The results obtained from the springs and springbrooks examined that discharge variability has a greater influence on macroinvertebrate community composition than the distribution and diversity of substrate types. A separate data analysis according to the areas springhead and springbrook would show a more significant influence of microhabitats to differentiate fauna communities of the eucrenal and hypocrenal. The characteristics of a springbrook (hypocrenal) are that here, significantly more lotic and crenoxene taxa are to be expected caused by a higher velocity flow than in the springhead (eucrenal) with a higher proportion of crenobionts within the fauna community [49]. The springhead should be seen as an autonomous ecotone with a complex of microhabitats, so that sampling and analyzing methods has to be performed using tools adapted to every microhabitat type [50].

In summary, the review of the state of research about fauna-microhabitat-relationships and an eco-faunistic substrate preference assessment to analyze research deficits shows that some structural hydromorphologically based water type subdivisions of springs using a variety of substrate types already exist, e.g. to differentiate existing spring typology approaches. The integrative joint consideration of the function and the ecological significance of the substratum as a hydromorphological element and as a microhabitat for invertebrates of springheads are lacking in assessment methods and analyses. In eco-faunistic studies that interpret the fauna-microhabitat-relationship in the eucrenal of springs, combined quantitative and qualitative investigations and analysis of the substrate preferences of invertebrate taxa regarding the springhead as an ecotone are still missing. Thereby faunistic research focuses mostly on the aquatic taxa only, rarely on terrestrial organisms. The scientific deficits described are the motivation for new research about fauna-habitat-relationships in springs. The results of the prospective study presented here were conducted in order to answer the following main research questions:

1. Is there a substrate preference for specific taxa considering the ecotone characteristics of springs? (Quantitative Structural Analysis);
2. Which functions of microhabitat types of springs could be characterized with the investigation of the substrate preference of specific taxa? (Qualitative Functional Analysis);

3. How strong is the relationship (or correlation) between microhabitat diversity (substrate type richness) and biodiversity? (Structure-Function Synthesis).

3. Study area and methods

The selection of study sites (Figure 3) was based on two main criteria. First, only forest springs have been investigated in order to ensure a wide range of possible selection of different substrate types. Therefore, certain categories of protected areas were deliberately chosen in forest landscapes as forest reserve, national park, and core zone of the biosphere reserve or nature reserve to identify an equally wide variety of hydromorphological structures. Different land uses or management strategies in forests implicated anthropogenic influences such as artificial water control structures and were included consciously. Second, study sites were selected that were as little as possible or not studied to close regional gaps in knowledge for species inventory and for locational eco-faunistic characterization. The study sites are located in the central area of the German subdued mountains. Table 3 gives an overview to their natural physical geographic characteristics.
In this study, a total number of 152 springs are surveyed and analyzed. Related to the natural area classification of the Federal Republic of Germany [51] the study sites can be grouped in 4 different landscapes (thick lined frames in Table 3) regarding geological subsoil characteristics (Groundwater Body in Table 3). On the landscape scale the study sites can be aggregated into two main groups concerning chemical groundwater criteria: 1) study sites with siliceous springs (Niddahänge, Schafstein, Burgwald, Kellerwald and Krofdorfer Forst); 2) study site with limestone springs (Hainich).

The investigation approach based on a hierarchical spatial framework for spring habitats to aid the illustration and understanding of functional, structural and process relationships on different scales [15]. The springscape (Figure 4) is a theoretical concept concerning specific geographical dimensions as levels of habitat filters that operate to influence species distribution and abundance within the landscape [52]. This implies that hierarchically nested environmental factors like substrate type influences the assemblage of species at progressively more localized spatial scales (e.g. at the microhabitat scale) [13].

Every substrate type is arranged at the microhabitat scale (or nano scale) and can be seen as the smallest habitat unit as a relatively homogeneous minor area where species occur. It is
similar to the habitat scale of the patch dynamic concept [16]. These substrate types form a mosaic-structured complex, which determine the entire substratum within the ecotone of a springhead on the habitat scale. The arrangement of substrate types corresponds to the patch scale of the patch dynamic concept [16]. Springhead (eucrenal) and springbrook (hypocrenal) consolidated the spring area at the meso scale within the stream system [31]. Several spring areas are part of headwater catchments, which are taken together in a higher-level system of a river catchment. Spring and river catchments are part of the landscape scale of the patch dynamics concept [16]. Finally, such stream systems can be a part of major, continent-scale river basins. For the microhabitat scale a new method to detect substrate types within springhead ecosystems and to sample the invertebrate fauna of each substrate type within an ecotone approach was developed. It is a multi-habitat sampling technique with a 2-layer approach (Figure 5).

The principle is similar to the AQEM/STAR approach to assess the riverbed of river segments [33,53], but with basic changes in the procedure considering essential springhead environmental characteristics. The inorganic and organic layers are considered individually in a 2-layer approach by taking the area of the whole springhead habitat as a reference surface (5-10 square meters). The appraisement of substrate type coverage was documented in a record sheet. The number of sub-samples taken in each layer corresponds to the fraction of the substrate types of the reference surface that layer has, with one sample taken per 5 percent coverage. On the example of the substrate type microlithal (coarse gravel in Figure 5) a coverage ratio of 40 percentages was estimated, 8 separate samples of fauna collections have to be performed. For each sample, a substrate specific sampling technique (e.g., sampling by net, collecting with tweezers) is performed for 2 minutes over a 10 cm by 10 cm reference area. A specific handheld net sampler was used with a mesh width of 100 µm. For taxonomic
determination invertebrates were preserved in ethanol alcohol (90 %) and stored in small (6 ml) Wheaton polyethylene jars. The samples are archived in the laboratory of the Biospeleological Register maintained by the Hesse Federation for Cave and Karst Research [54] and are available for genetic research by the Bavarian State Collection of Zoology in Munich. Some taxonomic groups were passed on to specialist taxonomists for detail determination: Dr. Peter Martin (Kiel, Germany) for Halacaridae and Hydrachnellae of the order Acari (water mites); Dr. Axel Schönhofer (Mainz, Germany) for Opiliones (harvestmen); Christoph Bückle (Tübingen, Germany) for Auchenorrhyncha (cicadas) and Andreas Allspach (Frankfurt / Main, Germany) for Trichoniscidae (woodlice, isopods). Mapping and sampling were taken once a time for 152 springs in 2008. In 2009 a control sample in 4 representative helocrene springs carried out to identify possible changes in substrate coverage. As a descriptive statistics method the relative frequency \( f_i \) was calculated to compare the habitat type occurrence of the different substrate types for the quantitative structural analysis (Equation 1):

\[
f_i = \frac{n_i}{N}
\]

Equation 1. Calculation of the relative frequency \( f_i \) of a taxon within a substrate type (=substrate preference); \( n_i \): absolute frequency of a taxon within a substrate type; \( N \): total number of samples of a substrate type.

The SIMPER analysis (similarity percentages) was executed (Equation 2) to test the validity of aggregated microhabitat types with specific taxa as statistical descriptors by ranking similarity in fauna community pattern [55-56].

\[
S_{jk} = \sum_{i=1}^{p} I S_{jki}
\]
Equation 2. Calculation of the SIMPER analysis. S: Group within a pair of samples j, k; i: ith term of $S_{jk}$; $l S_{jk}(i)$: Bray-Curtis coefficient, see [57].

Therefor a similarity coefficient with a standard deviation regarding the abundances of taxa was calculated. Most commonly occurring taxa with high abundances are the best descriptors to identify ecological relevance (or validity) in microhabitat types. The qualitative functional analysis of diet types was performed using existing feeding type valence values [58-59] and new established values for water mites in cooperation with Dr. Peter Martin [10]. To calculate a metric for the biodiversity of the invertebrate fauna the Shannon Index was used as a basis for the Structure-Function Synthesis [60]. In addition, the Evenness Index was performed as a structure metric to analyze the statistical distribution of the Shannon Index [61]. The interpretation of the relationship between structure and function within the context of analyzing hydromorphological structures and biodiversity the Pearson correlation coefficient was applied for statistical calculation. A multivariate statistical method was applied using a principal component analysis (PCA) to characterize variables to differentiate springheads.

A modeling of aggregated microhabitat types was performed using a new and specially developed three-step decision scheme to subdivide hydromorphological based habitat types for springheads (Figure 6).

![Figure 6. Decision Scheme for modelling microhabitat types of springheads. See [10].](http://dx.doi.org/10.5772/59072)
4. Results

In this study 11.663 individuals (single organisms) in 639 sampling jars were sampled and determined, which corresponds to an average value of 76 individuals per springhead. Arthropoda accounted for the largest share (81%), followed by Mollusca (11%), Annelida (4%), Nemathelminthes (2%) and Plathelminthes (1%) regarding the phylum of invertebrates. The dominant class is Insects (51%), followed by Arachnida (13%), Crustacea (12%), Gastropoda (8%), Clitellata (4%), Entognatha (4%), Bivalvia (3%), Nematoda (1%), Turbellaria (1%) and Others (1%). The major group within the order is Diptera (24%), followed by Coleoptera (15%), Trichoptera (8%), Araneae (7%), Plecoptera (5%), Stylommatophora (5%), Oligochaeta (5%), Amphipoda (4%), Veneroida (3%), Acari (3%), Isopoda (3%), Cyclopoida (3%), Hemiptera (3%), Neotaenioglossa (3%), Harpacticoida (2%), Basammothorpha (2%), Seriata (2%), Opiliones (1%), Hymenoptera (1%), Gordiida (1%), Diplopora (1%), Lepidoptera (1%) and Others (1%; 11 further groups). A detailed presentation of results regarding the taxonomic rank of families is given by [10]. The taxonomic ranks of Genus and Species are considered in the results of the substrate and microhabitat preferences. The species composition of the six different study areas is very similar, regarding a cluster analysis and a nonmetric multidimensional scaling. Using mean abundances a separation is possible corresponding to a differentiation based on natural physiogeographic characteristics (Table 3). The average value of springhead specific genus and species is about 28 percentages for all study areas.

4.1. Results of the quantitative structural analysis

Relative presence within all investigated springs means the percentages of the occurrence of a substrate type in 152 springs as the statistical main unit (Example: Psammopelal is present in 61 springs of 152 total springs, that results 40%). The relative coverage ratio was calculated as the mean value of the related substrate type of all studied springheads. The results in Table 4 showing the presence and the coverage of the investigated substrate types of all 152 studied springs.

The most present substrate type is psammopelal with a ratio of 40 percentages. This mineral substrate type represents 51 percentages of the coverage in comparison to all substrate types. The second common substrate type is coarse particular organic matter (CPOM) with 21 percentages presence and 37 percentages coverage. The most common coarse mineral substrate type is microlithal with 18 percentages presence and 20 percentages coverage. Further representative organic substrate types are xylal (coarse woody debris), emergent macrophytes and moss cushions. Artificial substrates are of minor importance. Most of the springheads can be characterized as structurally undisturbed and non-degraded habitats. Nevertheless, the results of the found substrate types represent diverse microstructures related to forestland cover.

The results of the aggregated microhabitat types performed using the decision scheme (Figure 6) is documented in Table 5. In comparison to the findings of the substrate types (Table 4) it is to ascertain that the most common habitat types of all studied springs are organic dominated (74%). Here, the dominant microhabitat type is the organic-dominated, fine-material-
Abounded microhabitat type (43%), which represents the importance of fine mineral substrate. Exclusively mineral habitat types are less representative (11%). However, their presence and importance as habitat types had not been sufficiently documented by previously existing mapping methods, because of the non-regarding of overlapped substrates. Here the application of the 2-layer approach is a benefit for ecological characterization and classification.

| Substrate Type       | Presence (relative) | Coverage (relative) | Presence (relative) | Coverage (relative) |
|----------------------|---------------------|---------------------|---------------------|---------------------|
| Argyllal             | 2 %                 | Fine Sediment       | 2 %                 | Fine Sediment       |
| Psammal              | 4 %                 | 47 %                | 2 %                 | 55 %                |
| Psammopelal          | 40 %                | 51 %                |                     |                     |
| Akal                 | 10 %                | Coarse Sediment     | 4 %                 | Coarse Sediment     |
| Microlithal          | 18 %                |                     | 20 %                |                     |
| Mesolithal           | 10 %                | 49 %                | 5 %                 | 38 %                |
| Macrolithal          | 7 %                 |                     | 4 %                 |                     |
| Megalithal           | 5 %                 |                     | 5 %                 |                     |
| Open Construction    | 2 %                 | Technolithal        | 3 %                 | Technolithal        |
| Closed Construction  | 2 %                 | 4 %                 | 4 %                 | 7 %                 |
| Emergent macrophytes | 15 %                | Organic Matter      | 20 %                | Organic Matter      |
| Submerged macrophytes| 1 %                 |                     | 1 %                 |                     |
| Moss cushions        | 19 %                | 100 %               | 10 %                | 85 %                |
| Xylal                | 22 %                |                     | 12 %                |                     |
| CPOM                 | 21 %                |                     | 37 %                |                     |
| Coniferous litter    | 4 %                 |                     | 3 %                 |                     |
| FPOM                 | 17 %                |                     | 2 %                 |                     |
| Algae                | 1 %                 |                     | 0 %*                |                     |
| Without organic substrates | 15 % | 15 % |                     |                     |

Table 4. Presence and coverage of substrate types within the investigated springheads. * 0.4% rounded down=0%. First layer: Mineral and artificial substrate types (100%); Second layer: Organic substrate types and coverage without substrates (100%).
### Table 5. Ecohydrological microhabitat types for springheads within the investigated springheads.

| Habitat Type       | Percentages | Microhabitat Type (HT)                                    | Percentages |
|--------------------|-------------|----------------------------------------------------------|-------------|
| Organic dominated  | 74%         | Organic-dominated, fine-material-abounded HT (O)          | 43%         |
|                    |             | Organic-dominated, coarse-material-abounded HT (Oc)       | 24%         |
|                    |             | Organic-dominated, fine- to coarse-material-abounded HT (Oc) | 7%          |
| Mineral dominated  | 11%         | Mineral-dominated, fine-material-abounded HT (Mf)         | 6%          |
|                    |             | Mineral-dominated, coarse-material-abounded HT (Mc)       | 3%          |
|                    |             | Mineral-dominated, fine- to coarse-material-abounded HT (Mc) | 1%          |
| Mixed Type         | 7%          | Mixed type (organic/mineral), fine-material-abounded HT (O/Mf) | 3%          |
|                    |             | Mixed type (organic/mineral), coarse-material-abounded HT (O/Mc) | 4%          |
|                    |             | Mixed type (organic/mineral), fine- to coarse-material-abounded HT (O/Mc) | 0%          |
| Artificial         | 7%          | Technolithal with open construction (To)                 | 3%          |
| (Technolithal)     |             | Technolithal with closed construction (Tc)               | 4%          |
| Special Type       | 1%          | Special Type (S)                                         | 1%          |
| Total              | 100%        | Total                                                    | 100%        |

### Table 6. Assessment Scheme to classify the substrate preference. See [62].

| Relative Frequency ($f_i$) | Preference Classification |
|----------------------------|---------------------------|
| ≥ 50%                      | strong                    |
| 25 – 49%                   | common                    |
| < 25%                      | rare                      |
| 0 %                        | absent                    |
| Fauna Area | Taxon                   | Mineralic Substrates | Organic Substrates |
|------------|------------------------|----------------------|--------------------|
|            |                        | Fine Sediment | Coarse Sediment |     |
|            |                        | Arg  | Psa  | Psp  | Aka  | Mic  | Mes  | Mac  | Meg  | eMp  | sMp  | Moss | Xyl | CPOM | CoL |
| Agabus sp. | -                      | -    |      |      | -    |      |      |      |      | -    |      |      |     |      |     |
| Arr font.  | -                      | -    |      |      |      | -    |      |      |      |      |      |      |     |      |     |
| Bezzia sp. | -                      |      | +    |      |      |      |      |      |      |      |      |      |     |      |     |
| Byt com    | +                      | -    |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Byt dun    | -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Cord bid   | +                      |      | -    |      |      |      |      |      |      |      |      |      |     |      |     |
| Cren alp   | -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Galba tr   | -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Gamm fos   | -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Gams pul   | -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Habrol con | -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Helop sp.  | -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Hydrov pla | ++                     |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Hygrob nor | +                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Leuctra sp.| -                      | +    |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Loboh web  | ++                     |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Nemoura sp.| -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Niph aqu   | -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Niph schell| -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Partn steinm| -                     |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Pisidium sp.| -                     |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Polyc fel  | +                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Protn sp.  | -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Protz squ squ| +                     |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Seric sp.  | -                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Sold chap  | ++                     |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Sold mon   | +                      |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Sperchon sp.| ++                    |      |      |      |      |      |      |      |      |      |      |      |     |      |     |
| Fauna Area | Taxon         | Mineralic Substrates | Organic Substrates |
|------------|---------------|----------------------|-------------------|
|            |               | Fine Sediment        | Coarse Sediment   |
|            | Arg | Psa | Psp | Aka | Mic | Mes | Mac | Meg | eMp | sMp | Moss | Xyl | CPOM | CoL |
| Velia sp.  | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | +    | -   |
| Anac sp.   | -   | -   | -   | -   | -   | -   | -   | -   | -   | +   | +    | -   | +    | -   |
| Crano irr  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Dixa sp.   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Carych sp. | -   | +   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | +    | +   |
| Carych trid| -   | +   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Cicad vir  | +   | ++  | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | -   |
| Discus rot | -   | -   | -   | -   | -   | -   | -   | -   | -   | +   | -    | -   | +    | -   |
| Eisen tetr | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | +    | +   | +    | -   |
| Ligid hyp  | -   | -   | +   | +   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Monac inc  | -   | -   | ++  | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Oligol trid| ++  | ++  | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Oniscus as | -   | -   | ++  | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Paran quadrip | +   | ++  | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Polydesmus sp. | ++ | ++  | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Trichoniscus sp. | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Bryo pter  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | -   |
| Eucon fulv | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | -   |
| Euconulus sp. | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Ixodes sp. | ++  | ++  | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Leiob blackw | ++ | ++  | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Lithobius sp. | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Neob carc  | -   | +   | ++  | +   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Neob sim  | ++  | ++  | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |
| Stenod hols | ++ | ++  | -   | -   | -   | -   | -   | -   | -   | -   | -    | -   | -    | +   |

*Table 7.* Substrate preference. Fauna Area: *hygp* – hygropetric; *ln* – liminaria. See assessment scheme in Table 6. Abbreviation: Arg: Argyllal; Psa: Psammal; Psp: Psammopelal; Aka: Akal; Mic: Microlithal; Mes: Mesolithal; Mac: Macrolithal; Meg: Megalithal; eMp: Emergent Macrophytes; sMp: Submerged Macrophytes; Moss: Moss cushions; Xyl: Xylal; CPOM: Coarse particular organic matter; CoL: Coniferous litter.
The classification of the substrate preference of the found taxa was performed using the assessment scheme showing in Table 6. Taxa with a relative frequency of 25 and more percentages are classified as good descriptors for substrate type preference. The results of the substrate preference analysis are shown in Table 7. Generally, we found 30 taxa with a substrate preference for CPOM, 17 taxa with a substrate preference for psammopelal, 12 taxa with a substrate preference for xylal, 8 taxa with a substrate preference for emergent macrophytes, 5 taxa with a substrate preference for moss cushions, 4 taxa with a substrate preference for microlithal and 1 taxa with a substrate preference for megalithal. For all other substrate types we cannot found a substrate preference. These results represent not only the quantity of the methodological approach, because of the more intensive sampling of fauna in more representative substrate types. The results are also characterizing qualitative aspects like choosing a specific food source. In usually oligotrophic springheads organic matter is an important substrate as a food basis. That means, the most representative substrate type psammopelal (mineral substrate) is not the substrate type with the most fauna preference value for spring related invertebrates. Taxa are more present in organic substrates like CPOM or xylal. In FPOM and algae no taxa were found.

The results of the microhabitat type preference are documented in Table 8. The SIMPER test shows a differentiation in the microhabitat preference for the most abundant taxa. We analyzed an excerpt of the most representative organic and mineral microhabitat types (Table 5). Although organic substrates clearly dominate (74 % mean coverage), also a substrate preference of mineral substrates (11 % mean coverage) can be recognized. It is also interesting that not only aquatic taxa contribute to the characterization of the faunal relevance of these aggregated microhabitats. We found also hygropetric fauna (Anacaena sp., Crunoecia irrorata) and terrestrial-hygropilous fauna (Trichoniscus sp.) in the species pool. Already two taxa (Pisidium sp., Anacaena sp.) describe almost half (46 %) the contribution to the substrate preference of the organic-dominated, fine-material-abounded microhabitat type (O). The organic-dominated, coarse-material-abounded microhabitat type is signified by 4 taxa (Sericostoma sp., Crunoecia irrorata, Anacaena sp., Trichoniscus sp.) with more than the half of there contribution (52 %). Here, the caddisfly Sericostoma sp. seems to be a good taxon to differentiate organic dominated microhabitats with coarse mineral abounded substrates, because of the preferential occurrence in such substrate types (substrate preference: microlithal). Therefor a precise quantitative analysis of the substrate preference (Table 7) is implicitly necessary to interpret SIMPER test results. The faunistic relevance of the less representative mineral dominated microhabitats (Mf, Mc) is partial uncertain. The most dominant taxon is the stonefly Leuctra sp., which characterizes fine mineral substrates (psammopelal) and the organic substrate type CPOM. A similar uncertainty can be observed for the water scavenger beetle Anacaena sp. as the second most representative taxa for the mineral-dominated, coarse-material-abounded microhabitat type. This taxon occurs mostly in organic substrates like CPOM and xylal. However, other faunistic findings are very plausible to interpret microhabitat preferences. For example, the pea clam Pisidium sp. prefers organic and fine mineral substrates and determines the mineral-dominated, fine-material-abounded microhabitat type. Although, spring taxa are normally not very abundant, it is possible to statistically validate modeled microhabitat types within a SIMPER analysis and to differentiate microhabitat preferences by taxon related contributions.
Table 8. Results for the contribution of the SIMPER analysis. Oᵗ: organic-dominated, fine-material-abounded; Oᵝ: organic-dominated, coarse-material-abounded; Mᵗ: mineral-dominated, fine-material-abounded; Mᵝ: mineral-dominated, coarse-material-abounded.

It is to summarize that there is a significant substrate preference of certain taxa within separate fauna areas of the spring ecotone. A quantitative determination of indicator taxa of aquatic, hygropetric, liminarian, terrestrial-hygropilous and terrestrial fauna areas can be given as a basis for an eco-faunistic substrate preference assessment in forest springs of the German subdued mountains.

4.2. Results of the qualitative functional analysis

The qualitative function of substrates as microhabitats is related to the life strategy of an animal, which means the question about the use of a substrate type by a specific taxon. Can we qualitatively validate a specific quantitative assessed substrate preference by regarding autecological information about a taxon? Life strategies are diverse to characterize (movement type, diet type), however, they can all lead to a certain adaptation to the habitat [63]. Therefore, a suitable variable to analyze microhabitat functions is to typify the feeding group of a taxon. It allows the classification whether the taxon occurs for a direct food intake (substrate as food basis), indirectly for food intake (e.g. predators follows taxa with direct food intake) or another reason is to describe. The result of the qualitative functional analysis is summarized in Table
Most aquatic insects, especially stoneflies (Plecoptera), caddies flies (Trichoptera) and mayflies (Ephemeroptera) are almost exclusively present as larvae. The aquatic and hygropetric beetles were only found as imago. For most of the taxa the microhabitat function can be interpreted as the area of food intake or the substrate itself is the food source. The latter means, e.g. shredder organisms occurred dominantly in CPOM (coarse particular organic matter), because leaf litter is the original food basis. Interesting is the fact, that CPOM is the most dominantly organic substrate type and the preferential substrate type while FPOM is not representative. Here, we can assume that fine particular organic matter is transported downwards into parts of the springbrook or the epirhithral of the headwater, because of a dominant activity of shredders in the springheads. Therefore, barely collectors were found which filters or catch FPOM. Here, we have to confirm the River Continuum Concept with respect to headwaters and the declaration that shredders play a major role [64]. An importance of emergent macrophytes is conspicuous for the fauna areas of the terrestrial-hygrophilous and terrestrial zones. Microhabitat function is also food intake, but here, non-aquatic plant suckers occur. Other functional feeding groups are also existent, e.g. xylophages on coarse woody debris (xylal) or detritus and/or sediment feeder in fine mineral substrates, while psammopelal is the dominant mineral substrate type. Predators also occur, partial there are the major feeding group regarding the equivalence values of feeding groups [10], as in the aquatic and terrestrial fauna area. The substrate type itself has no direct significance as a food basis, because predators using microhabitats as hunting grounds. Therefore, it is indirectly of importance that specific taxa from other functional feeding groups showing a distinctive substrate preference, because predators reproduce a similar substrate preference, as the prey seeks for special microhabitats. We can classify corresponding substrate preferences for CPOM or psammopelal considering predators. A similar conclusion can be made for parasites like the spring specific taxa group of most water mites, certainly with a possible specific host preference within certain microhabitats. Another function of substrates can be deduced without analyzing the trophic state of taxa. Microhabitats are refuge areas for different organisms within the whole ecotone. Aquatic taxa like the pea clam (Pisidium sp.) or the terrestrial non-spring specific ticks (Ixodes sp.) find an area to retreat suboptimal environmental conditions. Pea clams burrowed actively into fine wet sediment (psammopelal) to survive times without discharge in the springhead, while ticks waiting in more or less bodily immobilization for host organisms. For some taxa a certain interpretation about their diet type is not really possible, because autecological information is lacking. For example, we found biting midges of Bezzia sp. larvae (Ceratopogonidae) with a high abundance and a specific substrate preference for fine mineral sediment (psammopelal). The Taxon is not specified in common functional feeding group reference lists [58-59]. Adult animals are plant and bloodsuckers, so that for the larvae the aquatic environment of fine sediment is a refuge area or a nursery ground. The larvae also survive droughts in springheads in wet fine sediment [65], so that this taxon needs more attention as a substrate preference indicator for temporary springs. For the two marine mite species of Soldanellonyx we did not found any information about diet type, what makes an interpretation of the microhabitat function impossible.
| Fauna Area | Taxon  | Substrate Preference | Diet Type (Feeding Group) | Microhabitate Function              |
|------------|--------|----------------------|---------------------------|------------------------------------|
| Agabus sp. | CPOM   | Predator (9)         |                           | Hunting Ground                     |
| Arr. font. | CPOM   | Predator (7), Parasite (3) |                           | Hunting Ground                     |
| Bezzia sp. | Psammopelal | Not specified; Bezzia are plant and blood suckers (host insects) |                           | Refuge Area for larvae?             |
| Byt. com  | Psammopelal | Grazer (7), Sediment/Detritus Feeder (3) |                           | Area of food intake; Substrate as food source |
| Byt. dun  | CPOM   | Grazer (10)          |                           | Area of food intake; Substrate as food source |
| Cord. bid | Microlithal, Psammopelal, CPOM | Predator (10)         |                           | Hunting Ground                     |
| Cren alp  | CPOM   | Predator (10)        |                           | Hunting Ground                     |
| Galba tr  | CPOM   | Sediment/Detritus Feeder (4), Grazer (3), Shredder (3) |                           | Area of food intake; Substrate as food source |
| Gamm fos  | CPOM   | Shredder (7), Sediment/Detritus Feeder (2), Grazer (1) |                           | Area of food intake; Substrate as food source |
| Gans pal  | Psammopelal, CPOM | Shredder (7), Sediment/Detritus Feeder (2), Grazer (1) |                           | Area of food intake; Substrate as food source |
| Habrol con| CPOM   | Grazer (7), Sediment/Detritus Feeder (3) |                           | Area of food intake; Substrate as food source |
| Helop sp. | Moss, Xylal, CPOM | not specified         |                           | Larvae are predators (= Hunting Ground) |
| Hydrov pla| Psammopelal, Microlithal | Predator (7), Parasite (3) |                           | Hunting Ground                     |
| Hygrob nor| Psammopelal | Predator (7), Parasite (3) |                           | Hunting Ground                     |
| Leuctra sp.| Psammopelal, CPOM | Shredder (4), Sediment/Detritus Feeder (4), Grazer (2) |                           | Area of food intake; Substrate as food source |
| Loboh web | Psammopelal | not specified         |                           | Opiliones are predators (= Hunting Ground) |
| Nemoura sp.| CPOM   | Shredder (6), Sediment/Detritus Feeder (4) |                           | Area of food intake; Substrate as food source |
| Niph aqu  | CPOM   | Sediment/Detritus Feeder (10) |                           | Area of food intake; Substrate as food source |
| Taxon          | Substrate Preference | Diet Type (Feeding Group) | Microhabitat Function                        |
|---------------|----------------------|---------------------------|-----------------------------------------------|
| *Niph schell* | CPOM                 | Sediment/Detritus Feeder (10) | Area of food intake; Substrate as food source |
| *Partn steinn* | Psammopelal          | Predator (7), Parasite (3) | Hunting Ground                                |
| *Pisidium sp.* | Psammopelal, CPOM    | Filtering Collectors      | Area of food intake; Refuge                    |
|               |                      |                           | Area (dry period)                             |
| *Polye fel*   | Psammopelal, CPOM    | Predator (10)             | Hunting Ground                                |
| *Proton sp.*  | Xylal                | Shredder (6), Sediment/Detritus Feeder (2), Grazer (2) | Area of food intake; Substrate as food source |
| *Protz squ squ* | CPOM, Psammopelal    | Predator (7), Parasite (3) | Hunting Ground                                |
| *Seric sp.*   | Microlithal          | Shredder (7), Sediment/Detritus Feeder (1), Grazer (1), Predator | Area of food intake; Substrate as food source; Hunting Ground |
| *Sold chap*   | Psammopelal          | Not specified             | No interpretation possible                    |
|               |                      |                           | (Food: Bacteria, Algae; Plant suckers, Predators) |
| *Sold mon*    | Psammopelal          | Not specified             | No interpretation possible                    |
|               |                      |                           | (Food: Bacteria, Algae; Plant suckers, Predators) |
| *Sperchon sp.* | Psammopelal          | Predator (7), Parasite (3) | Hunting Ground                                |
| *Vela sp.*    | Microlithal          | Predator (10)             | Hunting Ground                                |

Table 9. Diet types and microhabitat functions of the investigated springheads for aquatic taxa. * see Table 5; † see [58-59]; (*) clear preference, but without value.
| Fauna Area | Taxon          | Substrate Preference | Diet Type (Feeding Group) | Microhabitate Function                      |
|-----------|---------------|----------------------|---------------------------|---------------------------------------------|
| terrestrial-hygrophilous | *Carych trid* | Psammopelal, CPOM    | Shredder (10)             | Area of food intake; Substrate as food source |
| terrestrial-hygrophilous | *Cicad vir* | Emergent Macrophytes | Plant Sucker (10)         | Area of food intake; Substrate as food source |
| terrestrial-hygrophilous | *Discus rot* | Xylal                | Shredder (*), Sediment/Detritus Feeder (*) | Area of food intake; Substrate as food source |
| terrestrial-hygrophilous | *Eisen tetr* | Xylal, CPOM          | Sediment/Detritus Feeder (10) | Area of food intake; Substrate as food source |
| terrestrial-hygrophilous | *Ligid hyp* | Emergent Macrophytes | Shredder (6), Xylophage (2), Sediment/Detritus Feeder (2) | Area of food intake; Substrate as food source |
| terrestrial-hygrophilous | *Monac inc* | Emergent Macrophytes | Xylophage (*), Shredder (*) | Area of food intake; Substrate as food source |
| terrestrial-hygrophilous | *Oligol trid* | Emergent Macrophytes | Predator (10)             | Hunting Ground                             |
| terrestrial-hygrophilous | *Oniscus as* | Xylal                | Shredder (6), Xylophage (2), Sediment/Detritus Feeder (2) | Area of food intake; Substrate as food source |
| terrestrial-hygrophilous | *Paran quadrip* | Xylal, Megalithal | Predator (10)             | Hunting Ground                             |
| terrestrial-hygrophilous | *Polydansmus sp.* | Xylal, CPOM         | Shredder (7), Xylophage (3) | Area of food intake; Substrate as food source |
| terrestrial-hygrophilous | *Trichoniscus sp.* | Moss, Xylal, CPOM | Shredder (8), Sediment/Detritus Feeder (2) | Area of food intake; Substrate as food source |
| terrestrial | *Bryo pter* | Emergent Macrophytes | Plant Sucker (10)         | Area of food intake; Substrate as food source (only ferns) |
| terrestrial | *Eucon fulv* | CPOM                | Shredder (*), Xylophage (?) | Area of food intake; Substrate as food source |
| terrestrial | *Euconulus sp.* | Xylal            | Shredder (*), Xylophage (?) | Area of food intake; Substrate as food source |
| terrestrial | *Ixodes sp.* | Moss                | Parasite (10)             | Refuge Area                                 |
| terrestrial | *Leiob blackw* | Emergent Macrophytes, CPOM | Predator (10) | Hunting Ground                             |
| terrestrial | *Lithobius sp.* | Emergent Macrophytes | Predator (10)             | Hunting Ground                             |
| terrestrial | *Neob carc* | CPOM, Xylal         | Predator (10)             | Hunting Ground                             |
| Fauna Area | Taxon       | Substrate Preference | Diet Type (Feeding Group) | Microhabitat Function                                |
|------------|-------------|----------------------|---------------------------|-------------------------------------------------------|
| Neob sim   | Moss, Xylal | Predator (10)        |                           | Hunting Ground                                        |
| Stenod hols| Emergent    | Plant Sucker (10)    |                           | Area of food intake; Substrate as food source          |
|            | Macrophytes |                      |                           |                                                       |
| Stenod laev| Emergent    | Plant Sucker (10)    |                           | Area of food intake; Substrate as food source          |
|            | Macrophytes |                      |                           |                                                       |

Table 10. Diet types and microhabitat functions of the investigated springheads for the other taxa. * see Table 5; † see [58-59]; (*) clear preference, but without value.

In general, there is a heterogeneous feeding group composition within the aquatic and hygropetric fauna areas of the spring ecotone (Figure 7), although only three different taxa could be indexed for the hygropetric fauna, but with diverse feeding type valence values. In contrast, for the Fauna liminaria just one feeding group (shredder) is dominant, because only the small air-breathing snail *Carychiun* with only one main feeding type valence value is indicated. The terrestrial-hygrophilous and terrestrial fauna is also characterized by a heterogeneous feeding group arrangement. Here, shredders and predators are of similar importance in comparison to the aquatic fauna area, but with different taxa and substrate preferences. That means, also the adjacent non-aquatic spring areas showing a high diversity concerning their trophic state. That underlines a basic necessity of sampling and indicating terrestrial invertebrates in spring ecotones. Thereby, we can interpret trophic functions within hydromorphological structures with the result, that for terrestrial non-aquatic spring invertebrates similar functions of microhabitats can be ascertained, but in comparison to the aquatic spring invertebrates within different hydromorphological structures (substrate types).
It is to summarize that we can identify specific trophic functions of different microhabitat types. Aquatic and terrestrial spring invertebrates using specific substrates as a food basis, so that the substrate type is the area of food intake. Otherwise microhabitats were used as hunting grounds and refuge areas.

4.3. Results of the structure-function synthesis

There is an important relationship between the diversity of substrates and species diversity. The statistical correlation ($R^2=0.88$) between substrate diversity and biodiversity is highly significant (Figure 8). It is remarkable that the trend of the two curves (substrate diversity, Shannon-Index) is very similar, i.e. an increase in the substrate diversity leads to an almost identical increase in the Shannon index as an indicator value for biodiversity. The evenness values are between 0.7 (study areas: KW, VB) and 0.9 (study area: H) and emphasize the good quality of the results with a normal distribution of the fauna data (evenness values for the study areas RH: 8.0; BW and KR: 8.3). A further univariate analysis of the Shannon-Index with other location parameters and a correlation between these parameters and the occurrence of spring related taxa showing that substrate diversity is a key parameter determining biodiversity in springheads (Figure 9 and Figure 10).

Figure 8. Substrate Diversity and Biodiversity. Study Areas: BW: Burgwald, H: Hainich, KR: Krofdorfer Forst, RH: Rhön (Schaftstein), VB: Vogelsberg (Niddahänge).
Figure 9. Univariate Correlation Shannon-Index and other location parameters.

Figure 10. Univariate Correlation spring fauna (occurrence) and other location parameters.
The substrate diversity is one of the most important discriminatory factors for biodiversity in springheads besides forest cover type and pH (Figure 9). It is also an essential key driver for the occurrence of the spring fauna (crenobionts), which means taxa with a very strong and exclusive relationship to the eucrenal (Figure 10).

Figure 11. Principal component analysis.

The importance of substrate diversity as a key parameter determining biodiversity in springheads is also confirmed by a statistical multivariate analysis (Figure 11).

Table 11. Biodiversity of undisturbed and artificial degraded springsheads. Data rounded off to whole numbers.

| Indices                  | Undisturbed | Artificial | Relative Tendency |
|--------------------------|-------------|------------|-------------------|
| Shannon-Index            | 2           | 1          | %48% Decrease     |
| No. of Species (mean)    | 8           | 3          | %57% Decrease     |
| No. of Individuals (mean)| 41          | 11         | %73% Decrease     |

The artificial degradation of springheads with open or closed technical constructions (spring tapping and/or piping) is an immense stressor for fauna species in the eucrenal (Table 11). This can be shown strongly on the detailed quantitative analysis; not only the Shannon-Index and
the number of species decrease significantly, especially the number of individuals' decreases sharply. The loss of biodiversity is significantly caused by spring tapping and is a consequence of the loss of substrate richness and microhabitat diversity. Here, the hydromorphological structure (substrate type diversity) is an important ecosystem service to preserve and develop biodiversity in springheads.

It is to summarize that there is a very strong relationship between microhabitat and substrate type diversity and biodiversity. The substrate diversity is one of the most significant discriminatory factors for biodiversity in springheads. The degradation of hydromorphological structures causes a substantial loss of species and abundance of species. Nature conservation strategies for spring ecotones have to consider the importance of substrate type richness and heterogeneity to protect and develop biodiversity in springheads.

5. Further research

The results of this study provide numerous starting points for further research. One of the most pressing issues is the question about the relevance of dynamics and resulting changes in the occurrence and coverage of substrate types on species presence and species composition. The investigated springs of the study areas are predominantly helocrene springs with low amplitudes of the annual discharge. Further research in karst springs with episodic and temporally very high discharge may abruptly change hydromorphological structures. Are these disturbances significant also to detect in a temporally or more long-term variation in species composition? How stable are such more or less dynamic hydromorphological springheads or is there a tendency to equilibrium conditions after substrates changing events? Hereby, it is to verify the transferability of the methodological approach of the research. In addition, generally long-term monitoring of hydromorphological and environmental monitoring is still lacking in studying spring ecology. A representative selection of already fewer objects of investigated springheads would determine a good approach to analyze long-term changes and trends in the future. Particularly, research is needed about the impact of land use change, which will require future projections of probably modifications of the occurrence and mosaic structures of substrates. What is the influence of a potential forest conversion to microhabitat heterogeneity and biodiversity in forest springheads? Regarding land use pattern comparison research is necessary to study substrate preferences of the spring fauna in non-forest areas, e.g. extensive wetland, grassland and springs in flood plains. Can we observe shifts of substrate preferences of known spring species caused by the absent of substrate types or can we characterize an absent of these known taxa or can we find complete different taxa? Beside the empirical study from field surveys also habitat modeling is crucial to answer those questions. Therefore, more detailed experimental research to strengthen the knowledge of autecological conditions, especially for non-aquatic spring species, but also for aquatic fauna with a recent not specified classification of the feeding type is needed. Especially, there is no robust information about the indexing of feeding groups for species of the Family Halacaridae.
(marine mites), which are a consistent part of springhead communities in the Meiobenthos (Mesofauna). Considering the background of future climate change conditions the importance of microhabitats like fine mineral substrates as refuge area (“moist islands”) caused by decreasing time periods of drought not only for aquatic organisms with adaption strategies, but also for terrestrial-hygrophilous of the adjacent areas of springheads should be investigated. Also applied research for ecological assessment procedures is an essential issue and would benefit the practical orientated outcome of this basic research in spring ecology. For the protection and management of springs it is useful to implement the quantitative results of the substrate preference in existing or new metrics to characterize the ecological quality of these freshwater habitats.

6. Conclusion

Springs are considered as unknown habitats, most notable the relationship between invertebrates and hydromorphological structures. Research about the ecological importance of substrates for the inhabitation of species and consequences for biodiversity is still necessary to improve the knowledge about the relationship between structures and functions in springheads. This is needed if effective protection strategies and ecologically worthwhile nature conservation shall stand on a scientifically founded basis. Therefore, a first and operable mapping, sampling and assessment method was developed and can be used for further research and methodologically advances and modifications. Mainly, the theoretically background of the 2-layer approach is meaningful to assess also biased, not representative substrate types. Nevertheless, it is practicable to classify and verify ecological valid microhabitat types within representative substrate types for springheads. Here, we use a common limnological substrate type nomenclature, similar used for running waters, to compare the results with other water types or segments of brooks and rivers (rhithral, potamal). A quantitative approach to categorize substrate preferences is possible and can use as a basis to characterize the importance of mineral and organic substrate types in spring ecosystems. For specific invertebrate taxa a significant substrate preference is notable. Therefore, springheads were analyzed regarding their ecotone characteristics. Springheads are both, firstly an interface between the subterranean groundwater and the surface freshwater, secondly an embedded aquatic ecosystem with transition zones to terrestrial ecosystems. Hence, the whole importance of substrate heterogeneity and complexity in relation to biodiversity can be illustrated, although springheads are small sized inland water ecosystems or sometimes classified within small water bodies. The results of the found fauna reflecting the ecotone and a separate consideration of the substrate preference by fauna areas like the aquatic, hygropetric, liminaria and adjacent terrestrial fauna zone can be conducted. A taxa specific substrate preference considering the ecotone characteristics of springs can be determined. A qualitative functional analysis was done concerning each categorization of the feeding group (diet type) of the specific taxa. Thereby, an interpretation of microhabitats functions shows, that most of the taxa are present, because the substrate itself is the food basis or the place of food intake, especially for shredders, but also as a hunting ground for predators or a refuge area to survive non-optimal environ-
mental conditions. To conclude the structure-function synthesis we can significantly prove a strong relationship between the diversity of substrates and species diversity. An increasing diversity of substrate types leads to a higher biodiversity. Hydromorphological degradation results in the distinctive decrease of invertebrate species and their abundances, especially caused by technical spring tapping. Substrate respectively substrate diversity is an important discriminatory factor to classify springhead ecosystems and their invertebrate fauna. It shows mainly the susceptibility and the need of nature conservation of these special habitats.

**Nomenclature**

We used abbreviations for taxa names in tables as listed below. (common name mentioned as far as applicable).

| Abbreviation | Taxon | Common Name |
|--------------|-------|-------------|
| Agabus sp.  | Agabus sp. | Aquatic Beetle |
| Arr font.   | Arrenurus fontinalis | Water Mite |
| Bezzia sp.  | Bezzia sp. | Biting Midge |
| Byt con     | Bythinella compressa | Spring Snail (Rhoen Spring Snail) |
| Byt dun     | Bythinella dunkeri | Spring Snail (Dunkers Spring Snail) |
| Cord bid    | Cordulegaster bidentata | Dragonfly (Sombre Goldenring) |
| Cren alp    | Crenobia alpina | Triclad (Turbellaria) |
| Galba tr    | Galba truncatula | Freshwater Snail |
| Gamm fos    | Gammarus fossarum | Scud (Amphipod Crustacean) |
| Gams pul    | Gammarus pulex | Scud (Amphipod Crustacean) |
| Habrol con  | Habroleptoides confusa | Mayfly |
| Helop sp.   | Helophorus sp. | Scavenger Beetle |
| Hydrov pla  | Hydrovolzia placophora | Water Mite |
| Hygrob nor  | Hygrobates norvegicus | Water Mite |
| Leuctra sp. | Leuctra sp. | Stonfly |
| Loboh web   | Lobohalacarus weberi | Marine Mite |
| Nemoura sp. | Nemoura sp. | Stonfly |
| Niph aqu    | Niphargus aquilex | Groundwater Amphipod (Crustacean) |
| Niph schell | Niphargus schellenbergi | Groundwater Amphipod (Crustacean) |
| Partn steinn| Partnunia steinnanni | Water Mite |
| Pisidium sp.| Pisidium sp. | Pea Clam |
| Abbreviation | Taxon                      | Common Name                              |
|--------------|----------------------------|------------------------------------------|
| Polyc fel    | Polycelis felina           | Planaria                                 |
| Proton sp.   | Protonemura sp.            | Stonefly                                 |
| Protz sq squ | Protzia squamosa squamosa  | Water Mite                               |
| Seric sp.    | Sericostoma sp.            | Caddisfly                                |
| Sold chap    | Soldanellonyx chappuisi    | Marine Mite                              |
| Sold mon     | Soldanellonyx monardi      | Marine Mite                              |
| Sperchon sp. | Sperchon sp.               | Water Mite                               |
| Velia sp.    | Velia sp.                  | Water Strider                            |
| Anac sp.     | Anacaena sp.               | Water Beetle                              |
| Cruno irr    | Crunoea irrorata           | Caddisfly                                |
| Dixu sp.     | Dixa sp.                   | Meniscus Midge                           |
| Carych sp.   | Carychium sp.              | Hollow-shelled Snails (Ellobiidae)       |
| Carych trid. | Carychium tridentatum      | Herald Snail                             |
| Cicad vir    | Cicadella viridis          | Leafhopper (Cicada)                      |
| Discus rot   | Discus rotundatus          | Rotund Disc                              |
| Eisen tetr   | Eiseniella tetradra        | Square Tail Worm (Earthworms)            |
| Ligid hyp    | Ligidium hypnorum          | Woodlouse                                |
| Monac inc    | Monachoides incarnatus     | Land Snail (“Incarnadine Snail”)         |
| Oligol trid. | Oligolophus tridens        | Harvestman (Arachnids)                   |
| Oniscus as   | Oniscus asellus            | Woodlouse                                |
| Paran quadrip| Paranemastoma quadripunctatum | Harvestman (Arachnids)                   |
| Polydesmus sp.| Polydesmus sp.            | Flat-backed Millipede                    |
| Trichoniscus sp.| Trichoniscus sp.        | Woodlouse                                |
| Bryo pter    | Bryocoris pteridis         | Bug                                      |
| Eucon fulv  | Euconulus fulvus           | Hive Snail (Land Snail)                  |
| Euconulus sp.| Euconulus sp.              | Hive Snail (Land Snail)                  |
| Ixodes sp.   | Ixodes sp.                 | Tick                                      |
| Leiof blackw | Leiobunum blackwalli       | Harvestman (Arachnids)                   |
| Lithobius sp.| Lithobius sp.              | Stone Centipede                          |
| Neob carc    | Neobisium carcinoides      | Pseudoscorpion                           |
| Neob sim     | Neobisium similne           | Pseudoscorpion                           |
| Stenod hols  | Stenodema holsata          | Bug                                      |
| Stenod laev  | Stenodema laevigata        | Bug                                      |
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