Author's response to editor's and referee's comments on hess-2020-151

"Groundwater fauna in an urban area: natural or affected?"

Dear Editor,

We would like to thank you for the opportunity to once more revise our manuscript, for your time and for the constructive comments. We are convinced that we have fully addressed now all comments and substantially improved the manuscript.

In general, our replies to the comments are highlighted in blue.

Best regards,

Fabien Koch, on behalf of all authors

Editor:

Comments to the Author:

The reviewers re-evaluated the revised manuscript. One of the reviewers was satisfied with most of the corrections and pointed out some details still needing revision (details see attached document). The second reviewer thoroughly commented on the manuscript pointing out some major weaknesses and even errors in the manuscript (see comments referee #2). Normally, this would justify a rejection of the manuscript at this point; after already having had a round of "major revisions". Still, the referee also pointed out that there is novelty in the dataset and gave good advice on how to improve the manuscript. Therefore, there is the very last chance to thoroughly and substantially revise the manuscript. If you think that all of the comments can be addressed and the manuscript can be substantially improved, a re-submission of a revised version is recommended as I will make clear decision on acceptance or rejection of the manuscript in the next round. Please also have again a look at the comments from the first round of revisions.

Response: We agree that the referees gave very helpful comments for improving the manuscript. Hence, we additionally performed a more sophisticated multivariate analysis in form of a dimensionality-reduction method for visualization, which revealed interesting insights into parameter relations and confirmed our previous findings about spatial differentiation. Moreover, we improved the manuscript's language and as suggested we looked again at the comments from the first round of revisions for improving the manuscript.

In detail, we added isohypses in Figure 2c of the manuscript to provide details about local groundwater flow conditions as was recommended in Comment#25 of Referee#2 from the first round. Moreover, the results of the additional multivariate test support the categorization of land use types for which we argued in the response of Comment#12 of Referee#1 and Comment#15 of Referee#2, as well as the hypothesis that the order *Bathynellacea* and the genus *Parastenocaris* are type species for urban situations (Referee#2 Comment#21 Round#1).

Dear Referee #1,

we would like to thank you for your time and the constructive comments, which helped to improve the quality of the manuscript. Please find our detailed replies on the comments below. We hope that we answer all your remarks.

In general, our replies to the referee's comments are highlighted in blue. To highlight the nature of our replies we use a traffic light system indicating agreement with the referee marked in green, partial agreement in yellow, and objections in red.

Best regards,

Fabien Koch, on behalf of all authors

Referee #1:

The paper has improved significantly since the first publication and is a novel manuscript, providing insight into groundwater biota beneath urban areas and the surrounding landscape. The work is very interesting, however more time needs to be invested in correcting issues highlight below to meet the high standards of HESS.

Response: We fully agree. Thus, we added clarifications and statements in the methodology, which are presented below in our replies to the 'general and specific comments'. Moreover, we removed grammatical and punctuation errors (see 'specific comments').

General comments

Comment #1: I have difficulties in establishing the types of landuses, some 'forested' areas appear very close to the urban areas on figure 3c. Further clarification and a statement that distinguishes or helps classify landuses would be helpful.

Response: We agree that a clarification is necessary to better understand the classification. Hence, we added the following information to the manuscript (lines 205-209):

"In order to allow a spatially differentiated assessment, the study site is classified in different zones based on land use types provided by the European seamless vector database of the CORINE Land Cover (CLC) inventory (GISAT, 2016). Based on this data the study site is subdivided into:(1) Forest area (local name: Hardtwald) and (2) Urban area containing industrial, commercial and residential areas (Figure 2a). A more detailed subdivision in the urban area did not appear reasonable due to the heterogeneous structure."

Comment #2: Did you consider looking at stygobite vs stygoexene ratios, this may have provided more insight into the biotic differences.

Response: As we already mentioned in in the last round of revision in Comment #26 of Referee #2, we partially agree that use of the ratio of stygobites/stygophiles vs. stygoxenes might be useful in the context of this study. We agree that this ratio could provide more insight into the biotic differences. Yet, we decided not to use it, because the required determination of the fauna is not part of the assessment scheme by Griebler et al. (2014) (Level 1). The information is therefore not added to our manuscript, yet we now mentioned this ratio as an important adaptation for future assessment schemes in the conclusion of the manuscript.

Comment #3: You mention using detritus as a measure, but there is not mention of methodology, unit of measurement is not included, and you have referred to this inconsistently throughout the results.

Response: We agree that information about measurement technique and unit of detritus was not mentioned in the methodology yet. Thus, we added the following paragraph (lines 157-161):

"Furthermore, the relative amount of sediment as an indication of the nutrient availability and the cavity system was measured. Before the fauna sample from the net sampler was passed over a sieve with a mesh size of 74 µm, the sediment is separated and classified in different categories (sand, fine sand, ochre, detritus, silt). It should be noted that the detritus content is not recorded

quantitatively but on the basis of estimated frequency classes. The estimation of the relative amounts of sediment per sample is based on Table S1 in the supplement."

Table S1. Estimation of the relative amounts of sediment per sample (modified after Hahn, 2006)

Scale	Description	Characterisation
0	Absent	No sediments in the sampling vessel
1	Little	Bottom of the sampling vessel (Ø ¼ 7.6 cm) slightly covered by sediment
2	Much	Bottom of the sampling vessel covered by several millimetres of sediment
3	Very much	Bottom of the sampling vessel covered by one or more centimetres of sediment

Comment #4: It would be good to get an indication of the flow of GW particularly in the areas where forest and urban areas are close. This would help the statement made in line 233 (see below).

Response: We agree that a more detailed indication of the groundwater flow would help the reader to follow this statement. In addition to the statement about flow velocity (lines 260-261) and the general flow direction already indicated in Figure 2c and Figure S1b, we added a groundwater contour map in Figure 2c providing more details about local groundwater flow conditions.

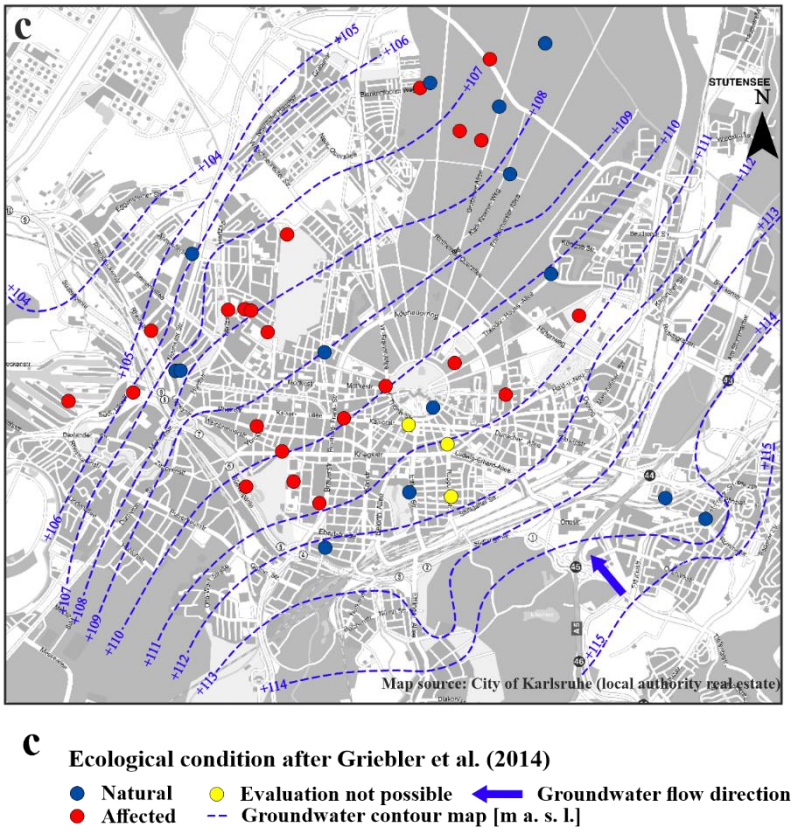

Figure 2: Overview map city area of Karlsruhe: … (c) faunistic evaluation after Griebler et al. (2014) and groundwater contour map in metres above sea level (modified after the local authority real estate of Karlsruhe).

Comment #5: Whilst language and grammar have improved since the first version, this still requires a good proof-read to remove grammatical and punctuation errors.

Response: We agree. Hence, we again thoroughly checked the manuscript to remove grammatical and punctuation errors.

Specific comments

Comment #6: Line 51-53: incorrect grammar. Remove ‘etc’ and combine sentence on line 53 to above paragraph.

Response: We agree. Done.

Comment #7: Line 104: it would be great to see a hypothesis here…

Response: We agree. Hence, we added the following sentence to the paragraph (line 113):

“The objective of this study is to investigate specifically the groundwater fauna beneath an urban area in comparison to a natural forest to determine whether land use has an impact on groundwater organism communities.”

Comment #8: Line 225: One sentence doesn’t make a paragraph, combine with previous paragraph.

Response: We agree. Done. We combine the sentence with the previous paragraph by adding, i. a. the conjunction ‘moreover’ at the beginning of the sentence.

Comment #9: Line 233: ‘no impact of GW originating from the urban areas on the wells in forest areas is observer’ how does reader interpret this as we do not know flow direction of aquifer?

Response: We agree and added further information to this paragraph and a groundwater contour map in Figure 2c:

“Moreover, no impact of groundwater originating from the urban area on the wells in the forest area is observed, as the groundwater flow direction in Karlsruhe is northwest (see Chapter 2.1 and Figure 2c).”

Comment #10: Line 238-241: this seems to be in the wrong section. Talk about biota in the below section.

Response: We agree and moved the two sentences to the below section (lines 336-338).

Comment #11: Line 244: The ‘biotic’ communities sounds better.

Response: We agree. We replaced the word organism by biotic.

Comment #12: Line 274: Also need to clarify that n=8 in ? forested areas. (this should also be stated in the methods section ie 8 wells in forested areas X wells in urban areas)

Response: We agree. Thus, we added this information in the brackets in line 300. Moreover, we added the following sentence in the methodology (line 143):

“From 2011 to 2014, samplings of groundwater parameters and fauna were performed in 39 groundwater monitoring wells in Karlsruhe, of which eight wells are in the forest and 31 in the urban area.”

Comment #13: Line 295: missing a comma

Response: We agree. Added.

Comment #14: Line 306: How did you measure detritus (should be in methodology) and what does (>2) mean? What are the units here?

Response: We agree. Thus, we added this information in the methodology (see Comment #3).

Comment #15: Line 381-383: mention 31 wells in urban and 8 wells in forested areas.

Response: We agree. Done.

Comment #16: Line 385: I would mention that Ampiphod were much more abundant in forested wells than in urban areas.

Response: We agree. Done. We added the following sentence in the conclusion (lines 475-476):

“Moreover, Amphipods are more abundant in wells in the forest than in urban area.”

Comment #17: Line 403: remove the ‘etc’

Response: We agree. Done.

Dear Referee #2,

we would like to thank you for your time and the constructive comments, which helped to improve the quality of the manuscript. Please find our detailed replies on the comments below. We hope that we answer all your remarks.

In general, our replies to the referee's comments are highlighted in blue. To highlight the nature of our replies we use a traffic light system indicating agreement with the referee marked in green, partial agreement in yellow, and objections in red.

Best regards,

Fabien Koch, on behalf of all authors

Referee #2:

The study of Koch et al. has now been revised and individual sections of the manuscript improved considerably. And while I still think there is substantial novelty in this data set, there is numerous issues that would need to be seriously addressed before publication. In fact, the manuscript contains 'scientific errors' that must be removed and draws conclusions that are not supported by the outcome of the study (see below). […] I am very sorry to disappoint the authors, after putting efforts in the revision of the original submission, but the manuscript to my opinion is still far from being ready to be published in HESS. I recommend another round of major revision.

Response: Thank you for the critical assessment of our study. We address your specific comments in detail below.

Specific comments

Comment #1: To my opinion, nitrate values found in urban groundwater are comparably low and to my opinion do not point to a strong contamination. Moreover, there is numerous studies that underline that nitrate at concentrations below 50mg/L does not directly affect groundwater fauna. In consequence, one cannot expect much of an outcome in that respect. Indeed, correlations with nitrate have been shown but through indirect effects in agricultural areas. Since only a few physical-chemical parameters have been measured, and only temperature and land use, that show clear alterations to a 'natural' reference situation, I would put my focus on these two 'impacts'.

Response: We agree to put the focus on temperature and land use as major impacts, and modified the manuscript accordingly. In line 266, we deleted "and nitrate concentration" and in line 332 "and the highest nitrate concentrations (> 6 mg/l)". Furthermore, we added the following sentences to the discussion of Chapter 3.2:

"In case of nitrate, numerous studies underline that nitrate at concentrations below 50 mg/l does not directly affect groundwater fauna (Fakher el Abiari et al., 1998; Mösslacher and Notenboom, 2000; Di Lorenzo and Galassi, 2013; Di Lorenzo et al., 2020). As the highest average nitrate content per well is below 15 mg/l in this study, a direct negative effect of the nitrate concentration on the groundwater fauna is unlikely. Thus, nitrate is only mentioned as one measured parameter and is not discussed as a potential anthropogenic impact in this study."

Comment #2: I fully agree that groundwater fauna is temperature sensitive and in central Europe stygobionts are almost exclusively (with some exceptions) cold stenothermic. I do not agree with the thresholds mentioned in the manuscript and the sources cited. It is stated (P2-L37) that groundwater fauna 'cannot withstand' water temperatures over 16°C (Brielmann et al. 2009) or rather 14°C (Spengler et al. 2017) for an extended period. This is definitely not true. I went back into the cited sources and what is stated there is as follows: Brielmann et al. (2009) says "True groundwater invertebrates (stygobites) are assumed to be cold stenotherm and can hardly persist at water temperatures exceeding 16°C for extended periods of time (T.Weber & S.I. Schmidt, unpublished data)." It says 'hardly' and cites work 'not

published' and the paper is 10 years old. The study itself found that "… faunal abundance showed no relation to impacted groundwater temperatures, but faunal diversity decreased with temperature, possibly emphasizing the sensitivity of individual groundwater invertebrates towards heat discharge." No relationship between temperature and faunal abundance! In Brielmann et al. (2011) it is stated that „Niphargus inopinatus (groundwater amphipod) when allowed to move freely in a temperature gradient preferred a temperature between 8 and 16°C; in 77% of the observations the speciemen were found there, but in consequence in 23% of the cases the animals were outside this range. For the isopod Proasellus cavaticus, specimen were in 66% of the observations found between 8 and 16 °C. In Glatzel (1990) a species-specific critical threshold temperature of 19°C is mentioned for Parastenocaris phyllura (harpacticoid copepod) beyond which a significantly higher mortality occurred. A study on groundwater microbes and fauna in local aquifers below basins collecting surface runoff during extreme rain events found that groundwater fauna was almost absent at spots that were impacted by significant temperature dynamics, with maximum temperatures of up to 22°C (Foulquier et al. 2011). Spengler et al. (2017) reports about declining fauna biodiversity at temperatures above 14°C. In fact, there is species found that start to disappear from the communities at higher temperatures while others are still found.

If we summarize all this information, then it is clear that there is a variability in temperature tolerance among groundwater faunal groups and species. No clear threshold at 14°C or 16°C appears proven, more likely individual thresholds are somewhere between 14°C and 18-20°C, based on what has been reported so far. It is really essential to carefully interpret findings from other studies and data published.

Response: We fully agree. We reformulated the paragraph carefully and added more studies as follows (lines 36ff):

"Hence, in Central Europe they are assumed to be cold stenotherm which means that they prefer cold temperatures. A variability in temperature tolerance among groundwater faunal groups and species is reported in various studies, which explains why the use of individual temperature thresholds is more useful to capture different preferences. According to Spengler (2017) faunal diversity is generally declining at a temperature above 14 °C. Various authors reported species specific temperature preferences between 8 and 16 °C (for individuals of the species *Niphargus inopinatus* and *Proasselus cavaticus* (Brielmann et al., 2009, 2011)) and a specific temperature

threshold of up to 19 °C (for *Parastenocaris phyllura* (Glatzel, 1990)). Above these thresholds the mortality of individuals raises until groundwater fauna is almost absent, for example at 22 °C in the study of Foulquier et al. (2011). However, temperature sensitivity is not only an issue at species level, but also for the communities as a whole. Spengler (2017) reported 12 °C to be a temperature threshold value indicated by a shift in community structure for faunal communities of groundwater of the Upper Rhine Valley."

Comment #3: To my very personal opinion there is two ways to publish scientific results and findings. First, to do the minimum necessary. Second, to explore the data best possible. My feeling is, and this was already said in the first round of review, that the data set has not yet been explored and analyzed in a proper way. Although there was substantial criticism from both reviewers because of a lack of statistical analyses, the only change that was done is applying now a simple Withney-Mann-U-Test to all data. That is sad and boring, and to my opinion does not deserve publication in a high ranked journal. Only from the papers cited, the authors could have derived ideas about the application of additional, more sophisticated multivariate tests like PCA, CCA, … Sorry to be so direct.

Response: We agree that the dataset should be explored in the best possible way. Thus, we added an additional, more sophisticated multivariate analysis in form of PHATE analysis. The rationale for the selection as well as the description of the method were added in the methodology, the results of the analysis are described in the new chapter 3.4. Moreover, the detailed results of the PHATE analysis were added to the supplement of the manuscript (Figure S1b, S3 & S4 and Table S45 & S5).

"To better understand large-scale relationships as well as fine structures of high-dimensional biological data, the PHATE (potential of heat diffusion for affinity-based transition embedding) analysis introduced by Moon et al. (2019) (https://github.com/KrishnaswamyLab/PHATE) was used. This dimensionality-reduction method generates a low-dimensional embedding specific for visualization, which provides an accurate, denoised representation of both local and global structures of a dataset without imposing strong assumptions on the structure of the data. The PHATE algorithm computes the pairwise distances from the data matrix and transforms the

distances to affinities to encode local information by applying a kernel function, which is developed to Euclidian distances. By using diffusion processes, global relationships are learned and encoded using the potential distance. Finally, the potential distance information is embedded into low dimensions for visualization by using metric Multi-Dimensional-Scaling (MDS) (Moon et al., 2019). Objects that are close to each other in the final graph therefore have similar characteristics."

"A PHATE analysis is conducted using the following 15 input parameters: depth, GWT, nitrate and phosphate content, relative amount of detritus, geological unit, numbers of taxa, number of individuals, Shannon diversity, amount of Crustaceans and Oligochaetes (according to Griebler et al., 2014) and the abundance of Amphipods as well as of individuals of the order *Cyclopoida*, *Bathynellacea* and the genus *Parastenocaris*. The content of dissolved oxygen is not considered in this analysis, since it was always above the limit of 1 mg/l, except for in one case. Thus, dissolved oxygen is not expected to have an influence on the groundwater fauna in our study area.

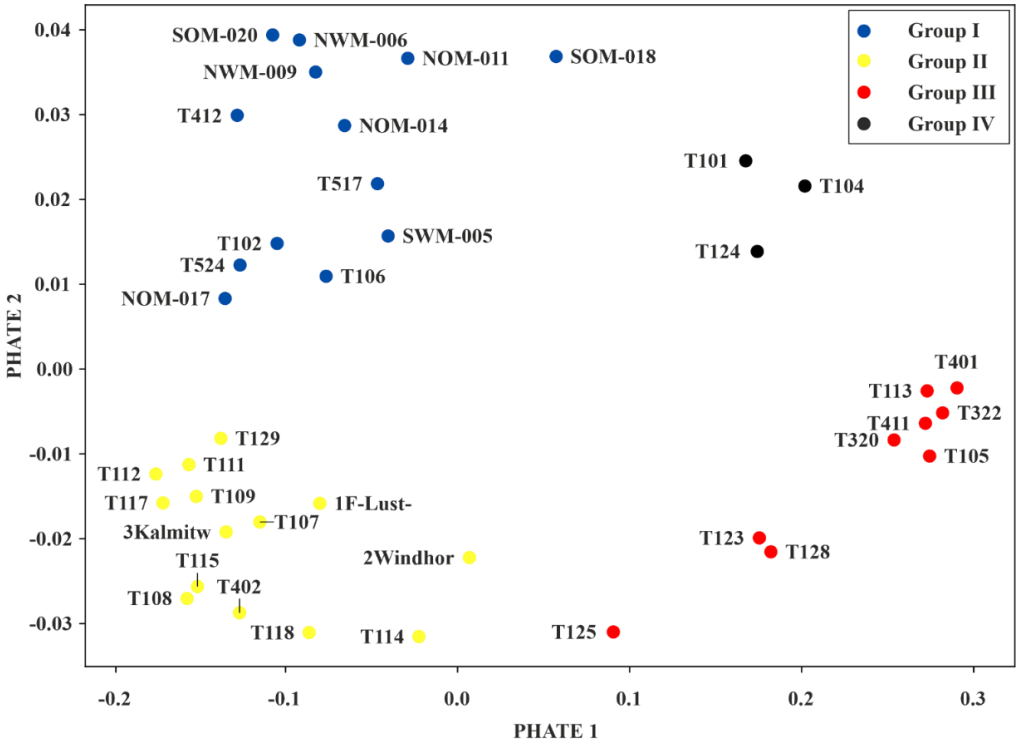

Figure 5: PHATE visualization showing similarities between measurement wells. Different colours indicate the four clearly separable groups.

Four groups, which can be assigned predominant characteristics, can be distinguished in the PHATE visualization (Figure 5, Figures S3-S4). Three measurement wells (Group IV) contain neither Oligochaetes nor Crustaceans, indicating unfavourable living conditions. In contrast, the nine wells of Group III contain high amounts of Oligochaetes (100 % Oligochaetes according to the scheme of Griebler et al. (2014), and an average GWT of 14.3 °C. However, diversity and abundance was found to be low in Group III.

An even higher average GWT of 15.0 °C was found for Group II, which mostly consists of wells drilled in drifting sand sediments. Surprisingly, these wells also show the highest diversity (≥ three Taxa per well), the highest Shannon diversity (see Supplement), highest amount of individuals in total, as well as of individuals of the genus *Parastenocaris*. Individuals of this genus are often found isolated in altered areas (Spengler, 2017). Moreover, in five wells of Group II individuals of the order *Bathynellacea*, which can tolerate temperatures up to 18 °C and typically inhabit interstitial groundwater (Stein et al., 2012), were found.

The presence of individuals of the genus *Parastenocaris* and the order *Bathynellacea* in Group II suggests that they may act as type species for urban situations. The observation that Group II shows the highest GWT and the highest Shannon diversity is in contrast to findings of previous studies that noticed decreased diversity at elevated temperatures (Brielmann et al., 2009). These diverging observations suggest that faunal quantities, such as diversity or abundance, are not always suitable indicators for changes within organism communities. For example, if species disappear due to increased temperatures and are substituted by more tolerant species, the difference in diversity may be marginal and the change in the community may not be noticeable.

Wells of Group I (blue) are drilled predominantly in Würm gravel (geological unit of Group I vs. Group II: U-test: p-value = 8.2×10^{-3}, n = 13; 14), while having the lowest GWT (GWT of Group I vs. Group II: U-test: p-value = 2.0×10^{-5}, n = 13; 14). These wells show a moderate diversity and amount of individuals, yet the highest average amount of Crustaceans as well as the highest amount of Amphipods and individuals of the order *Cyclopoida*. Considering these findings and the U-Test results (see Table S5), the grouping of the measurement wells seems to be influenced

by the composition of the groundwater organism communities, the faunal diversity (numbers of taxa and amount of individuals), as well as the geological unit and the GWT (see Figure S3-S4). Considering the spatial distribution of the grouped wells in the study area, it becomes apparent that all wells in the forest area fall within Group I (Figure 5). Those wells which are located outside the forest area are in locations with nearby green areas (parks, recreational areas, etc.). In contrast, the wells of the other three groups are heterogeneously distributed within the urban area. Many of the measurement wells of Group III and IV are associated with suspected or known contaminated sites (see Figure S1b).

Overall, a spatial pattern of abiotic groundwater characteristics (GWT, nitrate content) and occurrence of particular species (*Parastenocaris*) within the study area is apparent in the PHATE analysis, which confirm the classification according to land use. Yet again, no clear spatial pattern regarding faunal diversity in the study area could be identified. Although, a tendency of clustering of wells from Group III with higher diversity and amount of individuals can be seen in the northwest city area."

Comment #4: I like the idea of testing the ecological assessment schemes of Hahn (2006), Griebler et al. (2014) and Korbel & Hose (2017) in an urban setting. However, such an application needs to be done with some care. In the first tier (step) of the scheme described in Griebler et al. (2014) which is somehow similar to what was published by Korbel & Hose (2011), it is recommended to choose five or more criteria with a minimum of 3 biological ones. If criteria are selected that are partly dependent to each other, e.g. proportion of crustaceans and proportion of oligochaetes, then the resolution of the assessment is very low. Surprisingly, although several assessment indices have been considered by the authors (GHI, GESI, GFI), results of none are presented in the paper. Obviously, as I got from the reply to reviewers' comments, things have not worked out as clear as expected. I would have liked to read in the discussion about the 'pitfalls' of the individual assessment schemes. Again, an assessment scheme cannot compensate the lack in use of multiple sensitive criteria. Finally, although, the prerequisite to sample stations more than once is fulfilled, sites that are compared have been sampled in different years, a fact that should at least be discussed.

Response: We absolutely agree that results from additional assessments, which are not presented in the manuscript (but in the supplement of the manuscript), and the fact that wells have been sampled in different years should be discussed. Hence, we added the following sentences to the manuscript (lines 378-380 & 409-423):

"Care should also be taken when interpreting faunistic results of sites that were sampled in different years. To improve comparison of the biotic communities, a consistent sampling period of every well is necessary in the future."

"Furthermore, the integration of additional biological criteria might help to improve the results of the assessment according to Griebler et al. (2014), as well as the application of different assessments, such as the similarly structured GHI or wGHI^{N} (Korbel and Hose, 2017; Di Lorenzo et al., 2020b). Moreover, there are a couple of newly developed indexes, like the D-A-C-Index, which is based on microbiological indicators and shows whether groundwater reserves deviate from natural references (Fillinger et al., 2019), which can be used in the future. As mentioned in the introduction, another way to quantify the relevant ecological conditions in the groundwater is the GFI (Hahn, 2006). During the preparation of this study, the GFI was applied to the data (see Supplement), however, it did not provide any additional information or valuable insights. The influence of multiple stressors, such as the pollution of the groundwater by industrial plants etc., and their effects on the governing parameters are likely to bias the GFI. In general, the GFI seems to be suitable only for unpolluted and anthropogenically undisturbed groundwater with sufficient oxygen concentrations (> 1 mg/l). Moreover, in urban areas changes in GWT are caused by anthropogenic heat inputs (Menberg et al., 2013b, 2013a; Benz et al., 2014; Tissen et al., 2018), rather than being related to surface water influences. Hence, the GFI appears to be unsuitable for the assessment of the groundwater fauna in an urban setting. The same outcome emerges for the Shannon diversity index, which was also tested during the preparation of the study and showed no clear distribution pattern according to faunal diversity."

Comment #5: I guess, we all agree that this first study of groundwater fauna and assessment of the groundwater ecological status in an urban setting was accompanied by some limitations. There have been

only a few physical-chemical parameters measured, the number of wells sampled werde very different for the two land use categories, and regional and local reference conditions for the groundwater fauna were missing, to give just three examples. This is normal, and one can nicely build on this first experience. And yes, the reply of the authors to several of the reviewer recommendations was: “The aim of this study was to provide a first overview of the ecological groundwater conditions of the study area”. What I really disliked is that although the results are of limited validity and transferability, and need to be confirmed in follow-up investigations, at the end of the discussion section it is stated that: “Areas with no or little groundwater fauna could be used for to store thermal energy at higher temperatures.” and “HT-ATES could be established in urban environments.” How can this conclusion be drawn from the findings presented?

Response: We agree that this conclusion cannot be drawn from the findings anymore. Thus, we removed this paragraph.

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