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 revise our manuscript, for your time and for the constructive comments. We hope that we answer all your remarks.

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:

Two reviewers evaluated the manuscript and gave very detailed and very helpful comment for improving the manuscript. Both reviewers appreciated the general topic of the study but requested a more thorough data analysis including statistics, justification of some of the statements, and a more detailed discussion. Further, the structure of the introduction was recommended to be improved as well as the manuscript's language.

The authors answered in detail to the comments and mainly agreed to the main points raised. They have to be implemented into a revised version of the manuscript next. I further recommend that those comments, where the authors (partially) disagree, should still be taken up - in an objective way – in the discussion, because they are very useful.

The manuscript will be reconsidered for publication after major revisions.

Response: We agree that the referees gave very helpful comments for improving the manuscript. Therefore, we performed a more profound statistical analysis and provided further details in the discussion.

Moreover, we improved the structure of the introduction and the manuscript's language. The manuscript was carefully revised by a native speaker to ensure correct English.

Furthermore, we agree that the comments, where we (partially) disagree, should be taken up in the discussion. Thus, we added this information in the discussion to take into account the very useful comments (see comment #22 Referee #1, comment #9 Referee #2 and marked manuscript).

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:

This article is an application of an existing method to assess groundwater ecological condition. The article utilises a classification scheme based on a single threshold of proportion of crustaceans and oligochaetes within sample wells, with varying success. The manuscript acknowledges several limitations of using this single method suggesting that multiple methods should be used to fully understand impacts of humans on groundwater ecology. The research presented increase awareness of groundwater ecosystems and the threats facing them, however requires further analysis to justify some of the claims made. As such, I recommend major revision, purely because of the requirement for further statistical analysis.

> Response: We partially agree. Thus, we performed a more profound statistical analysis (e.g. U-tests), which are presented below in our replies to the 'specific comments'.

General comments

Generally, the sections flow well and it is easy to understand. The manuscript needs to be thoroughly edited as there are multiple issues with grammar, and the manuscript can be reduced in length particularly in the introduction. The figures and table are well presented. The methods and results section needs to have some aspects clarified. There is a lack of statistical analysis throughout the manuscript which detracts from the quality of the paper. The results show some interesting trends in the distribution of biota, however without the necessary statistical analysis of this data, it is difficult to establish if there are significant differences between landuses, or if these trends are just due to differences in sample size (n8-n31) between the two landuses. This needs to be addressed, as currently there are speculations that differences in means indicates differences between landuses without any specific statistical analysis. A simple ANOVA or t-test would, in most cases, suffice and allow a more thorough analysis of this useful data.

Response:

We agree on the grammar issues. Hence, the manuscript was carefully revised by a native speaker to ensure correct English.

We agree on the length of the introduction. Several sentences were shortened or even deleted (for more details see the marked manuscript attached).

We partially agree on the statistical analysis as already mentioned earlier, which is detailed below (Comment #12).

Specific comments

Comment #1: Introduction: In general the introduction is a little too long and can be made more concise. Eg paragraph starting line 45 and line 50 could be compressed and merged.

Response: We agree on the length of the introduction and the conciseness. Thus, we shortened or deleted sentences, e.g. we condensed the paragraph starting in line 47: "The study by Hahn and Fuchs (2009) focuses on defining stygoregions based on different hydrogeological units located in Baden-Württemberg, Germany. They conclude that the observed patterns of groundwater communities reflect a high spatial and temporal heterogeneity of aquifer types with respect to habitat structure, food, oxygen supply etc."

Comment #2: Line 35-37: Whilst these may be the usual temperatures for stygofauna within the region of this study, they exist in temperatures well over 14-16 deg on a global basis. This sentence needs to be rephrased.

Response: We agree on the reformulation of this sentence (lines 36-38). Nevertheless, it was not possible to find international values. Thus, we clarified the spatial reference of the values in this sentence: "Hence, in Central Europe they are assumed to be cold stenotherm, which means that they prefer cold temperatures and cannot withstand water temperatures over 16 °C (Brielmann et al., 2009) or rather 14 °C (Spengler, 2017) for an extended period."

Comment #3: Line 38: Remove 'the' from "the German and European legislation"

Response: We agree. Done.

Comment #4: Line 44 remove 'data recorded by' in the brackets

Response: We agree. Done.

Comment #5: Line 54: Typo error (Protocol for the Assessment: : :..

Response: We agree. Done.

Comment #6: Line 83: Korbel & Hose 2011 is correct reference, also consider Di Lorenzo et al. 2020 Ecological Indicators, 116, 106525.

Response: We agree and now consider this new study in the manuscript. Hence, we added the following paragraph (see lines 91-94 in the marked manuscript):

"This index is applied and tested by Di Lorenzo et al. (2020) in unconsolidated aquifers in Italy, which are located in nitrate vulnerable zones. They refined the index ($wGHI^N$) and demonstrated its applicability on shallow and deep aquifers and also revealed that this new index is limited due to low correlations between the indicators."

Methods

Comment #7: Line 116: replace ‘with’ to ‘was’

Response: We agree. Done.

Comment #8: Line 116-117: improve sentence structure ‘is mainly caused by’ is incorrect, consider ‘which’ is mainly caused by: : : or rewrite sentence appropriately.

Response: We agree. Done. We replaced ‘is mainly caused by’ by ‘which is mainly caused by’.

Comment #9: Line 120-124: condense and combine sentences

Response: We agree. Done. We condensed and combined sentences in this paragraph as follows: “A contaminant plume, which contains a polycyclic aromatic hydrocarbons concentration of up to 500 µg/l, of 200 m length over the entire aquifer thickness is located at a former gas plant in the east of Karlsruhe (Figure S1b) (Kühlers et al., 2012). Moreover, three parallel contamination plumes of 2.5 km length each, can be found in the southeast of Karlsruhe (Figure S1b), where highly volatile chlorinated hydrocarbons (7 µg/l – 26 µg/l) and their degradation products were detected (Wickert et al., 2006).”

Comment #10: Line 139-140: belongs in the results section not methods Section

Response: We agree. Done.

Comment #11: 2.3. I found this section hard to read, particularly due to some grammatical errors. This section is too long and verbose, it needs to be rewritten to make it clearer. The second paragraph starts well. I suggest removing the sentence start on line 160 “this requires to obtain also: : :.”

Response: We agree on the grammar and the length of this section. Thus, we rewrote the paragraph and deleted some sentences (for more details see marked manuscript, lines 161-174).

Comment #12: Statistical analysis: You do not mention any of the statistical analysis competed in this paper. To be able to distinguish between forest and urban areas, you should at a minimum be completing some statistical analysis of the water quality data you have collected, even if this is simple ANOVA or t-test analysis. This is a major issue that detracts from the quality of this paper. I understand that you have used average values of the sampling wells, however determine whether there are statistical differences between (for instance) temperature at forested areas in comparison with urban areas, and look at the relationships between temperature, well depth and landuses. This analysis would greatly improve the scientific credibility of this study.

Response: We partially agree. We therefore performed U-tests instead of the suggested t-test, due to the possibility that the abiotic data does not follow a normal distribution. The results are as follows:

- GWT forest vs. urban area: p-value = 3.3×10^{-5}, n = 8; 31 → significant
- Depth vs. GWT (forest): p-value = 1.6×10^{-4}, n = 8 → significant
- Depth vs. GWT (urban area): p-value = 5.5×10^{-5}, n = 31 → significant

Hence, we added the p-values of the U-tests to the manuscript (e.g. page 10, line 208). However, as various studies demonstrated (e.g. Amrhein et al., 2019) using p-values alone in a statistical analysis can lead to spurious interpretations, because p-values can exhibit wide sample-to-sample variability and therefore do not reliably indicate the strength of evidence against the null hypothesis (Halsey et al., 2015). Thus, we show the determined p-values, yet our focus remains on the presented spatial analysis using box-plots and other visual tools and comparisons.

Moreover, we added the following introductory sentence in Chapter 2.2. (line 148):

"Mann-Whitney-tests (U-tests) were applied to detect potential impacts of groundwater characteristics (physical-chemical parameters), geology and well design on the groundwater quality as well as on groundwater fauna. Samples were regarded as significantly different if the p-value was $< 5.0\times10^{-2}$."

Results/Discussion

Comment #13: Line 180: complete statistical analysis to indicate if there are significant differences in temperature between urban and forests areas- it appears that there are.

Response: We partially agree and added p-values of U-tests (see previous reply to comment #12).

Comment #14: Lines 192: while the box plots show that there are differences between forests and urban areas in DO and nitrate with landuses, these do not appear to be statistically different. I am not convinced that there are differences in DO and Nitrate between landuses this needs further discussion, as does the large differences in n values between the landuses.

Response: We partially agree (see previous reply to comment #12). The statistical analysis of the content of dissolved oxygen as well as of nitrate in the forest and urban area reveals significantly different distributions, which were added to the manuscript.

Furthermore, we agree that further discussion is needed with regard to the differences between both parameters and land use. Thus, the following paragraph was added in the manuscript (lines 218-223):

"In the urban area average nitrate concentrations are typically higher and correlate with the content of dissolved oxygen (U-test: p-value = 4.0×10^{-3}, n = 39) showing the link between nitrate content and oxygen consumption. Wells with a content of dissolved oxygen below 1.5 mg/l have an average content of nitrate of 1.5 mg/l, caused by nitrate reduction under anoxic conditions. Groundwater with reducing conditions (< 5 mg/l dissolved oxygen) has an average nitrate content of about 7 mg/l in contrast to groundwater with oxidising conditions with 9 mg/l, which is characterised by the oxidation of ammonium to nitrate."

We agree that the results would be more meaningful, if more measurements wells were considered. However, this is beyond the scope of this study and therefore should be part of a future large-scale study.

Comment #15: Line 196: References in chronological order

Response: We agree. Done.

Comment #16: Line 201: 'hold back' should be 'retained' or 'held back' Line 201: suggest these sentences are combined and reduced eg ': : :..where atmospheric nitrogen in retained by forest soils and fertilization is prohibited due to water protection regulations'

Response: We agree. Done.

Comment #17: Line 207-209: Again you cannot claim 'clear differences' without adequate statistical analysis of these factors. You need to run further analysis of the data for this statement.

Response: We partially agree (see previous reply to comment #12). Thus, we added *p*-values from U-tests to corroborate these observations (see also comment #13 & #14).

Comment #18: Line 231: I would not say that amphipods 'predominantly live within wells' rather they have a habitat preference for open spaces such as wells.

Response: We agree. We reformulated the sentence: "Stygobiotic Amphipods, i.e. large-bodied invertebrates which due to their size have a habitat preference for open spaces such as wells, …"

Comment #19: Paragraph starting line 231: It appears that amphipods are significantly higher in forested areas than urban areas, however without analysis this cannot be determined. This may be ecologically important and should be discussed. It is also worthwhile looking at the correlation between cyclopoida and amphipods as briefly mentioned in line 238.

Response: We partially agree (see previous reply to comment #12). Hence, we performed U-tests and added the corresponding *p*-values and the following sentences in the manuscript (line 268):

"Although statistical analysis with U-tests showed no significant differences between the abundance of Amphipods and land use (U-test: p-value = 1.5×10^{-1}, n = 8; 31), the higher number of individuals in the forest area can support the hypothesis that, as mentioned above, Amphipods indicate healthy groundwater ecosystems as…"

"The lack of a statistically significant correlation might also be related to the low number of wells (n = 8) and individuals (n = 46)."

Moreover, we agree that it is worthwhile looking at the correlation between the abundance of Amphipods and the order *Cyclopoida*. The following correlations were found and added in the manuscript (line 278). The results of the total statistical analysis are as follows:

- Abundance *Amphipoda* forest vs. urban area: p-value = 1.5×10^{-1}, n = 8; 31 → not significant
- Abundance *Cyclopoida* forest vs. urban area: p-value = 5.0×10^{-3}, n = 8; 31 → significant
- Abundance *Amphipoda* vs. *Cyclopida* (forest): p-value = 9.0×10^{-3}, n = 8 → significant
- Abundance *Amphipoda* vs. *Cyclopida* (urban area): p-value = 2.0×10^{-3}, n = 31 → significant
- Abundance *Amphipoda* vs. *Cyclopida*: p-value = 9.6×10^{-5}, n = 39 → significant

Comment #20: Line 238-240: Incorrect grammar: : : remove 'be'

Response: We agree. Done.

Comment #21: Line 248: Incorrect grammar

Response: We agree. We rewrote the sentence as follows:

"In addition, quantities of *Bathynellacea* (371 individuals) were found in five monitoring wells all located in the urban area in a depth of 9.0 to 13.5 m at a GWT of 12-15 °C (Figure 4b)."

Comment #22: Line 274- 280: The issue of purging wells needs further discussion as this is a limitation of your study. If you are looking at proportions of crustaceans to oligochaetes this is almost certainly affected by sampling method. The sentence on line 277 needs to indicate that relative abundances and proportions of crustaceans is likely to be impacted by the sampling methods, thus caution must be taken when interpreting the results.

Response: We partially agree that the sampling method is a limitation of our study. The standing water in the monitoring wells can host a larger number of individuals, caused by filtration effects. Yet, the proportional differences between the two groups are similar between wells and aquifers, as already demonstrated by various previous studies (Hahn and Gutjahr, 2014; Hahn and Matzke, 2005; Korbel et al., 2017). Moreover, only large Amphipods, which were found in three wells, prefer living in open space (well water) (Hahn and Matzke, 2005; Korbel et al., 2017). Other Crustaceans are smaller, like the order *Cylopoida*, and are not influenced by filtration effects.

We do not agree that the sentence in line 277 (now: 338) needs to indicate that relative abundances and proportions of crustaceans is likely to be impacted by the sampling methods, as justified by the explanation above.

However, we added the following sentences (line 334 & 341):

"Nevertheless, pumping can result in the selection of the taxa, especially in the presence of very fine sediments, and can result in changes of the sediment composition in the surrounding of wells and therefore in changes of habitat conditions."

"As the aim of this study is to provide an overview of the groundwater fauna community (assess biodiversity) and to receive a first impression of groundwater ecology, sampling the fauna by using a net sampler is sufficient."

Thus, we are confident about the appropriateness of the used sampling method, yet also mention that caution must be taken when interpreting the results (line 323).

Comment #23: Lines 295 -300: Could this also be due to organic carbon supply? Would level 2 assessment clarify these issues if it were undertaken?

Response:

We agree that the food supply is one of the most limiting parameters for the survival of groundwater fauna. Thus, we added the following sentence in the manuscript (lines 364-365):

"If the organic carbon supply varies on a small scale, this can influence microbiology and therefore groundwater fauna as well, although, short-term changes in nutrient supply can be compensated by groundwater fauna."

We agree that the application of Level 2 might help to get a better understanding of the living conditions of groundwater fauna and might explain why some measurement wells are not populated. The assimilable organic carbon is one indicator which can be chosen as criteria (of the category microbiology) for the evaluation according to Level 2. However, the application of Level 2 is time-consuming and cost-intensive.

Comment #24: Line 305: Could the high (35%) of urban areas displaying natural sites be due to the sample methodology; ie were they classified as good incorrectly due to high proportions of crustaceans that may be influenced by the lack of purging of the wells?

Response: We partially agree. The standing water of the monitoring wells can contain a larger numbers of individuals than the surrounding aquifer, because wells serve as traps for the groundwater fauna and filtration effects can occur. Yet, as mentioned above, the proportional difference between the two groups will be similar, which is the main criterion for good ecological conditions in this assessment. In addition, some smaller Crustacean (e.g. of the order *Cylopoida*), which were found in larger numbers in most wells, are not influenced by such effects.

Conclusion

Comment #25: The conclusions of this study to me indicate that the method you have adopted (ie net sample wells and use the proportions of oligo/crustacean populations to determine ecosystem condition) need to be investigated further. The disproportionate number of crustaceans in wells due to sampling methods may be impacting the assigning of “OK” condition to sites that are actually impacted. Potentially a wider range of indicators need to be used including expanding on the use of only oxygen concentration in the classification scheme. The Level 2 assessment (Figure 1) also needs to be discussed in the conclusion.

Response: We disagree that measurement wells are incorrectly classified as good due to the sampling method (see comment #24). This study is focusing on existing approaches to obtain an initial impression on this complex topic. However, we fully agree that there has to be a defined sampling method to achieve representative sampling and comparable assessment of groundwater fauna in the future (see line 344).

We agree that a wider range of indicators has to be used in such classification schemes. Thus, we added the following sentences to the conclusion (line 396):

“The Level 2 assessment from Griebler et al. (2014) can help to achieve a more reliable and quantitative ecological assessment of urban aquifers as it divides groundwater ecosystems in ecological grades according to the intensity of anthropogenic disturbance. It is based on the use of local reference values and the collaboration with experts, which is however challenging to apply. Therefore, further studies with large-scale and repeated measurement campaigns are needed to verify our findings. This should also include other cities and the determination of undisturbed local reference values which are required for a more reliable but also quantitative ecological assessment of urban aquifers. Moreover, a wider range of indicators should be considered in a classification scheme, such as temperature, porosity of the aquifer, groundwater flow, pollutants, nutrient supply, etc., especially when investigating urban areas. In addition, an important adaptation for an improved evaluation method is the determination of fauna at species level, which will provide more information (i.e. about Stygobionts, Stygophiles, Stygoxenes) and also consider the endemism of stygobiotic species. In this context, classification schemes should pay more attention to the different groundwater species and their potential use as indicator species."

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:

This study on the distribution of groundwater fauna in the shallow subsurface of urban (city of Karlsruhe) and rural (nearby forest) areas, as well as the use of groundwater fauna for the assessment of the ecological status in groundwater has considerable scientific novelty. To my knowledge, this is the first study that investigates groundwater ecological aspects in a city's subsurface. This strength by novelty, however, is kind of counteracted by serious weaknesses. While I like the study very much on one hand, it is a pity that the authors did not spend enough time to distill the best out of it. Besides obvious shortcomings in the study design (the selection of chemical parameters measured, the restriction to only well water), the authors did not dig at all into the data available in a 'statistical' sense. I do see more categories of land-use types. I think measures such as well depth and origin of groundwater (what if groundwater impacted in the urban area travels underneath the forest where it is sampled) would be interesting aspects to evaluate. Moreover, fauna data set comes along with further information that has not been used, i.e. the Shannon-Wiener biodiversity and the ratio of stygobites/stygophiles vs. stygoxenes. Not to talk about the determination of individual 'species' of Crustaceans and other groups of animals that could resolve the picture much more. Although, the basic water chemistry in the urban groundwater exhibits some differences to the groundwater sampled in the rural area, there is obviously no clear indication for a 'contamination' of the urban groundwater. An exception is only some temperature deviations. Thus, why it is expected that the groundwater fauna in the urban area is different. I would have loved to see a few hypotheses that are tested. When reading the preprint I also got the impression that most groups of groundwater fauna are described as quite temperature tolerant, however, other publications of the same authors claim the strong sensitivity of groundwater fauna upon groundwater warming. I really missed individual statistical testing of such questions. To be very honest, the paper addresses a really interesting topic that ghas ahrdly been studied to date but has not been properly prepared before submission. My feeling is also that some of the co-authors have not spend much time with the paper, otherwise it would not contain so many flaws. In the following, I will try to provide detailed comments that may help to improve the manuscript. Overall, I not sure if the paper, even when reworked properly, will satisfies the high standard of HESS.

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

Specific comments

Comment #1: P1 L19-20: How have the anthropogenic impacts be measured. I agree that elevated temperature may be seen as Impact. What else? The groundwater chemical analyses do not focus on any contaminants, with exception of nitrate; and nitrate concentrations are not elevated.

Response: We agree that our study is focusing on temperature and nitrate concentrations as important anthropogenic impacts on groundwater ecosystems. We now specifically mention these two proxies in the abstract and also cited the publication by Griebler et al. (2016) in the Introduction (see lines 77-79).

We are aware that there are more potential anthropogenic impacts, such as contaminants, which are not specifically considered in this study. We further agree that more investigations are necessary in the future, as there are likely to be more influencing factors on groundwater fauna distribution such as the sediment, groundwater flow, pollutants, nutrient supply, well design, etc.

Thus, we added further research in the supplement of the manuscript and a short summary about what the urban impact is in the summary of chapter 3.1 (see comment #26 and comment #25 Referee #1).

Comment #2: P1 L21: it is mentioned here that more comprehensive assessment methods are required to fully capture the different effects on groundwater fauna. I agree. However, you should mention, at least in the discussion section, what you think of.

Response: We agree that potential strategies for more comprehensive assessment methods should be discussed in more detail. Hence, we added some information to the manuscript, which is also presented in the reply to comment #25 by Referee #1 and in the marked manuscript.

Comment #3: P2 L44-50: This paragraph does not seem to be linked to the what is introduced before and after.

Response: We agree. Hence, we linked the paragraph by adding the following introductory sentence (lines 45-46):

"The availability of ecological criteria can only be increased by conducting a large number of studies dealing with the analyses of groundwater ecosystem health by investigating groundwater fauna."

Comment #4: P2 L51: There is a pile of studies dealing exactly with that. You should name some as examples. What is really new with your study is that there is hardly anything investigated in urban areas.

Response: We agree. We reformulated the sentence and added some studies as follows: "Accordingly, although there are various studies on this topic (e.g. Gibert and Deharveng, 2002; Malard et al., 2002; Deharveng et al., 2009; Dole-Olivier et al., 2009b) stygobiotic biodiversity is still likely to be underestimated."

Comment #5: P2 L53: Are you sure that the European Union (FP5) PASCALIS project focused on 7 North-American regions. Please check that again.

Response: We agree that the PASCALIS project is focusing on six European regions only. Gibert et al. (2009) focus on six European, as well as seven North-American regions. Thus, we deleted this part of the sentence.

Comment #6: P3 L57: If you state here that regional features have a stronger influence on groundwater fauna than local habitat features, you should test that with your data set. If this is true, maybe the anthropogenic impacts are not strong enough to overrule the regional selective forces. This point should also be discussed.

Response: We agree that this point should be tested with our data and discussed. Thus, we added the following information and results from an additional statistical analysis to this paragraph (lines 311-327), dealing with local habitat features:

"One important natural influence is the local geology, as fine sands and silts are typically rather harsh environments, resulting in an impoverishment of specific groundwater fauna such as *Crustacea* (Hahn, 1996). The city of Karlsruhe is located on carbonate ('Würm') gravel and river terrace sands, pervaded by bands of drifting sand and inland dune sands. These sediments are highly water-permeable and show almost exclusively vertical seepage of water movement. Flood sediments (on top of the river gravel) and bog formations, are located in the east and west of Karlsruhe (Regierungspräsidium Freiburg, 2019). This local geology limits the cavity size and therefore has impacts on the habitat of the groundwater fauna (Wirsing and Luz, 2007). For example, individuals of the genus *Parastenocaris* typically inhabit small-scale cavity systems (Spengler, 2017). Individuals of this genus can be found both in the wells drilled in gravel (4 wells) and in drifting sand sediments (3 wells) (abundance *Parastenocaris* vs. geological units: U-test: p-value = 1.4×10^{-9}, n = 39). Amphipods are predominantly found in measurement wells located in the 'Würm' gravels (in 5 of 7 wells) (abundance *Amphipoda* vs. geological units: U-test: p-value = 9.0×10^{-11}, n = 39). Moreover, it seems that differences in the geological units have an influence on the total amount of individuals (U-test: p-value = 1.7×10^{-9}, n = 39) and the relative amount of detritus (U-test: p-value = 3.0×10^{-3}, n = 39). As these results show, regional geology seems to have an influence on the occurrence of specific groundwater taxa and on the number of individuals as well as on food supply, in terms of available organic matter. However, it is not possible to give a reliable estimate of the strength of the anthropogenic impacts, e.g. if they are strong enough to overrule the regional selective forces. Hence, this should be investigated in more detail in future studies."

Comment #7: P3 L83: you could also have used a different approach to look at your results. What if you treat the forest samples as your local natural reference? Just an idea. Starting from there, you could evaluate which well downtown Karlsruhe match natural conditions and which not. Currently, you obviously use a German-wide reference conditions and thresholds (Crustaceans >50%, worms <20%) that may not 'absolutely' reflect the situation in the natural surroundings of Karlsruhe.

Response: We fully agree that it might be useful to define local thresholds by using the forest samples. Thus, we tested the proposed idea. We used the average values of all wells in the forest to define local 'natural' conditions. The calculated values are as follows:

- Chemical: 8 mg/l O_2, Nitrate: 2.7 mg/l
- Physical: 10.7 °C, a high content of detritus
- Faunistic: 28 % Oligochaetes, 66 % Crustaceans, 6 % other individuals, in average three taxa in each well, 131 individuals in each well, average Shannon Diversity Index: 0.7

The biggest issue with these conditions arises from the temperature (no well in the urban area is as cold as the wells in the forest) and the low nitrate concentrations in the forest. Without consideration of temperature and nitrate content, four wells in the urban area are in accordance with the new 'natural' reference values. As this number is similar to the original approach with the German-wide reference, it appears that these new thresholds cannot reflect the complex situation in the urban area of Karlsruhe. Thus, we decided not to adopt this idea for our paper.

Comment #8: In P3 L86: you say that the authors of the UBA study come to the conclusion that aquifer typology is more important than local features. Is this what you say? Why this is not properly discussed in your paper?

Response: We agree that this discussion is missing. Hence, we added a corresponding paragraph in the manuscript (lines 311-327, see comment #6 above).

Comment #9: P4 L113: If 56% of the city's area is covered by vegetation, doesn't it make sense to group the wells in the urban area according to their 'land type' on top and do some statistical analyses?

Response: We disagree to subdivide the wells in the urban area according to their land use due to the following reasons. The total city area of Karlsruhe contains not only the inner city centre and the neighbouring districts (termed "urban area" in this study), but also parts of the Hardtwald and several less built-up outskirts, which results in the high proportion of vegetation in the official 'city area'. The urban area itself however does not contain enough green spaces to justify a more detailed subdivision and statistical analysis. In our opinion, there is a risk of over-interpreting the results by following this approach. Instead, sampling of groundwater fauna and parameter measurements should be repeated before more emphasis is put on other influencing factors, such as land use.

To clarify the issue, we added the information about the definition of the city area in the manuscript (lines 118-119):

"Based on the land use plan of Karlsruhe, about 20 % of the area (i.e. urban area, city centre, neighbouring districts, as well as parts of the Hardtwald forest and several outskirts) is covered by buildings."

And we added the following note in the discussion (lines 372-373) to take into account this important aspect:

"In future, a further subdivision of a study area in more land use categories could be useful to specifically look at typical anthropogenic impacts."

Comment #10: P5 L127: It is mentioned here that the sampling took place between 2011 and 2014 and 39 wells have been sampled. But how often each well were sampled is not mentioned. Did I miss it. 3 times, as said in line 134? Or more often? You cannot have followed the recommendations of Hahn & Gutjahr published in 2014 when sampling took place between 2011 and 2014.

Response: We agree that this information is not mentioned explicitly, but would help to understand the framework of this study. We added this information in the part ‘Material and sampling’ (lines 142-144) and in Table S1 in the Supplements:

“Every well is sampled at least three times. From 2011-2012, 22 measuring wells (mainly in the Hardtwald and the North-West of Karlsruhe) were sampled six times at a minimum interval of two months. In 2014, 17 measurement wells, mainly located in the south/inner city, were sampled three times.”

Thus, our approach is in agreement with the recommendations given by Hahn and Gutjahr (2014).

Comment #11: P5 L134: what you mean with ‘integrative sampling’? Explain!

Response: By “integrative sampling” we mean taking multiple samples repeatedly over a period of time. We agree that this explanation should be in the manuscript and therefore added this information (line 140-141).

Comment #12: P5 L136: replace ‘groundwater ecology’ by ‘groundwater ecological status’ ; we ‘sampled’ the fauna…

Response: We agree. Done.

Comment #13: P6 L145: If this table shall stay in the paper then the information provided with the individual groups of organisms asks for a balancing. The provided information is very heterogeneous. Some of the terms used have not been explained before, e.g. ‘stygophile’.

Response: We agree that some terms are not explained before their use. Thus, we edited Table 1 and added a footnote, which explains the term ‘stygophile’ (see marked manuscript).

Comment #14: P7 L156: No, I do not agree at all. There is many natural groundwaters in good ecological shape that do not contain any dissolved oxygen, they may also produce ochre where they come in contact with oxygen. I guess we agree that these sites are not ‘good’ habitats for groundwater fauna. However, the absence of fauna does not necessarily mean a disturbed ecosystem status.

Response: We agree that the absence of fauna does not necessarily mean a disturbed ecosystem status. In our opinion, it is necessary to clarify that the ecological assessment takes place on the basis of groundwater fauna. Thus, we added the following sentences to Chapter 2.3 and edited the caption of Figure 1 in the manuscript:

"…If an ecological assessment of groundwater ecosystems based on the groundwater fauna takes place, some faunistic criteria must be considered. Invertebrates avoid habitats that are ochred or have a low content of dissolved oxygen. Thus, unstressed or natural habitats are defined as…".

Comment #15: P7 L170: Doesn't it make sense to further categorize the land use types, also within the city limits?

Response: We disagree. In our opinion, a further subdivision is not reasonable. The aim of this study is to give a first overview of the ecological groundwater conditions of the study area, so we decided to use only these two major categories (see also reply to the previous comment #9). Also, a subdivision of the urban area into "inner city" and "north-western city", including industrial areas, in an earlier version of the study lead to similar results.

Comment #16: P9 L177: did you also consider well depth in your data analysis. It si a big difference between 8.5 and 39m below land surface which may affect occurrence of fauna and the availability of dissolved oxygen.

Response: We agree that the depth of wells can have an impact on the availability of dissolved oxygen and the occurrence of fauna. In our study, only two wells have a depth of over 16 m (in detail: 27 m and 39 m). The deepest well is uninhabited and has a content of dissolved oxygen of 0.97 mg/l. As the statistical analysis shows, the correlation between well depth and the total amount of individuals is not significant (U-test: p-value = 1.7×10^{-1}, n = 39), but there exists a correlation between the depth and content of dissolved oxygen in the wells (U-test: p-value = $<10^{-13}$, n = 39).

Thus, we added the following sentences in the Chapter 'Physical and chemical parameters' (lines 210-215):

"Moreover, it seems that with a greater depth of the measurement wells the content of dissolved oxygen is increasing (U-test: p-value = $<10^{-13}$, n = 39). This can be explained by the fact that shallow wells can have a low water column in which oxygen can rapidly be consumed by groundwater microorganisms, chemical reactions and/or groundwater fauna. In the upper unscreened part of deeper wells, dissolved oxygen can be consumed while in the screened lower part oxygen is continuously refilled by oxic groundwater from the surroundings (Malard et al., 2002). Furthermore, reducing conditions in the overlaying soil can result in a low content of dissolved oxygen in groundwater."

This results shows that the content of dissolved oxygen depends on depth, but depth has no direct influence on groundwater fauna in this study.

Comment #17: P10 Figure3: why are there two lines (red and blue) indicading the percentage of wells with good and affected ecological status? Automaticly one looks if the box is above or below. However, the values of the individual physicaö-chemical parameters are not in line with the ecological status. I recommend to delete the lines.

Response: We tend to agree. However, we would like to keep both lines and the important information given by these lines. Hence, we reformulated the caption of Figure 3 as follows:

"Boxplots of the physical and chemical parameters for the forest and urban area in the study site and the proportion of wells in which ecological conditions are O.K. in percentage [%] indicated by the blue (forest area) and red (urban area) lines (secondary axis);…"

Comment #18: P10 L192: To my understanding, a concentration of 1 mg/L dissolved oxygen in wells water strongly indicates that there are anoxic conditions in groundwater. As is mentioned I the preprint well water is not representative for groundwater. To my opinion, since well water is open to the atmosphere, DO concentrations are likely to be overestimated.

Gw fauna may, at times of elevated DO in groundwater migrate through the local subsurface and enter wells. There, they may outlast times of no oxygen in the surrounding aquifer. Frankly speaking, I am not sure if the threshold of 1mg/L of DO mentioned before should refer to the surrounding groundwater.

Response: We partially agree that the content of dissolved oxygen (DO) in the well could differ from the content in the surrounding groundwater. In our study, the water and the groundwater fauna are sampled in the well swamp. In the upper unscreened part of the well, DO can be consumed. The lower screened part of the well can be continuously refilled with oxic or anoxic groundwater of the surrounding (Malard et al., 2002). In addition, in the study by Hahn and Matzke (2005) and Korbel et al. (2017) hydro-chemical data such as temperature, pH and DO of the sampled well water and the surrounding groundwater shows no significant differences.

For these reasons, we assume that the content of DO, as wells as the threshold of 1 mg/l, of sampled well water in our study is also representative for the aquifer. Nevertheless, to clarify this issue we already added a paragraph (see comment #16) and the information in brackets '(temperature, pH, dissolved oxygen, etc.)' in the corresponding paragraph (Chapter 3.2 in the end).

Comment #19: P10 L198: The study does not show high nitrate concentration! When stating that ⩽ 10 mg/L is natural, then in consequence nitrate concentrations between 1.3 and 14mg/l are not high!

Response: We agree. We therefore replaced 'high' by 'higher'.

Comment #20: P10 L199: In general, their relationship between DO and nitrate is not inversely correlated. Only when the oxygen is gone nitrate is reduced. As such, low or no oxygen goes along with low or no nitrate. I do not get the 'link' between oxygen and pollution claimed here (P10 L200).

Response: We agree that the relationship is not inversely correlated in general, and that the link between oxygen concentrations and pollution is not explained. Thus, we added the following sentences to the manuscript:

"In the urban area average nitrate concentrations are generally higher and correlate with the content of dissolved oxygen (U-test: p-value = 4.0×10^{-3}, n = 39) showing the link between nitrate content and oxygen consumption. Wells with a content of dissolved oxygen below 1.5 mg/l have an average content of nitrate of 1.5 mg/l, caused by nitrate reduction under anoxic conditions. Groundwater with reducing conditions (< 5 mg/l dissolved oxygen) has an average nitrate content of about 7 mg/l in contrast to groundwater with oxidising conditions with 9 mg/l, which is characterised by the oxidation of ammonium to nitrate."

Moreover, we added the *p*-values (dissolved oxygen vs. nitrate concentration) for the forest area in the manuscript (line 226).

Comment #21: P10 L199: P11 L220: does this mean that Parastonocaris and Bathynellacea are 'type'-species (groups) for urban situations? Such a possibility is not discussed in the paper. There are groundwater ecology experts in the list of authors. I miss an in depth interpretation of the ecological data.

Response: We partially agree that an in-depth interpretation of the ecological data is missing in the study. However, making a statement about a type species for urban areas on the basis of a single study area with a limited number of measurement wells does not seem reasonable. For this reason, we only hint at the possibility that these two species might be indicators of disturbed and stressed habitats.

Comment #22: P13 L231: Is it that stygobiont amphipods live predominantly within wells? This is to my opinion not a correct interpretation of what is published in Hahn & Matzke (Hahn is co-author of this preprint) and Korbel et al.

Response: We disagree that the interpretation is incorrect. In our opinion, the studies of Hahn and Matzke (2005) and Korbel et al. (2017) indicate that stygobiotic Amphipods have a habitat preference for open spaces, such as wells, and therefore can be found predominantly within wells. However, to eliminate any misunderstandings and in accordance with comment #18 of Referee # 1, we rewrote this sentence (see comment #18 Referee #1 and marked manuscript).

Comment #23: P13 L264: When the authors write about 'groundwater quality' it is not straightforward what is meant. Only very basic water chemistry (e.g. selected nutrients, pH, DO) and temp was measured. There is no indication for a 'bad' or 'impacted' groundwater quality (with the exception in temperature), so why should the groundwater fauna show associated distribution patterns.

Response: We agree that it is not straightforward what is meant by groundwater quality here. Therefore, we substitute the word ‘groundwater quality’ by ‘groundwater chemistry’ in this sentence. Also, we carefully checked the manuscript and clarified the differentiation between groundwater chemistry (i.e. chemical parameters) and groundwater quality.

Comment #24: P13 L235: Does the study of Brielmann et al. 2011 really state that amphipods react sensitive to a gw temperature of 11 ± 5°C (which is natural gw temp in central Europe) or do they refer to a change of ambient gw temp by 11°C? Check that carefully.

Response: We agree that this sentence can be misinterpreted. Hence, we re-checked the literature and edited the sentence (line 267) carefully as follows:

“Although, statistical analysis showed no clear correlation between the abundance of Amphipods and land use (U-test: p-value = 1.5×10^{-1}, n = 8; 31), the higher number of individuals in the forest area could support the hypothesis that Amphipods indicate healthy groundwater ecosystems as they react most sensitively to disturbances such as pollutants (Korbel and Hose, 2011) and groundwater temperature. In laboratory experiments with a thermal tank, Brielmann et al. (2011) found that 77 % of the individuals of the studied Amphipods (*Niphargus inopinatus*) preferred areas with a temperature between 8 and 16 °C. In addition, Spengler (2017) and Issartel et al. (2005) observed maximum temperatures of up to 17 °C.”

Comment #25: P14 L266: It would help the reader if you indicate the general groundwater direction in one of your maps (Fig. 2). If the groundwater flow direction in the area is north-west, then it is very likely that groundwater originating from the urban area is travelling below the forest. This point should be discussed as well.

Response: We agree. Hence, we indicate the groundwater flow direction in Figure 2. The groundwater flow direction in the study area is north-west towards the river Rhine.

Indeed, there is a certain likelihood that groundwater originating from the urban area can travel below the forest, although the whole area north-east in the forest area is water protection area. Nevertheless, the “Waldstadt” settlement in the north-east of the city might affect groundwater fauna in the forest area. Thus, we looked up measured chemical parameters of wells provided by the continuous monitoring program of the LUBW. One measurement well is located in the "Waldstadt", next to the wells T411 and T412 of this study. This well shows values in the range of the local background or threshold of the drinking water ordinance of Germany.

Hence, to clarify this issue we added the following sentences (lines 233):

"Moreover, no impact of groundwater originating from the urban area on the wells in the forest area is observed."

Comment #26: P14 L285: Why only the two criteria (>70% Crustaceans and <20% of oligochaetes were use for the evaluation of the ecological status. There are more criteria mentioned in the UBA report and in the international literature, some of which have been used or even developed by the co-authors, i.e. the Groundwater Fauna Index, the ratio of stygobites/stygophiles vs. stygoxenes, etc. Making use of these additional measures could provide a much clearer picture.

Response: We agree that using additional measures could provide more comprehensive information. Actually, we tested more methods during the preparation of this study, which we now present in the supplement of the manuscript.

The GFI however did not provide any additional information or valuable insights and was therefore excluded. The influence of multiple stressors, such as the pollution of the groundwater through industrial plants etc., and their effects on the governing parameters can bias the GFI. Moreover, under 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. We added this information to the supplement of the manuscript.

We partially agree that use of the ratio of stygobites/stygophiles vs. stygoxenes is useful in the context of this study. We agree that this ratio will provide more information on the endemism of stygobiotic species. Yet, we decided not to use it, because the required determination of the fauna cannot be done by untrained persons, which was in the sense of the UBA project (Level 1). This information is therefore also not added to our manuscript. The same applies to the GHI, where the microbiological analyses are beyond the purpose of a first tier assessment.

Comment #27: P15 L300-301: ‘as expected, this indicates anthropogenically influenced groundwater ecosystems…’. Again, the physical-chemical data provided do not hint at a seriously ‘impacted’ groundwater quality. The only exception is the temperature. It would have been worth to expand the list of chemical parameters analyzed and include ‘contaminants’ besides nitrate which is more of an issue in agricultural land. I ask the authors to make clear in the paper ‘what exactly the urban impact’ is.

Response: We agree that the focus on nitrate and temperature as anthropogenic impacts in this study has to be clarified (see also reply to comment #1).

In order to expand the list of chemical parameters, we conducted further analysis using data provided by a continuous monitoring system. This information is now given in the supplement and a short summary is presented in chapter 3.1 (line 234-241):

“Further investigations demonstrated that besides one larger and two smaller contaminated sites (however, still with concentrations below the threshold values, Figure S1), only minor groundwater pollution is documented in Karlsruhe (see Supplement). The chemical and physical parameters considered in the long-term monitoring system are within the range of local background and below threshold values of the drinking water ordinance of Germany (see Supplement for more information). In addition, groundwater fauna can usually cope well with short-term changes of chemical-physical parameters (Griebler et al., 2016). Previous studies showed that some species can even benefit from pollutants (Matzke, 2006; Zuurbier et al., 2013). Thus, the main documented impacts on groundwater quality in the study area are related to temperature, oxygen and nitrate concentration.”

Comment #28: P15 L304-306: This sentence needs an explanation. Why do the results you obtained lead to the ‘offer’ of using groundwater for heating and cooling? This sentence is not in line with what has been discussed right before.

Response: We agree that this sentence needs further explanation. Thus, we rewrote the sentence in the manuscript (lines 373-377) as follows:

"The observed spatial heterogeneity in ecological conditions and the existing heat anomalies in the urban area of the study also call for an adapted usage for shallow geothermal energy systems. Areas with no or little groundwater fauna (i.e. affected habitats) could also be used to store thermal energy at higher temperatures. Thus, high-temperature aquifer thermal energy storage (HT-ATES) could be established in urban environments (e.g. Fleuchaus et al., 2018), where the demand is high."

Comment #29: Discussion section in general: I miss proposals for improvement, i.e. the use of additional parameters, more sampling, more wells, other sampling techniques (here I could find 1 sentence), …

Response: We agree. Done (see comment #1, #22 and #25 of Referee #1, see marked manuscript).

Technical comments

Comment #30: P1 L12: 'scarce' not 'scare'

Response: We agree. Done.

Comment #31: P1 L15: If I am correct, then the classification is from German Federal Environment Agency (UBA) but the result from a UBA funded research project. This makes an important difference. The funding agency not necessarily identifies itself with the outcome of funded projects. The 'invention' and 'responsibility' is with the authors from the study. As such, I would not call the scheme used, and UBA classification scheme. Same applies to P2 L31.

Response: We agree. Done.

"For classification we apply the scheme of Griebler et al. (2014), on behalf of the Federal Environmental Agency (UBA),…"

Comment #32: P1 L16: wrong wording: 'fine' ecological conditions. Replace by 'good', 'natural' or something similar. Best you use the terminology used with the assessment scheme you used.

Response: We agree. Done.

Comment #33: P2 L26: HESS is an international journal. I would cite ‘German’ and ‘grey’ literature only if there is not similar publication in international journals. This is my very personal opinion.

Response: We agree. However, as the study site is located in Germany, it is sometimes necessary to cite ‘German’ literature, e.g. to get data on regional geology, which is often not available in the international literature. We carefully assessed the cited literature again and found that the mentioned study cannot be replaced and is thus kept in the manuscript.

Comment #34: P2 L28: ‘retention’ is the wrong term here! What you mean is ‘degradation’ or ‘mineralization’.

Response: We disagree. We mean retention of organic matter by groundwater ecosystems, which react like a buffer and storage zones.

Comment #35: P2 L30: delete ‘valuable’. What do you mean with ‘tied’? Reword.

Response: We agree. Done.

We mean “to bind”, e.g. organic matter, by biological processes/microbial activity. For a better understanding ‘tied’ is replaced by ‘bound’.

Comment #36: P2 L34: change ‘relatively’ to ‘typically’ or ‘naturally’. Typo: Brielmann et al. 2011 not 20011.

Response: We agree. Done.

Comment #37: P2 L35: change âA˘Žstygobiote‘ to âA˘Žstygobite’ or ‘stygobiont’.

Response: We agree. Done, ‘stygobiote’ is replaced by ‘stygobiont’

Comment #38: P2 L38: … groundwater … is not yet recognized as a protected habitat … reword this part of the sentence. Wat you probably mean is that gw is not yet recognized as an ecosystem that deserves protection.

Response: We agree. Done.

"Nevertheless, in German and European legislation, as in many countries globally, groundwater is not yet recognized as a habitat which is worthy of protection and there is no common understanding on the best practice of assessing the ecological status of groundwater (Hahn et al., 2018; Spengler and Hahn, 2018)."

Comment #39: P2 L39: change 'assessing groundwater ecology' into ' assessing groundwater ecological status'.

Response: We agree. Done.

Comment #40: P3 L59: delete 'Unfortunately'. Not needed.

Response: We agree. Done.

Comment #41: P3 L65: do the temp fluctuations range between 4°C and 20°C or is there a temp fluctuation with a temp range between 4°C and 20°C. Try to be more precise with your wording.

Response: We agree. We have rewritten the sentence:

"According to Brielmann et al. (2011) annual temperature fluctuations in aquifers, caused by shallow geothermal energy systems, range between 4 °C in winter and ≤ 20 °C in summer."

Comment #42: P3 L73: change 'clearly increasing' to 'increased and 'usually decreases' to 'decreased'.

Response: We agree. Done.

Comment #43: P3 L75: Brielmann et al. 2011 not 20011!

Response: We agree. Done.

Comment #44: P3 L79: The UBA did not develop anything! The UBA funded a research project in which these tools you refer to were developed. Rephrase this sentence.

Response: We agree. Hence, we rephrased this sentence:

"Commissioned by the Federal Environmental Agency of Germany (Umweltbundesamt, UBA), Griebler et al. (2014) developed a concept for an ecologically based assessment scheme for groundwater ecosystems."

Comment #45: P4 L96: change 'waterside filtration' to 'river bank filtration'.

Response: We agree. Done.

Comment #46: P4 L98: 'beneath' an urban area

Response: We agree. Done.

Comment #47: P4 L99: you can not sample thermal properties. You collected or sampled gw fauna and 'analyzed' gw chemistry and measured gw temp.

Response: We agree. Thus, we rewrote the sentence:

"Hence, in 39 groundwater monitoring wells in Karlsruhe, Germany, the groundwater fauna is sampled, groundwater temperatures measured and chemical properties are analysed."

Comment #48: P4 L100: Again, it is not the classification scheme of the UBA.

Response: We agree. Thus, we rewrote the sentence:

"In our study the classification scheme developed by Griebler et al. (2014) is applied."

Comment #49: P4 L101: 'state of ecosystem quality' sounds weird.

Response: We agree. Thus, we replaced 'state of their ecosystem quality' by 'state of their ecosystem'

Comment #50: P4 L116-117: annual mean LST! Is this what you mean?

Response: We agree. We mean annual mean land surface temperature (LST). Thus, we added 'annual mean'.

Comment #51: P4 L120: Didn't you specifically 'analyze' statistically if wells in the area of known contaminations show different features than others?

Response: We partially agree. Wells in the area of known contaminations can indeed show different features than others, yet in this study only two measurement wells are close to a known contamination, which makes a statistical analysis infeasible. Hence, we added some information in the manuscript (see our reply to comment #27).

Comment #52: P7 L149: …classification scheme in the framework of a research project funded by the…

Response: We agree. Done.

Comment #53: P7 L152: O.K. is an improper term in this connection

Response: We agree, but we would like to keep the original phrase and meaning of the original document by Griebler et al. (2014).

Comment #54: P11 L207: chemical characteristics do not distribute! There is distribution patterns.

Response: We agree. Done.

Comment #55: P13 L242: 'Larger'!

Response: We agree. Done.

Comment #56: P13 L252: 8.3 mg/l is 'not' a rather high nitrate content! Same applies to P13 L257.

Response: We agree. Thus, we reformulated the sentence:

"…and a rather high nitrate content (8.3 mg/l) compared to the wells in the forest area…"

"…and nitrate concentrations up to 14 mg/l, which is above the geogenic concentration of 10 mg/l and higher compared to the wells in the forest area."

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Groundwater fauna in an urban area: natural or affected?

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Abstract. In Germany 70 % of the drinking water demand is met by groundwater, whose quality is the product of multiple physical-chemical and biological processes. As healthy groundwater ecosystems help to provide clean drinking water, it is necessary to assess the ecological conditions of these ecosystems. This is particularly true for densely populated, urban areas, where faunistic groundwater investigations are still scarce. The aim of this study is therefore to provide a first-tier assessment of the groundwater fauna in an urban area. Thus, we assess the ecological condition of an anthropogenically influenced aquifer by analysing the groundwater fauna in 39 groundwater monitoring wells in Karlsruhe (Germany) and a nearby forest. For classification, we apply the scheme of Griebler et al. (2014), in which a threshold of more than 70 % of Crustaceans and of less than 20 % of Oligochaetes serves as an indication for good ecological conditions. In our study it is revealed that only 35 % of the wells in the urban area, and 50% of wells in the forest fulfil these criteria. While the assessment shows that ecological conditions in the studied urban area are not in a good ecological state, there is no clear spatial pattern with respect to land use and other anthropogenic impacts, in particular, groundwater temperature and nitrate concentrations. However, there are noticeable differences in the spatial distribution of species and abiotic groundwater characteristics between wells in the forest and the urban area, which indicates that more comprehensive assessment methods are required to fully understand the different effects on groundwater fauna.

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1. Introduction

In Germany 70 % of the drinking water demand is met by groundwater, whose quality is the product of multiple physical-chemical and biological processes (Avramov et al., 2010). Groundwater ecosystems are responsible for several services that help to provide clean drinking water, which is a vital resource for humanity (Griebler and Avramov, 2015). Bacteria and fauna also play an important role in the biological self-purification of groundwater by the retention of organic matter, natural attenuation of pollutants, storing and buffering of nutrients as well as the elimination of pathogens. Organic matter and pollutants can be degraded and converted to biomass or bound by microbial activity. Protozoa and higher organisms can graze resulting biofilms, loosen the substrate and therefore stimulate the biological self-purification (Hancock et al., 2005; Avramov et al., 2010).

Healthy groundwater ecosystems can provide clean drinking water however they are sensitive to external influences, such as chemical and thermal disturbances. The latter drives hydro-geochemical and biological processes in groundwater systems, which are typically isothermal (Brielmann et al., 2009; 2011). Groundwater fauna mainly consists of stygobiont species, which spend their entire life in groundwater and are adjusted to this habitat (Hahn, 2006). Hence, in Central Europe they are assumed to be cold stenotherm, which means that they prefer cold temperatures and cannot withstand water temperatures over 16 °C (Brielmann et al., 2009) or rather 14 °C (Spengler, 2017) for an extended period.

Nevertheless, in German and European legislation, as in many countries globally, groundwater is not yet recognized as a habitat which is worthy of protection and there is no common understanding on the best practice of assessing the ecological status of groundwater (Hahn et al., 2018; Spengler and Hahn, 2018). The assessment of surface water is typically based on biological, physical-chemical and supported by hydro-morphological criteria (European Water Framework Directive and German legislation article 5 of the 'Regulation on the Protection of Surface Water'). While groundwater quality is mostly assessed by physical-chemical and quantitative criteria, very few quantifiable ecological criteria are available for the assessment of the health of groundwater ecosystems. The availability of ecological criteria can only be increased by conducting a large number of studies dealing with the analyses of groundwater ecosystem health by investigating groundwater fauna. Results from previous faunistic groundwater analyses are contained in a Germany-wide data record (Hahn, 2005; Berkhoff, 2010; Stein et al., 2012; Gutjahr, 2013; Spengler, 2017; Spengler and Hahn, 2018). The study by Hahn and Fuchs (2009) focuses on defining stygoregions based on different hydrogeological units located in Baden-Württemberg, Germany. They conclude that the observed patterns of groundwater communities reflect a high spatial and temporal heterogeneity of aquifer types with respect to habitat structure, food, oxygen supply etc.

Accordingly, although there are various studies on this topic (e.g. Gibert and Deharveng, 2002; Malard et al., 2002; Deharveng et al., 2009; Dole-Olivier et al., 2009b) stygobiotic biodiversity is still likely to be underestimated.

Regional investigations on the spatial variation of groundwater fauna, i.e. stygobiont occurrences, and corresponding environmental parameters, such as geological site characteristics and altitude, are rare (Dole-Olivier et al., 2009a; Gibert et al., 2009). An approach to elucidate groundwater biodiversity patterns in six European regions was conducted in the PASCALIS

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project (Protocol for the Assessment and Conservation of Aquatic Life In the Subsurface) (Gibert et al., 2009), which aimed at mapping biodiversity and endemism patterns (Deharveng et al., 2009) and shows that regional processes, such as hydrological connectivity, in a specific habitat (e.g. river floodplains as in Ward and Tockner, 2001) have a much stronger influence on species composition than local habitat features such as permeability and saturation. Within a region, hydrogeology, altitude, palaeographical factors and human activities can interact in complex ways to produce dissimilar patterns of species compositions and diversity (Gibert et al., 2009). The PASCALIS sampling protocol recommends selecting hydro-geographic basins that are not strongly affected by human activities such as groundwater pollutions (Malard et al., 2002), and does not biogeographically classify a groundwater system (Stein et al., 2012).

In urban areas, anthropogenic impacts such as a dense building development, underground car parks, open geothermal systems and injections of thermal wastewater from industry, result in local thermal alteration of groundwater up to several degrees (e.g. Taylor and Stefan, 2009; Zhu et al., 2011; Menberg et al., 2013b; Tissen et al., 2019). According to Brielmann et al. (2011) annual temperature fluctuations in aquifers, caused by shallow geothermal energy systems, range between 4 °C in winter and ≤ 20 °C in summer. In 2000, the European Union (EU) (Water Framework Directive) defined the release of heat in the groundwater as a pollution, whereas the cooling of the groundwater is not mentioned. Until now, there are no scientifically derived threshold values for groundwater temperature in the case of thermal (heat) pollution (Hähnlein et al., 2010; 2013). This results in a tension between conservation, exploitation and thermal use of groundwater. However, as seen in an aquifer ecosystem downstream from an industrial facility in Freising (Germany), where groundwater is used for cooling resulting in a warm thermal plume, no relation between faunal abundance and groundwater temperature could be identified (Brielmann et al., 2009). Investigation of hydro-geochemical parameters, microbial activities, bacterial communities and groundwater faunal assemblages indicates that bacterial diversity increased with temperature, while faunal diversity decreased with temperature (Brielmann et al., 2009). Similar results are provided by Griebler et al. (2016), where potential impacts of geothermal energy use and storage of heat on groundwater are investigated. Temperature changes in groundwater correspond with changes in groundwater chemistry, biodiversity, community composition, microbial processes and function of the ecosystem. How exactly groundwater communities react to changes in temperature and concentration of nutrients, dissolved organic carbons and oxygen, is not yet fully understood (Brielmann et al., 2009, 2011; Spengler, 2017; Sánchez et al., 2020).

Several approaches exist that allow a local assessment of the ecological state of groundwater based on different faunistic, hydro-chemical and physical parameters. Commissioned by the Federal Environmental Agency of Germany (Umweltbundesamt, UBA), Griebler et al. (2014) developed a concept for an ecologically based assessment scheme for groundwater ecosystems. This two-step scheme characterizes groundwater on two different levels by using the most important physico-chemical parameters, such as content of dissolved oxygen, as well as microbiological and faunistic characteristics such as amount of Oligochaetes and Crustaceans, and comparing these to reference values for natural, undisturbed and ecologically intact groundwater ecosystems (Griebler et al., 2014). Moreover, Korbel and Hose (2017) introduced the Groundwater Health Index (GHI), which is a tiered framework for assessing the health of groundwater ecosystems. Here, both biotic and abiotic attributes of groundwater ecosystems are used as benchmarks for ecosystem health. Their study shows that

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ecosystem health benchmarks are probably more associated with aquifer typology, than being applicable for local areas. This index is applied and tested by Di Lorenzo et al. (2020) in unconsolidated aquifers in Italy, which are located in nitrate vulnerable zones. They refined the index ($wGHI^N$) and demonstrated its applicability on shallow and deep aquifers and also revealed that this new index is limited due to low correlations between the indicators.
Furthermore, the Groundwater-Fauna-Index (GFI), introduced by Hahn (2006), quantifies the relevant ecological conditions in the groundwater as a result of hydrological exchange between surface and groundwater. It incorporates ecologically important groundwater parameters such as relative amount of detritus, variation of groundwater temperature and concentration of dissolved oxygen (Hahn, 2006). Gutjahr et al. (2014) used the GFI as part of a proposal for a groundwater habitat classification on a local scale, which introduce five types of faunistic habitats as a result of surface water influence, content of dissolved oxygen and amount of organic matter. Moreover, in the study of Berkhoff (2010) the GFI was used to examine the impact of the surface water influence on groundwater with the aim to develop a faunistic monitoring concept for hydrological exchange processes in the surrounding river bank filtration plants. Spengler and Hahn (2018) argued for the definition of a regional and ecological temperature threshold and an ecology based assessment of thermal stress in groundwater.
The objective of this study is to investigate specifically the groundwater fauna beneath an urban area in comparison to a natural forest. Hence, in 39 groundwater monitoring wells in Karlsruhe, Germany, the groundwater fauna is sampled, groundwater temperatures measured and chemical properties are analysed. In our study the classification scheme developed by Griebler et al. (2014) is applied. The wells are characterized regarding the state of their ecosystem. Hence, we finally aim to distinguish areas with natural groundwater ecology from anthropogenically disturbed areas.

2. Material and methods

2.1 Study site

The study is performed in Karlsruhe, a city in the Upper Rhine Valley in south-western Germany. The urban region covers an area of 173 km^2 and has about 310,000 inhabitants (Amt für Stadtentwicklung - Statistikstelle, 2018). The Cenozoic continental rift valley is filled with Tertiary and Quaternary sediments, which are dominated by sands and gravels with minor contents of silt, clay and stones (Geyer et al., 2011). Sporadic layers with lower permeabilities lead to a separation of up to three aquifer levels (Wirsing and Luz, 2007). The upper aquifer is unconfined with a water table between 2 and 10 m below the ground. The flow direction is northwest of the Rhine River with groundwater flow velocities ranging between 0.5 and 1.5 m/d (Technologiezentrum Wasser, 2018).
Based on the land use plan of Karlsruhe, about 20 % of the area (i.e. urban area, city centre, neighbouring districts, as well as parts of the Hardtwald forest and several outskirts) is covered by buildings. The rest is vegetation (~ 56 %) and artificial surface covers (~ 24 %), showing the complexity and heterogeneity of the urban environment. According to Benz et al. (2016), the annual mean groundwater temperature (GWT) in Karlsruhe in the years 2011 and 2012 was 13.0 ± 1.0 °C. Distinct temperature hotspots occur mainly below the city centre, where building densities are highest. In the north-western part of Karlsruhe, the

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increase of GWT was about 3 K warmer than the annual mean land surface temperature (LST), which is mainly caused by several groundwater reinjections of thermal wastewater (Benz et al., 2016).

In general, groundwater in the region of Karlsruhe is of good quality and the local drinking water supplier (Stadtwerke Karlsruhe) only needs to remove oxidised iron and manganese from the pumped groundwater. However, two main contaminations, which affect groundwater quality, are known in the urban area (Stadt Karlsruhe, 2006). A contaminant plume, which contains a polycyclic aromatic hydrocarbons concentration of up to 500 µg/l, of 200 m length over the entire aquifer thickness is located at a former gas plant in the east of Karlsruhe (Figure S1b) (Kühlers et al., 2012). Moreover, three parallel contamination plumes of 2.5 km length each, can be found in the southeast of Karlsruhe (Figure S1b), where highly volatile chlorinated hydrocarbons (7 µg/l - 26 µg/l) and their degradation products were detected (Wickert et al., 2006).

2.2 Material and sampling

From 2011 to 2014, samplings of groundwater parameters and fauna were performed in 39 groundwater monitoring wells in Karlsruhe. At the beginning of each sampling process, temperature and electrical conductivity were measured with an electric contact gauge (Type 120-LTC, Hydrotechnik) at a depth interval of 1 m. Using a bailer (Aqua Sampler, Cole-Parmer), water from the bottom of the groundwater monitoring wells was sampled and the pH value (Multiline Type 3430; WTW GmbH, Weilheim Germany) as well as the contents of dissolved oxygen (Multiline Type 3430; WTW GmbH, Weilheim Germany), iron, nitrate (NO_3^-) and phosphate (PO_4^{3-}) (RQflex® plus 10 Reflectoquant®; Merck Millipore KGaG, Darmstadt Germany) were measured.

In accordance with the suggestion made by Hahn and Gutjahr (2014), several integrative samplings (i.e., repeated samples taken over a period of time) were conducted to capture an ecological representation of groundwater fauna which reflects the occurring species at a community level. Every well is sampled at least three times. From 2011-2012, 22 measuring wells (mainly in the Hardtwald and the North-West of Karlsruhe) were sampled six times at a minimum interval of two months. In 2014, 17 measurement wells, mainly located in the south/inner city, were sampled three times (see Table S1). As the aim of this study is to provide a first-tier screening of the groundwater ecological status, we sampled the fauna in the monitoring wells in accordance with the sampling manual of the European PASCALIS Project (Malard et al., 2002) and the procedure described by Hahn and Fuchs (2009), using a modified Cvetkov net.

Mann-Whitney-tests (U-tests) were applied to detect potential impacts of groundwater characteristics (physical-chemical parameters), geology and well design on the groundwater quality as well as on groundwater fauna. Samples were regarded as significantly different if the *p*-value was $< 5.0\times10^{-2}$.

Crustaceans, especially Amphipods and Copepods represent the majority of groundwater fauna. The identification keys from the following studies were used to identify the different groups in the samples: Einsle (1993), Janetzka et al. (1996), Meisch (2000), Schellenberg (1942) and Schminke et al. (2007). The sampled fauna for this study can be assigned to the subphylum *Crustacea* and four other subordinate taxa (Table 1).

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Kommentiert [FK37]: Referee#2 Comment#11
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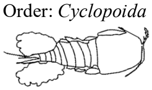
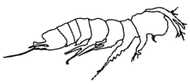
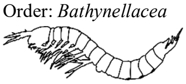
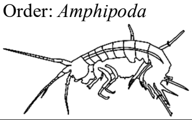
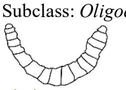
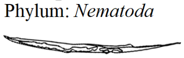
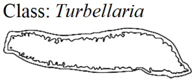
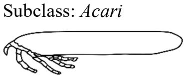
Table 1: Overview of the sampled fauna, divided into the subphylum *Crustacea* and other subordinate taxa.

Subphylum: *Crustacea*	**Size [mm]**	**Habitats**	**Species number**
Order: *Cyclopoida* 	0.4 - 0.7[1]	Fresh and marine water, groundwater[1]	298 species and subspecies worldwide[2], 8 stygobiotic species in Germany[3]
Order: *Harpacticoida* 	< 0.5[4]	Marine, freshwater, semi-terrestrial environments, groundwater[5]	599 (sub-)species worldwide[2], 20 stygobiotic species in Germany[3], 17 stygophile* & stygobiotic species in Baden-Württemberg[6])
Genus: *Parastenocaris*	0.3 - 0.5[1]	Tertiary relict living in cavity rooms of streams, in groundwater and moss[1]	206 (sub-)species worldwide[2] (16 stygophile & stygobiotic species in Baden-Württemberg[1])
Order: *Bathynellacea* 	0.5 - 5.4[7]	Cavity systems[7] and in groundwater[8] (foreign tropical origin)[9]	Exclusively 160 stygobiotic species worldwide[9], 8 species in Germany[3]
Order: *Amphipoda* 	0.5 – 30[1]	Sea, fresh water[1] and in healthy groundwater ecosystems (important ecosystem service providers[10] & biodiversity indicators in Europe[11])	321 stygophile and stygobiotic species in Europe[12], 24 stygobiotic species in Germany[3]
Other subordinate taxa	**Size [mm]**	**Habitats**	**Species number**
Subclass: *Oligochaeta* 	< 1 – 3[13]	Colonise every habitat, groundwater[13]	27 stygobiotic species in Europe[13] and 100 species worldwide[14]
Phylum: *Nematoda* 	1 – 3[9]	Colonise every habitat[9], can live under unfavourable conditions[15]	20,000 species worldwide[16], 60 stygobiotic species in Europe, 6 in Germany[3]
Class: *Turbellaria* 	0.4 – 5[17]	Sea, brackish and fresh water and groundwater[17]	3,400 species worldwide[17], 7 stygobiotic species in Germany[3]
Subclass: *Acari* 	a few mm[9]	Colonize every habitat, also groundwater, have high demands on water quality[9]	< 5,000 water mite species wordlwide[18], 10 stygobiotic species in Germany[3]

[1] Fuchs et al. (2006)
[2] Galassi (2001)
[3] Zenker et al. (2020)
[4] Hahn (1996)
[5] Galassi et al. (2009)
[6] Fuchs (2007)
[7] Sauermost and Freudig (1999a)
[8] Camacho (2006)
[9] Hunkeler et al. (2006)
[10] Boulton et al. (2008)
[11] Stoch et al. (2009)
[12] Botosaneanu (1986)
[13] Sauermost and Freudig (1999b)
[14] Batzer and Boix (2016)
[15] Hahn et al. (2013)
[16] Eckert et al. (2008)
[17] Sauermost and Freudig (1999c)
[18] di Sabatino et al. (2000)

*Stygophile organisms are found primarily in surface water, but they can survive in shallow groundwater for a while (Preuß and Schminke, 2004).

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2.3 Classification scheme by Griebler et al. (2014)

Commissioned by the Federal Environmental Agency of Germany (UBA), Griebler et al. (2014) developed a two-step ecologically based classification scheme for characterization of groundwater ecosystems and also defined spatially dependent reference values of ecologically intact groundwater ecosystems. In order to enable a statement about the exposure of the groundwater at a specific site, biotic and abiotic parameters, which are determined and compared with reference values, are used to distinguish locations with very good or good (i.e. O.K.) ecological conditions or locations which fail these criteria, i.e. affected areas (Figure 1). If an ecological assessment of groundwater ecosystems based on the groundwater fauna takes place, some faunistic criteria must be considered. Invertebrates avoid habitats that are ochred or have a low content of dissolved oxygen. Thus, unstressed or natural habitats are defined as areas with a content of dissolved oxygen > 1.0 mg/l, that are not ochred and have an existing fauna, i.e. an amount of > 50 % of Stygobites, of > 70 % of Crustaceans and of < 20 % of Oligochaetes (Figure 1). This allows a qualitative interpretation of the ecological condition of the groundwater system. If the results indicate affected ecological conditions, i.e. one or more biological/ecological indicators are out of the reference range, an assessment according to the Level 2 scheme is necessary. This requires a determination of reference values at local reference locations, which are protected and have a weak surface influence, and a subsequent comparison of these values with measured data. As our aim is a first-tier screening of an urban area, we only apply Level 1 in our study.

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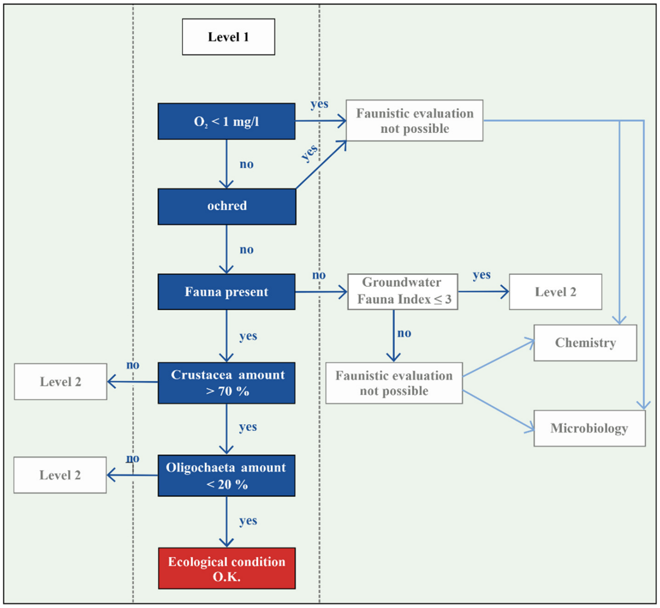

Figure 1: Classification scheme by Griebler et al. (2014) according to Level 1 for groundwater ecosystems on the basis of groundwater fauna (modified after Griebler et al. (2014)).

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3. Results and discussion

3.1 Physical and chemical parameters

First, the groundwater conditions in the study site are evaluated by their physical-chemical characteristics. The following values are average values of the individual samplings from each monitoring well. In order to allow a spatially differentiated assessment, the study site is classified into two separate zones based on land use type: (1) Forest area (local name: Hardtwald), (2) Urban area containing industrial, commercial and residential areas (Figure 2a).

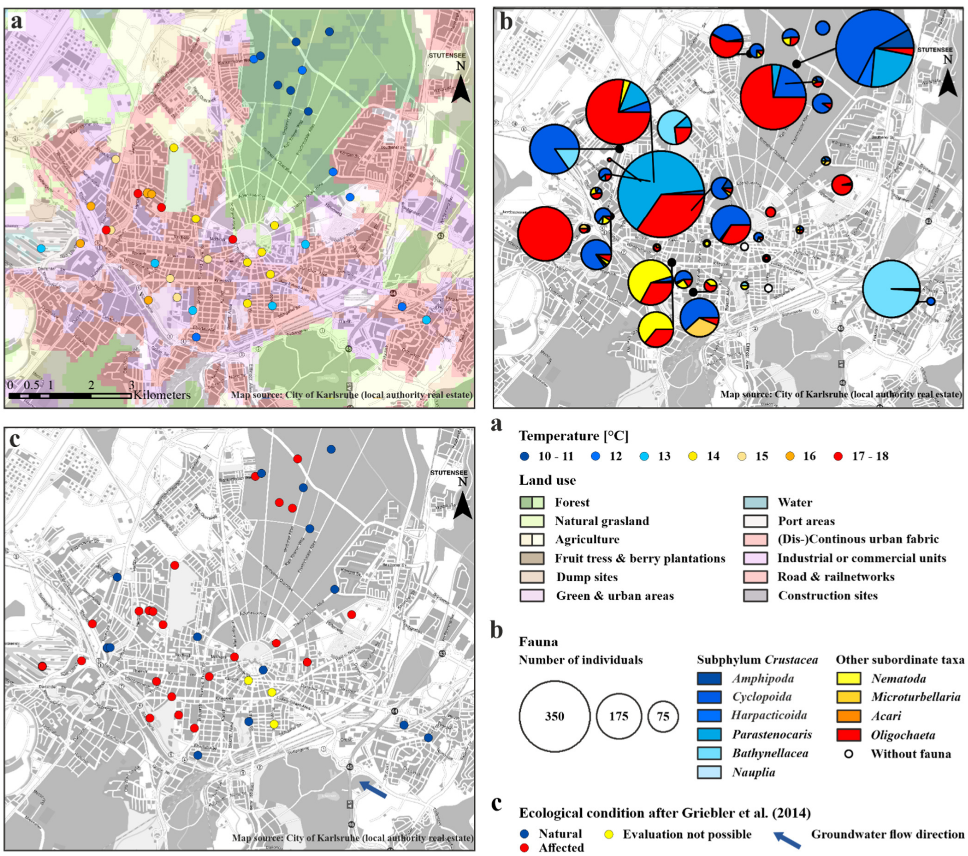

Figure 2: Overview map city area of Karlsruhe: (a) land use plan (GISAT, 2016) and average groundwater temperature of the multiple measurements [°C] at the bottom of the monitoring wells; (b) detailed groundwater fauna: colours of the circles shows the different taxa in the sample [%], the size indicates the number of individuals; (c) faunistic evaluation after Griebler et al. (2014).

As expected, measured GWT at the bottom of the wells, in 8.5 to 39.0 m depth, are mainly constant over the repeated measurements. The lowest GWT ranging between 10.5 and 10.9 °C were measured in the eight wells of the forest area (Table S1). In contrast, the highest average GWT with 17.5 °C was measured in a well near the city hospital (T113) (Figure 2a). The mean value of all wells is 13.5 ± 2.1 °C, which is similar to the results from Benz et al. (2015) with 13.0 ± 1.0 °C. According to Benz et al. (2017), annual shallow GWT vary between 6 and 16 °C in the area of Karlsruhe, which is in line with the temperatures measured during fauna sampling (Figure 3a). For the urban area in the north-western part of the city, Figure 2a shows a clear warming trend, which was also observed by Menberg et al. (2013a,b). The increased GWT in this area can be traced back to effects of urban infrastructures and industries, which use groundwater for cooling purposes.

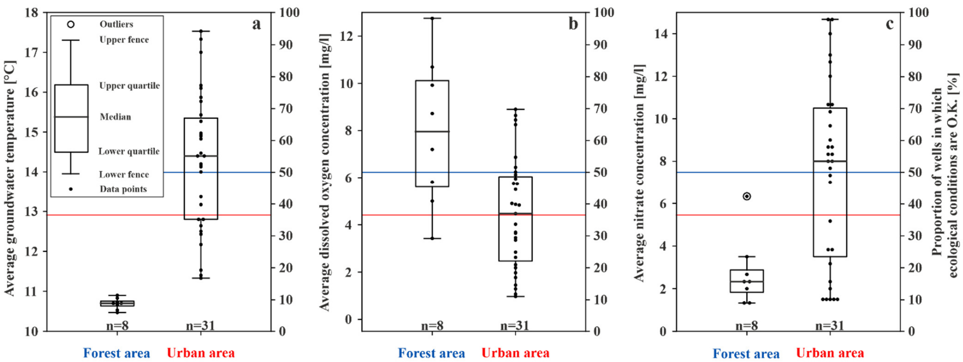

Figure 3: Boxplots of the physical and chemical parameters for the forest and urban area in the study site and the proportion of wells in which ecological conditions are O.K. in percentage [%] indicated by the blue (forest area) and red (urban area) lines (secondary axis); (a) average temperature of the repeated measurements [°C] at the bottom of the monitoring wells; (b) average content of dissolved oxygen [mg/l] of the monitoring wells; (c) average nitrate content [mg/l] of each monitoring well. (n = number of wells)

The content of dissolved oxygen acts as a limiting factor for groundwater fauna, since groundwater is usually under-saturated with a varying oxygen content between 0 and 8 mg/l (Griebler et al., 2014; Kunkel et al., 2004). In this study, the average content of dissolved oxygen in all wells is between 1.0 and 12.8 mg/l (Figure 3b and Figure S1a). As expected, the monitoring wells located in the forest area (Hardtwald) show the highest content, while the lowest values are found in urban areas and is is likely linked to aquifer contamination and other anthropogenic effects (content of dissolved oxygen of forest vs. urban area: U-test: *p*-value = 5.3×10^{-3}, n = 8; 31). Urban water can be polluted in multiple ways, which affects the chemical and biological oxygen consumption in the groundwater. The higher the pollution and/or biological activity, the lower the dissolved oxygen (Kunkel et al., 2004; Griebler et al., 2014). Moreover, it seems that with a greater depth of the measurement wells the content of dissolved oxygen is increasing (U-test: *p*-value = $<10^{-13}$, n = 39). This can be explained by the fact that shallow wells can have a low water column in which oxygen can rapidly be consumed by groundwater microorganisms, chemical reactions and/or groundwater fauna. In the upper unscreened part of deeper wells, dissolved oxygen can be consumed while in the lower screened part oxygen is continuously refilled by oxic groundwater from the surroundings (Malard et al., 2002). Furthermore, reducing conditions in the overlaying soil can result in a low content of dissolved oxygen in groundwater.

Nitrate is often named as an important pollutant in groundwater. The natural and geogenic concentrations of nitrate in groundwater is usually under 10 mg/l (Griebler et al., 2014). In our study area, the average nitrate content of all wells varies between 1.3 and 14.7 mg/l. In the urban area average nitrate concentrations are generally higher and correlate with the content

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Kommentiert [FK48]: Referee#1 Comment#15

Kommentiert [FK49]: Referee#2 Comment#16

Kommentiert [FK50]: Referee#2 Comment#19

Kommentiert [FK51]: Referee#2 Comment#20

of dissolved oxygen (U-test: p-value = 4.0×10^{-3}, n = 39) showing the link between nitrate content and oxygen consumption. Wells with a content of dissolved oxygen below 1.5 mg/l have an average content of nitrate of 1.5 mg/l, caused by nitrate reduction under anoxic conditions. Groundwater with reducing conditions (< 5 mg/l dissolved oxygen) has an average nitrate content of about 7 mg/l in contrast to groundwater with oxidising conditions with 9 mg/l, which is characterised by the oxidation of ammonium to nitrate. The lowest nitrate concentrations are found in the forest area (Figure 3c and Figure S1c), where atmospheric nitrogen is held back by forest soils (U-test: p-value = 1.7×10^{-3}, n = 8) and fertilization is prohibited due to water protection regulations in the forest area (Aber et al., 1998; Schönthaler and von Adrian-Werburg, 2008).

Within the study, the average concentration of iron and phosphate are low and in most cases below the detection limit of the test (Figure S1d, e) and also below the natural and geogenic concentrations (phosphate: 0.05 mg/l (Griebler et al., 2014) and iron: 3.3 mg/l (Kunkel et al., 2004)).

Considering these findings, clear differences in the spatial distribution patterns of abiotic groundwater characteristics are noticeable. The rural forest area shows lower average GWT than the urban area (U-test: p-value = 3.3×10^{-5}, n = 8; 31), lower nitrate concentrations (U-test: p-value = 4.1×10^{-3}, n = 8; 31) and higher dissolved oxygen concentrations (U-test: p-value = 5.3×10^{-3}, n = 8; 31), which indicates a correlation between abiotic groundwater characteristics and land use in the study area. Moreover, no impact of groundwater originating from the urban area on the wells in the forest area is observed. Further investigations demonstrated that besides one larger and two smaller contamination sites (however, still with concentrations below the threshold values, Figure S1), only minor groundwater pollution is documented in Karlsruhe (see Supplement). The chemical and physical parameters considered in the long-term monitoring system are within the range of local background and below threshold values of the drinking water ordinance of Germany (see Supplement for more information). In addition, groundwater fauna can usually cope well with short-term changes of chemical-physical parameters (Griebler et al., 2016). Previous studies showed that some species can even benefit from pollutants (Matzke, 2006; Zuurbier et al., 2013). Thus, the main documented impacts on groundwater quality in the study area are related to temperature, oxygen and nitrate concentration.

3.2 Groundwater fauna

The organism communities of the groundwater consist of microorganisms and invertebrates (in particular Crustaceans) (Griebler et al., 2014). In the pool of samples, 3,666 individuals were detected in 37 of 39 wells (Table S2). With 2,014 individuals, the group of *Crustacea* was found to be the most abundant (56 %). 976 individuals (27 %) of the order of *Cyclopoida* dominated this group, followed by the genus *Parastenocaris* with 599 individuals (16 %), by the order of *Bathynellacea* (371), *Amphipoda* (66), *Harpacticoida* (33) and *Nauplia*. The communities of the monitoring wells also frequently contained Oligochaetes (1,343 individuals, 37 %). Furthermore, individuals of the phylum *Nematoda* (228 individuals) and *Microturbellaria* (46 individuals) were also often present.

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Kommentiert [FK53]: Referee#2 Comment#54

Kommentiert [FK54]: Referee#1 Comment#12 & #13

Kommentiert [FK55]: Referee#2 Comment#27 & #51

Kommentiert [FK56]: Referee#1 Comment#10

Overall, there is a noticeable difference in the spatial distribution of species within the study area. Individuals of the subphylum *Crustacea* were found in larger numbers, with regard to the number of wells, in the monitoring wells in the forest area (660 individuals in eight wells) compared to those in the urban area (1,354 individuals in 31 wells). Furthermore, no individuals of the order *Bathynellacea* and only 135 individuals of the genus *Parastenocaris* were found in the forest area. In contrast, larger numbers of the latter species as well as of Oligochaetes are characteristically found in the wells in the urban area. However, in contrast to the abiotic characteristics, no clear pattern of faunal diversity and land use was observed as Crustaceans and individuals of other subordinate taxa were found both in the rural forest and in the urban area.

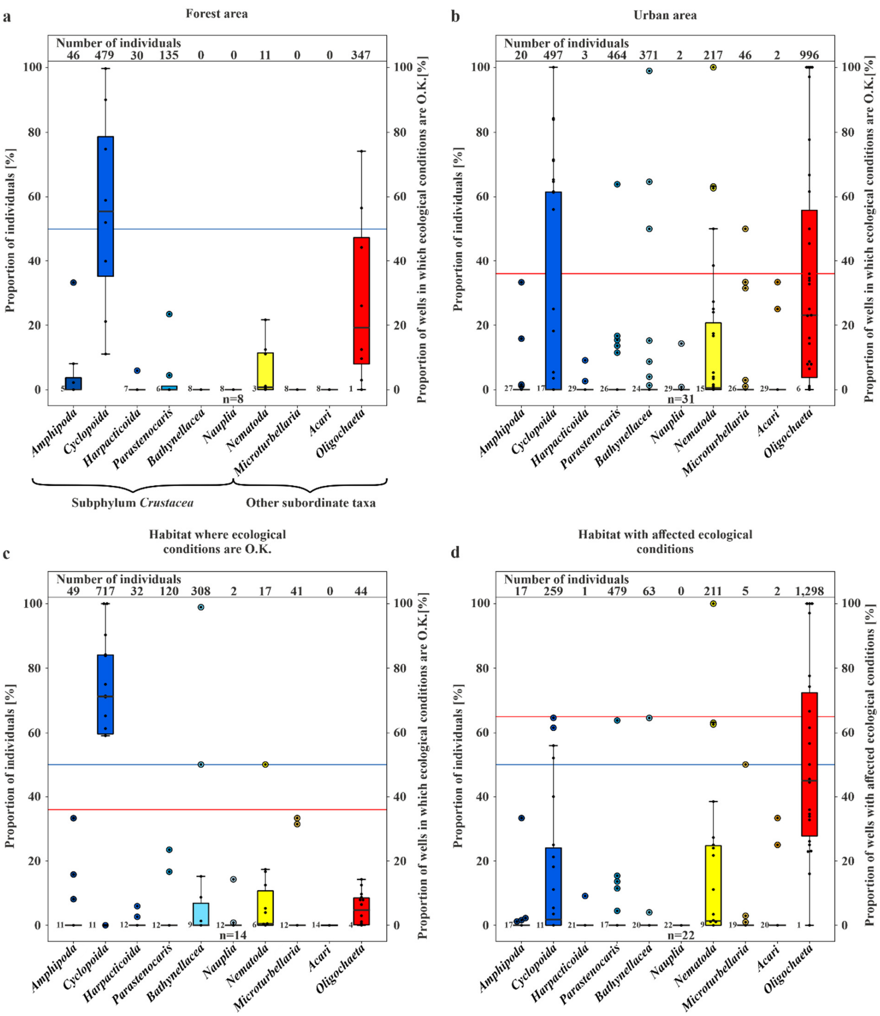

Figure 4: Boxplots of the amount of fauna [%]: (a) proportion of individuals and of wells in which ecological conditions are O.K. (secondary axis) [%] of the forest area; (b) proportion of individuals and of wells in which ecological conditions are O.K. [%] of the urban area; (c) proportion of individuals and of wells in which ecological conditions are O.K. [%] divided based on the results of the UBA classification scheme; (d) proportion of individuals and of wells with affected ecological conditions [%] divided based on the results of the UBA classification scheme. The colour of the boxes shows the different taxa in the samples. (n = number of wells)

Stygobiotic Amphipods, i.e. large-bodied invertebrates which due to their size have a habitat preference for open spaces such as wells (Table 1) (e.g. Hahn and Matzke, 2005; Korbel et al., 2017), were found only in three wells (Figure 2c). 46 individuals of this order were detected in the forest and 20 individuals in the urban area (Figure 4a,b). Although statistical analysis showed no clear differences between the abundance of Amphipods and land use (U-test: p-value = 1.5×10^{-1}, n = 8; 31), the higher number of individuals in the forest area could support the hypothesis that Amphipods indicate healthy groundwater ecosystems as they react most sensitively to disturbances such as pollutants (Korbel and Hose, 2011) and groundwater temperature. In laboratory experiments with a thermal tank, Brielmann et al. (2011) found that 77 % of the individuals of the studied Amphipods (*Niphargus inopinatus*) preferred areas with a temperature between 8 and 16 °C. In addition, Spengler (2017) and Issartel et al. (2005) observed maximum temperatures of up to 17 °C. The lack of a statistically significant correlation might also be related to the low number of wells (n = 8) and individuals (n = 46). Amphipods are important ecosystem service providers in terms of bioturbation and organic decomposition (Boulton et al., 2008). As observed in laboratory experiments (Smith et al., 2016), they actively move with migration speeds between 1.7 and 3.5×10^{4} m per year. In most cases when Amphipods were found, higher concentrations of individuals of the order *Cyclopoida* were also identified (Abundance *Amphipoda* vs. *Cyclopoida*: U-test: p-value = 9.6×10^{-5}, n = 39). Individuals of the latter order were generally found in larger quantities in the majority of the wells (479 in the forest area and 497 in the urban area), as they are the largest group of Crustaceans in this environment (Fuchs et al., 2006) and can tolerate a wide temperature range (e.g. upper thermal limit of 26.9 ± 0.2 °C in laboratory tests by Sánchez et al. (2020))(Spengler, 2017).

The order *Harpacticoida*, which includes the genus *Parastenocaris*, have an elongated body shape and a stem-chiselling movement, which is why they are predestined for living in cavities and groundwater (Hahn, 1996; Fuchs, 2007), preferring sand and gravel as a substrate (Galassi et al., 2009). Larger numbers of *Parastenocaris* (464 individuals), which can tolerate GWT from 8 to > 20 °C (Fuchs et al., 2006) (e.g. *Parastenocaris phyllura* up to 22.5 °C in laboratory tests; Glatzel, 1990), were found in the urban area, especially in the northwest area (Figure 2b). This area is characterised by GWT between 16 and 18 °C, the highest at the study site. This observation is comparable with previous studies (Hahn, 2006; Hahn et al., 2013; Spengler, 2017), which showed that the genus *Parastenocaris* is particularly non-competitive and can often be found isolated in structurally burdened and physico-chemically altered areas. Accordingly, only 135 individuals were detected in the forest area.

In addition, quantities of *Bathynellacea* (371 individuals) were found in five monitoring wells all located in the urban area in a depth of 9.0 to 13.5 m at a GWT of 12-15 °C (Figure 4b). This order typically inhabits the interstitial groundwater, which is characterised by a dominant exchange with the surface water and high variations in GWT and can tolerate temperatures up to 18 °C (Stein et al., 2012). Interestingly, one location in the southern city area with 272 individuals is characterised by a high fluctuation in GWT (standard deviation of 3.4 °C) and a rather high nitrate content (8.3 mg/l) compared to wells in the forest area which are both indications for a disturbed and stressed habitat.

Besides the group of Crustaceans, Oligochaetes, which can tolerate a wide temperature range, were also found in large abundance in the study site. A significant amount of the subclass *Oligochaeta* (996 individuals) was found in the urban area

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Kommentiert [FK58]: Referee#2 Comment#24

Kommentiert [FK59]: Referee#1 Comment#20

Kommentiert [FK60]: Referee#2 Comment#55

Kommentiert [FK61]: Referee#1 Comment#21

Kommentiert [FK62]: Referee#2 Comment#56

(Figure 4b), compared to an overall number of 1,343 individuals. In general, the number of Oligochaetes is larger in locations with high GWT (12.6 -17.3 °C) and nitrate concentrations up to 14 mg/l, which is above the geogenic concentration of 10 mg/l and higher compared to wells in the forest area.

Finally, Nematodes and Microturbellarians were found at locations with unfavourable living conditions, such as a low content of dissolved oxygen, or a high amount of fine substrates, as also reported by Hahn et al. (2013), both can tolerate high temperature ranges (*Turbellaria*: 2 – 20°C (Herrmann, 1985), *Acari*: 9.1 – 18.5 °C (Więcek et al., 2013)). Here, both were found in larger quantities in the urban area of Karlsruhe (Figure 4b). This area has the lowest content of dissolved oxygen, relatively higher amount of detritus (> 2) and the highest nitrate concentrations (> 6 mg/l).

Eventually, correlation analysis between groundwater fauna and the chemical parameters showed that Stygobites are only slightly affected by groundwater chemistry (Hahn, 2006; Schmidt et al., 2007; Stein et al., 2010). Only the Spearman's rank correlation coefficient ρ between the number of taxa and the content of dissolved oxygen is significant with a value of $\rho = 0.55$ (p-value = 3.0×10^{-4}, n = 39). The natural influence on porosity, groundwater flow and nutrient delivery were also discussed as a primary influence on natural Stygobites distribution in previous studies (Hahn, 2006; Korbel and Hose, 2015). One important natural influence is the local geology, as fine sands and silts are typically rather harsh environments, resulting in an impoverishment of specific groundwater fauna such as *Crustacea* (Hahn, 1996). The city of Karlsruhe is located on carbonate ('Würm') gravel and river terrace sands, pervaded by bands of drifting sand and inland dune sands. These sediments are highly water-permeable and show almost exclusively vertical seepage of water movement. Flood sediments (on top of river gravel) and bog formations, are located in the east and west of Karlsruhe (Regierungspräsidium Freiburg, 2019). This local geology limits the cavity size and therefore has impacts on the habitat of the groundwater fauna (Wirsing and Luz, 2007). For example, individuals of the genus *Parastenocaris* typically inhabit small-scale cavity systems (Spengler, 2017). Individuals of this genus can be found both in the wells drilled in gravel (4 wells) and in drifting sand sediments (3 wells) (abundance *Parastenocaris* vs. geological units: U-test: p-value = 1.4×10^{-9}, n = 39). Amphipods are predominantly found in measurement wells located in the 'Würm' gravels (in 5 of 7 wells) (abundance *Amphipoda* vs geological units: U-test: p-value = 9.0×10^{-11}, n = 39). Moreover, it seems that differences in the geological units have an influence on the total amount of individuals (U-test: p-value=1.7×10^{-9}, n = 39) and the relative amount of detritus (U-test: p-value = 3.0×10^{-3}, n = 39). As these results show, regional geology seems to have an influence on the occurrence of specific groundwater taxa and on the number of individuals as well as on food supply, in terms of available organic matter. However, it is not possible to give a reliable estimate of the strength of the anthropogenic impacts, e.g. if they are strong enough to overrule the regional selective forces. Hence, this should be investigated in more detail in future studies.

Limitations regarding the sampling method must be considered when interpreting the faunistic results. In this study, a simple basic screening of well water was conducted using net sampler and bailer to assess conditions in the groundwater monitoring wells (39 wells with an average diameter of 132.5 mm, which corresponds to an area of 0.003 ‰ of the total urban area). According to the sampling manual of the PASCALIS Project 'the use of a phreatobiological net alone is considered as a satisfactory method for sampling groundwater fauna in large diameter wells' (Malard et al., 2002). Yet, several studies (e.g.

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Kommentiert [FK65]: Referee#2 Comment#6 & #8

Scheytt, 2014) report that scooped samples of wells are not representative, and therefore the water remaining in a well has to be purged and discarded before sampling. Nevertheless, pumping can result in the selection of the taxa, especially in the presence of very fine sediments, and can result in changes of the sediment composition in the surrounding of wells and therefore in changes of habitat conditions. Other studies, on the other hand, found no significant differences in hydro-chemical values (temperature, pH, dissolved oxygen, etc.) between the surrounding groundwater and the standing water in a well (Hahn and Matzke, 2005; Korbel et al., 2017). The sampled groundwater fauna of corresponding wells and aquifers were also shown to be similar with respect to the types of faunal communities. However, in terms of total abundance, as well as the numbers of individuals per litre, monitoring wells appear to exhibit larger numbers caused by filtration effects (Hahn and Matzke, 2005; Hahn and Gutjahr, 2014; Korbel et al., 2017). As the aim of this study is to provide an overview of the groundwater fauna community (assess biodiversity) and to receive a first impression of groundwater ecology, sampling the fauna by using a net sampler is sufficient. In order to achieve a representative sampling of groundwater fauna in the aquifer and to reflect the occurring species at a community level a more comprehensive sampling method is required, e.g. the use of a defined standard sampling method using a pump to collect animals (Malard et al., 2002).

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Kommentiert [FK67]: Referee#2 Comment#18

Kommentiert [FK68]: Referee#1 Comment#22

3.3 Classification scheme by Griebler et al. (2014)

In three wells evaluation with the classification scheme by Griebler et al. (2014) was not possible due to ocherous conditions in two monitoring wells and low content of dissolved oxygen (<1 mg/l) in the third well. According to the classification scheme by Griebler et al. (2014), unstressed (meaning no natural or anthropogenic stressors), or natural groundwater habitats have an amount of more than 70 % of Crustaceans and less than 20 % of Oligochaetes. In 36 % of the sampled wells, i.e. 14 out of 39, these criteria were fulfilled, indicating O.K. ecological conditions or in other words a natural groundwater habitat (Figure 4c). These natural areas tend to contain more individuals of the orders *Amphipoda, Cyclopoida* and *Bathynellacea*. Monitoring wells, which do not fulfil these criteria and are accordingly defined as affected areas not having natural ecological conditions, contain more Oligochaetes and also Nematodes, which is partly explained by the used criteria of this classification scheme (Figure 4d).

Surprisingly, only 50 % of the wells in the rural forest, which is also the catchment area of the drinking water supply of Karlsruhe, are described as natural groundwater habitats. An identical number of wells yielded habitats with affected ecological conditions. The main difference between natural and affected wells in the forest area arises from the occurrence of specific species. 86 to 100 % of species found in natural wells are Crustaceans, in contrast to affected wells with only 33-67 % (Table S1 and Table S2). However, the abiotic parameters scarcely differ between natural and affected wells (average values for GWT: 10.8 and 10.6 °C, dissolved oxygen: 7.1 and 8.8 mg/l, nitrate: 2.5 and 3.0 mg/l), indicating that there are other processes or parameters that influence the groundwater fauna in these wells. One reason could be the varying local geology as mentioned above. Moreover, food supply is one of the most limiting parameters for the survival of groundwater fauna (Datry et al., 2005; Hahn, 2006). If the organic carbon supply varies on a small scale, this can influence microbiology and therefore groundwater fauna as well, although, short-term changes in nutrient supply can be compensated by groundwater fauna.

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In contrast to the forest land, the majority of wells (65 %) in the urban area are categorised as affected habitats. As expected, this indicates anthropogenically influenced groundwater ecosystems beneath the studied urban area. Once more, no significant differences between the abiotic parameters of natural and affected wells are observed (e.g. median of dissolved oxygen: 4.7 and 5.8 mg/l, median of nitrate: 7.2 and 7.8 mg/l). On the other hand, the remaining 35 % of the wells in the urban area show natural ecological conditions even though some of them are located in areas with anthropogenic impacts such as increased groundwater temperatures. Hence, no distinct spatial pattern of the ecological condition with respect to land use could be identified. In future, a further subdivision of a study area in more land use categories could be useful to specifically look at typical anthropogenic impacts. The observed spatial heterogeneity in ecological conditions and the existing heat anomalies in the urban area of the study also call for an adapted usage for shallow geothermal energy systems. Areas with no or little groundwater fauna (i.e. affected habitats) could also be used to store thermal energy at higher temperatures. Thus, high-temperature aquifer thermal energy storage (HT-ATES) could be established in urban environments (e.g. Fleuchaus et al., 2018), where the demand is high.

Kommentiert [FK70]: Referee#2 Comment#9 & #15

Kommentiert [FK71]: Referee#2 Comment#28

4. Conclusion

The aim of this study is to provide a first-tier assessment of the ecological state of groundwater in an urban area and to distinguish areas with a natural state of groundwater ecology from anthropogenically affected areas. To achieve this, we examine the groundwater fauna, as well as abiotic parameters in 39 groundwater monitoring wells in the urban area of Karlsruhe, Germany, and a nearby forest land using the simple classification scheme by Griebler et al. (2014) to characterise the sampled monitoring wells.

We found a noticeable difference in the spatial distribution of abiotic groundwater characteristics and special species within the study area. The rural forest area shows lower GWT, lower nitrate concentrations and higher dissolved oxygen concentrations, which indicates a correlation between abiotic groundwater characteristics and land use. However, no clear spatial pattern regarding faunal diversity and land use was found, as both in the rural forest and in the urban area Crustaceans and individuals of other subordinate taxa were widely found. In terms of faunal quantity, Crustaceans were found in larger numbers, with respect to the number of wells, in the monitoring wells in the forest area than in the urban area. Larger amounts of the genus *Parastenocaris* as well as of Nematodes and Oligochaetes were found to be characteristics for wells in the urban area.

Furthermore, no clear spatial pattern of ecological groundwater conditions according to the classification scheme by Griebler et al. (2014) could be observed. Surprisingly, only 50 % of the sampled wells in the rural forest were described as natural (undisturbed) groundwater habitats, while the other four were characterised as habitats with affected ecological conditions. Yet, the majority of wells (65 %) in the urban area were classified as affected locations suggesting that there are noticeable differences in the groundwater ecosystems between the surrounding rural and urban areas. The Level 2 assessment from Griebler et al. (2014) can help to achieve a more reliable and quantitative ecological assessment of urban aquifers as it divides groundwater ecosystems in ecological grades according to the intensity of anthropogenic disturbance. It is based on the use of local reference values and the collaboration with experts, which is however challenging to apply. Therefore, further studies with large-scale and repeated measurement campaigns are needed to verify our findings. This should also include other cities and the determination of undisturbed local reference values which are required for a more reliable but also quantitative ecological assessment of urban aquifers. Moreover, a wider range of indicators should be considered in a classification scheme, such as temperature, porosity of the aquifer, groundwater flow, pollutants, nutrient supply, etc., especially when investigating urban areas. In addition, an important adaptation for an improved evaluation method is the determination of fauna at species level, which will provide more information (i.e. about Stygobionts, Stygophiles, Stygoxenes) and also consider the endemism of stygobiotic species. In this context, classification schemes should pay more attention to the different groundwater species and their potential use as indicator species.

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Finally, city and energy planning should seriously consider urban groundwater ecosystems as they provide valuable information for a sustainable use of the subsurface.

Data availability

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PB and HJH provided the topic and supervised the work, together with KM. SS and CS executed the field work and evaluated the samples. FK evaluated the collected data and interpreted as well as visualised the results and wrote the first draft of the paper. KM, CS, HJH and PB participated in editing the paper.

Competing interests

The authors declare that they have no conflict of interest.

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Review statement

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