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Correlations between the increase in atmospheric CO_2 and temperature, and the subsequent increase in silica, and groundwater organisms

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Abstract

Rising atmospheric temperature and CO_2 impact all freshwater systems. In groundwater, one such impact is CO_2- and temperature-induced weathering, which leads to more intense weathering of silicate rocks. Here, we tested whether the increased CO_2 levels, the weathering, or rather the increasing temperature, impacted on fauna and prokaryotes in the groundwater ecosystem. We also conducted the analyses separately for two groups of wells, one of which contained wells that were secluded from the surface (and often rather deep), and wells tapping the quaternary aquifers (often rather shallow) which exchange with the surface more intensely. Organism abundances and relative composition did not correlate with temperature or CO_2 levels. While many organisms rely on silica, in contrast, we found negative correlations between silica concentrations and fauna. The increases in silica concentrations over time, i.e. temporal trends, also partly correlated negatively with organisms. We hypothesize that the unexpected negative correlations are not direct effects, but indirectly indicate that groundwater communities do not adapt rapidly enough to changes in silica concentrations, but also more generally to changes for which silica might only be a proxy. Groundwater fauna take part in the ecosystem service of water self-cleaning and are thus considered beneficial for sustainable raw water for drinking water production. The propensity of groundwater fauna to decrease with increases in silica, jeopardizes future drinking water production.

Keywords: groundwater ecosystem, weathering, geological thermostat, fauna, prokaryotes, silica, Earth's Critical Zone

1. Introduction

Atmospheric CO_2 levels and temperature have risen globally, particularly in the past 30 years, largely due to industrialization (IPCC, 2022) and/or anthropogenically increased CO_2 (Penman et al., 2020). Consequently, by diffusion into water and a temperature-induced increase in biotic production and respiration, aqueous CO_2 concentrations have risen (Cole, 2013). Sea water is recognized to be one major sink for CO_2 (Sabine et al., 2004), and in rivers, HCO_3^- concentrations, most probably derived from increased CO_2, have increased (Kwon et al., 2021; Meybeck, 1993; Powley et al., 2024). Recent modelling shows that the global increase depends mainly on temperature and latitude (Lehmann et al., 2023). Soil is a source for global CO_2 levels, because increased temperatures have led to increased organic matter degradation and outgassing CO_2 (Ferdush & Paul, 2021). However, it is much less clear whether groundwater is a sink or source for global CO_2 (Boerner & Gates, 2015; Nydahl et al., 2020). More than likely, the net import of CO_2 into groundwater has risen, via the increased CO_2 and HCO_3^- concentrations in soil (Bakalowicz, 1994) that are subsequently recharged into groundwater. This increased CO_2 import will immediately lead to increased acidity which, in turn, will dissolve minerals, and thus, fuel weathering (for a summary of CO_2-driven weathering, see Supplementary Information SI 1). In fact, in parallel to sea water acting (still) as a CO_2 sink, silicate rock weathering due to CO_2 is regarded as one mechanism stabilizing earth's climate (Brantley et al., 2023; Li et al., 2016). Such geochemical processes are estimated to have absorbed the equivalent of about half of the CO_2 that was emitted each year in the 2012-2021 decade (Friedlingstein et al., 2022). While the effects of the increased CO_2 on freshwater surface organisms are unclear (Weiss et al., 2018), the effects on groundwater organisms which differ from surface organisms in many traits (Hose et al., 2022), are even less obvious. More is known about groundwater temperature: while microbial numbers and diversity increased with temperature, faunal abundances and diversity decreased

(Brielmann et al., 2009), and above 12°C, a fauna community shift in mid-European groundwater assemblages was observed (Spengler & Hahn, 2018, see also below). A further influence on groundwater pH, and thus impact on the CO_2 and HCO_3^- concentrations, comes from acid atmospheric deposition which have also increased weathering.

Climate change in general threatens groundwater ecosystems, but we do not know yet, how groundwater ecosystems will react, and which will be the driving impacts (Mammola et al., 2019). Thus, we do not know what will happen to the ecosystem functions that the groundwater ecosystems deliver, e.g. self-cleaning (Griebler & Avramov, 2015). A decline of groundwater ecosystem functions may threaten the availability of drinking water for around 50% of the world's population (UNESCO World Water Assessment Programme, 2022).

Increased CO_2 levels, in contrast to the clear direct effects from temperature, might have contradictory effects. They might provide the electron acceptor and/or carbon substrates for microbial reactions, such as the Calvin cycle, or the oxidation of reduced sulphur and nitrogen compounds, such as sulphide and ammonia (Herrmann et al., 2015; Schmidt et al., 2017). Primary production in groundwater, without light, is chemolithoautotrophic. Increased CO_2 might boost this primary production. Most groundwater food webs are likely bottom-up controlled and thus rely on organic import via recharge, or, sometimes in parallel (Herrmann et al., 2020; Kellermann et al., 2012), on primary production. Groundwater food webs might thus benefit from increased CO_2 import and one might expect increased prokaryote and consequently fauna (i.e. grazers') biomasses. In contrast, Crustacea, which dominate groundwater systems, can be negatively affected by increased CO_2 in surface waters (Hasler et al., 2017; Langer et al., 2017; Weiss et al., 2018). Fauna might thus not be able to make use of the increased prokaryote primary production. This would lead to higher-than-expected prokaryote biomass, because grazing pressure is missing.

In parallel to the anticipated direct effects of groundwater temperature and the unclear direct effect of groundwater CO_2 on fauna, indirect effects must be considered. One major indirect effect of increased CO_2 may be the increased silicate weathering ("The gradual removal of atmospheric CO_2 through dissolution of silicate and carbonate rocks"; IPCC, 2022), and consequently, increased silica concentrations in soil from which groundwater is recharged, and their potential impact on all organisms in groundwater, not only fauna. Classen et al. (2015) showed direct and indirect impacts from climate change on the soil microbial and soil microbial-plant interactions. Since soil and groundwater are hydrologically connected, similar interplay of direct and indirect effects can be expected for groundwater. One major indirect effect of increased CO_2 may be the increased silicate weathering, and consequently, increased silica concentrations, and their potential impact on all organisms, not only fauna (Sentenac et al., 2023). Where exactly the weathering occurs, is less important than the net effect of increased silica on groundwater fauna. Here, we use silica to mean generically, the dissolved element Si (silicon) and ionic forms, such as SiO_4^{2-} etc., while we use silicate to indicate the undissolved rock mineral. Increased silica concentrations are not expected to harm fauna communities. Silicon, Si, is the second-most abundant element on earth. If it was harmful in itself, we would expect a lot less fauna anywhere near silica rock outcrops. However, silica may be a (limiting) nutrient for fauna and flora that rely on it, such as diatoms, foraminifera, and radiolaria (Lampert & Sommer, 2007; Moore et al., 2013). An increase in dissolved silica may therefore benefit fauna. Fauna, and thus the ecosystem functions they deliver, evolved alongside the silica weathering since geological times. However, it is unclear whether fauna stay unfazed during comparably rapid changes of the environment, including an increase of the silica weathering, particularly when evidence is increasing (see the first paragraphs of the introduction and SI 1) that the weathering has been accelerating recently. Sheldon (2006) related extinction events to periods with major changes in rock weathering. If such extinction

117 happened in groundwater, we would lose its self-cleaning capacity, and this might jeopardize
118 drinking water production. This is because the prokaryotes directly involved in self-cleaning
119 are grazed upon by protozoa and fauna which may reduce (Kota et al., 1999) but may also
120 advance (Mattison et al., 2005) bacterial biodegradation, even under groundwater conditions.
121 Fauna may even enhance prokaryote transport and thus diversity (Smith et al. 2016). It is thus
122 inadequate to look at self-cleaning merely from a prokaryote perspective – the whole
123 groundwater ecosystem is involved in self-cleaning, and the interactions between prokaryotes
124 and grazers are insufficiently known. Feeding, grazing, and degradation rates have rarely been
125 measured for groundwater fauna (Di Lorenzo et al., 2016; Hervant et al., 1997; Wilhelm et
126 al., 2006). Fauna clearly regulates microbial biodegradation and thus plays a part in the self-
127 purification that we can so far describe neither qualitatively nor quantify.
128 We tested the effects of current (averaged over the years 2019 until 2021) temperature, redox
129 potential, CO_2, HCO_3^-, and silica on prokaryote numbers which are at the bottom of the food
130 web, and on fauna, i.e. organisms being held back by 70 µm mesh size net, which are largely
131 at the top of the food web, in a set of Western Czech aquifers. Organisms were collected
132 between 2019 and 2021 – earlier data on fauna from the investigated wells is not available.
133 We classed fauna into high level taxa and calculated biomasses from the abundances which
134 we also correlated to the current physical and chemical values. The current (averaged over
135 2019 until 2021) values at the period of sampling may be, however, the result of changes that
136 occurred in the past decades. We assumed that where such changes occurred, we would
137 observe trends in temperature, CO_2, HCO_3^-, and silica concentrations over the past decades.
138 Therefore, we used these trends as proxies for the magnitude of (climate) change in soil, or
139 subsequently by recharge, in the aquifers. Current fauna assemblage structure might reflect
140 the changes that occurred in the past decades, since fauna integrates over a longer time (Thorp
141 & Covich, 2001). We therefore related the trends of the past decades to current organism
142 numbers, to check whether the changes having happened since 1985 (depending on the well)

predicted current (averaged over 2019 – 2021) fauna assemblages better than nowadays physical and chemical conditions do. We also related the relative composition of the fauna assemblages to the current values and the trends.

Shallow, often quaternary, aquifers are more vulnerable than deep, secluded ones, because they are in more intensive exchange with the surface and the atmosphere (Calmels et al., 2011). Effects from climate change are likely to trickle down only slowly from the surface into deeper realms. These deeper aquifers usually also receive less import from the surface and thus, support lower numbers of less diverse organisms (Schmidt & Hahn, 2012).
The actual depth below ground is hydrogeochemically not decisive, since geochemical properties depend more on hydrogeological patterns than on the fact how much rock or soil is piled above the aquifer. In addition to the clear-cut groups of quaternary and deep aquifers, the redox potential may be seen as an indicator for the vulnerability, or the degree of exchange with the surface, of an aquifer: usually, electron acceptors such as oxygen, nitrate, and sulphate are reduced during groundwater recharge and along the groundwater flow path, mainly by prokaryotes, while electron donors may be released from minerals (Meckenstock et al., 2015). Thus, the redox potential decreases and can be used to confirm the grouping into quaternary, high-exchange, and deep, low-exchange aquifer groups. With the redox potential being an indicator for import from the surface, we suspected less organisms at lower redox potential. Since redox reactions at lower redox potentials yield less free energy (Aquilina et al., 2023), we expected prokaryote numbers, and consequently, faunal numbers, to be lower at lower redox potentials. However, fauna is not only limited by resources, but also by oxygen. Some protozoa are anaerobic (Fenchel & Finlay, 2008) and avoid oxygen. In the current contribution, however, we focus on metazoa, i.e. multicellular organisms, and use the term "fauna" for them. Groundwater fauna is much better adapted to low oxygen values than surface fauna (Malard & Hervant, 1999), but 1 mg L^{-1} DO seems to be a limit below which

169 the vast majority of metazoa cannot survive, unless they undergo tight symbioses, which was
170 so far only observed in the deep ocean, not in groundwater (Danovaro et al., 2010).
171
172 Our hypotheses were the following: 1) over the past decades, the increases in temperature,
173 CO_2, HCO_3^-, and silica, were significant in the investigated wells, while redox potential and
174 DO stayed unchanged; 2) the increases were greater in quaternary aquifers which are more
175 vulnerable and exchange more intensely with the atmosphere; 3) there were direct correlations
176 between current temperature, DO, CO_2, HCO_3^-, redox potential, and silica, and current
177 organism assemblages, with higher numbers of prokaryotes with higher temperature, DO,
178 CO_2, HCO_3^-, redox potential, and silica, and lower numbers and biomasses of fauna with
179 higher temperature, CO_2, HCO_3^-, but higher numbers with higher levels of DO, redox
180 potential, and silica; 4) the higher the respective absolute slope of the trend was over the past
181 decades, i.e. data within the database covering 1985 – 2021, the lower were current, i.e.
182 between 2019 and 2021, organism numbers and fauna biomass; the reason for this is that the
183 more distinct the trends were, the more rapid the change must have occurred this would have
184 been detrimental to groundwater organisms which are not adapted to rapid changes, 5) current
185 (2019 – 2021) faunal assemblage structure reflects not only current levels, but also the trends
186 of the past decades.
187
188
189 **2. Methods**
190
191 Thirty eight of the wells monitored by CHMI (Czech Hydrometeorological Institute) were
192 sampled during the respective spring and autumn campaigns during the years 2019 to 2021
193 (for a partial, preliminary account, see Schmidt et al., 2021), and we refer to this period 2019
194 to 2021 as “current”. The wells were chosen according to geographical situation and

according to logistic restraints. The coordinates of the location of the wells are given in Table S1, and the position is indicated in the overview maps Figure S1, in the Supplementary Information SI 2. We used the Czech Hydrometeorological Institute classification into "deep", i.e. secluded, and "shallow", i.e. quaternary, wells, tapping aquifers interacting regularly with the surface (Table S1). This is only a rough and categorical division which can only help understanding the reality to a certain extent, of course. However, where more detailed knowledge of the real exchange patterns is lacking, this may help us understand subsurface processes and may help to direct future, more detailed investigations. The division into "shallow", i.e. quaternary, and "deep" wells was based on expert judgement and was largely the same as the division into "recharge zone" or "no recharge zone", which was also based on expert judgement (Table S5). Except for VP1855, VP1857, VP1877, and VP7718, all "deep" wells were confined. In contrast, except for VP0814, VP0903, VP1115, VP1136, VP1143, VP1617, and VP1854, the "quaternary" wells were unconfined. The confinement also largely reflected whether an aquifer was recharging (Table S1). We considered the distinction between "deep" and "quaternary", as more informative than the confinement for organisms since deep and quaternary wells, which coincided with recharge (Tables S1 and S5), translates into food sources for fauna.

For the whole Czech Republic, the increase in air temperature was between 0.037 °C yr^{-1} and 0.027 °C yr^{-1} for the period 1961 – 2019 (Zahradníček et al., 2021). In two small Šumava catchments close to some of the wells studied here, air temperature had increased by 0.039 °C yr^{-1} between 1981 and 2012 (Turek et al., 2014). Soil temperature in these catchments related well to air temperature (Kopáček et al., 2024). Thus, increase in soil and subsequently groundwater temperature was to be expected as well for the whole studied area.

220 The chemical and physical results for those wells where fauna was sampled, were extracted
221 from the CHMI database. Some of the wells have been monitored for chemical and physical
222 properties as early as 1985 (VP0814, VP0903, VP1103, VP1117, VP1614, VP1617), while
223 one well (VP1977) was only included in the monitoring in 2021 (Table S1 provides periods of
224 sampling) and was thus excluded from the trend analyses. We used all data available in the
225 database until autumn 2021. Details on pumping the wells prior to sampling for physical and
226 chemical characteristics are given in Table S1. Wells were purged prior to taking the samples
227 for chemical and physical analyses. For the evaluation of CO_2, see Supplementary
228 Information SI 3. Molar ratios of Na/Ca versus Mg/Ca were calculated to show which type of
229 rock weathering might have dominated (Gaillardet et al., 1999; Négrel et al., 1993).
230
231 For the analyses of trend over time, the complete data set, covering the whole monitored
232 period (since 1985; see paragraph above), was used, while for the analyses of the recent or
233 current environment of the organisms, the chemical and physical values were averaged over
234 the years 2019 – 2021. That was the "current" period during which we sampled fauna and the
235 chemical and physical values were thus those that the sampled organisms experienced
236 directly. These current chemical and physical conditions were correlated to faunal
237 abundances, faunal biomasses, and prokaryote numbers, and linear regression parameters
238 were calculated.
239
240 The slopes of the linear trends of the chemical and physical variables over the past decades
241 (since 1985; see above) were calculated as yearly slope, taking the first and last fitted value
242 per variable and well, and dividing by the number of years that this well was sampled for, for
243 this variable. These slopes were also correlated to faunal abundances, biomass, prokaryote
244 numbers, and relative assemblage composition (see below: environmental fitting "envfit()",

245 from the R package “vegan” (Oksanen et al., 2022), to reflect whether recent developments
246 had shaped organisms.
247
248 Fauna was sampled first with a 70 µm pore net (manufactured by Institut für
249 Grundwasserökologie GmbH, Landau, Germany) lowered down the well and drawn up at
250 arm’s length for ten times to scoop up bottom-living fauna (Bou, 1974; Matzke & Hahn,
251 2002; Schmidt et al., 2004). This is the most common method of sampling fauna. However, it
252 was not always possible, in contrast to pumped samples. Pumping was chosen as the default
253 method for this study. Subsequently, from the pumped water stream (see Table S1 for details
254 on pumping), 50 L aliquots of pumped water were taken and sieved through the same net. It
255 was checked whether there was an obvious difference in numbers of fauna sampled by
256 pumping or with the net draws, related to the volume that was estimated to have passed
257 through the net (Further information on sampling is given in Supplementary Information SI
258 4). Fauna biomasses were estimated from abundances based on biomass per fauna taxonomic
259 relationships given in Marxsen et al. (2021). Prokaryote numbers were estimated by flow
260 cytometry in samples stained with the fluorochrome Syto13 (Molecular Probes, Eugene,
261 Oregon, U.S.A.) using the FACScalibur flow cytometer (Becton Dickinson, Franklin Lakes,
262 New Jersey, U.S.A.) as detailed in Gasol & del Giorgio (2000).
263
264 Normality was tested by the Shapiro-Wilk normality test (Royston, 1984), using the base R
265 command “shapiro.test()”. The data failed normality for the prokaryote and fauna data sets
266 and for most physical and chemical properties and the variances were heterogeneously
267 distributed. Therefore, regressions were calculated with the the nonparametric Kendall–Theil
268 Sen Siegel linear regression using the R command “mblm” from the package “mblm”
269 (Komsta, 2019). Comparisons among boxplots were calculated with the nonparametric
270 Wilxox test using the command “stat_compare_means()” from the R package “ggpubr”.

A multivariate analysis, nonmetric Multidimensional Scaling (NMDS), was conducted on the faunal assemblages. It is not possible to calculate the Bray–Curtis coefficient when there are samples consisting only of 0 abundances for all taxa. In such cases, a dummy species is added with an abundance of 1 in all samples (Clarke et al., 2006). Since there were many zeros in the faunal assemblages' data, the distance matrix for the NMDS was calculated using the zero-adjusted Bray–Curtis coefficient (Clarke et al., 2006), after Naupliae abundances were excluded. Since well assemblages with only this zero abundance which was raised to 1 for calculation purposes, fell into one coordinate, the dots with which they were represented were drawn apart for visualization of hydrogeological basins and were encircled with a grey circle. Environmental fitting of variables to the NMDS was done by the "envfit()" procedure in the vegan package (Oksanen et al., 2022). This procedure correlates the values of the characteristics to the first two dimensions of the NMDS, and it is possible to only draw those variables overlying the NMDS which correlated significantly – here, we used the significance level $p = 0.05$. In parallel to temperature, redox potential, CO_2, HCO_3^-, and silica, further chemical and physical characteristics were chosen for the correlation with organism data. We focused on those that were measured most frequently, are most telling about hydrogeological conditions, and/ or are known to limit organisms: alkalinity, chemical oxygen demand (COD), Ca^{2+}, Cl^-, dissolved oxygen (DO), dissolved organic carbon (DOC), dissolved reactive phosphorus (DRP), electrical conductance (EC), F^-, dissolved iron, hardness, Mg^{2+}, Mn(II), NH_4^+, NO_2^-, NO_3^-, Na^+, SO_4^{2-}, pH. Also, their standard deviations, i.e. variability, and the slopes of the linear trends over time (see third paragraph of the Methods section) were included in the analyses, as well as geographic coordinates. The variability that standard deviations indicates, may be due to interannual variability, or to the trend over time. This meant 52 individual regressions. With that number of tests, the likelihood that some of the tests might by chance be significant ("false positive"), or insignificant ("false negative"),

297 increases, because of multiple testing. To compensate for such effect, the *p*-values were
298 Benjamini-Hochberg-Yekutieli-corrected for multiple testing (Benjamini & Hochberg, 1995).
299 Only environmental fitting ("envfit()") regressions with an adjusted *p*-value below 0.05 were
300 marked as significant and plotted in the MDS. All analyses, where not marked otherwise,
301 were performed using R 4.3.2 (R Core Team, 2023).
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303
304
305
306 **3. Results**
307
308 **3.1 Environmental conditions**
309 The averages, minima and maxima of those variables that are assumed to be most relevant for
310 weathering are listed in Table 1. In the lower part, the respective statistics are given separately
311 for quaternary, i.e. high exchange, and deep, i.e. low exchange, wells.
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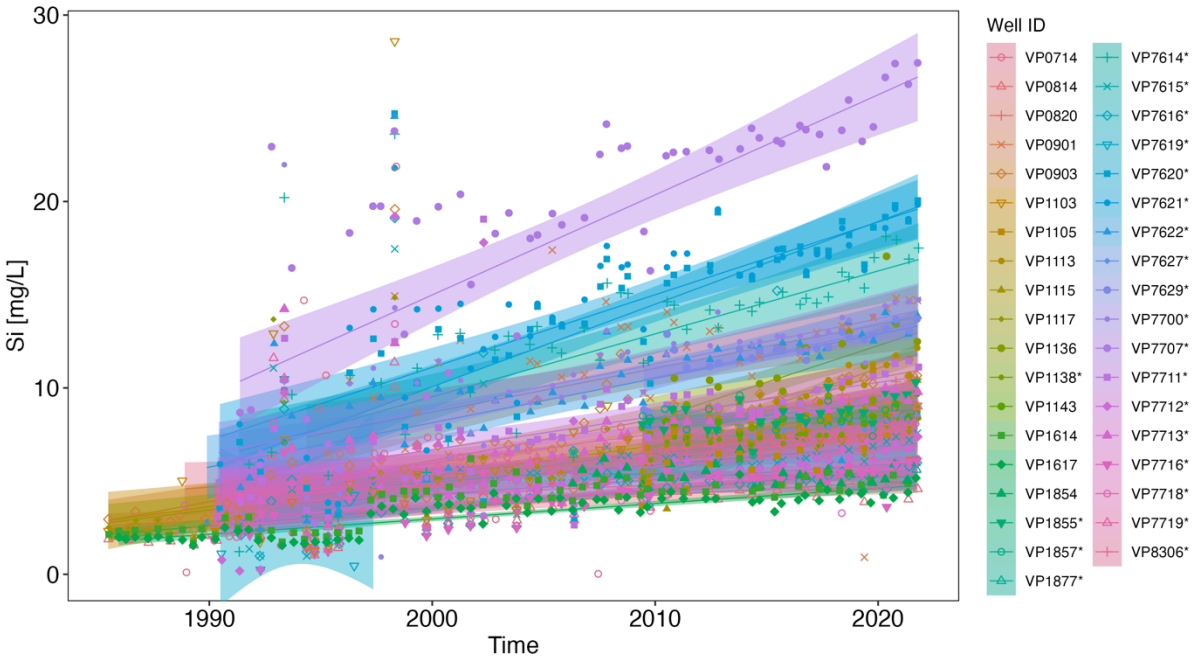
Table 1: Measured values for the most important variables, average over all individual measurements, minima, and maxima for the total data set, and separately for deep, i.e. low exchange, and quaternary, i.e. high exchange and recharge wells.

Measured values		**Tempera-ture (°C)**	**pH**	**Redox (mV)**	**DO (mg L^{-1})**	**pCO_2 (µatm)**	**HCO_3^- (mg L^{-1})**	**Si (mg L^{-1})**
Total	Average	10.25	6.42	36.43	2.12	46506	126.64	7.62
	Minimum	2.70	4.40	-181.00	0.01	0	0	0.03
	Maximum	21.60	12.40	963.00	12.70	4641203	521.00	28.59
Quater-nary	Average	10.45	6.52	64.07	2.51	42516	165.20	6.17
	Minimum	2.70	4.40	-175.00	0.01	0	0	0.03
	Maximum	21.60	8.40	436.00	12.30	4641203	521.00	28.59
Deep	Average	10.11	6.35	16.98	1.85	44974	98.72	8.67
	Minimum	2.80	4.50	-181.00	0.05	0	0	0.18
	Maximum	16.80	12.40	963.00	12.70	800483	112.00	27.44

The developments over time of those variables most relevant for weathering are shown in SI 5, Figures S4 to S9, and Figure 1, and the full statistics, including the Shapiro test for normality, are given in Table S2. There were 3269 slopes of a variable at a well that deviated from normality, while 954 were distributed normally at a level of $p < 0.05$. However, to keep the statistics comparable, we treated all values as non-normal and only used the nonparametric regression model subsequently.

The nonparametrically calculated slopes of the trends over time of those variables that we assumed most influential on weathering are summarized in Table 2. In the lower part, the respective statistics are also given separately for quaternary and deep wells. Except for one

335 well (quaternary well VP1854), silica concentrations increased significantly in all wells
336 (Figure 1) significantly (Table S2).
337 In contrast, trends in the other variables varied, with increases and decreases regardless of
338 whether the wells were deep or quaternary (SI 5 and Table S2).
339



340

341 *Figure 1: Silica, reported as Si concentration, over time in the wells that were sampled for fauna. Measurements, linear trend*
342 *(in darker colour), and confidence interval (in lighter colour around the linear trend lines) are coloured according to the well.*
343 *Well identificators of deep wells are marked with a star.*

344

345 Molar ratios of Na/Ca versus Mg/Ca indicated that samples were closely related to value
346 ranges known for silicate rock weathered water (Gaillardet et al., 1999; Négrel et al., 1993),
347 as shown in Supplementary Information SI 6a, Figure S10. While almost all wells can be
348 considered to tap silicate rock type aquifers, the mean concentrations of silica of the current
349 period, i.e. years 2019 – 2021, differed among geological strata (Figure S14), but not among
350 soil types (Figure S15). The redox potential and DO were lower in deep, than in shallow,
351 quaternary wells (Wilcoxon rank sum test with continuity correction: W= 148521, p-value <

0.001, and W = 224719, p-value < 0.001, respectively; see also the correlation matrix in Figure S12). We also tested the effects of soil or well installation type on current temperature or silica concentration and on the trends of temperature or silica concentration. None of the responses was clearly different in different soils or different well installation types (SI 6b). While the silica trends over time differed among geological strata, they were almost always positive (Table 2; Figure S19).

Table 2: Fitted slopes (nonparametric model) of the trend lines over time for the most important variables, average, minima, and maxima for the general nonparametric trends. In the lower part: separate for deep, i.e. low exchange, and quaternary, i.e. high exchange wells.

Slope over time		Tempe-rature (°C y^{-1})	pH (y^{-1})	Redox (mV y^{-1})	DO (mg L^{-1} y^{-1})	pCO_2 (µatm y^{-1})	HCO_3^- (mg L^{-1} y^{-1})	Si (mg L^{-1} y^{-1})
Total	Average	0.30	0.00	0.57	-0.03	212.29	0.27	0.19
	Minimum	-4.38	-0.03	-4.38	-0.45	-1848.41	-6.41	-0.02
	Maximum	7.89	0.07	7.89	0.10	2460.32	3.32	0.45
Quater-nary	Average	0.05	0.00	0.32	-0.06	306.76	0.44	0.19
	Minimum	-0.13	-0.03	-4.38	-0.45	-641.12	-6.41	-0.02
	Maximum	0.13	0.02	7.89	0.10	2460.32	3.20	0.39
Deep	Average	0.03	0.00	0.76	-0.02	144.81	0.15	0.19
	Minimum	-0.05	-0.02	-3.27	-0.12	-1848.41	-0.59	0
	Maximum	0.39	0.07	5.29	0.10	961.71	3.32	0.45

3.2 Organisms in their environment

There was no obvious geographical or hydrogeological pattern with which fauna was found (SI 7, Figure S30), nor were there patterns with the geological stratum (Figure S32) – wells where high numbers were found were often close to wells where no fauna was found (Figure S30), and fauna abundances did not clearly depend on the geological stratum (Figure S32). Deep wells harboured significantly fewer fauna and prokaryotes. There were deep and

quaternary wells where fauna was not found (compare the map Figure S30 in SI 7 and the results of the test in Figure S31 in SI 8 with the map in SI 2, Figure S1; for the fauna occurrence per sample, refer to Table S3). When aquifers tapped recharge zones, at least partially, the numbers of fauna (Figure S33a) and prokaryotes (Figure S33b) were significantly higher than in no-recharge zones.

Fauna depended on prokaryote numbers (Figure 2), for abundances and for biomass, but the slope, particularly for biomass, was close to 0.The regressions separated in quaternary, or those for deep aquifers, were not significant and/or the slope was 0 (SI 9, Figure S34).

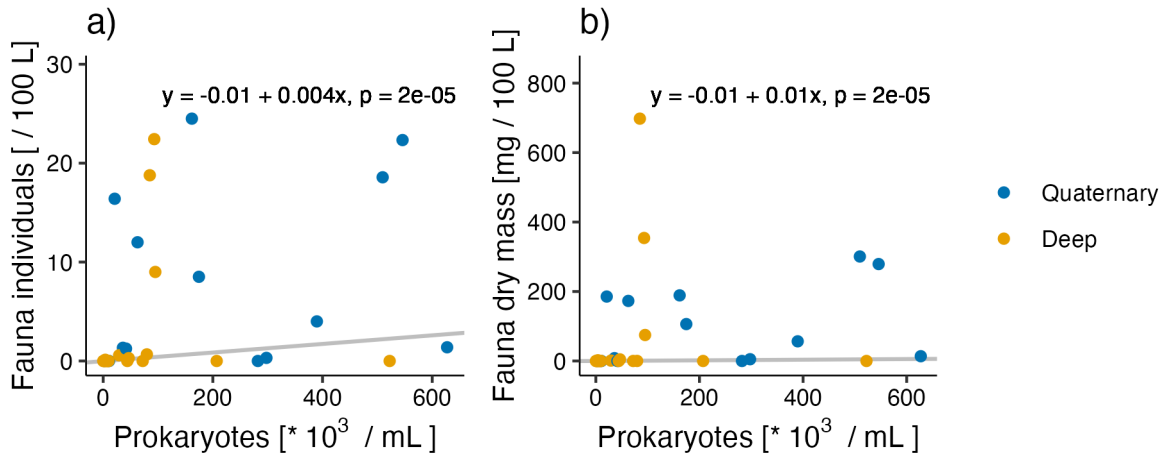


Figure 2: Fauna abundances (a) and fauna biomass (b) in dependence on prokaryote numbers. Given are the individual measurements (dots), the regression line (grey line), and the confidence interval (lighter grey area around the regression line), the regression equation ("y = … "), and the significance level p. For separate fits per aquifer type see Supplementary Information SI 9, Figure S34.

Fauna individuals and biomass did not show a dependency on temperature (p-value < 0.05 and/or zero slope), but highest abundances and biomasses seemed to be reached at temperatures between 10 and 11 °C in the quaternary aquifer, and between 9 and 10 °C for the deeper aquifers (SI 9, Figure S35 a-f). For prokaryotes, the temperature which yielded the

390 highest cell numbers was in the low and high ranges for the deep aquifers, and close to 11 °C
391 for prokaryotes from the quaternary aquifers (SI 9, Figure S35 e, f).
392
393 The current redox potential was not correlated with abundances and biomass for all aquifers
394 taken together (Figure S36 a, c), and was positively related forquaternary abundances, but not
395 for quaternary biomasses and deep and quaternary abundances (Figure S36 b, d). The
396 prokaryote numbers were positively related to redox potential over all aquifers, but not
397 separately for deep and quaternary aquifers (Figure S36 e, f). The patterns for the current
398 dissolved oxygen (DO) concentrations were similar to those for redox potential (Figure S37),
399 although redox potential and DO were only slightly correlated (Spearman rank correlation r_s =
400 0.51; Figure S12). Neither CO_2 nor HCO_3^- concentrations and organisms were correlated
401 (Figures S38, S39), except for prokaryotes depending on CO_2 and HCO_3^-, separately in the
402 deep and quaternary aquifers.
403

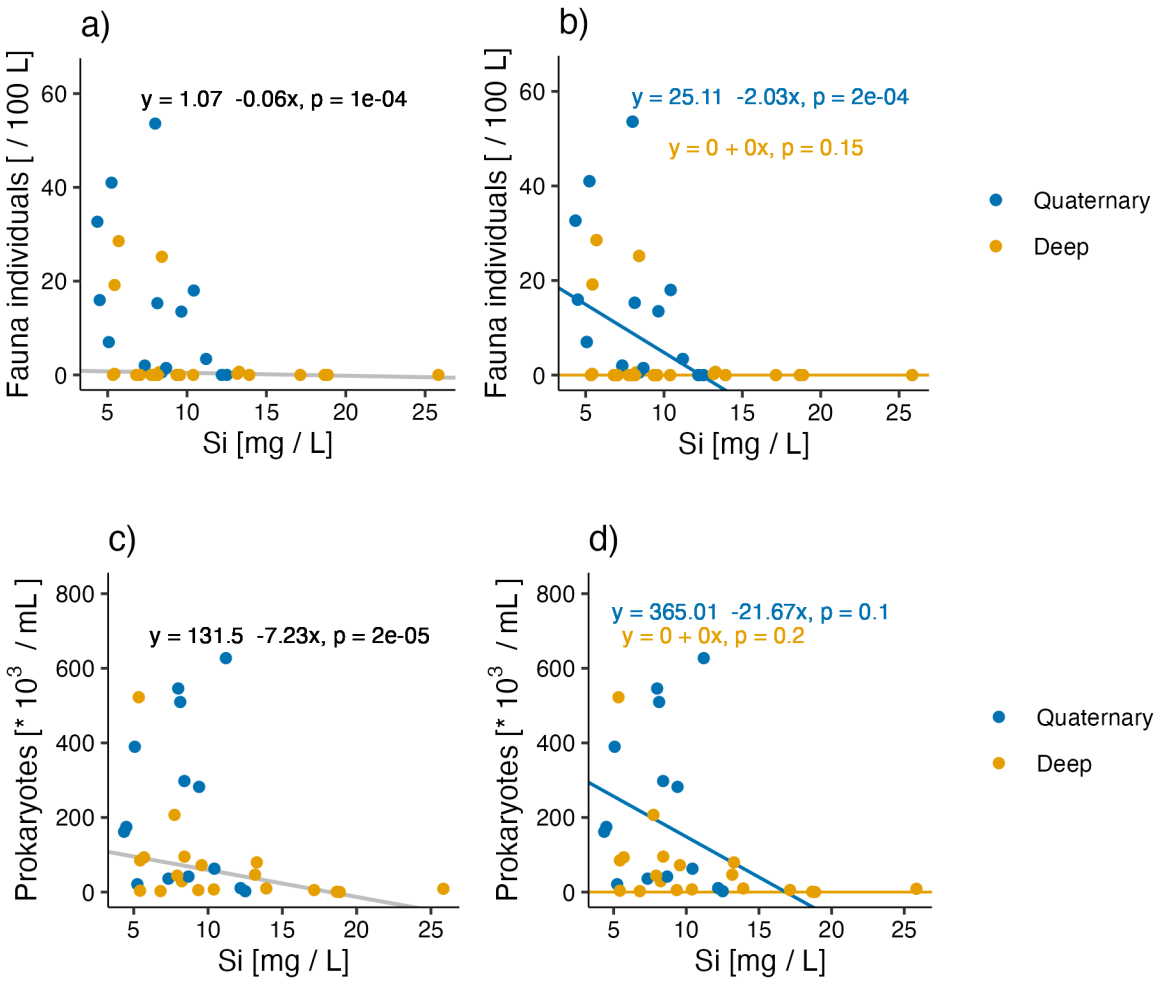
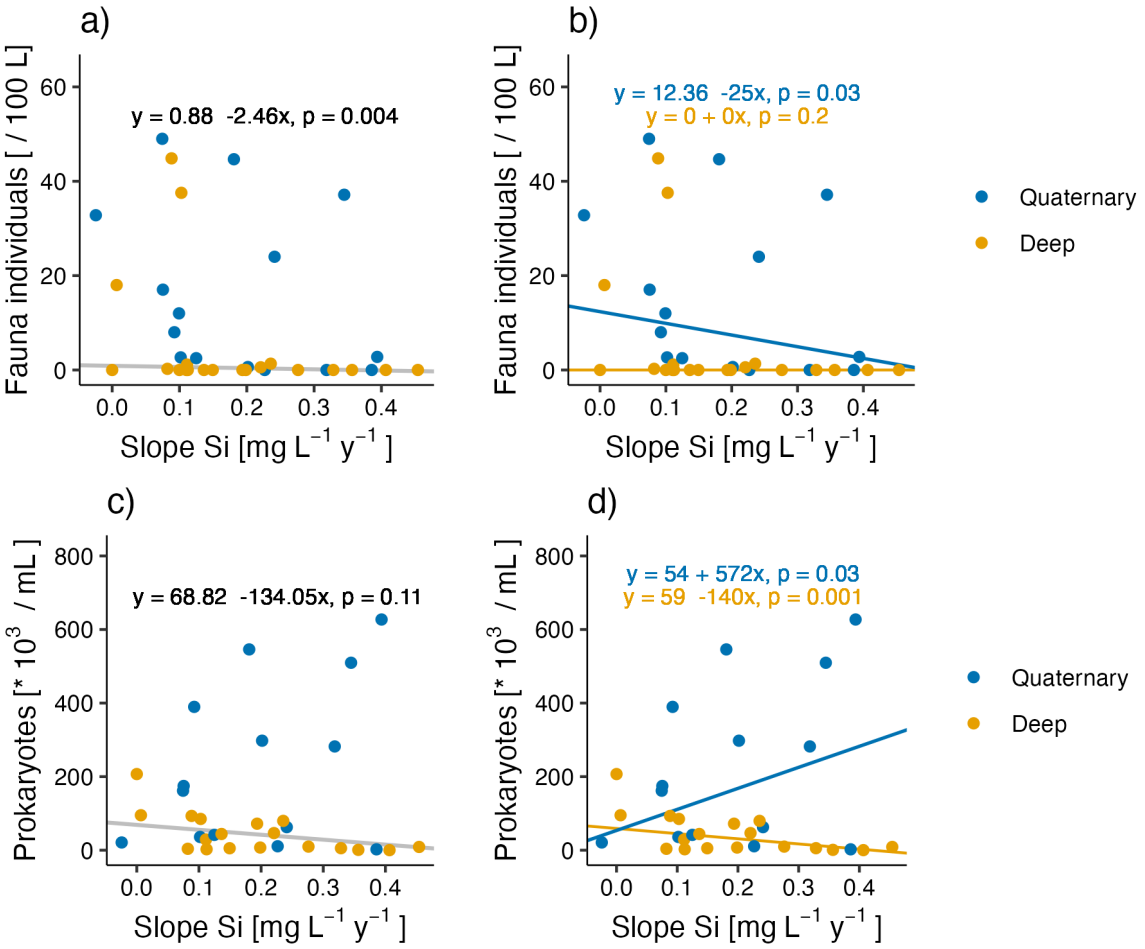


Figure 3: Organisms in dependence on silica concentrations. Top row: fauna abundances. a) Total trends; b) trends separately for deep (yellow) and quaternary (blue) aquifers; Bottom row: prokaryotes. c) Total trends; d) trends separately for deep (yellow) and quaternary (blue) aquifers. Plots for fauna biomass are in the SI 9, Figure S40. The equations for the trends are given above the respective plot.

Fauna and prokaryotes reacted in a similar way to increasing silica concentrations (Figure 3). In contrast to the expectation, fauna abundances (Figure 3a, b) and biomass (Figure S40), were negatively correlated to silica concentrations, except for deep aquifers. Prokaryote numbers were negatively correlated to silica concentrations when considering all wells (Figure 3c), but not when considering quaternary and deep wells separately (Figure 3d).



417

418

419 *Figure 4: Dependence of fauna individuals (a, b), and prokaryotes (c, d) on the slope in silica concentration change over time.*

420 *Regressions are drawn separately for quaternary and deep aquifers in b) and d). Plots for fauna biomass are in the SI 9,*

421 *Figure S46.*

422

423 Like the current measured values for temperature, and CO_2 and HCO_3^- concentrations, also

424 the trends of temperature (Figure S41), redox potential (Figure S42), DO (except for fauna in

425 quaternary wells; Figure S43), CO_2 (except for fauna abundances and prokaryotes; Figure

426 S44), and HCO_3^- (except for fauna individuals, biomass, and prokaryotes in quaternary wells;

427 Figure S45) concentrations were not related to organisms' biomass and numbers. Fauna

428 individuals slightly decreased with increasing slopes in silica change over time, i.e. between

429 1985 and 2021, except for deep wells (Figure 4a, b; Figure S46 for fauna biomass).

430 Prokaryote numbers, in contrast, decreased in deep, and increased in quaternarywells (Figure

431 4d).

Nonmetric Multidimensional Scaling (NMDS) of the faunal assemblages averaged over the wells showed that there was no pattern among sampled wells. A pattern would have been if e.g. some points, standing for the average faunal assemblages, would be closer to each other, and if close points would be representative of the same hydrogeological basin, i.e. of the same colour in Figure 5a. In contrast, here, the points were evenly distributed within the two dimensions that the NMDS reduces the multivariate space to. Points close to each other were not of the same colour, i.e. not of the same hydrogeological basin. Thus, fauna did not favour certain hydrogeological basins over others, nor were fauna assemblages fundamentally different in some hydrogeological basins – the colours for the hydrogeological basins are well distributed within Figure 5a, and wells where no fauna was found were situated in diverse hydrogeological basins. These wells where no fauna was found were characterized by high silica ("Si") and dissolved iron ("Fe_d") concentrations, and were negatively correlated with Redox potential ("Eh"), nitrate ("NO3_N"), and the variability in nitrate ("sd_NO3"; Figure 5b, SI 10, Table S8). Independently of each other, i.e. vertically to each other, dissolved oxygen concentrations ("DO") and the variability in temperature ("sd_T") shaped faunal assemblages (Figure 5b, Table S8).

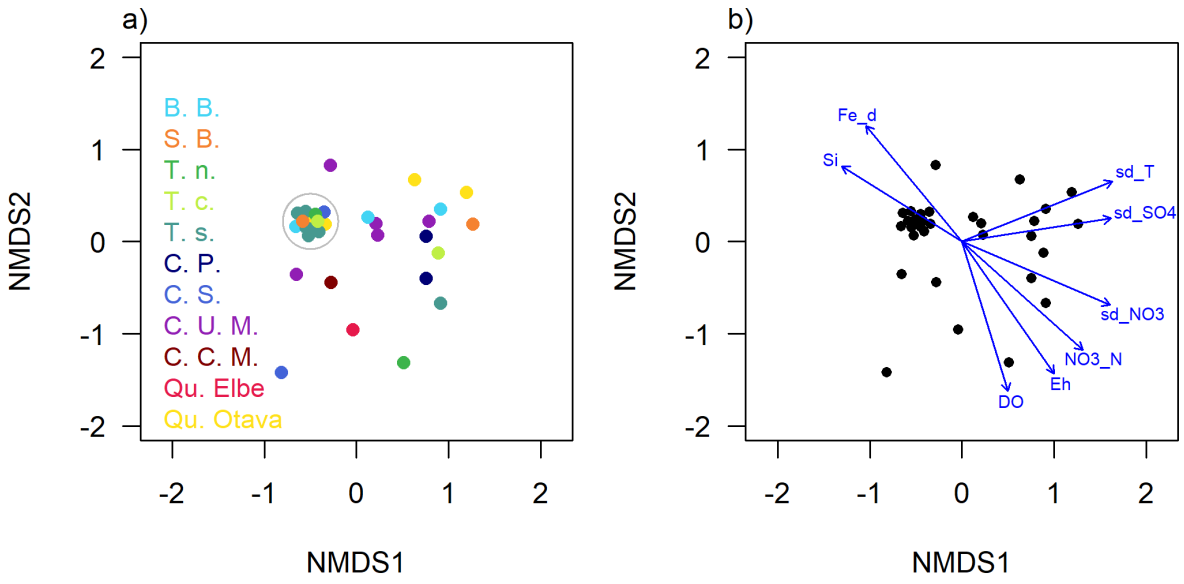


Figure 5: Nonmetric Multidimensional Scaling (NMDS) of the zero-adjusted faunal assemblage. Since well assemblages with only this artificial abundance fell into one coordinate, they were drawn apart for visualization of hydrogeological basins and were encircled with a grey circle. a) Sites coloured according to hydrogeological basin. "B. B." = Budweis basin, "S. B." = Sokolov basin, "T. n." = Třebon basin north, "T. c." = Třebon basin centre, "T. s." = Třebon basin south, "C. P." Crystalline Proterozoic, "C. S." = Crystalline Smrčín and southern parts of Ore Mountains, "C. U. M." = Crystalline Upper Vltava, "C. C. M." = Crystalline Central Vltava, "Qu. Elbe" = Quaternary of the Labe to the Vltava, "Qu. Otava" = Quaternary River Otava. b) The same plot as in a), but overlain by significant correlations with chemical and physical properties, identified by the "envfit" procedure of environmental fitting. "sd_" = standard deviation of the variable, "T" = temperature; "NO2_N" = nitrite nitrogen, "SO4" = sulphate, "NO3" or "NO3_N" = nitrate nitrogen, "COD" = chemical oxygen demand, "proks" = prokaryotes, "Si" = silica, "NH4_N" = ammonia nitrogen, "Fe_d" = dissolved iron; "_slope" = slope over time as yearly slope, "DO" = dissolved oxygen. Labels in b) slightly pulled apart to increase readability.

4. Discussion

Temperature and CO_2 levels may be seen as the main indicators of a changing climate in aqueous systems (Kasting, 2019; Penman et al., 2020; Xiong et al., 2022; Zhang et al., 2021). In the investigated aquifers, neither did they show clear trends, nor did they correlate with organism abundance or biomass. The clearest trend, in contrast, was exhibited by silica, i.e. the result of weathering which was likely induced by increased temperature and CO_2, either in

groundwater or, more likely, in soil and then recharged into groundwater. We cannot clarify the effect of surface vegetation period with just two samples per year, but the exact source of the silica is not the issue here. What we instead want to draw the attention to, is that this increased silica impacted on fauna, correlations that were clearer than all other correlations, except maybe redox potential. These are likely indirect effects, as will be clarified throughout this discussion.

4.1 General physical and chemical conditions in the study area

The temperatures, pH, redox potential, and concentrations of DO, CO_2, HCO_3^- and silica were within the ranges typical for groundwater, with some values of HCO_3^- being at the upper end of usual values (Fitts, 2012). As expected, the redox potential was lower in deeper, low-exchange aquifers. One oversimplified explanation might be that these strata may have undergone various cycles of biogeochemical reactions, reducing oxidized compounds. This underlines the importance of studying deep and quaternary aquifers separately. However, more complete explanations will have to consider lithologies, stratification, recharge situation at each well individually to clarify for each well how exactly the redox potential developed. This was beyond the scope of this contribution which focuses on regional patterns of a lack in increasing temperature, but an increase in silica. The high HCO_3^- values could be due to increased CO_2 recharge, as observed by Macpherson et al. (2008). The estimated CO_2 itself was up to three orders of magnitudes higher in this study than the values given in (Macpherson et al., 2008). The pH which was lower in the Czech aquifers than in the limestone aquifer studied by Macpherson et al. (2008) would have moved the carbonate equilibrium towards higher CO_2 at the expense of HCO_3^- in most aquifers studied here.

4.2 Trends in physical and chemical conditions

In contrast to the first hypothesis, and despite clear increases in air temperature over the past 40 years in the Czech Republic (Zahradníček et al., 2021) and a high correlation of air and soil temperature (Kopáček et al., 2024), groundwater temperature only increased in ca. 80% of aquifers over the previous ca. 30 years. This is also in contrast to Neidhardt & Shao (2023), Noethen et al. (2023), and Riedel (2019) who collated temperature increases in a range of groundwater aquifers. However, groundwater temperature is the result of conduction and advection, and groundwater temperature increase must be studied on a site-by-site basis – it is not possible to summarize temperature developments over several groundwater wells. Heat is conducted along soil and aquifer material along the temperature gradient according to the thermal conductivity of the local material. The transport of heat with water recharging from the surface into groundwater is called heat advection. When trending over time, one has to take into account the depth of sampling, seasonality (i.e. more than two samples per year in order to increase the chance to see the minima and maxima; Benz et al., 2017), and the properties of the geological material. The idea that groundwater temperature just follows surface temperature, is too simplistic – several processes are involved (Rau et al., 2015), and islands around industrial settlements have been observed (Menberg et al., 2013). In neighbouring Bavaria, Hemmerle & Bayer (2020) also found one out of 32 groundwater wells to have decreased in temperature between 1994 and 2019, although air temperature had increased considerably. Increased recharge may move the surface temperatures which are still cooler than the water temperatures in tens of meters of depth, to deeper areas. This would be further exacerbated by intense abstraction in deeper aquifer layers which may draw cooler groundwater into depths where warmer water prevailed, so that in a specific depth, the temperature trend is negative (Bense et al., 2020).

Likewise, CO_2 and HCO_3^- concentrations did not increase in all aquifers (Supporting Information Table S2 and SI 4, Figures S8 and S9), in contrast to the hypothesis – some

aquifers showed decreasing trends. Groundwater is often supersaturated in CO_2, particularly where it is under pressure. Groundwater CO_2 is also high where there was considerable microbial production (Chapelle et al., 1987). Import beyond saturation might not be physically possible (Bailey, 1991). However, rivers are also often supersaturated and still showed an increasing trend in the recent decades (e.g. Raymond & Cole, 2003), attributed to increased atmospheric CO_2, increased water flow, and changing land use in the Mississippi River. The reason for the lack in clear patterns in Western Czechia might lie in the fact that HCO_3^- is in a pH-dependent equilibrium with CO_2 and CO_3^{2-} and the HCO_3^- concentration may change in response to changes in CO_2 import into groundwater, pH-dependent transformation, carbonate precipitation, and uptake by organisms. The atmospheric acid deposition that was strongest in the eighties and still shows vestiges in some surface waters (Schmidt et al., 2022), may further impact on the pH-dependent carbonate equilibrium.

Kump et al. (2000) assumed that soil pCO_2 was determined by microbial activity, and not directly by the atmospheric concentrations. This may hold true for some aquifers where microbial productivity is high, as well. However, Kump et al. (2000) also concluded indirect effects of atmospheric pCO_2 on the soil, via the plant productivity which increases with pCO_2. This increased plant productivity may have effects on at least the quaternary groundwater. Also, since local heterogeneity is probably much higher than usually accounted for (Schmidt et al., 2017), it is not unreasonable to expect that locally, on the micro scale, a groundwater pore becomes undersaturated in CO_2 due to e.g. intense small-scale chemolithoautotrophy using CO_2 as a reaction partner. There will thus be a micro scale mosaic of over- and undersaturation, which the average reflects depending on local conditions. The overall supersaturation might increase on average in some aquifers where CO_2 levels did overall increase.

548 Decreasing CO_2 concentrations in other aquifers, while atmospheric CO_2 increased, probably
549 involved complex (biogeo)chemical reactions. These processes of import, transformation, or
550 export were not measured themselves during the monitoring presented here – this would have
551 required flux meters, isotopes, and related techniques, not only for the groundwater, but also
552 for the soil zones through which groundwater is recharged. Increase of CO_2, as mentioned
553 above, is known from soils (Bakalowicz, 1994; Nydahl et al., 2020), and thus the soil and
554 land use play a role for the hydrochemical and biological composition of soil water. Where
555 soil water recharges into groundwater, they play a role also for the groundwater.
556 Unfortunately, the exact mechanisms of CO_2 in soil and their recharge into groundwater are
557 not available for the sampled wells. Here, we were interested in the net effect of such changes
558 on groundwater communities. However, when and where the exact mechanisms of climate
559 change impacting on groundwater are to be clarified, soil processes need to be studied to the
560 same extent as groundwater processes, since weathering and pedogenesis in the surface soils
561 are influenced by climate change and may release increasing amounts of elements that are
562 then prone to be transported to the groundwater.
563
564 In contrast to CO_2 and HCO_3^- concentrations, silica concentrations rose in all but one aquifer,
565 and the rise was significant (Figure 1, Table S2). This is most likely a consequence of silicate
566 weathering which would have transformed CO_2 to HCO_3^- (Walker et al., 1981; Zhang et al.,
567 2021), another possible explanation for the lack in patterns in CO_2 and HCO_3^- concentrations
568 (for a more complete discussion, see Supplementary Information 1). It is thus possible that the
569 CO_2 import had temporarily increased in more aquifers than where it is now measurable, but
570 that the CO_2 was consumed in weathering, and we only look at the net CO_2 concentrations,
571 but can estimate the extent of increased weathering by the increase in silicate. Macpherson et
572 al. (2008) observed similar patterns of CO_2 partly being consumed by weathering in a
573 limestone Konza prairie aquifer. The net increase of silica observed in groundwater might

have been due to *in situ* weathering, or equally well might have originated in the soil and might subsequently been recharged into groundwater. The exact mechanism is the topic of parallel studies – here, we are interested in the effect that weathering, regardless of whether it took place in soil or *in situ* in groundwater, has on groundwater organisms, and thus on groundwater ecosystem services.

In agreement with the second hypothesis, quaternary and deep aquifers differed in the direction and steepness of trends, particularly trends of silica concentrations, with deep aquifers harbouring generally a steeper increase than quaternary aquifers (Table S2). The data series only spans up to almost 40 years, and many wells in this investigation had only been monitored for about a decade. It is possible that there had been an increase in silicate weathering in quaternary aquifers until one or two decades ago and – like the effects from acidification (Schmidt et al., 2022) – what we observed is a plateau which might still change with progressing climate change. One indicator for such a pattern is that the current silica concentrations are higher in shallower aquifers, which are quaternary, than in deep ones, despite the presumably shorter residence and younger age of the former. Usually, weathering rates depend inherently on the residence time (Bakalowicz, 1994; Maher, 2010) and silica concentrations are known to be higher in older aquifers (Marçais et al., 2018). Quaternary aquifers with a lower age but higher silica concentrations may reflect an interim stage that the deeper groundwater will pass beyond, given time. Again, whether the weathering took place in soil and was then recharged into groundwater, or whether the weathering took place in situ in groundwater, is secondary to the question what the new equilibrium for deeper aquifers will be (Mammola et al., 2024), and what this will mean for organisms and the ecosystem services they deliver (Griebler & Avramov, 2015).

While increases in CO_2 and HCO_3^- are known from rivers (Macpherson & Sullivan, 2019), increases in silica are rarely mentioned and net increases might not have occurred in surface waters (e.g. Macpherson & Sullivan, 2019). This is due to the fact that silicate is used by stream organisms, mainly diatoms (Lampert & Sommer, 2007), which may be washed into groundwater, but cannot survive long enough to build or maintain populations. Rivers where such groups as diatoms grow are thus not ideal for studying the effect of weathering on silica concentrations since the newly dissolved silica may be used up immediately. It makes more sense to study weathering effects directly in soil or groundwater.

4.3 Did organisms reflect the current physical and chemical conditions?

In contrast to the third hypothesis, neither aquifer temperature, nor current CO_2 and HCO_3^- concentrations, were significantly correlated with organism numbers, neither for fauna nor for prokaryotes (Figure 3; SI 8). Thus, temperature, CO_2 or HCO_3^- neither seemed to harm nor promote organisms. Regarding temperature, this apparently contrasts the findings by Brielmann et al. (2009) and Spengler & Hahn (2018), but in the cited references, wells were compared that were under varyingly intense anthropogenic influence, while the wells sampled in this study, were not directly influenced by human activity beyond global influences such as rising CO_2 and increasing land use – wells were neither in highly industrialized areas, nor in high-productivity agricultural areas or large cities. This might mean that the increases of air temperature have not begun to impact on groundwater ecosystems yet, or these effects were cancelled out by other factors and developments. However, the temperature ranges were different – temperatures in Brielmann et al. (2009) ranged from 8.5 to 17.8 °C, and the temperatures in Spengler & Hahn (2018) from 5.7 to 18.1 °C, and the investigations are thus not comparable with each other.

624 The positive relationship between redox potential and DO on the one hand, and fauna on the
625 other hand, were to be expected since most multicellular fauna require at least minute
626 concentrations of dissolved oxygen (Malard & Hervant, 1999). In addition, if the redox
627 potential, which was not strongly correlated to DO, indeed was an indicator for the
628 vulnerability of the aquifers or for the degree of exchange with the surface (Meckenstock et
629 al., 2015), then the positive relationship between redox potential and fauna indicates the
630 dependence of fauna on exchange, as postulated earlier (Schmidt & Hahn, 2012). Contrary to
631 expectation, prokaryote abundances were neither positively correlated with DO nor redox
632 potential. Microbial productivity is usually directly positively related to redox conditions,
633 since more oxidized compounds provide more energy in biogeochemical reactions and thus
634 may lead to more growth. The reason for the apparent lack of the expected positive
635 relationship between redox potential and microorganisms may be due to the measured
636 microorganism numbers not being representative for the actual microbial production. The
637 expected higher microbial biomass might have been grazed by fauna to a higher degree.
638 Fauna itself then increased in numbers and biomass along the redox gradient. Fauna may have
639 thereby reduced prokaryote stock to a minimum below which prokaryote production would
640 not support fauna production. We propose that fauna relied on prokaryote growth more in
641 deep wells than in quaternary wells where exchange with the surface might deliver sufficient
642 palatable food sources (compare Datry et al., 2004; but see the discussion in Karwautz et al.,
643 2022, on alpine, low organic input aquifers). This may indicate that deeper wells are generally
644 more high-quality carbon and energy-limited than the quaternary wells where exchange with
645 the surface is bound to be more intense. We suggest that chemolithoautotrophic carbon
646 fixation, which is likely to have been considerable in the deep, low-exchange wells, is indeed
647 an important carbon and energy source for food chains as coined by Overholt et al. (2022),
648 but all the more so, the lower the exchange with the surface, as shown in Herrmann et al.
649 (2017, 2020). The observed prokaryote numbers may thus represent a base production below

650 which ecosystem productivity does not fall, unless disturbed e.g. by agriculture (Marxsen et
651 al., 2021). In general, it needs to be noted that prokaryote numbers cannot be equated with
652 prokaryote production. Changing environmental conditions change the relative abundances of
653 e.g. aerobics to anaerobics, or e.g. heterotrophs to autotrophs. Even taxa fulfilling the same
654 physiological reaction do so in different intensities (Schmidt et al., 2018). Molecular and
655 physiological studies are needed to clarify the relationships between fauna and prokaryotes
656 further. Here, we use the prokaryote abundances merely as an indicator for food source for
657 fauna.
658
659 The lack in correlation between organisms and CO_2 is surprising, given that the values ranged
660 from 0 to 4 864 077 µatm, which is almost 60 times as high as the concentration tested by
661 Ramaekers et al. (2022) who found lethal effects for daphnids, ostracods, and rotifers at
662 83 000 µatm CO_2. Already at 18 000 µatm, Weiss et al. (2018) found that the increased CO_2
663 impaired the ability to sense predators in surface zooplanktonic *Daphnia* and their ability to
664 form the inducible defences in the form of helmets. Increased CO_2 thus rendered the daphnids
665 more prone to predation. The authors predicted impacts on the whole food chain. Crustacea
666 dominate groundwater fauna biomass and it is thus not unreasonable to expect similar
667 impairments to groundwater fauna where CO_2 concentrations increase more clearly. However,
668 groundwater taxa are characterized by different traits than surface water organisms (Hose et
669 al., 2022), and it is thus possible that groundwater organisms are better adapted to higher CO_2
670 concentrations than surface water organisms. The lack in correlations between temperature
671 and inorganic carbon concentrations (CO_2 and HCO_3^-) on the one hand and organisms on the
672 other hand might point to the single effects on organisms being cancelled out by each other.
673 Tracer and physiological experiments might shed light into the interactions between
674 temperature and inorganic carbon on organisms (Hose et al., 2022).
675

676 The negative effect of silica on organisms was unexpected and unlikely to be a direct effect,
677 since silica is not known to be harmful to organisms – on the contrary, it is a micronutrient for
678 e.g. diatoms, foraminifera, and radiolaria (Lampert & Sommer, 2007) – which, however, are
679 only washed accidentally into groundwater, but do not grow to populations. One explanation
680 for the negative correlation between fauna abundances and silica may be that silica
681 concentrations are higher, the older the aquifer is (Marçais et al., 2018). We saw fewer fauna
682 in higher silica concentrations, which usually stand for older aquifers, thus we seemed to see
683 fewer fauna in older aquifers. Fauna may thus be negatively correlated with the age of the
684 aquifer. This is underlined by some of the few evidences published so far: In the 3,000–
685 12,000-year-old, ca. 1 km deep palaeometeoric water of South African gold mine, only one
686 species of a very specialized nematode was found (Borgonie et al., 2011), while assemblages
687 near the surface which are only up to a few years old, consist of many species in comparably
688 high abundances and biomasses (e.g. Schmidt et al., 2007). The reason for this may lie in the
689 quality of carbon, in terms of degradability, decreasing over time, which is not reflected in the
690 total concentrations of DOC or TOC (Hofmann et al., 2020). However, the patterns of silica
691 having risen to higher levels in shallow aquifers and not rising as much anymore in shallow
692 aquifers than in deep aquifers, point to another possible explanation, i.e. weathering. The
693 increasing silica concentrations indicate the increased weathering. Whether the weathering
694 took place in soil, and the weathering products were then imported into groundwater, or
695 whether the weathering happened *in situ* in groundwater, is of secondary importance here and
696 needs to be elucidated in other investigations with a different research question. Weathering
697 itself is not harmful to fauna – some taxa are actively involved in silica weathering without
698 any obvious sign of damage (Vicca et al., 2022). However, through weathering, other
699 chemical compounds might have become bioavailable (Rue & McKnight, 2021) that might
700 have been harmful to organisms (Jones & Bennett, 2014), such as aluminum which is a

common companion in silica minerals (Amann et al., 2020). Aluminum is toxic to many organisms in the microgram per Litre range (Botté et al., 2022).

4.4 Do organisms reflect the trends in physical and chemical conditions?

Largely, the trends of temperature, CO_2 and HCO_3^- over time were not reflected in nowadays organism numbers in contrast to hypothesis 4, nor in the distribution of species among the aquifers, in contrast to hypothesis 5. However, we do not know when exactly the changes in groundwater began, i.e. how much change had already taken place before the monitoring started, nor what the populations were before the changes began. Also, we do not know how much more the aquifers will change and what that will mean to organisms, i.e. whether the ecosystems are at risk. It was hypothesized that fauna would be negatively correlated to an increase in silica concentrations, i.e. anthropogenically accelerated silica outflow rate, because that will reflect change which the groundwater organisms might not be able to withstand. However, this correlation was not significant, in contrast to the correlation with the current concentrations (see previous paragraphs).

One reason for CO_2 neither showing temporal trends, nor being clearly related to organisms, may be that the additional CO_2 import into groundwater is divided into pathways – parts of it may have been involved in weathering of silicate rock, while another part may have (and maybe increasingly so over the past decades) taken part in chemolithoautotrophic processes.

4.5 The geological thermostat

The uptake of CO_2 during bio-erosion of geological structures, which leads to increased aqueous concentrations of silica, reduces the greenhouse gas CO_2 in the atmosphere and thus reduces global air temperatures. This phenomenon has been called the geological thermostat

(Li et al., 2016). On the long-term time scales, but also on the shorter-term, this extends habitability of the planet Earth (Brantley et al., 2023). Schwartzman & Volk (1989) estimated that without biogenic weathering, the earth would be tens of degrees warmer, than it currently is. However, if one of the side effects of this thermostat is the loss of organisms and loss of the ecosystem functions they perform, this may negatively impact on habitability of the planet. We do rely on self-cleaning within aquifers and rivers for drinking water production (Griebler & Avramov, 2015). Prague, for example, receives most of the water studied in this contribution, is among the cities that are groundwater-dependent for their drinking water; in the case of Prague, drinking water production relies to 25% on groundwater (Vejvodová et al., 2006).

5. Conclusion

Climate change in terms of increased temperatures was not correlated with groundwater organism occurrence and fauna communities. However, the impacts from climate change are still in progress and patterns might change when surface temperatures have increasingly reached subsurface realms. More important than the current temperature, seems to have been the effect of increased CO_2 the net concentrations of which did not increase measurably in the studied wells, but which seems to have been responsible for increased silicate weathering in either soil through which aquifers were recharged, or in the aquifers themselves. The fauna individuals and biomass in total, and separately in quaternary wells, fauna biomass in total and in quaternary wells, and prokaryotes both in deep and quaternary wells, correlated negatively with current silica concentrations. Recent increases in silica concentrations correlated positively with quaternary prokaryotes, but negatively with fauna individuals in total and separately in quaternary aquifers, fauna biomass in total, and prokaryote numbers in

753 deep aquifer. It might not have been CO_2 produced in, or transported into, groundwater that
754 led to the increased weathering, but CO_2 produced in soil which was recharged into
755 groundwater. Maybe even the weathering itself did not happen in the groundwater, but in the
756 soil. The net effect though, i.e. increased silica concentrations in groundwater, seem to be
757 detrimental to groundwater fauna, and this is what attention needs to focus on. That the
758 process is on-going, can be deduced from the observation that the silica concentrations were
759 partly higher in quaternary aquifers with presumably a younger age, while one might have
760 expected that silica is highest in oldest, i.e. deepest aquifers. This seems to indicate that the
761 aquifers are still in development towards a new equilibrium. It remains to be seen what the
762 new equilibrium for deeper aquifers will be, and what this will mean for organisms and
763 ecosystem services that we rely on for e.g. drinking water production. It is also likely that
764 while temperature and CO_2 on their own did not correlate with organism abundances, their
765 interaction might have done. Lastly, this study focused on standing stocks. Transfer rates of
766 the abiotic and biotic components are necessary to further clarify processes.
767
768
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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors did not use any AI or AI-assisted technology in the writing process apart from spell and grammar checker. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

Amann, T., Hartmann, J., Struyf, E., de Oliveira Garcia, W., Fischer, E. K., Janssens, I., Meire, P., & Schoelynck, J. (2020). Enhanced Weathering and related element fluxes – a cropland mesocosm approach. *Biogeosciences*, *17*(1), 103–119. https://doi.org/10.5194/bg-17-103-2020

Aquilina, L., Stumpp, C., Tonina, D., & Buffington, J. M. (2023). Chapter 1—Hydrodynamics and geomorphology of groundwater environments. In F. Malard, C. Griebler, & S. Rétaux (Eds.), *Groundwater Ecology and Evolution (Second Edition)* (pp. 3–37). Academic Press. https://doi.org/10.1016/B978-0-12-819119-4.00014-7

Bailey, R. A. (1991). *Chemistry of the Environment*. Elsevier.

Bakalowicz, M. (1994). 4 Water geochemistry: Water quality and dynamics. In J. Gibert, D. L. Danielopol, & J. A. Stanford (Eds.), *Groundwater Ecology* (pp. 97–127). https://doi.org/10.1016/B978-0-08-050762-0.50011-5

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

805 Bense, V. F., Kurylyk, B. L., de Bruin, J. G. H., & Visser, P. (2020). Repeated subsurface
806 thermal profiling to reveal temporal variability in deep groundwater flow conditions.
807 *Water Resources Research*, *56*(6), e2019WR026913.
808 https://doi.org/10.1029/2019WR026913
809 Benz, S. A., Bayer, P., & Blum, P. (2017). Global patterns of shallow groundwater
810 temperatures. *Environ. Res. Lett.*, *12*, 034005.
811 Bermúdez, R., Feng, Y., Roleda, M. Y., Tatters, A. O., Hutchins, D. A., Larsen, T., Boyd, P.
812 W., Hurd, C. L., Riebesell, U., & Winder, M. (2015). Long-term conditioning to
813 elevated pCO2 and warming influences the fatty and amino acid composition of the
814 diatom Cylindrotheca fusiformis. *PLOS One*, *10*(5), e0123945.
815 https://doi.org/10.1371/journal.pone.0123945
816 Boerner, A. R., & Gates, J. B. (2015). Temporal dynamics of groundwater-dissolved
817 inorganic carbon beneath a drought-affected braided stream: Platte River case study.
818 *Journal of Geophysical Research: Biogeosciences*, *120*(5), 924–937.
819 https://doi.org/10.1002/2015JG002950
820 Borgonie, G., García-Moyano, A., Litthauer, D., Bert, W., Bester, A., van Heerden, E.,
821 Möller, C., Erasmus, M., & Onstott, T. C. (2011). Nematoda from the terrestrial deep
822 subsurface of South Africa. *Nature*, *474*(7349), 79–82.
823 https://doi.org/10.1038/nature09974
824 Botté, A., Zaidi, M., Guery, J., Fichet, D., & Leignel, V. (2022). Aluminium in aquatic
825 environments: Abundance and ecotoxicological impacts. *Aquatic Ecology*, *56*(3), 751–
826 773. https://doi.org/10.1007/s10452-021-09936-4
827 Bou, C. (1974). Recherche sur les eaux souterraines. Les méthodes de récolte dans les eaux
828 souterraines interstitielles. *Ann. Spéolo.*, *29*(4), 611–619.
829 Brantley, S. L., Shaughnessy, A., Lebedeva, M. I., & Balashov, V. N. (2023). How
830 temperature-dependent silicate weathering acts as Earth's geological thermostat.

Science, *379*(6630), 382–389. https://doi.org/10.1126/science.add2922

Brett, M. T., Bunn, S. E., Chandra, S., Galloway, A. W. E., Guo, F., Kainz, M. J., Kankaala, P., Lau, D. C. P., Moulton, T. P., Power, M. E., Rasmussen, J. B., Taipale, S. J., Thorp, J. H., & Wehr, J. D. (2017). How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? *Freshwater Biology*, 1–21. https://doi.org/10.1111/fwb.12909

Brielmann, H., Griebler, C., Schmidt, S. I., Michel, R., & Lueders, T. (2009). Effects of thermal energy discharge on shallow groundwater ecosystems. *FEMS Microbiology Ecology*, *68*(3), 273–286. https://doi.org/10.1111/j.1574-6941.2009.00674.x

Calmels, D., Galy, A., Hovius, N., Bickle, M., West, A. J., Chen, M.-C., & Chapman, H. (2011). Contribution of deep groundwater to the weathering budget in a rapidly eroding mountain belt, Taiwan. *Earth and Planetary Science Letters*, *303*(1), 48–58. https://doi.org/10.1016/j.epsl.2010.12.032

Chapelle, F. H., Zelibor, J. L., Grimes, D. J., & Knobel, L. L. (1987). Bacteria in deep coastal plain sediments of Maryland: A possible source of CO2 to groundwater. *Water Resources Research*, *23*(8), 1625–1632.

Clarke, K. R., Somerfield, P. J., & Chapman, M. G. (2006). On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient of denuded assemblages. *Journal of Experimental Marine Biology and Ecology*, *330*, 55–80.

Classen, A. T., Sundqvist, M. K., Henning, J. A., Newman, G. S., Moore, J. A. M., Cregger, M. A., Moorhead, L. C., & Patterson, C. M. (2015). Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere*, *6*(8), art130. https://doi.org/10.1890/ES15-00217.1

Cole, J. (2013). Freshwater in flux. *Nature Geoscience*, *6*(1), Article 1. https://doi.org/10.1038/ngeo1696

857 Danovaro, R., Dell'Anno, A., Pusceddu, A., Gambi, C., Heiner, I., & Kristensen, R. M.
858 (2010). The first metazoa living in permanently anoxic conditions. *BMC Biology*, *8*(1),
859 30. https://doi.org/10.1186/1741-7007-8-30
860 Datry, T., Malard, F., & Gibert, J. (2004). Dynamics of solutes and dissolved oxygen in
861 shallow urban groundwater below a stormwater infiltration basin. *The Science of the*
862 *Total Environment*, *329*(1–3), 215–229. https://doi.org/10.1016/j.scitotenv.2004.02.022
863 Di Lorenzo, T., Cannicci, S., Spigoli, D., Cifoni, M. (2016). Bioenergetic cost of living in
864 polluted freshwater bodies: respiration rates of the cyclopoid *Eucyclops serrulatus*
865 under ammonia-N exposures. *Fundam. Appl. Limnol*., *18*, 147–156.
866 http://dx.doi.org/10. 1127/fal/2016/0864.
867 Fenchel, T., & Finlay, B. (2008). Oxygen and the spatial structure of microbial
868 communities. *Biological Reviews of the Cambridge Philosophical Society*, *83*(4), 553–
869 569. https://doi.org/10.1111/j.1469-185X.2008.00054.x
870 Ferdush, J., & Paul, V. (2021). A review on the possible factors influencing soil inorganic
871 carbon under elevated CO2. *CATENA*, *204*, 105434.
872 https://doi.org/10.1016/j.catena.2021.105434
873 Fitts, C. R. (2012). *Groundwater Science* (Second Edi). Elsevier.
874 Friedlingstein, P., O'Sullivan, M., Jones, M. W., Andrew, R. M., Gregor, L., Hauck, J., Le
875 Quéré, C., Luijkx, I. T., Olsen, A., Peters, G. P., Peters, W., Pongratz, J.,
876 Schwingshackl, C., Sitch, S., Canadell, J. G., Ciais, P., Jackson, R. B., Alin, S. R.,
877 Alkama, R., … Zheng, B. (2022). Global Carbon Budget 2022. *Earth System Science*
878 *Data*, *14*(11), 4811–4900. https://doi.org/10.5194/essd-14-4811-2022
879 Gaillardet, J., Dupré, B., Louvat, P., & Allègre, C. J. (1999). Global silicate weathering and
880 CO2 consumption rates deduced from the chemistry of large rivers. *Chemical Geology*,
881 *159*(1), 3–30. https://doi.org/10.1016/S0009-2541(99)00031-5
882 Gasol, J. M., & del Giorgio, P. A. (2000). Using flow cytometry for counting natural

883 planktonic bacteria and understanding the structure of planktonic bacterial
884 communities. *Scientia Marina*, *64*(2), Article 2.
885 https://doi.org/10.3989/scimar.2000.64n2197
886 Griebler, C., & Avramov, M. (2015). Groundwater ecosystem services: A review.
887 *Freshwater Science*, *34*(1), 355–367. https://doi.org/10.1086/679903
888 Hasler, C. T., Hannan, K. D., Jeffrey, J. D., & Suski, C. D. (2017). Valve movement of three
889 species of North American freshwater mussels exposed to elevated carbon dioxide.
890 *Environmental Science and Pollution Research*, *24*(18), 15567–15575.
891 https://doi.org/10.1007/s11356-017-9160-9
892 Hemmerle, H., & Bayer, P. (2020). Climate change yields groundwater warming in Bavaria,
893 Germany. *Frontiers in Earth Science*, *8*. https://doi.org/10.3389/feart.2020.575894
894 Herrmann, M., Geesink, P., Yan, L., Lehmann, R., Totsche, K. U., & Küsel, K. (2020).
895 Complex food webs coincide with high genetic potential for chemolithoautotrophy in
896 fractured bedrock groundwater. *Water Research*, *170*, 115306.
897 https://doi.org/10.1016/j.watres.2019.115306
898 Herrmann, M., Opitz, S., Harzer, R., Totsche, K. U., & Küsel, K. (2017). Attached and
899 suspended denitrifier communities in pristine limestone aquifers harbor high fractions
900 of potential autotrophs oxidizing reduced iron and sulfur compounds. *Microbial*
901 *Ecology*, *74*(2), 264–277. https://doi.org/10.1007/s00248-017-0950-x
902 Herrmann, M., Rusznyák, A., Akob, D. M., Schulze, I., Opitz, S., Totsche, K. U., & Küsel,
903 K. (2015). Large fractions of CO_2-fixing microorganisms in pristine limestone aquifers
904 appear to be involved in the oxidation of reduced sulfur and nitrogen compounds.
905 *Applied and Environmental Microbiology*, *81*(7), 2384–2394.
906 https://doi.org/10.1128/AEM.03269-14
907 Hervant, F., Mathieu, J., Barre, H., Simon, K., Pinon, C. (1997). Comparative study on the
908 behavioural, ventilatory, and respiratory responses of hypogean and epigean

crustaceans to long-term starvation and subsequent feeding. *Comp. Biochem. Physiol., 118A*, 1277–1283. http://dx.doi.org/10.1016/S0300-9629(97)00047-9.

Hofmann, R., Uhl, J., Hertkorn, N., & Griebler, C. (2020). Linkage between dissolved organic matter transformation, bacterial carbon production, and diversity in a shallow oligotrophic aquifer: Results from flow-through sediment microcosm experiments. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.543567

Hose, G. C., Chariton, A., Daam, M. A., Di Lorenzo, T., Galassi, D. M. P., Halse, S. A., Reboleira, A. S. P. S., Robertson, A. L., Schmidt, S. I., & Korbel, K. L. (2022). Invertebrate traits, diversity and the vulnerability of groundwater ecosystems. *Functional Ecology*, *39*(9), 2200–2214. https://doi.org/10.1111/1365-2435.14125

IPCC. (2022). Climate Change 2022: Impacts, Adaptation and Vulnerability. *Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (Eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA*, 1–3056. https://doi.org/10.1017/9781009325844

Jones, A. A., & Bennett, P. C. (2014). Mineral microniches control the diversity of subsurface microbial population. *Geomicrobiology Journal*, *31*(3), 246–261. https://doi.org/10.1080/01490451.2013.809174

Karwautz, C., Zhou, Y., Kerros, M.-E., Weinbauer, M. G., & Griebler, C. (2022). Bottom-up control of the groundwater microbial food-web in an alpine aquifer. *Frontiers in Ecology and Evolution*, *10*. https://www.frontiersin.org/article/10.3389/fevo.2022.854228

Kassambara, A., 2023. ggpubr: "ggplot2" Based Publication Ready Plots. R package version 0.6.0. https://CRAN.R-project.org/package=ggpubr; accessed 3 October 2024.

Kasting, J. F. (2019). The Goldilocks Planet? How silicate weathering maintains earth "just

right.” *Elements*, *15*(4), 235–240. https://doi.org/10.2138/gselements.15.4.235

Kellermann, C., Selesi, D., Lee, N., Hügler, M., Esperschütz, J., Hartmann, A., & Griebler, C. (2012). Microbial CO_2 fixation potential in a tar-oil-contaminated porous aquifer. *FEMS Microbiology Ecology*, *81*(1), 172–187. https://doi.org/10.1111/j.1574-6941.2012.01359.x

Komsta, L. (2019). mblm: Median-Based Linear Models. R package version 0.12.1. https://CRAN.R-project.org/package=mblm. Accessed 1 October 2024.

Kopáček, J., Bače, R., Grill, S., Hejzlar, J., Kaňa, J., Porcal, P., & Turek, J. (2024). Dynamics and variability of microclimate in an unmanaged mountain forest after a bark beetle outbreak. *Agricultural and Forest Meteorology*, *344*, 109824. https://doi.org/10.1016/j.agrformet.2023.109824

Kota, S., Borden, R.C., Barlaz, M.A. (1999). Influence of protozoan grazing on contaminant biodegradation. *FEMS Microbiol. Ecol*., *29*, 179–189. http://dx.doi.org/10.1016/S0168-6496(99)00010-0.

Kump, L. R., Brantley, S. L., & Arthur, M. A. (2000). Chemical Weathering, Atmospheric CO2, and Climate. *Annual Review of Earth and Planetary Sciences*, *28*(1), 611–667. https://doi.org/10.1146/annurev.earth.28.1.611

Kwon, E. Y., DeVries, T., Galbraith, E. D., Hwang, J., Kim, G., & Timmermann, A. (2021). Stable carbon isotopes suggest large terrestrial carbon inputs to the global ocean. *Global Biogeochemical Cycles*, *35*(4), e2020GB006684. https://doi.org/10.1029/2020GB006684

Lampert, W., & Sommer, U. (2007). *Limnoecology*. Oxford University Press.

Langer, J. A. F., Sharma, R., Schmidt, S. I., Bahrdt, S., Nam, B., Achterberg, E. P., Riebesell, U., Horn, H. G., Boersma, M., Thines, M., & Schwenk, K. (2017). Community barcoding reveals little effect of ocean acidification on the composition of coastal plankton communities: Evidence from a long-term mesocosm study in the

961 Gullmar Fjord, Skagerrak. *PLoS ONE*, *12*(4), 1–20.
962 https://doi.org/10.1594/PANGAEA.864598
963 Lehmann, N., Stacke, T., Lehmann, S., Lantuit, H., Gosse, J., Mears, C., Hartmann, J., &
964 Thomas, H. (2023). Alkalinity responses to climate warming destabilise the Earth's
965 thermostat. *Nature Communications*, *14*(1), Article 1. https://doi.org/10.1038/s41467-
966 023-37165-w
967 Li, G., Hartmann, J., Derry, L. A., West, A. J., You, C.-F., Long, X., Zhan, T., Li, L., Li, G.,
968 Qiu, W., Li, T., Liu, L., Chen, Y., Ji, J., Zhao, L., & Chen, J. (2016). Temperature
969 dependence of basalt weathering. *Earth and Planetary Science Letters*, *443*, 59–69.
970 https://doi.org/10.1016/j.epsl.2016.03.015
971 Liu, H., Chen, M., Zhu, F., & Harrison, P. J. (2016). Effect of diatom silica content on
972 copepod grazing, growth and reproduction. *Frontiers in Marine Science*, *3*.
973 https://www.frontiersin.org/articles/10.3389/fmars.2016.00089
974 Macpherson, G. L., Roberts, J. A., Blair, J. M., Townsend, M. A., Fowle, D. A., & Beisner,
975 K. R. (2008). Increasing shallow groundwater CO_2 and limestone weathering, Konza
976 Prairie, USA. *Geochimica et Cosmochimica Acta*, *72*(23), 5581–5599.
977 https://doi.org/10.1016/j.gca.2008.09.004
978 Macpherson, G. L., & Sullivan, P. L. (2019). Watershed-scale chemical weathering in a
979 merokarst terrain, northeastern Kansas, USA. *Chemical Geology*, *527*, 118988.
980 https://doi.org/10.1016/j.chemgeo.2018.12.001
981 Maher, K. (2010). The dependence of chemical weathering rates on fluid residence time.
982 *Earth and Planetary Science Letters*, *294*(1–2), 101–110.
983 https://doi.org/10.1016/j.epsl.2010.03.010
984 Malard, F., & Hervant, F. (1999). Oxygen supply and the adaptations of animals in
985 groundwater. *Freshwater Biology*, *41*(1), 1–30. https://doi.org/10.1046/j.1365-
986 2427.1999.00379.x

Mammola, S., Altermatt, F., Alther, R., Amorim, I. R., Băncilă, R. I., Borges, P. A. V., Brad, T., Brankovits, D., Cardoso, P., Cerasoli, F., Chauveau, C. A., Delić, T., Di Lorenzo, T., Faille, A., Fišer, C., Flot, J.-F., Gabriel, R., Galassi, D. M. P., Garzoli, L., … Malard, F. (2024). Perspectives and pitfalls in preserving subterranean biodiversity through protected areas. *Npj Biodiversity*, *3*(1), 2. https://doi.org/10.1038/s44185-023-00035-1

Mammola, S., Cardoso, P., Culver, D. C., Deharveng, L., Ferreira, R. L., Fišer, C., Galassi, D. M. P., Griebler, C., Halse, S., Humphreys, W. F., Isaia, M., Malard, F., Martinez, A., Moldovan, O. T., Niemiller, M. L., Pavlek, M., Reboleira, A. S. P. S., Souza-Silva, M., Teeling, E. C., … Zagmajster, M. (2019). Scientists' Warning on the Conservation of Subterranean Ecosystems. *BioScience*, *69*(8), 641–650. https://doi.org/10.1093/biosci/biz064

Marçais, J., Gauvain, A., Labasque, T., Abbott, B. W., Pinay, G., Aquilina, L., Chabaux, F., Viville, D., & de Dreuzy, J.-R. (2018). Dating groundwater with dissolved silica and CFC concentrations in crystalline aquifers. *Science of The Total Environment*, *636*, 260–272. https://doi.org/10.1016/j.scitotenv.2018.04.196

Marxsen, J., Rütz, N. K., & Schmidt, S. I. (2021). Organic carbon and nutrients drive prokaryote and metazoan communities in a floodplain aquifer. *Basic and Applied Ecology*, *51*, 43–58. https://doi.org/10.1016/j.baae.2020.12.006

Mattison, R.G., Taki, H., Harayama, S. (2005). The soil flagellate Heteromita globosa acceler- ates bacterial degradation of alkylbenzenes through grazing and acetate excretion in batch culture. *Microb. Ecol*. *49*, 142–150. http://dx.doi.org/10.1007/s00248-003-0226-5.

Matzke, D., & Hahn, H. J. (2002). *Vergleich der Grundwasserfauna in Lockergesteins- und in Kluftgrundwasserleitern unter vergleichender Anwendung unterschiedlicher Sammeltechniken*. Final Report Project Az HA 3214 / 1-1.

1013 Meckenstock, R. U., Elsner, M., Griebler, C., Lueders, T., Stumpp, C., Aamand, J.,
1014 Agathos, S. N., Albrechtsen, H.-J., Bastiaens, L., Bjerg, P. L., Boon, N., Dejonghe, W.,
1015 Huang, W. E., Schmidt, S. I., Smolders, E., Sørensen, S. R., Springael, D., & van
1016 Breukelen, B. M. (2015). Biodegradation: Updating the concepts of control for
1017 microbial cleanup in contaminated aquifers. *Environmental Science & Technology*,
1018 *49*(12), 7073–7081. https://doi.org/10.1021/acs.est.5b00715
1019 Menberg, K., Bayer, P., Zosseder, K., Rumohr, S., & Blum, P. (2013). Subsurface urban
1020 heat islands in German cities. *The Science of the Total Environment*, *442*, 123–133.
1021 https://doi.org/10.1016/j.scitotenv.2012.10.043
1022 Meybeck, M. (1993). Riverine transport of atmospheric carbon: Sources, global typology
1023 and budget. *Water, Air, and Soil Pollution*, *70*, 443–463.
1024 Moore, C. M., Mills, M. M., Arrigo, K. R., Berman-Frank, I., Bopp, L., Boyd, P. W.,
1025 Galbraith, E. D., Geider, R. J., Guieu, C., Jaccard, S. L., Jickells, T. D., La Roche, J.,
1026 Lenton, T. M., Mahowald, N. M., Marañón, E., Marinov, I., Moore, J. K., Nakatsuka,
1027 T., Oschlies, A., … Ulloa, O. (2013). Processes and patterns of oceanic nutrient
1028 limitation. *Nature Geoscience*, *6*(9), Article 9. https://doi.org/10.1038/ngeo1765
1029 Négrel, P., Allègre, C. J., Dupré, B., & Lewin, E. (1993). Erosion sources determined by
1030 inversion of major and trace element ratios and strontium isotopic ratios in river water:
1031 The Congo Basin case. *Earth and Planetary Science Letters*, *120*(1), 59–76.
1032 https://doi.org/10.1016/0012-821X(93)90023-3
1033 Neidhardt, H., & Shao, W. (2023). Impact of climate change-induced warming on
1034 groundwater temperatures and quality. *Applied Water Science*, *13*(12), 235.
1035 https://doi.org/10.1007/s13201-023-02039-5
1036 Noethen, M., Hemmerle, H., & Bayer, P. (2023). Sources, intensities, and implications of
1037 subsurface warming in times of climate change. *Critical Reviews in Environmental*
1038 *Science and Technology*, *53*(5), 700–722.

https://doi.org/10.1080/10643389.2022.2083899

Nydahl, A. C., Wallin, M. B., Laudon, H., & Weyhenmeyer, G. A. (2020). Groundwater carbon within a boreal catchment: Spatiotemporal variability of a hidden aquatic carbon pool. *Journal of Geophysical Research: Biogeosciences*, *125*(1), e2019JG005244. https://doi.org/10.1029/2019JG005244

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., … Weedon, J. (2022). *Vegan: Community Ecology Package. R package version 2.6-2* [Computer software]. https://CRAN.R-project.org/package=vegan

Overholt, W. A., Trumbore, S., Xu, X., Bornemann, T. L. V., Probst, A. J., Krüger, M., Herrmann, M., Thamdrup, B., Bristow, L. A., Taubert, M., Schwab, V. F., Hölzer, M., Marz, M., & Küsel, K. (2022). Carbon fixation rates in groundwater similar to those in oligotrophic marine systems. *Nature Geoscience*, 1–7. https://doi.org/10.1038/s41561-022-00968-5

Penman, D. E., Caves Rugenstein, J. K., Ibarra, D. E., & Winnick, M. J. (2020). Silicate weathering as a feedback and forcing in Earth's climate and carbon cycle. *Earth-Science Reviews*, *209*, 103298. https://doi.org/10.1016/j.earscirev.2020.103298

Powley, H. R., Polimene, L., Torres, R., Al Azhar, M., Bell, V., Cooper, D., Holt, J., Wakelin, S., & Artioli, Y. (2024). Modelling terrigenous DOC across the north west European Shelf: Fate of riverine input and impact on air-sea CO_2 fluxes. *Science of The Total Environment*, *912*, 168938. https://doi.org/10.1016/j.scitotenv.2023.168938

R Core Team. (2023). *R: A Language and Environment for Statistical Computing* [Computer software]. The R Foundation for Statistical Computing. https://www.R-project.org/

Ramaekers, L., Pinceel, T., Brendonck, L., & Vanschoenwinkel, B. (2022). Direct effects of

elevated dissolved CO_2 can alter the life history of freshwater zooplankton. *Scientific Reports*, *12*(1), Article 1. https://doi.org/10.1038/s41598-022-10094-2

Rau, G. C., Cuthbert, M. O., Andersen, M. S., Baker, A., Rutlidge, H., Markowska, M., Roshan, H., Marjo, C. E., Graham, P. W., & Acworth, R. I. (2015). Controls on cave drip water temperature and implications for speleothem-based paleoclimate reconstructions. *Quaternary Science Reviews*, 1–18. https://doi.org/10.1016/j.quascirev.2015.03.026

Raymond, P. A., & Cole, J. J. (2003). Increase in the Export of Alkalinity from North America's Largest River. *Science*, *301*(5629), 88–91. https://doi.org/10.1126/science.1083788

Riedel, T. (2019). Temperature-associated changes in groundwater quality. *Journal of Hydrology*, *572*, 206–212. https://doi.org/10.1016/j.jhydrol.2019.02.059

Royston, P. (1982). An extension of Shapiro and Wilk's W W test for normality to large samples. *Applied Statistics*, *31*, 115–124. https://doi.org/10.2307/2347973.

Rue, G. P., & McKnight, D. M. (2021). Enhanced rare earth element mobilization in a mountain watershed of the Colorado mineral belt with concomitant detection in aquatic biota: Increasing climate change-driven degradation to water quality. *Environmental Science & Technology*, *55*(21), 14378–14388. https://doi.org/10.1021/acs.est.1c02958

Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., Wanninkhof, R., Wong, C. S., Wallace, D. W. R., Tilbrook, B., Millero, F. J., Peng, T.-H., Kozyr, A., Ono, T., & Rios, A. F. (2004). The oceanic sink for anthropogenic CO_2. *Science*, *305*(5682), 367–371. https://doi.org/10.1126/science.1097403

Schmidt, S. I., Cuthbert, M. O., & Schwientek, M. (2017). Towards an integrated understanding of how micro scale processes shape groundwater ecosystem functions. *Science of the Total Environment*, *592*, 215–227. https://doi.org/10.1016/j.scitotenv.2017.03.047

1091 Schmidt, S. I., & Hahn, H. J. (2012). What is groundwater and what does this mean to
1092 fauna? – An opinion. *Limnologica*, *42*(1), 1–6.
1093 https://doi.org/10.1016/j.limno.2011.08.002
1094 Schmidt, S. I., Hahn, H. J., Hatton, T. J., & Humphreys, W. F. (2007). Do faunal
1095 assemblages reflect the exchange intensity in groundwater zones? *Hydrobiologia*,
1096 *583*(1), 1–19. https://doi.org/10.1007/s10750-006-0405-8
1097 Schmidt, S. I., Hahn, H. J., Watson, G. D., Woodbury, R. J., & Hatton, T. J. (2004).
1098 Sampling fauna in stream sediments as well as groundwater using one net sampler.
1099 *Acta Hydrochimica et Hydrobiologica*, *32*(2), 131−137.
1100 https://doi.org/10.1002/aheh.200300522
1101 Schmidt, S. I., Hejzlar, J., Kopáček, J., Paule-Mercado, Ma. C., Porcal, P., Vystavna, Y., &
1102 Lanta, V. (2022). Forest damage and subsequent recovery alter the water composition
1103 in mountain lake catchments. *Science of The Total Environment*, *827*, 154293.
1104 https://doi.org/10.1016/j.scitotenv.2022.154293
1105 Schmidt, S. I., Kodeš, V., Svátková, M., & Blabolil, P. (2021). Ekologie podzemních vod,
1106 po stopách Františka Vejdovského (Czech with English abstract: Groundwater ecology,
1107 on Frantisek Vejdovsky's tracks). *Vodní Hospodářství*, *71*(2), 13–19.
1108 Schmidt, S. I., Kreft, J.-U., Mackay, R., Picioreanu, C., Thullner, M. (2018). Elucidating the
1109 impact of micro-scale heterogeneous bacterial distribution on biodegradation. *Advances*
1110 *in Water Resources, 116*, 67–76. https://doi.org/10.1016/j.advwatres.2018.01.013
1111 Schwartzman, D. W., & Volk, T. (1989). Biotic enhancement of weathering and the
1112 habitability of Earth. *Nature*, *340*(6233), Article 6233.
1113 https://doi.org/10.1038/340457a0
1114 Sentenac, H., Loyau, A., Zoccarato, L., Jassey, V. E. J., Grossart, H.-P., & Schmeller, D. S.
1115 (2023). Biofilm community composition is changing in remote mountain lakes with a
1116 relative increase in potentially toxigenic algae. *Water Research*, *245*, 120547.

https://doi.org/10.1016/j.watres.2023.120547

Sheldon, N. D. (2006). Abrupt chemical weathering increase across the Permian–Triassic boundary. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *231*(3), 315–321. https://doi.org/10.1016/j.palaeo.2005.09.001

Smith, R.J., Paterson, J.S., Launer, E., Tobe, S.S., Morello, E., Leijs, R., Marri, S., Mitchell, J.G. (2016). Stygofauna enhance prokaryotic transport in groundwater ecosystems. *Scientific Reports, 6*, 32738. https://doi.org/10.1038/srep32738

Spengler, C., & Hahn, J. (2018). Thermostress: Ökologisch begründete, thermische Schwellenwerte und Bewertungsansätze für das Grundwasser. *KW Korrespondenz Wasserwirtschaft*, *11*(9), 521–525. https://doi.org/10.3243/kwe2018.09.001

Thorp, J. H., & Covich, A. P. (2001). *Ecology and classification of North American freshwater invertebrates.* Academic Press.

Turek, J., Fluksová, H., Hejzlar, J., Kopáček, J., & Porcal, P. (2014). Modelling air temperature in catchments of Čertovo and Plešné lakes in the bohemian Forest back to 1781. *Silva Gabreta*, *20*(1), 1–24.

UNESCO World Water Assessment Programme. (2022). *The United Nations World Water Development Report 2022: Groundwater: Making the invisible visible* [UNESCO Digital Library]. https://unesdoc.unesco.org/ark:/48223/pf0000380721

Vejvodová, J., Hušková, R., Sklenář, M., Nesměráková, E., Růžička, P., Schinkmanová, H., & Pospíšilová, J. (2006). B2.2 Drinking Water. In *Yearbook Prague Environment 2006*. https://envis.praha.eu/rocenky/Pr06_htm/aB2_02.htm#B2_021

Vicca, S., Goll, D. S., Hagens, M., Hartmann, J., Janssens, I. A., Neubeck, A., Peñuelas, J., Poblador, S., Rijnders, J., Sardans, J., Struyf, E., Swoboda, P., van Groenigen, J. W., Vienne, A., & Verbruggen, E. (2022). Is the climate change mitigation effect of enhanced silicate weathering governed by biological processes? *Global Change Biology*, *28*(3), 711–726. https://doi.org/10.1111/gcb.15993

1143 Walker, J. C. G., Hays, P. B., & Kasting, J. F. (1981). A negative feedback mechanism for
1144 the long-term stabilization of Earth's surface temperature. *Journal of Geophysical*
1145 *Research*, *86*(C10), 9776. https://doi.org/10.1029/JC086iC10p09776
1146 Weiss, L. C., Pötter, L., Steiger, A., Kruppert, S., Frost, U., & Tollrian, R. (2018). Rising
1147 pCO_2 in freshwater ecosystems has the potential to negatively affect predator-induced
1148 defenses in Daphnia. *Current Biology*, *28*(2), 327-332.e3.
1149 https://doi.org/10.1016/j.cub.2017.12.022
1150 Wilhelm, F.M., Taylor, S.J., Adams, G.L. (2006). Comparison of routine metabolic rates of
1151 the stygobite, *Gammarus acherondytes* (Amphipoda: Gammaridae) and the stygophile,
1152 *Gammarus troglophilus*. *Freshw. Biol*., *51*, 1162–1174.
1153 http://dx.doi.org/10.1111/j.1365-2427.2006.01564.x.
1154 Xiong, L., Bai, X., Zhao, C., Li, Y., Tan, Q., Luo, G., Wu, L., Chen, F., Li, C., Ran, C., Xi,
1155 H., Luo, X., Chen, H., Zhang, S., Liu, M., Gong, S., Xiao, B., Du, C., & Song, F.
1156 (2022). High-resolution data sets for global carbonate and silicate rock weathering
1157 carbon sinks and their change trends. *Earth's Future*, *10*(8), e2022EF002746.
1158 https://doi.org/10.1029/2022EF002746
1159 Zahradníček, P., Brázdil, R., Štěpánek, P., & Trnka, M. (2021). Reflections of global
1160 warming in trends of temperature characteristics in the Czech Republic, 1961–2019.
1161 *International Journal of Climatology*, *41*(2), 1211–1229.
1162 https://doi.org/10.1002/joc.6791
1163 Zhang, S., Bai, X., Zhao, C., Tan, Q., Luo, G., Wang, J., Li, Q., Wu, L., Chen, F., Li, C.,
1164 Deng, Y., Yang, Y., & Xi, H. (2021). Global CO_2 consumption by silicate rock
1165 chemical weathering: Its past and future. *Earth's Future*, *9*(5), e2020EF001938.
1166 https://doi.org/10.1029/2020EF001938
1167