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„Distribution patterns of the groundwater fauna in the River Mur Valley, Austria“

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Zusammenfassung

Die Fauna des Grundwassers spielt eine wichtige Rolle als Bioindikator und bei der Grundwasserreinigung, wird jedoch bei der Bewertung von Grundwassersystemen zumeist vernachlässigt. Für einen integrativen Ansatz ist es zunächst notwendig, Zusammenhänge zwischen Verbreitungsmustern der Fauna, sowie abiotischen Schlüsselfaktoren und der Struktur der Lebensgemeinschaften zu identifizieren. Die Grundwasserfauna ist in weiten Teilen Österreichs bislang nicht untersucht. Bisherige Erhebungen beschränkten sich auf naturbelassene Gebiete, während diese Untersuchung eine Erstaufnahme der Verteilung der Stygofauna in unterschiedlich beeinflussten Regionen darstellt. Bei einer Bestandsaufnahme im Frühsommer 2020 wurden in den glazio-fluvialen Aquiferen des steirischen/salzburgischen Murtals in Österreich 45 Grundwasserproben gesammelt und alle gefundenen Tiere in 11 taxonomische Großgruppen unterschieden. Der Großteil der Organismen konnte dem Subphylum Crustacea zugeordnet werden. Speziell die Verteilung der Copepoden und Oligochaeten ließ darauf schließen, dass diese beiden Gruppen als Indikatoren für eine große taxonomische Vielfalt herangezogen werden können. Durch Vergleich mit ausgewählten Umwelt- und Einflussfaktoren konnte gezeigt werden, dass die Vielfalt der Grundwasserfauna und der Anteil an besiedelten Grundwasser-Messstellen in natürlich belassenen Regionen höher war, als in urbanen Arealen oder landwirtschaftlich genutzten Gebieten. Wenige Messstellen waren unbesiedelt (18 %) und hydrochemische Faktoren zeigten kaum Einfluss auf die Verteilung der Grundwasserfauna. Zwischen den oberen und unteren Regionen des Murtals offenbarten sich Unterschiede in der Zusammensetzung und Populationsstruktur der Grundwasserfauna, die vor allem durch Unterschiede in der Höhenlage, Grundwasser-Temperatur, Aquifer-Struktur, Tiefe des Grundwasserspiegels und durch historische Einflüsse (Vergletscherung während der Würm-Eiszeit) verursacht sind. Die Bestimmung der gefundenen Tiergruppen auf Artniveau, sowie die Berücksichtigung jahreszeitlicher Schwankungen sollten in zukünftigen Untersuchungen miteinbezogen werden.

Schlüsselwörter

Grundwasserfauna, Diversität, Verteilungsmuster, Landnutzung, Höhenlage, Murtal

Abstract

Groundwater fauna plays a major role as a bioindicator and for water purification, but is mostly neglected in the assessment of groundwater systems. For an inclusive approach, it is important to identify connections between the distribution patterns of groundwater fauna, abiotic key factors and community structures. In large parts of Austria, groundwater fauna is not yet investigated and previous research studied exclusively natural regions. Therefore, this is a baseline survey that evaluates the distribution of stygofauna in differently influenced regions. In the glacio/fluvial aquifers of the River Mur Valley, Styria/Salzburg (Austria) 45 groundwater wells were sampled in June 2020 and the collected groundwater animals were divided into 11 taxonomic groups. The majority of the sampled organisms belonged to the subphylum Crustacea. Especially the distribution of Oligochaeta and Copepoda suggested, that these two groups could act as indicators for high faunal richness. By comparing a selected set of environmental/influencing parameters, it was shown that the fauna richness and the rate of inhabited groundwater wells were higher in natural regions, than in urban or agricultural areas. Few wells were not inhabited (18 %) and hydrochemical factors indicated no impact on the fauna distribution. Between the lower and upper regions of the Mur Valley, differences in community structures and fauna distribution were observed, which result from variations in altitude, groundwater temperature, aquifer structure, depth of the groundwater table and historical influence (e.g. glacial periods). Further taxonomic identification of the sampled fauna as well as seasonal changes should be considered in prospective investigations.

Keywords

Groundwater fauna, richness, distribution pattern, land use, altitude, Mur Valley

1. Introduction

Subsurface water constitutes for about 97 % of all freshwater that is not bound in glaciers, ice and snow on the continental earth, with surface waters like rivers, lakes and swamps only representing two percent of the world's unfrozen freshwater (Gibert & Deharveng, 2002). Fresh groundwater is one of the most important resources for human kind as we not only depend on it as a main source of drinking water, but also as a crucial resource for agricultural purposes, for the use of its thermal energy storage and for its general property to sustain the integrity of the world's water cycle/climate and most surface aquatic and terrestrial ecosystems (Nace, 1960; Stein et al., 2010; Brielmann et al., 2009; Batelaan et al., 2003). Alongside with the challenges of climate change and the anthropogenic alteration of surface freshwater systems like rivers and lakes, human influences on groundwater systems are increasing.

Anthropogenic stressors do not only have physical (temperature changes, sediment replacement etc.) or chemical (pollutants, fertilizers, sewage etc.) impacts on groundwater, but also implicate biological (pathogens, local extinctions, etc.) influences. The assessment and management of groundwater ecosystems to date consists almost exclusively of the analysis of physicochemical variables, other abiotic factors and in some cases basic bacteriological and hygienic tests. However, faunal community structures and distribution patterns also play a major role in the functionality of groundwater systems and furthermore can not only impact surface ecosystems, but have a back-coupling effect on physicochemical factors. For a holistic monitoring, or the development of a 'Groundwater Health Index' as proposed by Korbel and Hose (2017), biotic criteria need to become part of an integrative assessment/management strategy (Stein et al., 2010; Cairns et al., 1993). Since groundwater fauna is an important contributor to ecosystem functions and can be assumed to be a biological sentinel for aquifer condition (Schmidt & Hahn, 2012), knowledge of the faunal composition and distribution in Austria is essential.

Groundwater fauna and its distribution in Austria has to date mainly been investigated in areas close to the capital Vienna (Vienna Basin, Lobau) and in Lower Austria (aquifers associated with the river Danube and the river Piesting), as well as in protected, natural habitats (Danielopol, 1989; Danielopol & Pospisil, 2001; Hynes, 1983). Hence, there is little to no information on stygofauna for most of Austria, and least for anthropogenically altered regions. This current study is designed to act as a baseline survey for the distribution of groundwater fauna in differently influenced regions (natural, agricultural, urban) associated with the river Mur in the provinces Salzburg and Styria in Austria. Stygofauna in this area is expected to differ from the composition of groundwater fauna in the well-studied regions around Vienna, since it displays a more southern like the geographical location, a broad range of altitude (from lowlands to alpine regions), anthropogenic impacts, and geological events (last glacial period).

Although the fauna of the river Mur in Styria is well studied (Kirchengast, 1984), groundwater fauna in the River Mur Valley, which is expected to vastly differ in its composition, is poorly investigated. Exceptions are some caves upgradient of Graz (in the sub-regions Grazer Feld and Murdurchbruchstal), including the Lurgrotte, Drachenhöhle and Katerloch. Based on past observations in the Lurgrotte as well as in other caves in Styria and Salzburg, it is expected that we may find individuals of Turbellaria (stygobiont species recorded in the Lurgrotte [Neuherz, 1974]), Nematoda (common groundwater animals, but not well investigated in Austria [Hilberg & Eisendle-Flöckner, 2016]), Gastropoda (high levels of endemism in groundwater habitats [Christian & Spötl, 2010]), Annelida (stygobiont and/or stygophilic species as recorded in the Lurgrotte [Neuherz, 1974]), Acari (commonly found in Austrian groundwater [Christian, 1997]), Copepoda (stygobiont and stygophilic Cyclopoida and Harpacticoida are very commonly found in alluvial and porous groundwater ecosystems in Austria, some species are endemic [Christian & Spötl, 2010]), Ostracoda (stygobiont species have been recorded in Austria and a stygophilic species was found in the Lurgrotte [Gaviria & Pospisil, 2009; Neuherz, 1974]), Syncarida (Bathynellacea have been recorded in groundwater of Styria/Austria [Hasenhüttl, 1972; Christian & Spötl, 2010]), Amphipoda (especially the genus *Niphargus* is commonly found in Austrian groundwater and in the Lurgrotte [Neuherz, 1974; Kühnelt, 1962; Christian & Spötl, 2010]) and Isopoda (stygobiont and stygophilic species are recorded in Styria/Austria and Slovenia [Vornatscher, 1952; Christian & Spötl, 2010]).

Being located in the (sub-) alpine region of Central Europe, the Mur Valley lies between the southern, Mediterranean regions where richness, diversity and endemism of groundwater fauna are exceptionally high, and the northern, Baltic regions where groundwater fauna is scarce, richness/diversity are low and endemic species are rare. The main reason for this large-scale distribution pattern is presumed to be the last glacial period (Riss/Würm ice age), during which the majority of limnic species that were living in glaciated areas either got extinct, sought refuge in deeper areas of the groundwater or migrated southwards (Culver et al., 2006; Thienemann, 1950). Former glaciated areas are therefore either not well populated by fauna, or were re-colonized. In re-colonized regions, endemism normally is not high, but in some cases, relict species can occur (Martin et al., 2009; Deharveng et al., 2009). Despite these historically originated patterns, the distribution of faunal richness/diversity in the groundwater of Europe is very heterogenous, highly variable between regions and dependent on the local species pool. Especially the distribution of stygobiota is very patchy with few hotspots rather than clear distribution borders (Malard et al., 2009; Deharveng et al., 2009).

The Riss-Würm ice ages had a direct impact on the upper third of the Mur Valley, as these areas were covered by glaciers, and influenced the lower regions of the Mur Valley indirectly by sediment shifts, deformations and erosions as well as permafrost (Hewitt, 1999). Therefore, and because of the altitude/temperature gradient of the sample sites, we may expect the upper regions of the Mur Valley to have lower faunal richness, than the downstream regions, but could possibly include specialized sygobionts and/or relict species. Furthermore, it was anticipated, that a re-colonization pattern originating from lower, more southern regions could be observed.

The distribution pattern and interactions of stygobiont, stygophile and stygoxenous fauna may be heterogenous on a large scale, but regionally there are key factors, that affect these community compositions. The main factors are geology (sediment quality, pore- and fissure sizes), oxygen and nutrient availability. Since all three factors are highly dependent on, or collude with hydrological exchange, physical measures (depth of the groundwater table, temperature, electrical conductivity, oxygen concentrations, pH and distances to the surface water) were expected to unravel in which wells/regions there are high hydrological exchange rates. High hydrological exchange should be accompanied by the dominance of stygophile and stygoxenous fauna in the communities, while wells/regions with medium hydrological exchange rates are more likely inhabited by stygobiota and low hydrological exchange commonly indicates the absence of fauna. Aquifers that are influenced by medium hydrological exchange usually are populated by stygobiont as well as stygophile and stygoxenous taxa, but as opposed to low or high exchange rates, there is an equilibrium of oxygen and food availability that prevents dominant species from oppressing specialist taxa (Thulin & Hahn, 2008). Therefore, we may expect to find richer groundwater fauna communities in areas of the Mur Valley that are relatively shallow, oxygen rich and have medium hydrological exchange.

Furthermore, it will be evaluated, if any patterns emerge in the combined appearance of faunal groups and if the proposition of Stoch et al. (2009), that some taxonomic groups can be used as indicators for faunal richness in groundwater systems can be applied in the Mur Valley. A factor that will be also taken into account regarding the faunal distribution will be the altitude. There is a steep elevation gradient of around 850 m at the study area along the river Mur which (being linked to a temperature gradient) could have an impact on faunal distribution patterns. In fact, previous research could show a clear connection (Dole-Olivier et al., 2009; Mösslacher, 2003).

Another factor that is supposed to impact groundwater fauna, but is little investigated, i.e. land use. It is known, that agricultural land use has an impact on the chemical composition of groundwater, as pollutants, pesticides and fertilizers can stress the integrity of the groundwater (Hahn, 2002). Moreover, the relatively high levels of water discharge can lead to differences in hydrological exchange rate, low groundwater tables and disruption of bacterial communities (Di Lorenzo & Galassi, 2013). Although hydrochemisty is (in non-critical concentrations) assumed to have little influence on the faunal community composition and richness, the effect of agricultural use on hydrophysical parameters and therefore hydrological exchange is assumed to have a negative impact on the groundwater fauna (Dole-Olivier et al., 2009).

Groundwater systems in urban areas are also highly anthropogenically influenced. As well as in agricultural regions, the discharge of groundwater is usually relatively high and the water can be stressed by pollutants, sewage, heavy metals, and industrial effluents (Khatri & Tyagi, 2015; Hancock, 2002). Additionally, the soil sealing and storage of energy in aquifers can lead to raised temperatures, less hydrological exchange and depletion of dissolved oxygen, leading to changes in water quality and ecological patterns (Griebler et al., 2016). Furthermore, concentrations of phosphate, ammonium and nitrate are usually elevated in anthropogenically influenced areas, having a possible negative effect on stygofauna (Hickey, 2014; Gerhardt, 2020). These factors set wells in agricultural or urban areas apart from those in natural regions and due to these stressors, we expect to find less fauna and/or differently composed communities below agricultural land and urban areas, when compared to natural sites. Since anthropogenic stressors (e.g. elevated concentrations of nitrate and ammonium) are higher in downstream regions, faunal richness is at least locally expected to be lower there than in upstream areas.

In particular, this survey intended to provide a baseline of the taxonomic groups (macro- and meiofauna) present in the glacio/fluvial aquifers of the River Mur Valley. A selected set of physicochemical parameters was analysed in relation to faunal richness and differences in community structure. The impacts of land use, altitude and spatiotemporal differences on the distribution patterns of groundwater fauna was evaluated and is discussed.

2. Material & Methods

2.1 Site description

Groundwater samples were taken from pre-existing observation wells alongside the river Mur in the provinces Styria and Salzburg of Austria. Since the authorities of the provinces are monitoring quality and quantity factors of the groundwater using those observation wells, long-term data on temperature and hydraulic head (groundwater table) were available for us to select adequate sampling spots ([eHYD], 2020). 45 groundwater wells were chosen and sampled over a five-week time period in June and July 2020.

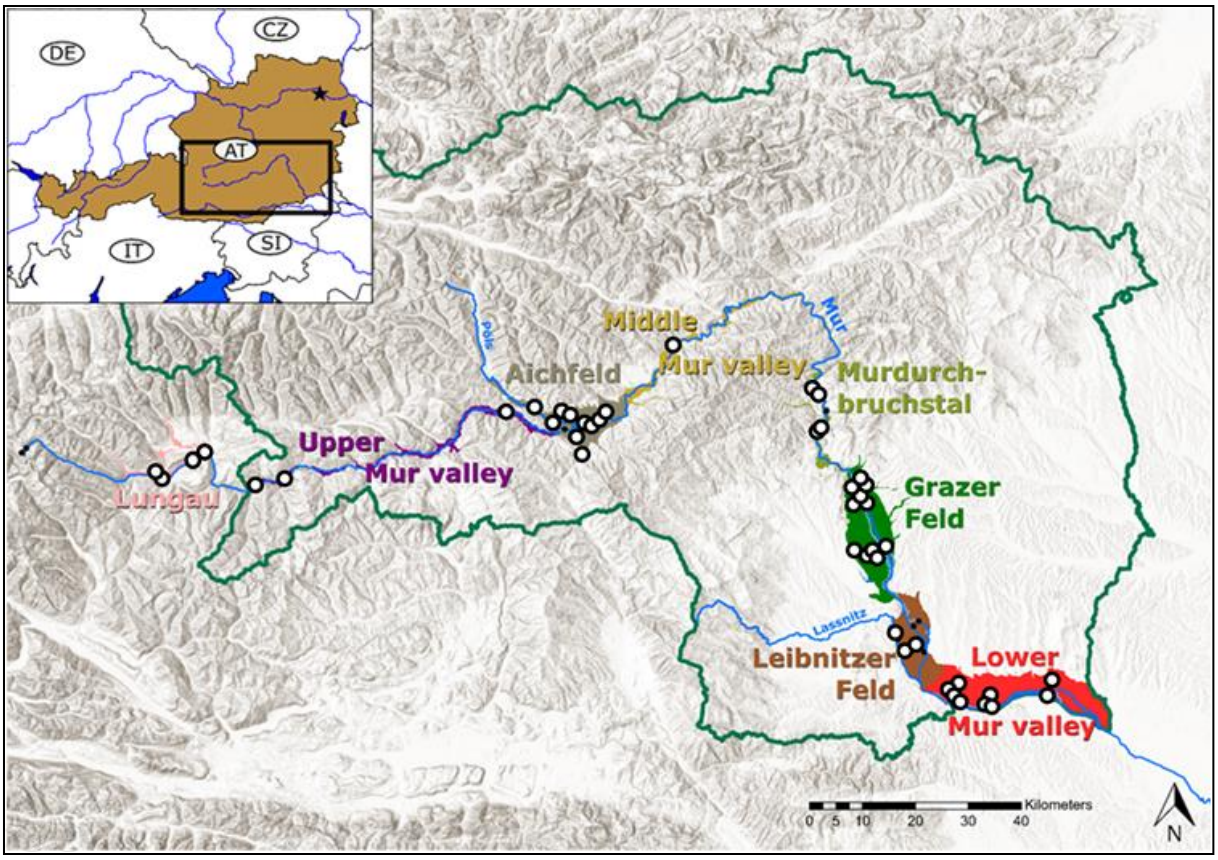

Figure 1: Location of the study site in Austria (upper left) and detailed positions of the sampling sites (white dots) along the river Mur, categorized into the eight sub-regions Lungau (province of Salzburg) and Upper Mur Valley, Aichfeld, Middle Mur Valley, Murdurchbruchstal, Grazer Feld, Leibnitzer Feld and Lower Mur Valley (province of Styria, indicated by green boundary line).

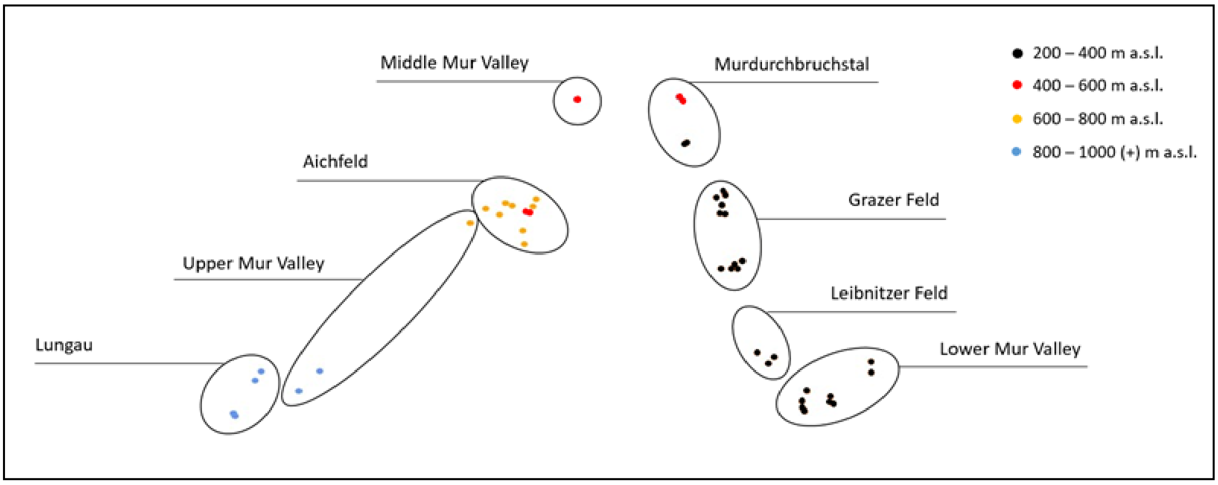

Figure 2: NMDS of the location of the 45 sampling sites (coloured dots), organized into the eight sub-regions Lungau, Upper Mur Valley, Aichfeld, Middle Mur Valley, Murdurchbruchstal, Grazer Feld, Leibnitzer Feld and Lower Mur Valley. Colours indicate the altitude of each site.

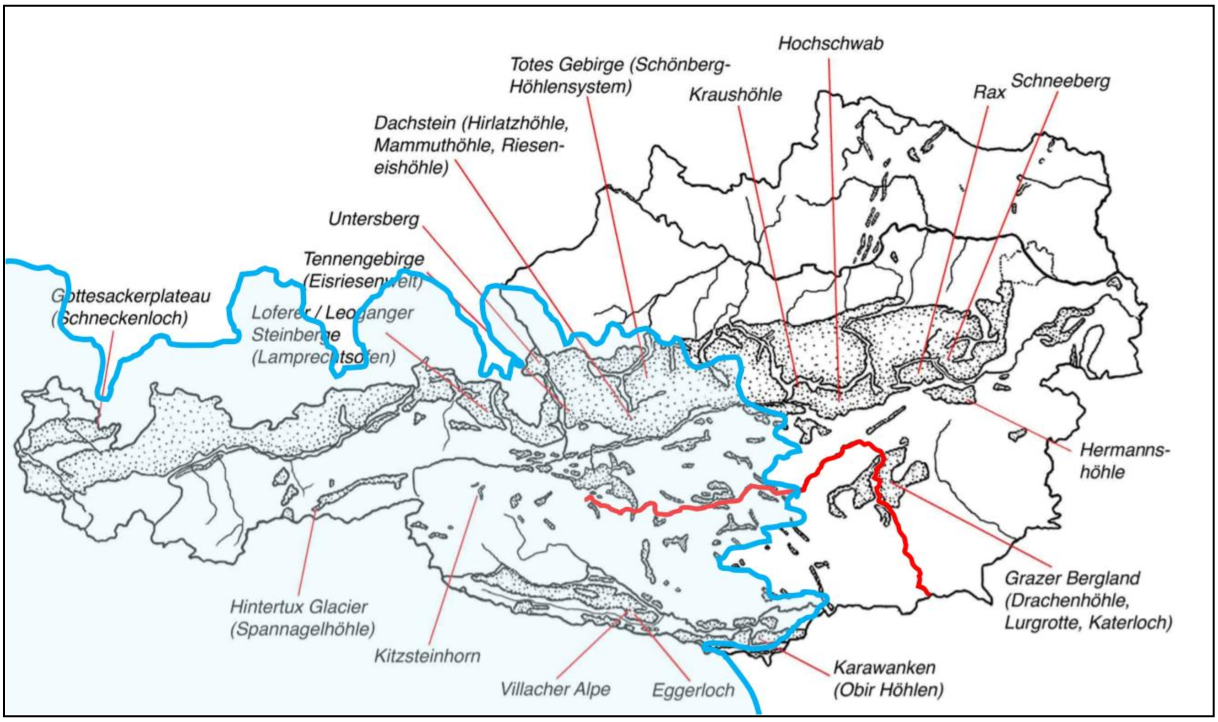

Figure 3: Illustration of main karst regions and the maximum extension of the Pleistocene glaciation in Austria (indicated in blue). Important cave systems (including Lurgrotte of the Grazer Highlands) and the river Mur (thick red line) are marked (map modified after Christian & Spötl, 2010).

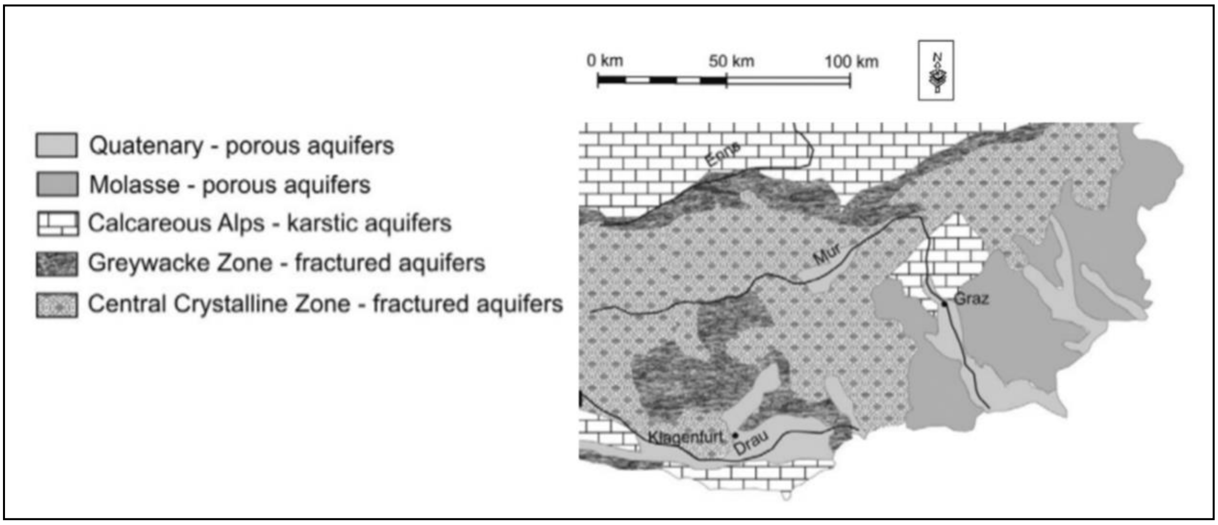

Figure 4: Overview of Aquifers in the River Mur Valley, Austria. Samples have been taken from shallow wells of the overlying quaternary Valley fillings (map simplified after Hilberg & Eisendle-Flöckner, 2016).

From its spring at approximately 1900 m a.s.l., the river Mur runs in an eastern direction through the province of Salzburg, until further passing across the province of Styria and ultimately proceeding in a southern direction, passing the border to Slovenia at about 200 m a.s.l. (Figure 1). The total stretch covers around 300 km with alternating geological and morphological settings, and an overall altitude gradient of approximately 850 m (excluding the spring). The sampled aquifers were differentiated into eight sub-regions with dissimilar hydrological and geological features, labelled as Lungau, Upper Mur Valley, Aichfeld, Middle Mur Valley, Murdurchbruchstal, Grazer Feld, Leibnitzer Feld and Lower Mur Valley (Haas & Birk, 2017). Starting at the highest altitudes above 1000 m a.s.l. at the Lungau, the sample site elevations were lower the more they were located downstream, with the lowest altitudes located at the Lower Mur Valley (Figure 2).

The river Mur originates at the periphery of the Tauern-window and is affected by mostly metamorphic rock as it runs through various units of Austro-alpine nappes, intermitted by Neogene sediments in some inner alpine basins, such as Aichfeld and south of the city of Graz. The aquifers alongside the river Mur can be categorized as glacio/fluvial valley fillings, mainly consisting of gravel and sand, with some areas including alluvial fan and rockslide sediments. In the lower parts of the Mur Valley (Grazer Feld, Leibnitzer Feld and Lower Mur Valley), and in Aichfeld, deeper aquifers are porous, while the underlying aquifers of the upper regions (Lungau, Upper Mur Valley and Middle Mur Valley) are mainly fractured. The subjacent aquifer of the Murdurchbruchstal and the upstream parts of the Grazer Feld is categorized as karstic and includes the cave system of the Grazer Highlands involving the Lurgrotte (Figure 4 & Figure 3). However, on top of these structures lay quarternary Valley fillings in the River Mur Valley, from whose shallow wells all samples were taken.

Table 1: Sample number, sampling date, coordinates (LAT & LON), altitude (m a.s.l.), geo-classification code, sub-region classification and land use type classification of each sampling site.

Sample name	Date	LAT	LON	Altitude [m a.s.l.]	Geo-class code	Sub-Region	Land use type
3	04.06.2020	46.71855	15.90023	221	C14	Lower Mur Valley	Forests and semi-natural areas
4	04.06.2020	46.73456	15.90251	231	C14	Lower Mur Valley	Urban areas
5	04.06.2020	46.70189	15.75395	237	C14	Lower Mur Valley	Forests and semi-natural areas
6	04.06.2020	46.69929	15.75902	238	C14	Lower Mur Valley	Forests and semi-natural areas
7	04.06.2020	46.71136	15.7522	249	C14	Lower Mur Valley	Agricultural use
8	17.06.2020	46.70709	15.65988	305	C14	Lower Mur Valley	Forests and semi-natural areas
9	05.06.2020	46.71236	15.6547	273	C14	Lower Mur Valley	Agricultural use
10	05.06.2020	46.72337	15.65283	275	C14	Lower Mur Valley	Agricultural use
11	05.06.2020	46.73486	15.67377	272	C14	Lower Mur Valley	Urban areas
15	05.06.2020	46.80074	15.54768	271	C14	Leibnitzer Feld	Agricultural use
19	11.06.2020	47.07745	15.44345	376	B20	Grazer Feld	Urban areas
21	11.06.2020	47.07269	15.44565	375	B20	Grazer Feld	Urban areas
22	11.06.2020	47.04605	15.43916	358	C14	Grazer Feld	Urban areas
24	11.06.2020	47.05	15.42218	367	C14	Grazer Feld	Urban areas
26	17.06.2020	47.07607	15.41315	380	C14	Grazer Feld	Urban areas
27	12.06.2020	47.06034	15.432	347	C14	Grazer Feld	Urban areas
28	12.06.2020	46.96718	15.48592	325	C14	Grazer Feld	Agricultural use
31	12.06.2020	46.96706	15.45948	338	C14	Grazer Feld	Urban areas
34	12.06.2020	46.96314	15.4461	332	C14	Grazer Feld	Urban areas
37	12.06.2020	46.95842	15.46766	326	C14	Grazer Feld	Agricultural use
38	17.06.2020	46.82376	15.51175	285	C14	Leibnitzer Feld	Agricultural use
45	17.06.2020	46.80567	15.56993	278	C14	Leibnitzer Feld	Agricultural use
49	18.06.2020	46.97002	15.4106	329	C14	Grazer Feld	Agricultural use
50	18.06.2020	47.17477	15.32404	384	C14	Murdurchbruchstal	Agricultural use
51	18.06.2020	47.17603	15.32611	384	C14	Murdurchbruchstal	Agricultural use
55	18.06.2020	47.23885	15.32486	419	C14	Murdurchbruchstal	Agricultural use
56	18.06.2020	47.24591	15.31498	421	C14	Murdurchbruchstal	Agricultural use
57	24.06.2020	47.18221	14.7553	565	C14	Aichfeld	Agricultural use
58	24.06.2020	47.17982	14.76385	574	C14	Aichfeld	Agricultural use
59	24.06.2020	47.13708	14.73891	624	C14	Aichfeld	Agricultural use
60	24.06.2020	47.15782	14.73747	603	C14	Aichfeld	Urban areas
64	24.06.2020	47.21434	14.61545	782	C11	Aichfeld	Agricultural use
66	24.06.2020	47.20882	14.68559	715	C14	Aichfeld	Urban areas
68	25.06.2020	47.19641	14.66202	683	C14	Aichfeld	Agricultural use
71	25.06.2020	47.20092	14.70705	702	C14	Aichfeld	Agricultural use
72	25.06.2020	47.18562	14.77964	636	C14	Aichfeld	Agricultural use
74	25.06.2020	47.19403	14.79267	643	C14	Aichfeld	Agricultural use
80	01.07.2020	47.31095	14.96158	584	C14	Middle Mur Valley	Agricultural use
81	01.07.2020	47.20431	14.55792	715	C14	Upper Mur Valley	Agricultural use
83	02.07.2020	47.09098	13.99708	874	H01	Upper Mur Valley	Agricultural use
86	02.07.2020	47.08818	13.68743	1035	C14	Lungau	Agricultural use
87	02.07.2020	47.08298	13.69211	1049	C14	Lungau	Agricultural use
90	02.07.2020	47.1208	13.77159	1024	C14	Lungau	Agricultural use
92	02.07.2020	47.12973	13.79537	1023	C14	Lungau	Forests and semi-natural areas
94	02.07.2020	47.07619	13.9194	912	C14	Upper Mur Valley	Agricultural use

Geo-classes:
B20 = Karpatium - Sarmatium
C11 = Quaternary and mostly crystalline sediments in the central alps
C14 = Quaternary, alluvium/alluvial cone/terraces alongside Mürz and Mur
H01 = Phyllite, mica slate, phyllonite and paragneiss

In the upper parts of the River Mur Valley and in the Murdurchbruchstal (where some sections of the valley are only a few hundred meters wide) the topology can be described as narrow valleys, alternating with, and changing into inner alpine basins that are deeper and wider. Southwards, the structure becomes broader and shallower, as foreland basins are dominating (Grazer Feld, Leibnitzer Feld and Lower Mur Valley). In the upper third of the Mur valley, alpine glaciation influenced the development of the aquifer directly, while the lower parts of the valley (downstream Aichfeld) were affected by indirect glacial impacts like erosion, formation and re-sedimentation of terraced sediments (Figure 3).

The selected groundwater wells covered different land use types, which were classified into three categories using the CORINE Land Cover Database from 2018 via the Digital Atlas provided by the province of Styria ([CORINE], 2018) . The category 'Agricultural use' includes most sample sites (29 sites) and is represented in all eight sub-regions, with areas of intense agriculture especially located in the Alpine foreland of southern Styria. 'Urban areas' resemble 12 sampling sites that are mainly located in and around the industrially and commercially used regions of the city of Graz, as well as spots at Aichfeld, the Lower Mur Valley and the Grazer Feld. Wells that were located in areas used for forestry and grassland farming were classified as 'Forests and semi-natural areas'. This category describes mainly locations in the alpine regions and peripheral mountain areas of Styria and Salzburg, including 5 sites at the Lower Mur Valley, the Lungau and the Murdurchbruchstal (Table 1).

2.2 Sampling methods

The depth of the wells, as well as the level of the groundwater table were measured with an electrical contact gauge (OTT HydroMet GmbH, Leibsdorf, Austria). Thereafter, fauna was extracted with a plankton net of 100 µm meshsize and a 5 cm diameter opening. The net was lowered to the bottom of the well with a fishing-rod and then was ten times moved 1 m up and down in a rapid manner to collect meio-, macrofauna and sediment in a 50 ml falcon tube. To withdraw groundwater samples, a Grundfos submersible MP1 pump (Eijkelkamp Soil & Water, Giesbeek, Netherlands) was used and positioned two meters below the groundwater table. To avoid sampling of stagnant well water, twice the volume of the well was replaced by pre-pumping until key parameters (T, EC, pH and O_2) had stabilized, using a pumping rate which allowed a maximum water level drop down of 0.5 m. The pumping rate was then lowered during the sampling process to prevent dislodgement of microbial biofilms and removal of fine sediments. Physico-chemical parameters including water temperature (T in °C), electrical conductivity (EC in µS/cm), pH and concentration of dissolved oxygen (O_2 in mg/L) were measured on site by using field sensors (WTW, Weilheim, Germany). The water that was withdrawn from the well was in addition sieved through a net of 63 µm mesh size to collect fauna passing the submersible pump.

Water samples for the analysis of hydrochemical parameters (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, PO_4^{3-}, NH_4^+, NO_2- and NO_3-) were filled in clean glass bottles. Groundwater samples for dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) analysis were filtered through a 0.45 µm PVDF syringe filter (STARLAB International, Hamburg, Germany) and filled into backed glass vials. Samples dedicated for DOC analysis were acidified with HCl to a pH ≤ 2 in the field.
All groundwater samples were stored in the dark at temperatures between 4-8 °C until further analyses. The fauna samples were fixed with 75 – 95 % ethanol (f. conc.) and coloured with eosin for further processing.

2.3 Analyses

After the fixation of the groundwater fauna with ethanol, the organic tissue was dyed with eosin and the animals were separated manually into groups of the same order using a binocular microscope.

The major ion concentrations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- and SO_4^{2-}) were analysed by ion chromatography (Dionex ICS-1100 RFIC; Thermo Scientific, Idstein, Germany), under the provisions of standard norms (Na^+, K^+, Ca^{2+} and Mg^{2+}: OENORM DIN EN ISO 14911, Cl^- & SO_4^{2-}: OENORM DIN EN ISO 10304-1). The concentrations of PO_4^{3-}, NH_4^+, NO_2- and NO_3- were evaluated by using photometric measurements. Phosphate was measured via the phosphomolybdic acid-method (filtrate as: α-phosphobolybdic blue), using ascorbic acid as a reduction agent ([λ = 890 nm]: OENORM DIN EN ISO 6878). The ammonium concentrations were found by using the indophenol blue-method (filtrate as [λ = 655 nm]: DIN 38406-5, OENORM ISO 7150-1). For NO_2-, the diazotization-method (filtrate as violet-red azo dye [λ = 542 nm]: OENORM DIN EN ISO 26777) and for NO_3-, the UV-method (filtrate as [λ = 220 nm]: APHA 4500-NO3/B) were applied.

Concentrations of DOC and DIC were measured via a TOC-L Analyzer (Shimadzu, Kyoto, Japan). In addition, quality was controlled by supplementary measurements of MilliQ water and control samples with defined DOC/DIC concentrations (25 ppm TOC curve standard).

The municipal wastewater indicator test (MWWI) compares the measured concentrations of typical municipal wastewater-derived compounds with mean concentrations of said compounds in effluents and literature values and ultimately classifies the samples into four categories (0 – no influence, 1- influence unlikely, 2 – influence likely, 3 – influenced by municipal waste water). Tested compounds included acesulfame, benzotriazole, carbamazepine, diclofenac, 10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-DiOH), metoprolol, sotalol and tolyltriazoles. All compounds were measured by direct injection in a LC-MS/MS system (Waters Xevo TQS) using a Waters Acquity UPLC BEH C18 1.7 µm 2.1x 50 mm column and MilliQ water with 0.1 % HFA as eluent. Acesulfame was analysed in negative mode, while benzotriazole, carbamazepine, 10, 11-dihydro-10, 11-dihydroxycarbamazepine (CBZ-DiOH), diclofenac, metoprolol, sotalol and tolyltriazole were analysed in positive mode.

For the statistical analyses of data and graphical presentation, MS Excel as well as PAST 4.03 (Hammer, Harper, & Ryan, 2001) and iNEXT (Chao, Ma, & Hsieh, 2016) were used.

3. Results

3.1 Fauna and diversity overview

In the groundwater wells (n = 45) sampled, a total of around 1500 individuals of macro- and meiofauna was collected. The fauna was distinguished into 12 main taxonomic groups containing only aquatic organisms, except for Collembola which are an edaphic group (Table 2). Not included in further analyses were single individuals of terrestrial fauna involving Apterygota, Cheliferidae, Coleoptera, Diptera, Hexapoda (-larvae), Myriapoda, and a Chironomidae-larva. The focus of this survey is on the observed stygobiont and stygophile fauna such as members of Acari, Amphipoda, Bathynellacea, Cyclopoida, Gastropoda, Harpacticoida, Isopoda, Oligochaeta, Ostracoda and Turbellaria, as well as stygoxen fauna like Collembola and Tardigrada.

Arthropoda was the most frequently represented phylum, with the subphylum of Crustacea being the most common/divers. Observed groups of Crustaceans included Ostracoda, Cyclopoida, Harpacticoida, Amphipoda, Bathynellacea and Isopoda. Other groups of Arthropoda found were the subclass Acari, belonging to the class Arachnida and the subclass Collembola, belonging to the class Hexapoda. From the phylum Mollusca, Gastropoda were found and the phylum Annelida was represented by Oligochaeta. In the case of Turbellaria (phylum Plathelminthes) as well as Tardigrada, only one individual was found respectively. The Turbellaria was found in sample 86 (Lungau) and the Tardigrada was found in sample 66 (Aichfeld). Furthermore, eight Nauplius-larvae were found in a single sample (Murdurchbruchstal, sample 51).

The group that was present in most of the samples was Cyclopoida (including copepodits and adults), followed by Acari and Oligochaeta (including cocoons, juveniles and adults). Amphipoda and Isopoda were also quite common since they were found in more than 10 wells, while Bathynellacea and Ostracoda were present in less than 10 sites. Gastropoda and Harpacticoida each were found in three samples, while Turbellaria, Tardigrada and Nauplius-larvae were only present in one sample respectively (Figure 6).

Table 2: Taxonomic affiliation of meio- and macrofauna found in the groundwater wells of the River Mur Valley. Highest taxonomic realization used in further data analyses are marked in bold.

Phylum	Class	Order
Platyhelminthes	**Turbellaria**	
Mollusca	**Gastropoda**	
Annelida	Clitellata	
	Oligochaeta	
Tardigrada		
Arthropoda		
Chelicerata	Arachnida	
	Acari	
Crustacea	Oligostraca	
	Ostracoda	
	Hexanauplia	
	Copepoda	Podoplea
		Cyclopoida
		Harpacticoida
	Malacostraca	
	Eumalacostraca	Peracarida
		Amphipoda
		Isopoda
		Syncarida
		Bathynellacea
Hexapoda	Entognatha	
	Collembola	

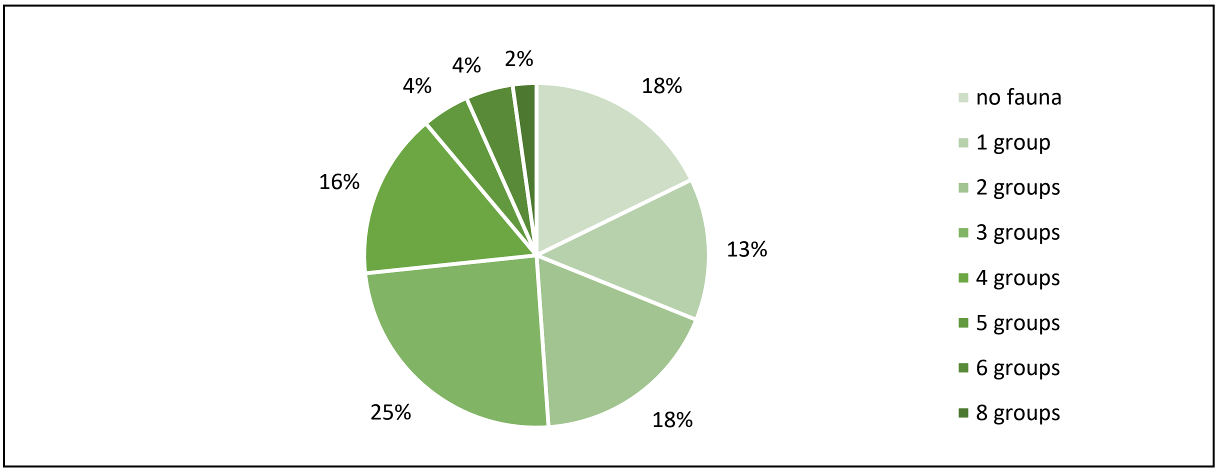

Figure 5: Richness of faunal groups (0 – 8 groups found in samples), described in percent of the entirety of 45 samples.

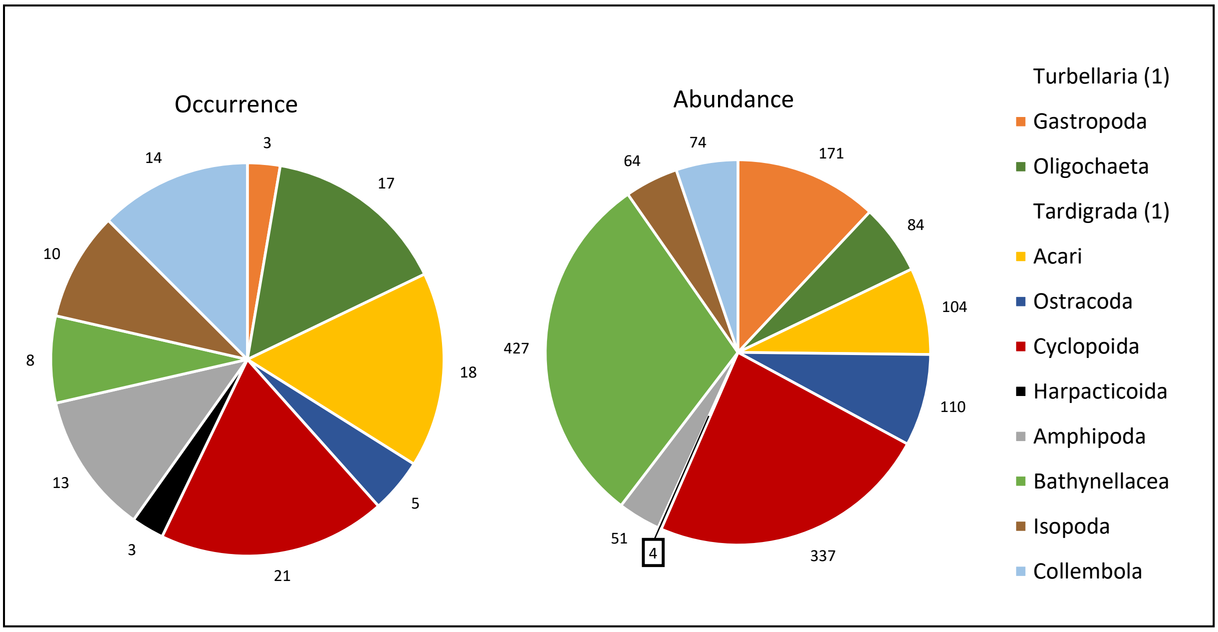

Figure 6: Occurrences and Abundances (total) of Turbellaria, Gastropoda, Oligochaeta, Tardigrada, Acari, Ostracoda, Cyclopoida, Harpacticoida, Amphipoda, Bathynellacea, Isopoda and Collembola in the River Mur Valley.

The five samples with the lowest absolute abundances over all ranged from one to two individuals per sample (samples with no fauna not taken into account), while the five samples with the highest absolute abundances over all contained 111 to 503 individuals per sample. In the specimen with the highest abundances, over 70 % of these abundances were described by a single dominant group (Dominating groups were Bathynellacea, Gastopoda and Cyclopoida) (Figure 6). Therefore, further analyses focused on presence and absence of the faunal groups (qualitative data) and number of groups present across the samples (richness). High abundances of dominant groups, as well as relatively extreme low/high concentrations of physico-/chemical parameters in single samples did not seem to have an impact on faunal community structures or diversity patterns (including differentiation by land use types, altitude, sub-regions or municipal waste water index).

Of the total 45 samples collected in spring, eight did not contain any macro- or meiofauna. In nearly a quarter of the samples, three faunal groups were found. The presence of four groups, two groups and one group was also common and roughly similar in respect to the total sample set. Samples with higher diversity (more groups present) were relatively rare, with five samples including more than five groups. Eight faunal groups were only found in one sample (Figure 5).

Regarding links between the co-occurrence of different groups, a correlation between the presence of Gastropoda and Ostracoda ($p = < 0.0001$, $r_S = 0.7908$, $r^2 = 0.6254$, $df = 43$, p being Bonferroni corrected) was found. There were no relationships indicated for the incidence between other faunal groups. Furthermore, a correlation was shown between faunal diversity (number of different faunal groups) and the occurrence of Cyclopoida (Bonferroni-corrected p = < 0.0001, r_S = 0.7086, r^2 = 0.5021, df = 43) as well as Oligochaeta (Bonferroni-corrected p = < 0.0011, r_S = 0.6028, r^2 = 0.3634, df = 43) (Figure 7). The composition of the faunal communities did not reveal a pattern for the different levels of diversity (especially between 2, 3 and 4 groups), however the sites with the highest diversity had more similar composition structures, than the sites with low diversity (Figure 8).

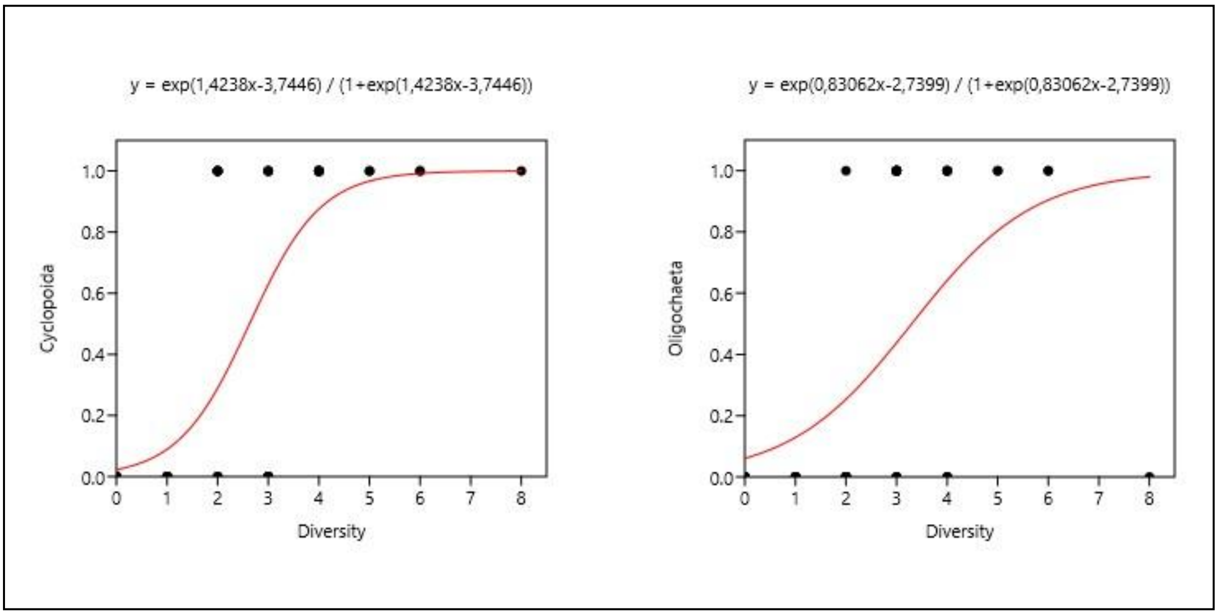

Figure 7: Occurrence frequencies of Cyclopoida [GLM results: p (slope=0) = < 0.0001, b_1 = 1.4238 ± 0.4248, b_0 = -3.7446 ± 1.1855, Log likelihood = -17.777, G = 26.629] and Oligochaeta [p (slope=0) = 0.0001, b_1 = 0.8306 ± 0.2776, b_0 = -2.7399 ± 0.8629, Log likelihood = -22.495, G = 14.677] in samples of different diversity values (0 – 8 faunal groups present in samples).

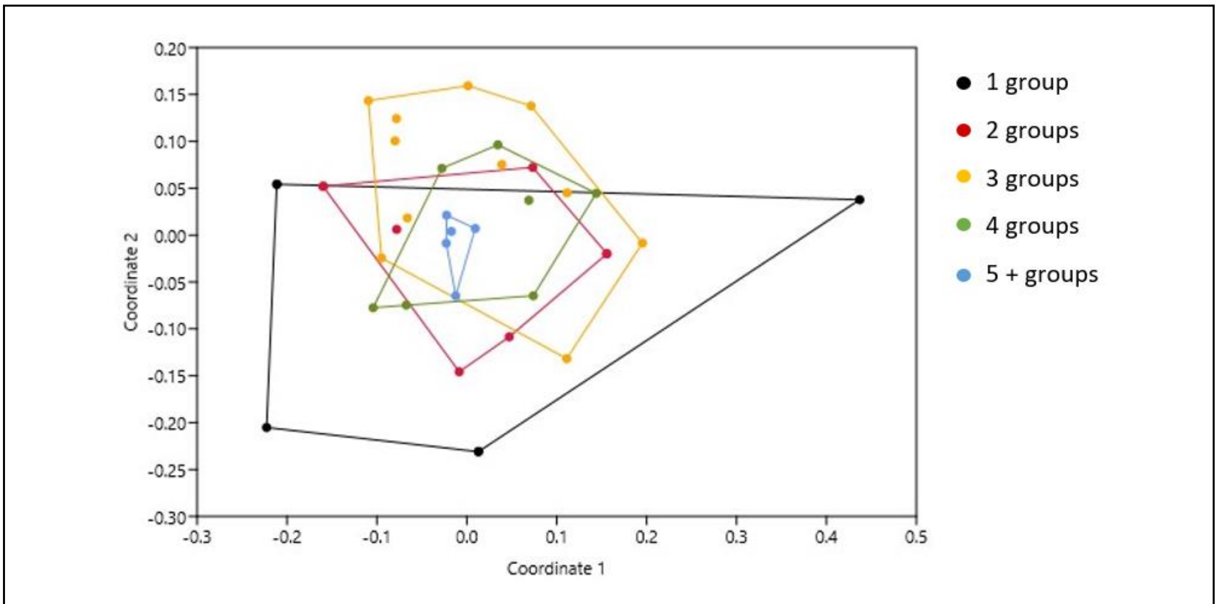

Figure 8: NMDS of the different faunal community compositions for each sample in comparison to the richness of the samples (indicated by colours).

3.2 Relationships between faunal groups and environmental factors

Environmental factors that were taken into account in the statistical analyses included the elevation (m a.s.l.), the depth of the well (m), the depth of the groundwater table (m), the height of the watercolumn (m) and the distance to the closest surface water (m) of each sampling site, as well as the temperature (°C), the pH, the electrical conductivity (µS/cm) and the concentration of dissolved oxygen (mg/L) in the groundwater. The parameters revealed a negative relationship between temperature and elevation (Bonferroni-corrected $p = < 0.0001$, $r = -0.7539$, $r^2 = 0.5684$, $df = 43$). The diversity had a weak, negative correlation with the depth of the groundwater table ($p = 0.0322$, $r_S = -0.3199$, $r^2 = 0.1023$, $df = 43$) and the well depth ($p = 0.0229$, $r_S = -0.3385$, $r^2 = 0.1146$, $df = 43$)(Figure 9). Although there was no correlation found between the occurrence of different faunal groups and the distance to surface water, 5 or more groups were exclusively found in wells which were less than 2 km away from the river Mur or its tributaries. Furthermore, for 50 % of the uninhabited wells the connection to surface water was assumed to be minimal.

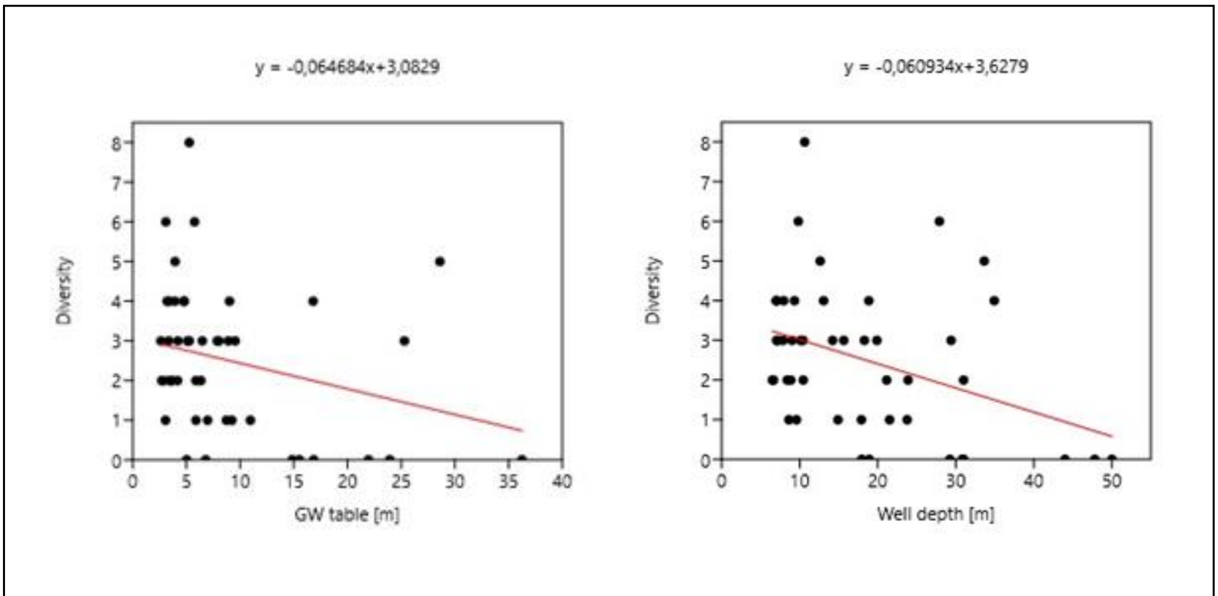

Figure 9: Diversity (Number of faunal groups) in relationship to the depth of the groundwater table (m) [GLM results: p (slope=0) = 0.0181, $b_1 = -0.0647 \pm 0.0227$, $b_0 = 3.0829 \pm 0.3488$, Log likelihood = -33.554, G = 5.5912] and the well depth (m) [p (slope=0) = 0.0071, $b_1 = -0.0609 \pm 0.0226$, $b_0 = 3.6279 \pm 0.4886$, Log likelihood = -21.5, G = 7.2562].

Chemical parameters that were considered are concentrations of sodium (mg/L), potassium (mg/L), calcium (mg/L), magnesium (mg/L), chloride (mg/L), sulphate (mg/L), phosphate (µg/L), ammonium (µg/L), nitrite (µg/L), and nitrate (mg/L). Moreover, concentrations of dissolved organic carbon (mg/L) and dissolved inorganic carbon (mg/L) were taken into account. None of these parameters showed unusual associations to each other and there was no relationship between either one of the measurements and the faunal diversity of the samples. However, it was noted that samples in which more DOC was detected respectively contained less O_2. Moreover, the electrical conductivity was lower in groundwater from wells which were closer to surface water, than wells that were more distant.

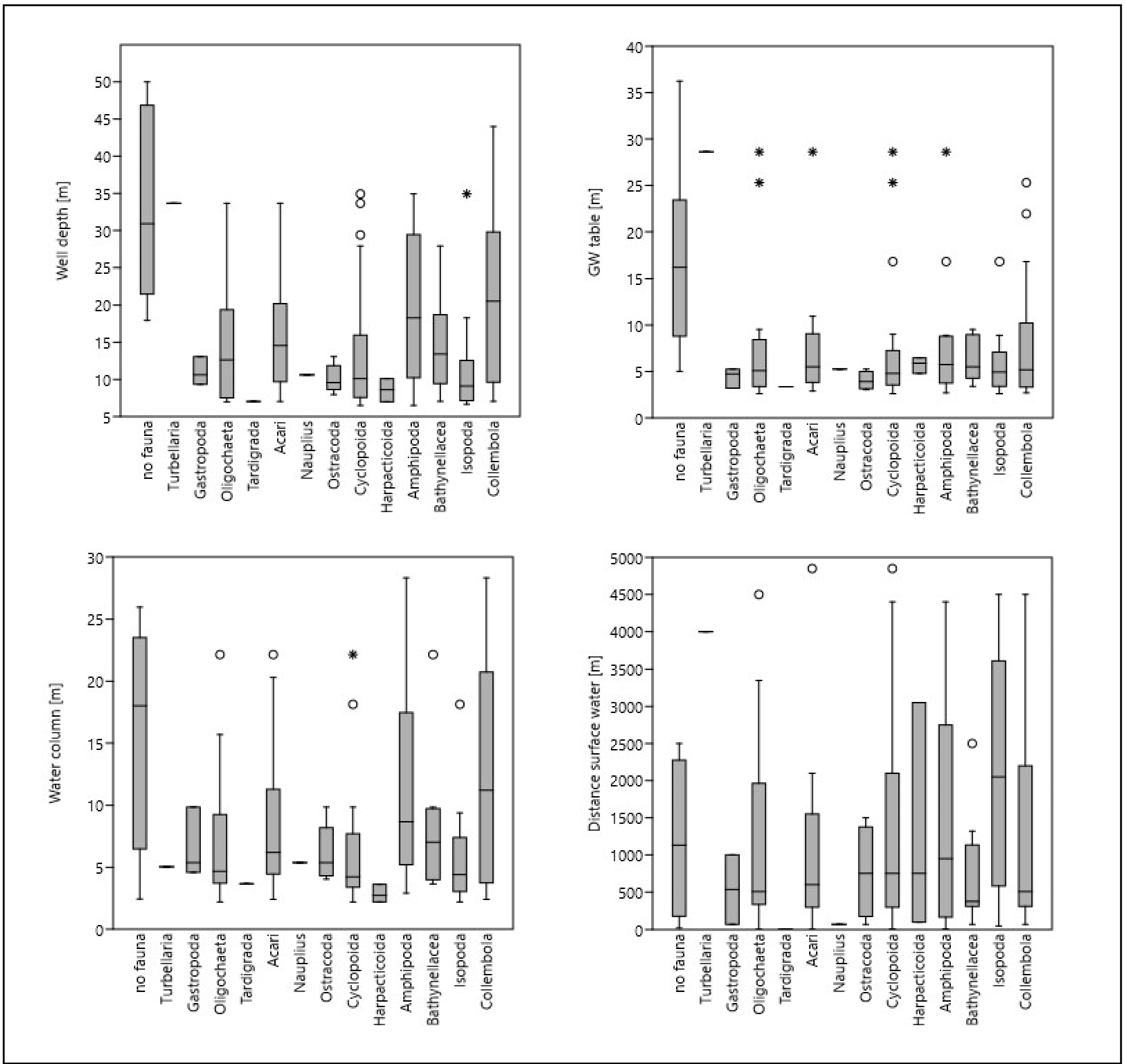

Figure 10: Differences in well depth (m) [ANOVA results: between groups: SS = 3.09, MS = 0.31, df = 10, within groups: SS = 9.37, MS = 0.09, df = 109, F = 3.59, p = 0.0004], groundwater table depth (GW table in m) [ANOVA results: between groups: SS = 3.41, MS = 0.34, df = 10, within groups: SS = 18.19, MS = 0.17, df = 109, F = 2.04, p = 0.0358], water column depth (m) and distance to surface water in relation to the presence/absence of fauna in general and specifically Turbellaria, Gastropoda, Oligochaeta, Tardigrada, Acari, Nauplius-Larvae, Ostracoda, Cyclopoida, Harpacticoida, Amphipoda, Bathynellacea, Isopoda and Collembola.

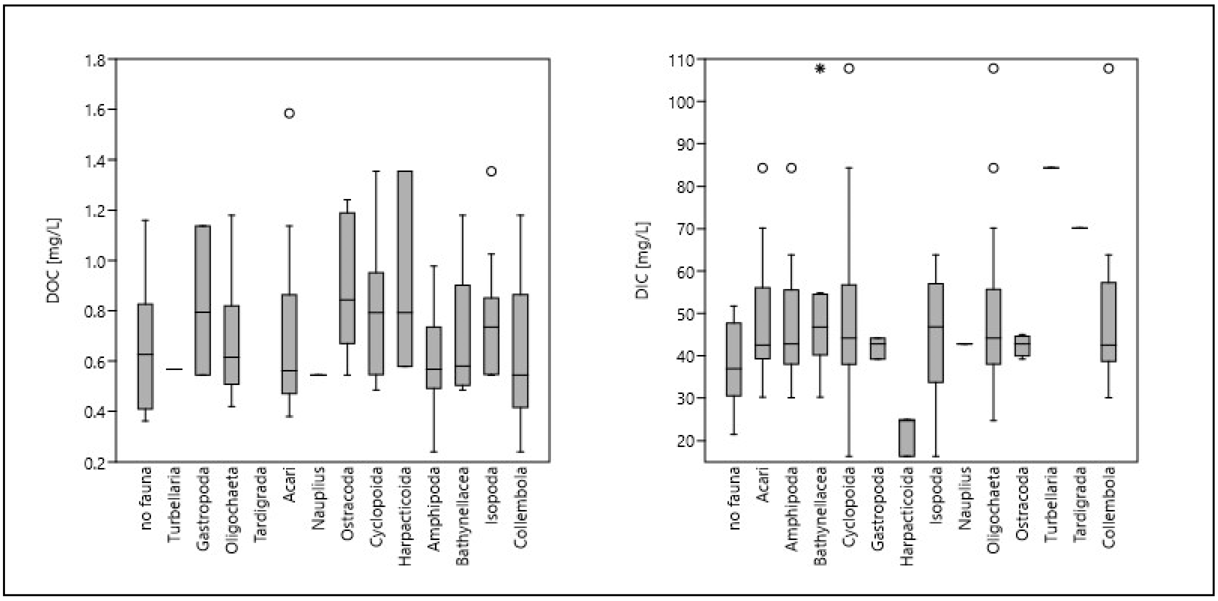

Figure 11: Incidence of Turbellaria, Gastropoda, Oligochaeta, Tardigrada, Acari, Nauplius-Larvae, Ostracoda, Cyclopoida, Harpacticoida, Amphipoda, Bathynellacea, Isopoda and Collembola as well as samples without fauna and their link to concentrations of dissolved organic carbon (DOC in mg/L) and dissolved inorganic carbon (DIC in mg/L) [ANOVA results: between groups: SS = 1.35, MS = 0.14, df = 10, within groups: SS = 7.47, MS = 0.07, df = 109, F = 1.98, p = 0.0428].

Comparing the environmental factors to the occurrence of the specific faunal groups, it was shown that Samples without fauna were mostly collected at sample sites with deeper wells (p = 0.0096, ANOVA) and deeper groundwater tables (p = 0.0297, ANOVA) than most of the wells where fauna was found. The majority of nearly all faunal groups was found at well depths of 5 – 20 m, groundwater table-depths of 2 – 10 m and water column depths of 2 – 12 m, besides Amphipoda and Collembola which were also found more frequently in deeper wells and deeper water columns. Regarding the distance of wells to surface water, no distinct differences in the distribution patterns of fauna could be found, however it should be noted that the data set was incomplete (Figure 10 & Table 3).

The majority of the observed animals were found in groundwater that had a temperature between 10 and 13 degrees Celsius. Gastropoda, Harpacticoida and Isopoda were only found in wells which had a minimum water temperature of 11 °C. The samples that did not contain fauna were not confined to a specific temperature span. Harpacticoida were the only faunal group (besides one Turbellaria) that were exclusively sampled from groundwater that had a pH below 7 (range 6.2 – 6.9) and differed therefore significantly from the groups Acari, Amphipoda, Bathynellacea and Collembola (p = 0.0208, ANOVA). All other groups (including samples without fauna) mainly were located in wells with a pH between 7 and 7.5. The range of electrical conductivity of the groundwater in which most of the macro- and meiofauna was found was 500 – 800 µS/cm. However, the samples that contained Gastropoda showed a narrower range between 500 and 600 µS/cm, and the Harpacticoida were taken from water of an electrical conductivity between 200 and 600 µS/cm. In the samples without fauna, the EC was between 200 and 900. Cyclopoida and Oligochaeta were equally distributed in wells with concentrations of dissolved oxygen between about 100 and 9000 mg/L. The majority of Acari, Amphipoda, Bathynellacea, Harpacticoida, Isopoda and Collembola were present in groundwater with O_2 concentrations above 4000 mg/L. Gastropoda occurred in rather oxygen-deficient sample sites and Ostracoda showed a distribution to oppositional sites, being present in sites with either respectively high or low oxygen concentrations. The samples containing spare fauna showed no pattern regarding the oxygen concentrations (Figure 12 & Table 3).

The samples showed no significant differences for the concentrations of DOC in the groundwater in regard to the establishment of fauna (except one Tardigrada). The majority of samples showed DOC levels between 0.4 and 1.2 mg/L (including samples without fauna). Gastropoda and Ostracoda were only found in samples of DIC concentrations between 40 and 45 mg/L. Harpacticoida occurred in wells with significantly lower DIC levels between 15 and 25 mg/L ($p = 0.0151$, ANOVA), while the majority of the remaining faunal groups showed a DIC range of 25 – 70 mg/L (Figure 11 & Table 4).

All sampled wells had phosphate concentrations below 0.5 mg/L and the majority of fauna was found in wells with concentrations below 0.2 mg/L. The values of ammonium were mostly below 15 µg/L in samples where Acari, Amphipoda, Bathynellacea, Cyclopoida, Collembola, Nauplii and one Turbellaria were found and in the samples in which no fauna was present. Regarding Gastropoda, Harpacticoida, Isopoda, Oligochaeta, Ostracoda and outlying Cyclopoida, there also were samples in which the ammonium concentration was up to 35 µg/L high. Single outlying samples with values between 100 and 650 µg/L were not considered. In virtually all observed wells the measured nitrite concentrations were below 4 µg/L. Few outliers showed values around 18 µg/L. The concentrations of nitrate generally were highly variable, showing no clear distinctive patterns regarding the faunal groups. The samples without fauna had rather low values (below 20 mg/L) and Gastropoda occurred only in wells with a low, narrow range between 5 and 10 mg/L. Harpacticoida and Isopoda were more frequent in groundwater of relatively high nitrate concentrations up to 60 mg/L. However, nearly all fauna was found in wells with nitrate concentrations below 50 mg/L (Figure 13 & Table 4).

For the majority of the faunal groups, the concentration of sodium lied between 5 and 30 mg/L. A narrower range was shown for Gastropoda and Ostracoda (7 – 17 mg/L). Samples without fauna were spread between minimum and maximum measured values. Potassium levels of 1 – 5 mg/L were linked to the appearance probability of all fauna groups, disregarding one Turbellaria and outliers. In samples where no fauna was detected, the potassium concentrations were comparatively higher (up to 14 mg/L). Harpacticoida occurred in groundwater that had calcium concentrations around 40 mg/L, while the rest of the fauna was found at calcium rates of about 50 to 110 mg/L. No fauna was primarily found below calcium concentrations of 90 mg/L. Regarding the concentrations of magnesium, Harpacticoida appeared in a very narrow range between 10 and 11 mg/L, as well as Gastropoda which were found in wells of 14 – 18 mg/L and Ostracoda (10 – 20 mg/L). The rest of the samples were in large parts describing Mg levels between 5 and 30 mg/L (including samples where no fauna was present). All samples were centered around chloride concentrations of 30 mg/L, from Gastopoda and Ostracoda having a narrower range (20 – 40 mg/L) to Bathynellacea and Oligochaeta having a wider range (0 – 100 mg/L) . The samples without fauna varied highly with minimum chloride concentrations of 7 mg/L up to maximum concentrations of 200 mg/L. The faunal distribution showed no relationship to measured concentrations of sulphate. The incidence of animals was scattered in groundwater with values between 0 and 75 mg/L (Figure 14 & Table 4).

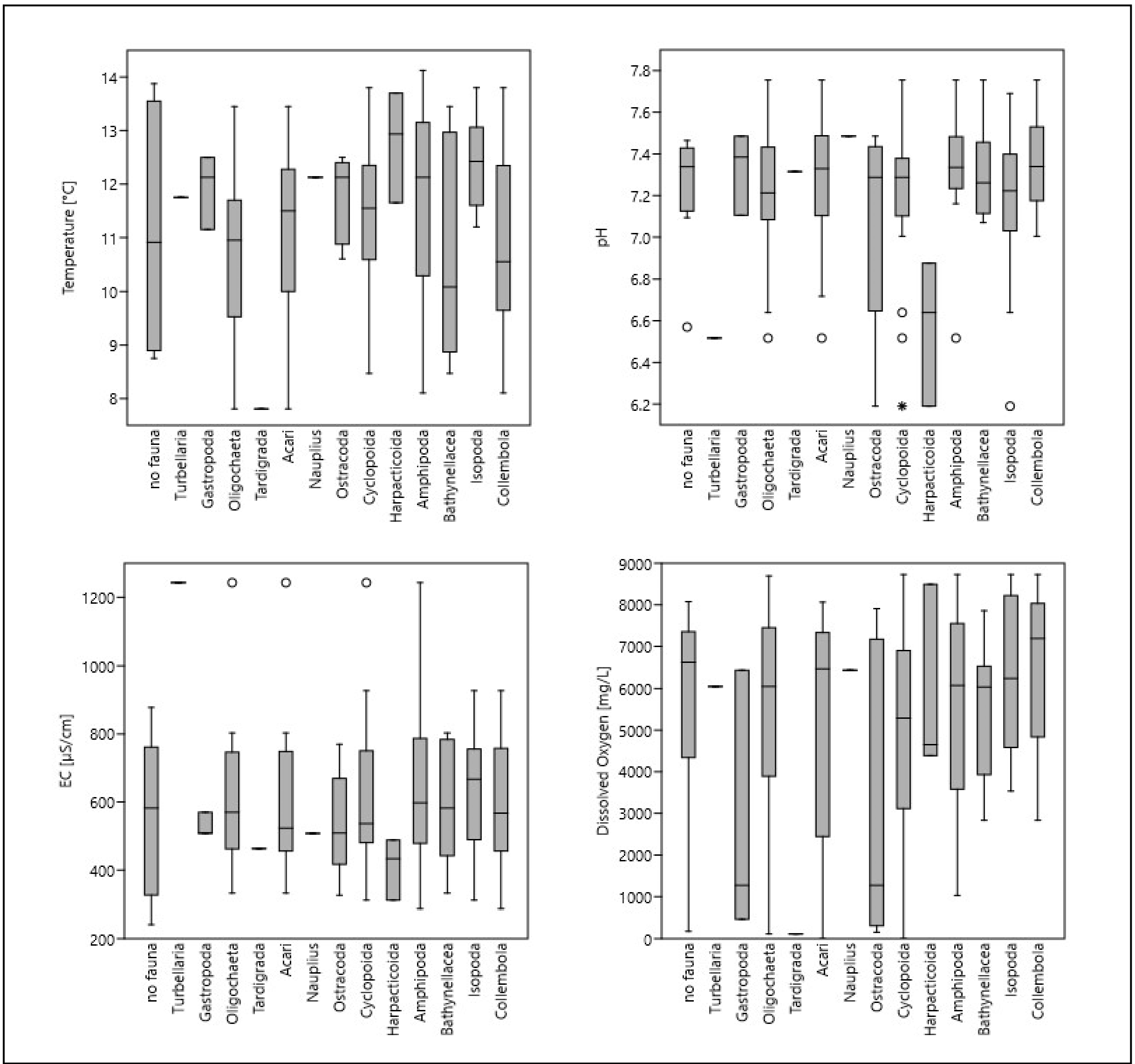

Figure 12: Uninhabited samples and samples inhabited by Turbellaria, Gastropoda, Oligochaeta, Tardigrada, Acari, Nauplius-Larvae, Ostracoda, Cyclopoida, Harpacticoida, Amphipoda, Bathynellacea, Isopoda and Collembola compared to the physico-hydrological parameters temperature (°C), pH [ANOVA results: between groups: SS = 0.02, MS = < 0.01, df = 10, within groups: SS = 0.11, MS = < 0.01, df = 109, F = 1.92, p = 0.0501], electrical conductivity (EC in µS/cm) and dissolved oxygen (mg/L).

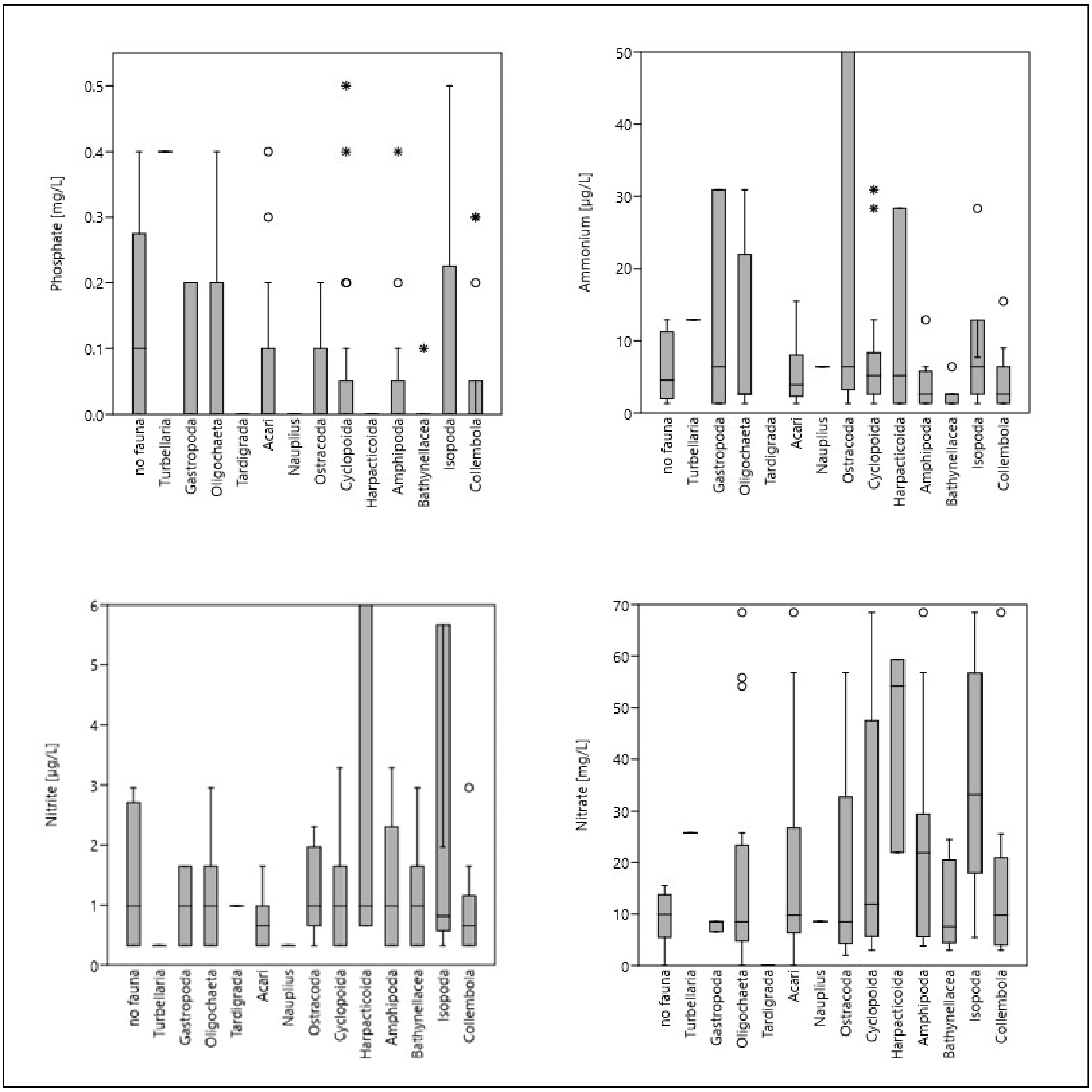

Figure 13: Incidence of Turbellaria, Gastropoda, Oligochaeta, Tardigrada, Acari, Nauplius-Larvae, Ostracoda, Cyclopoida, Harpacticoida, Amphipoda, Bathynellacea, Isopoda and Collembola as well as absence of fauna in relation to concentrations of the hydro-chemical parameters PO_4^{3-} (mg/L), NH_4^+ (µg/L), NO_2^- (µg/L) and NO_3^- (mg/L).

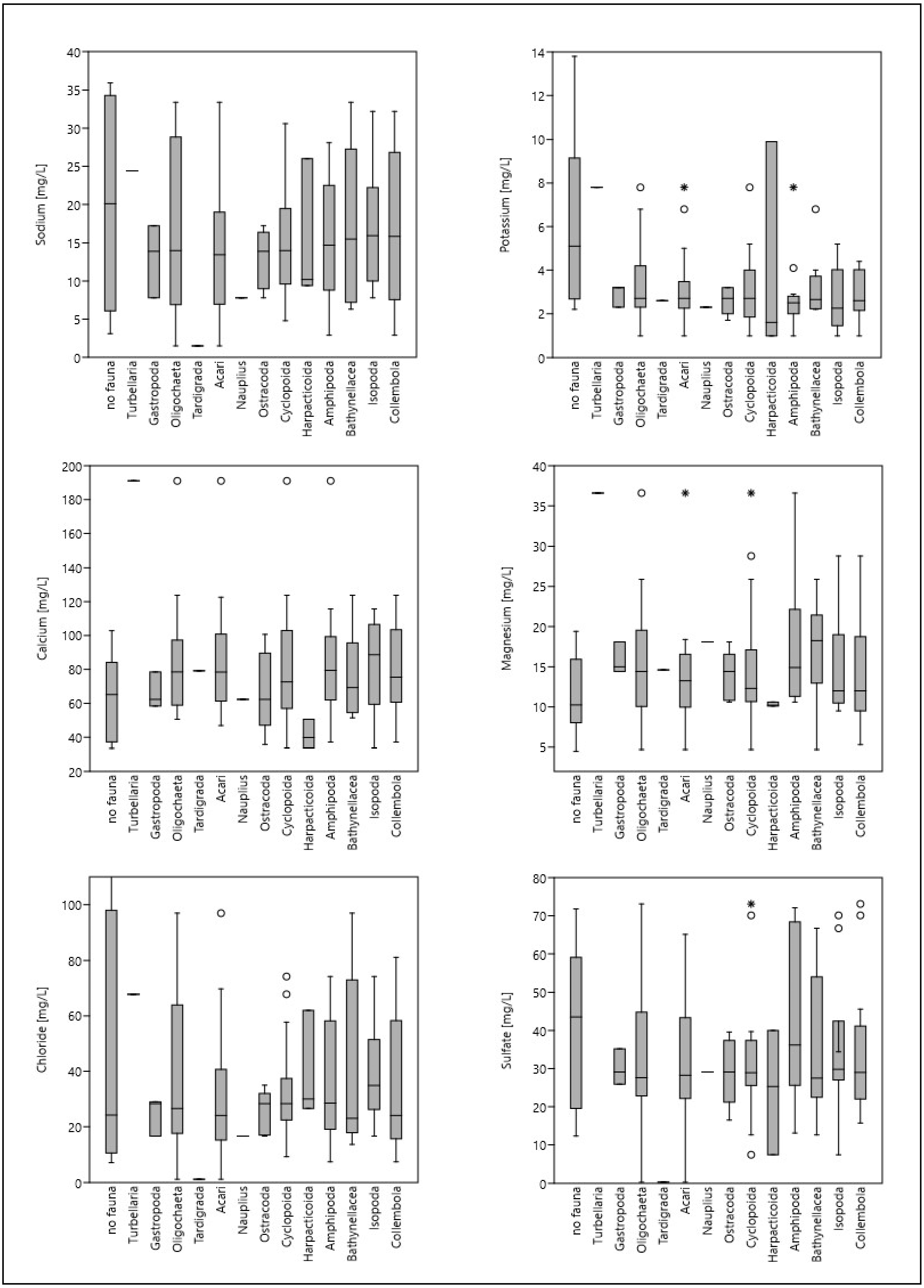

Figure 14: Concentrations of the major ions Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- and SO_4^{2-} (all in mg/L) in relationship to the presence/absence of fauna (Turbellaria, Gastropoda, Oligochaeta, Tardigrada, Acari, Nauplius-Larvae, Ostracoda, Cyclopoida, Harpacticoida, Amphipoda, Bathynellacea, Isopoda and Collembola) in the samples.

Table 3: Hydrophysical parameters of the sample sites, including the distance to surface water e.g. the river Mur or tributaries (Dist. SW), the well depth, the depth of the groundwater table (GW table), the water column depth (Wat. col.), the groundwater extraction depth (Extr. depth), the groundwater temperature (Temp.), the electrical conductivity (EC), the concentration of dissolved oxygen (O_2) and the pH.

Sample name	Dist. SW [m]	Well depth [m]	GW table [m]	Wat. col. [m]	Extr. depth [m]	Temp. [°C]	EC [µS/cm]	O_2 [mg/L]	pH
3	1000	9.33	4.74	4.59	7.04	12.50	509	1270	7.11
4	750	10.1	6.48	3.62	8.29	12.94	312	4650	6.19
5	3050	6.99	4.8	2.19	5.9	11.65	433.5	4385	6.64
6	10	6.5	3.59	2.91	5.05	10.55	488	1030	7.32
7	1600	9.82	3.08	6.74	6.45	11.45	597.5	8065	7.16
8	50	6.65	3.51	3.14	5.08	12.45	741.5	3535	7.29
9	3350	7.2	2.62	4.58	4.91	11.2	736	6405	7.21
10	1500	7.95	3.91	4.04	5.93	12.3	769.25	7910	7.29
11	500	9.58	3.05	6.53	6.32	10.6	326	156.5	6.19
15	4850	8.86	5.88	2.98	7.37	12.05	671	—	7.37
19	1320	14.22	9.54	4.68	11.88	13.45	803	6000	7.07
21	1600	18.9	14.86	4.05	16.88	13.88	877.8	6572	7.09
22	3500	23.76	6.97	16.79	15.37	13.05	469.5	3680	7.51
24	4400	34.93	16.8	18.13	25.87	13.8	927	8725	7.18
26	2500	44	21.95	22.05	32.98	13.25	618.5	7142.5	7.34
27	—	29.25	6.79	22.46	18.02	13.65	562.5	3947.5	7.45
28	—	13.06	3.2	9.86	8.13	11.15	570	465	7.38
31	2500	18.29	8.9	9.39	13.6	13.25	755.5	6075	7.37
34	—	15.64	7.86	7.78	11.75	12.93	697.5	7045	7.48
37	—	21.52	8.72	12.8	15.12	14.13	804.5	7190	7.34
38	1550	14.9	9.23	5.67	12.07	12.27	744.5	1160	7.34
45	4500	7.85	5.1	2.75	6.48	13	757	8695	7.24
49	100	8.62	5.89	2.73	7.26	13.7	488.33	8493.33	6.88
50	250	10.45	6.35	4.1	8.4	10.63	493	8475	7.52
51	70	10.63	5.26	5.37	7.95	12.13	508	6440	7.49
55	2100	10.43	8.03	2.4	9.23	11.55	760	7235	7.34
56	1050	8.4	4.17	4.23	6.29	12.4	536.5	5115	7.69
57	360	23.88	3.58	20.3	13.73	9.95	441.5	7625	7.64
58	—	17.9	10.95	6.95	14.43	10.95	366	6630	6.72
59	300	18.86	9.01	9.85	13.94	10.15	332.5	6570	7.1
60	—	30.82	16.84	13.98	23.83	10.73	241	7427.5	7.22
64	20	47.8	23.92	23.88	35.86	8.75	478.5	177	7.46
66	—	33.65	28.61	5.04	31.13	11.75	1243	6050	6.52
68	440	29.4	25.30	4.1	27.35	10.95	473.5	5290	7
71	—	50	36.24	13.76	43.12	11.1	602	8075	7.36
72	600	19.9	4.21	15.69	12.06	9.93	461	6505	7.68
74	120	21.15	2.9	18.25	12.03	10.15	537.5	8025	7.49
80	350	27.9	5.75	22.15	16.83	10.02	425	2835	7.76
81	400	12.62	3.94	8.68	8.28	9.12	494	5535	7.31
83	200	31	2.7	28.3	16.85	8.1	287	3480	7.41
86	5	7.03	3.36	3.67	5.2	7.81	463	113	7.32
87	—	17.95	15.52	2.43	16.74	9.32	809	6690	6.57
90	350	7.05	3.4	3.65	5.23	8.47	793	3395	7.21
92	570	9.02	5.26	3.76	7.14	8.79	656	7860	7.16
94	660	31	5.02	25.98	18.01	8.75	276	5525	7.34

Table 4: Hydrochemical composition of the 45 groundwater samples, including concentrations of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), orthophosphate (PO_4^{3-}), ammonium (NH_4^+), nitrate (NO_2^-), nitrate (NO_3^-), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-) and sulphate (SO_4^{2-}).

Sample name	DIC [mg/L]	DOC [mg/L]	NO_3^- [mg/L]	NO_2^- [µg/L]	NH_4^+ [µg/L]	PO_4^{3-} [mg/L]	SO_4^{2-} [mg/L]	Cl^- [mg/L]	Na^+ [mg/L]	K^+ [mg/L]	Ca^{2+} [mg/L]	Mg^{2+} [mg/L]
3	39.21	1.14	6.57	0.99	1.29	0.018	25.9	29.0	17.2	3.2	58.4	15.0
4	16.35	1.36	59.40	18.40	5.15	0.006	7.4	30.0	9.4	1.0	33.8	10.1
5	24.65	0.79	54.16	0.66	28.34	0.025	25.3	26.6	10.2	1.6	50.6	10.6
6	40.14	0.98	21.91	3.29	2.58	0.012	13.1	22.4	9.8	1.2	68.9	12.0
7	36.71	0.69	68.47	16.75	6.44	0.012	27.6	28.5	14.0	1.0	79.4	11.7
8	58.62	1.03	40.76	0.33	7.73	0.509	33.7	39.8	17.9	4.0	103.3	13.6
9	54.50	0.79	55.86	1.97	646.58	0.049	29.5	43.1	21.0	5.2	107.9	10.7
10	44.99	0.84	56.81	0.99	5.15	0.009	39.6	35.0	15.6	2.7	100.7	10.6
11	40.68	1.24	1.99	2.30	204.79	0.006	16.5	17.4	10.2	1.7	35.7	11.0
15	50.71	0.62	40.06	0.66	2.58	0.009	25.8	33.3	15.3	2.1	101.4	11.6
19	44.12	0.61	19.32	0.99	2.58	0.129	62.4	96.9	33.4	6.8	88.5	18.4
21	51.73	0.85	13.97	18.40	12.88	0.061	57.0	115.1	35.9	13.8	102.9	19.4
22	36.17	0.62	5.74	2.96	1.29	0.015	36.2	16.5	14.7	2.9	61.6	10.7
24	63.85	0.54	25.52	0.99	1.29	0.009	70.1	74.2	25.9	4.1	115.7	28.8
26	48.95	0.51	10.15	0.99	3.86	0.230	45.6	26.7	17.7	2.6	82.3	17.1
27	34.07	0.74	13.16	0.33	6.44	0.365	41.5	46.4	29.5	4.6	60.9	11.1
28	44.17	0.79	8.51	1.64	30.91	0.218	35.2	28.3	13.9	3.2	78.5	14.4
31	49.45	0.78	24.52	1.64	2.58	0.031	66.7	48.6	20.2	2.2	98.0	21.7
34	41.91	0.42	5.93	0.33	2.58	0.227	54.4	69.7	28.1	2.5	83.4	17.9
37	56.23	0.58	33.04	1.31	2.58	0.083	72.1	46.5	20.6	2.5	92.1	22.6
38	64.06	1.58	12.84	0.33	7.73	0.055	65.1	15.7	12.4	1.1	122.6	14.2
45	56.46	0.60	21.06	0.66	2.58	0.254	30.1	60.1	32.2	2.4	106.1	9.5
49	24.86	0.58	21.98	0.99	1.29	0.012	40.0	62.0	26.0	9.9	39.8	10.2
50	45.88	0.55	5.87	0.99	2.58	0.009	25.8	9.2	4.8	1.9	62.2	21.4
51	42.77	0.54	8.60	0.33	6.44	0.037	29.1	16.6	7.8	2.3	62.3	18.1
55	59.76	0.92	11.92	1.64	9.02	0.037	39.7	57.7	25.8	2.0	102.6	16.1
56	44.17	0.55	5.50	0.66	6.44	0.233	34.4	25.0	12.0	1.8	72.6	12.3
57	39.29	0.38	8.66	0.33	2.58	0.037	15.7	11.7	6.0	3.0	70.7	7.4
58	32.50	0.55	29.58	0.66	1.29	0.015	23.1	21.4	8.2	5.0	46.9	7.8
59	30.19	0.52	6.52	0.33	1.29	0.046	12.6	13.6	7.0	2.9	51.4	4.7
60	21.53	0.37	6.18	0.33	5.15	0.297	12.3	8.9	5.2	2.9	33.5	4.5
64	36.04	1.16	0.03	1.97	182.90	0.144	59.9	15.3	8.7	5.6	69.4	7.7
66	84.37	0.57	25.77	0.33	12.88	0.414	256.7	67.8	24.4	7.8	191.1	36.6
68	32.79	0.84	3.53	0.33	2.58	0.018	73.1	22.9	30.6	4.4	55.5	5.3
71	44.01	0.62	9.73	0.99	1.29	0.009	71.8	21.7	22.5	5.7	84.7	9.4
72	40.23	0.43	9.88	0.33	15.46	0.258	19.3	12.8	6.8	3.1	73.1	9.5
74	42.30	0.38	9.66	0.66	5.15	0.040	38.6	24.4	13.6	4.3	77.7	10.1
80	39.32	0.50	4.06	0.99	1.29	0.003	28.9	23.7	13.3	2.6	51.9	12.3
81	54.85	0.48	5.46	1.64	2.58	0	23.6	21.6	6.3	2.7	70.6	14.9
83	30.05	0.24	3.80	0.33	1.29	0.015	21.8	7.5	2.9	1.8	37.1	10.9
86	70.17	4.37	0.02	0.99	543.54	0.003	0.3	1.2	1.5	2.6	79.2	14.6
87	37.85	0.64	15.53	2.96	3.86	0.015	19.3	199.7	84.6	10.3	37.2	12.4
90	107.80	1.18	2.95	0.33	2.58	0.009	26.1	22.4	17.7	4.0	123.8	25.9
92	53.48	0.94	20.92	2.96	1.29	0.006	22.1	81.1	29.6	2.2	68.0	20.7
94	29.30	0.36	5.24	0.33	1.29	0.018	20.3	7.2	3.1	2.2	37.2	9.0

3.3 Fauna distribution with regard to land use types

The 45 sample sites have been categorised into three land use types named ‘Urban areas’ (Urban), ‘Agricultural use’ (Agricultural) and ‘Forests and semi-natural areas’ (Natural). The category Urban includes 12 samples, Agricultural involves with 28 sites the most samples and the smallest category Natural covers five wells. The sites where no fauna was found were either classified as Urban (4 samples) or Agricultural (4 samples), the wells of the land use type Natural were all populated. Therefore, nearly 33 % of the Urban sample sites did not contain any macro- or meiofauna, while in the Agricultural wells only about 14 % were not inhabited (Figure 16). The composition of the faunal communities regarding the land use types differed slightly. In the Natural sites, Cyclopoida were the most present group, followed by Isopoda and Oligochaeta. Thereafter, all other groups were equally common. Turbellaria, Nauplius-larvae and Tardigrada were not found in Natural wells. The Agricultural samples contained foremost Acari and Cyclopoida. Oligochaeta and Collembola occurred also very frequently, followed by Amphipoda, Bathynellacea and Isopoda. Less prevalent in the Agricultural wells were Ostracoda, Gastropoda, Harpacticoida and Nauplii. With one single, additional Tardigrada, Agricultural is the land use type that represents the most different faunal groups. At the Urban sites, the group that most occurred were Amphipoda. Also frequent were Acari, Cyclopoida, Isopoda and Oligochaeta, followed by Bathynellacea and Collembola. Less common groups in the Urban land use type included Harpacticoida, Turbellaria and Ostracoda, while Nauplii and Tardigrada were not present at all (Figure 15).

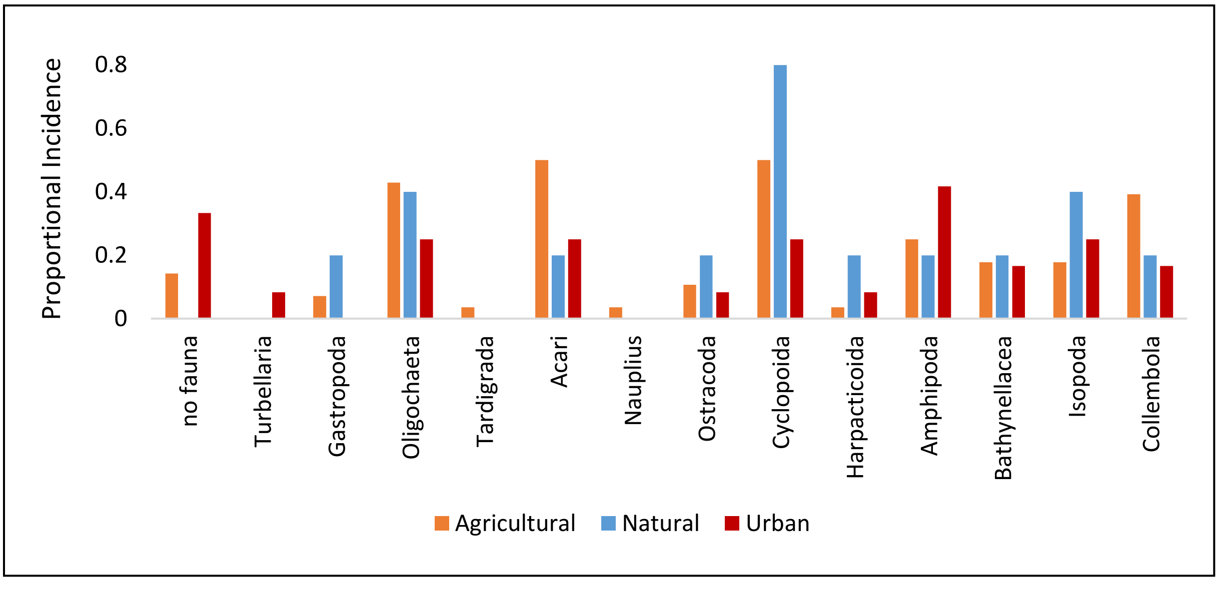

Figure 15: Distribution of fauna (Turbellaria, Gastropoda, Oligochaeta, Tardigrada, Acari, Nauplius-Larvae, Ostracoda, Cyclopoida, Harpacticoida, Amphipoda, Bathynellacea, Isopoda and Collembola) and occurrence of uninhabited wells relating to the three land use types Agricultural use (Agricultural), Urban areas (Urban) and Forests and semi-natural areas (Natural).

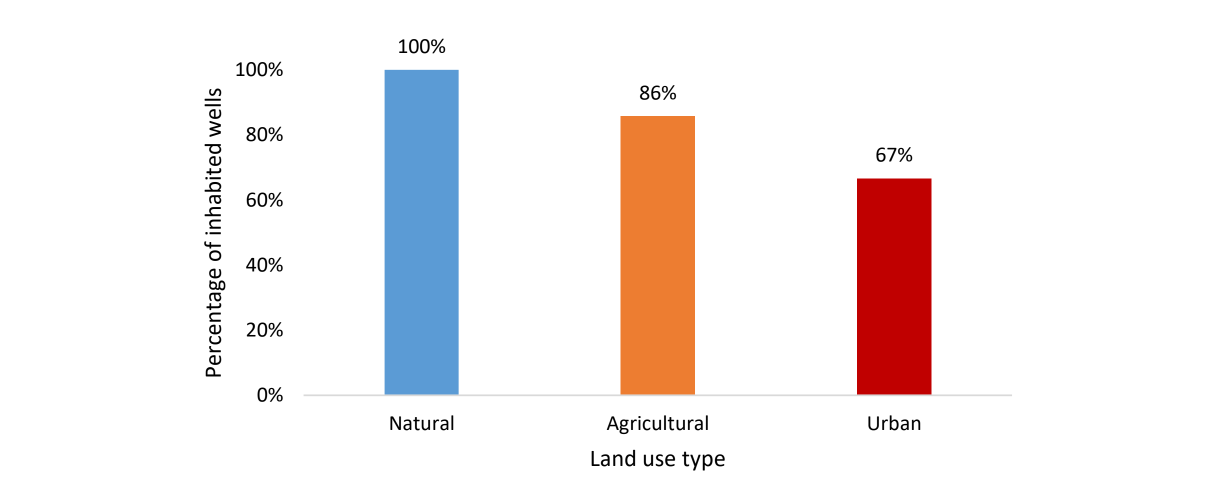

Figure 16: Portion of wells populated by groundwater fauna with respect to land use type (Natural, Agricultural and Urban).

The distribution pattern of the single fauna groups revealed, that Acari were more likely to occur in Agricultural areas and less likely to occur in Natural areas. Amphipoda were most commonly found in Urban wells and less common in Natural wells. The distribution of Bathynellacea differed insignificantly between land use types. Cyclopoida appeared mostly in Natural habitats and less in Urban areas, as well as Ostracoda and Gastropoda (the latter were not found in Urban wells at all). Harpacticoida and Isopoda were most commonly found in Natural wells and least present in Agricultural sites. Oligochaeta and Collembola occupied more likely Agricultural wells and less likely Urban wells. Tardigrada and Nauplius-larvae were only found in the Agricultural land use type and one Turbellaria was found in an Urban site (Figure 15).

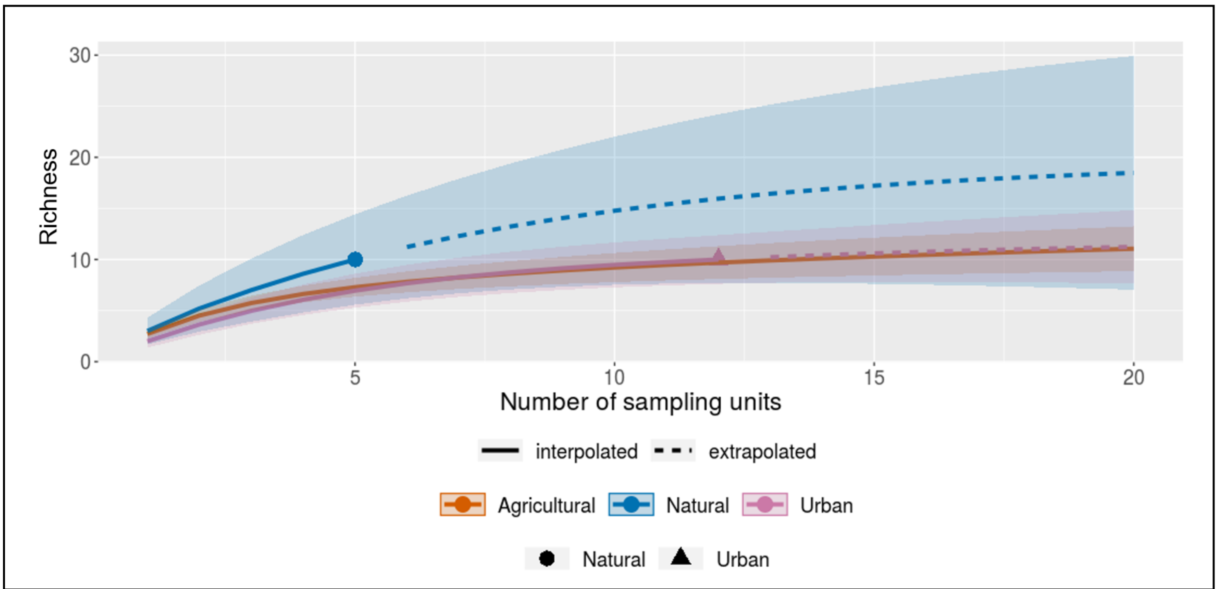

Figure 17: Combined rarefaction-extrapolation curve of richness (faunal group incidence) respective to sampling units, comparing the three land use types Agricultural use (Agricultural), Urban areas (Urban) and Forests and semi-natural areas (Natural).

Comparing the environmental parameters between the different land use types, it was shown, that the well depth in the Natural sites was significantly less deep than in the Agricultural ($p = 0.0201$, Tukey's pairwise) and Urban areas ($p = 0.0013$, Tukey's pairwise). Furthermore, the groundwater temperature was higher in Urban areas (difference Urban/Agricultural $p = 0.0013$, Tukey's pairwise) and in urban regions the wells appeared to be more distant to surface waters than in agricultural or natural areas. Further differences or distribution patterns between the land use types and the environmental factors or chemical parameters were not identified (Figure 19).

Based on the incidence data of the faunal groups, diversity was compared between the land use types, which showed, that the Natural sites indicated the highest diversity, although the more samples would be needed for a clear discrimination. Over all, the difference of diversity between the land use types (especially between Agricultural and Urban) is not vital (Figure 17). The distribution of the faunal communities did not show any patterns in relation to the three land use types.

The geographical positions of the wells categorized by land use types revealed the locations of Urban, Agricultural and Natural areas across the eight defined sub-regions. Sample sites categorized as Urban areas (e.g. the city of Graz) were situated at the Aichfeld, the Grazer Feld and the Lower Mur Valley. Natural areas could only be identified at the Lower Mur Valley and the Lungau, while wells of the category Agricultural areas were present in all eight aub-regions (Figure 18).

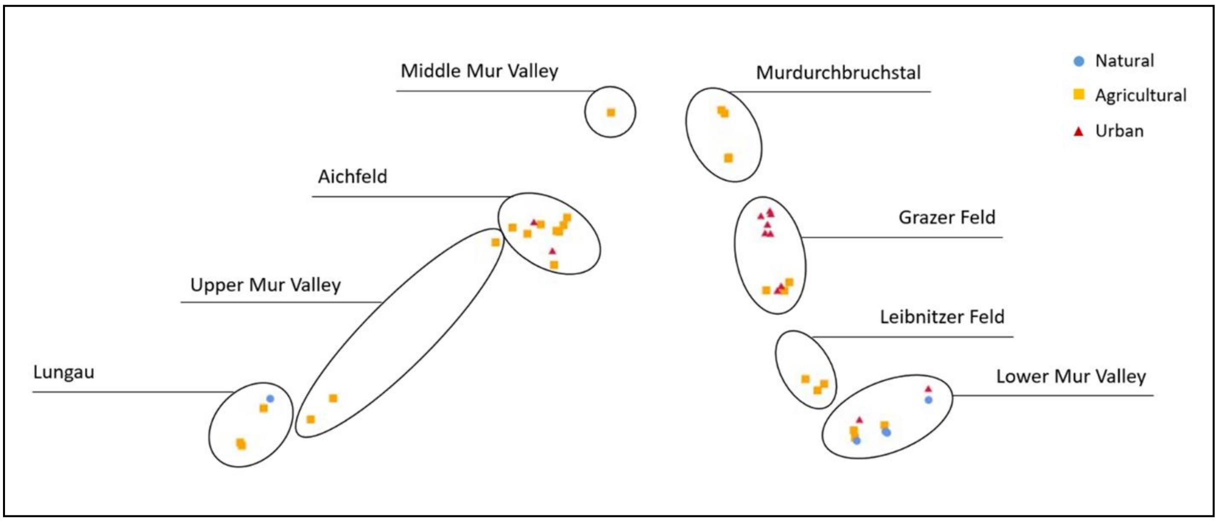

Figure 18: Geographical positions of the 45 sampling sites in relation to the eight sub-regions (Lungau, Upper Mur Valley, Aichfeld, Middle Mur Valley, Murdurchbruchstal, Grazer Feld, Leibnitzer Feld and Lower Mur Valley. Colour-labels indicate each land use type.

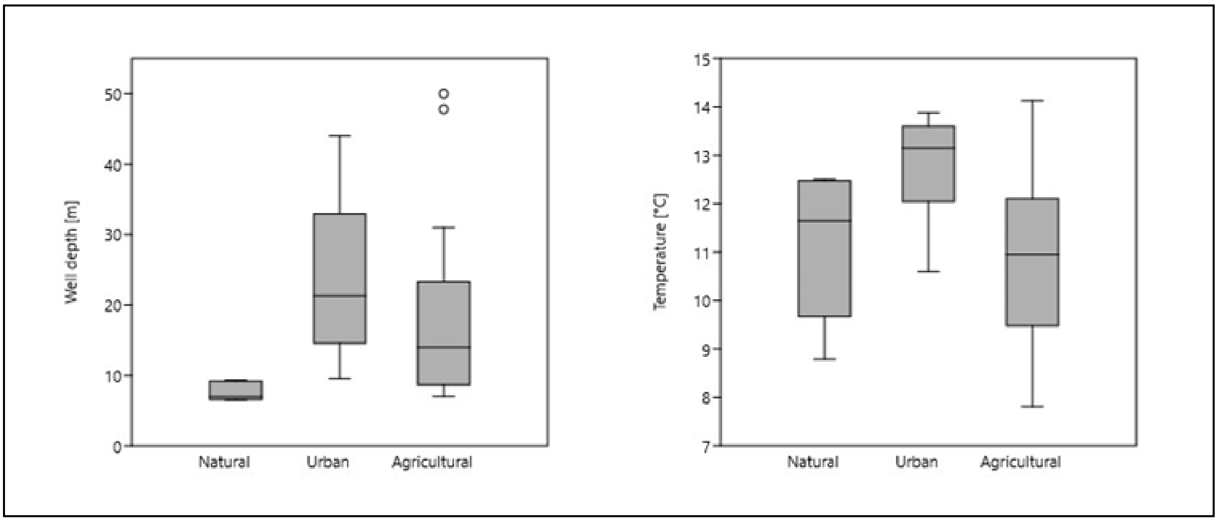

Figure 19: Comparison of the well depths (m) (ANOVA results: values between groups: SS = 0.82, MS = 0.41, df = 2, values within groups: SS = 2.38, MS = 0.06, df = 42, F = 7.202, p = 0.0020) and temperatures (°C) (ANOVA results: values between groups: SS = 33.90, MS = 15.95, df = 2, values within groups: SS = 97.68, MS = 2.33, df = 42, F = 7.29, p = 0.0019) between the three land use types (Natural, Agricultural and Urban).

The three land use types were differently influenced by domestic wastewater, shown via the municipal wastewater indicators approach (MWWI), which varied from the categories 0 (no influence) to 3 (influenced by municipal wastewater). All Natural sites were mostly not influenced by municipal wastewater or at least very unlikely to be influenced. The majority (nearly 80 %) of the Agricultural wells were not influenced or unlikely to be influenced by municipal wastewater. However, one fifth of the observed Agricultural sites were evaluated to be likely influenced or be influenced by municipal wastewater. The majority of the Urban wells scored a MWWI of 2 or 3. In less Urban sites (34 %) a MWWI of 0 or 1 was assessed (Figure 20).

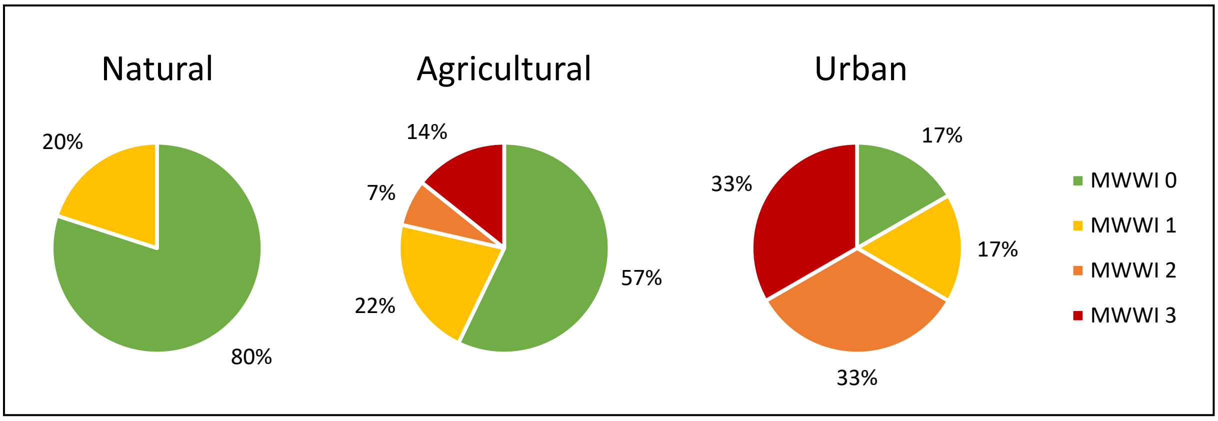

Figure 20: Portions of wells influenced by municipal waste water (MWWI 0 – 3) at Forests and semi-natural areas (Natural), Agricultural used areas (Agricultural) and Urban areas (Urban).

3.4 Fauna distribution patterns with respect to altitude (m a.s.l.)

Of 45 sampled wells, 25 (and therefore more than half) were lying between 200 and 400 m a.s.l., 5 wells were located between 400 and 600 m a.s.l., 9 sites were located between 600 and 800 m a.s.l. and 6 sampling sites were situated above 800 and up to 1049 m a.s.l. While all the samples of the category 400 – 600 contained fauna, in 12 % of the wells of the category 200 – 400 no fauna was found. In the class of 600 – 800, as well as in the category 800 – 1000 (+), about one third of the wells did not contain any macro- or meiofauna and therefore the wells in which no fauna was present were comparatively higher elevated than wells that were inhabited (Figure 21). The communal structures differed between the categories of elevation, especially since some faunal groups were only present in a single class. Most of the fauna in general was found below 600 m a.s.l., especially Gastropoda, Harpacticoida, Isopoda, Ostracoda and Nauplii were restricted to sites below 400 m a.s.l., while Acari, Amphipoda, Cyclopoida, Oligochaeta and Collembola were revealed to have had wider ranges. Bathynellacea on the other hand were mostly found in areas above 400 m a.s.l. The most common group between 200 and 400 m a.s.l. were Cyclopoida, followed by Amphipoda, Isopoda and Acari as well as Oligochaeta. Between 400 and 600 m a.s.l., Acari appeared most frequently, and Cyclopoida as well as Collembola also were well represented. Further equally common groups included Amphipoda, Bathynellacea, Isopoda and Oligochaeta. The most present groups in the elevation between 600 and 800 m a.s.l. were Acari and Oligochaeta, followed by Cyclopoida and Collembola. Also found in this category were Amphipoda, Bathynellacea and the only observed Turbellaria-individual. In wells above 800 m a.s.l., Oligochaeta and Collembola were found above all, and furthermore Bathynellacea, Acari, Amphipoda and Cyclopoida were present. A Tardigrada also was found exclusively in this category. Generally, the most different groups (11) were found between 200 and 400 m a.s.l., while all other categories included 7 different faunal groups (Figure 22).

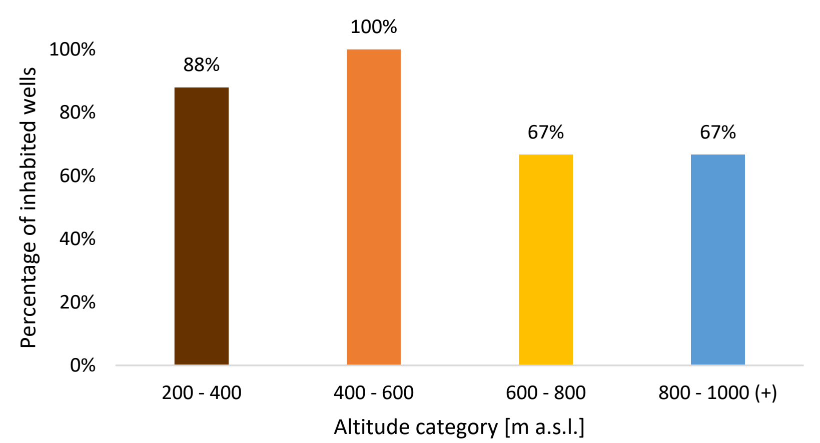

Figure 21: Portions of wells where groundwater fauna was found with regard to altitude (categories: 200 – 400 m a.s.l., 400 – 600 m a.s.l., 600 – 800 m a.s.l. and 800 – above 1000 m a.s.l.).

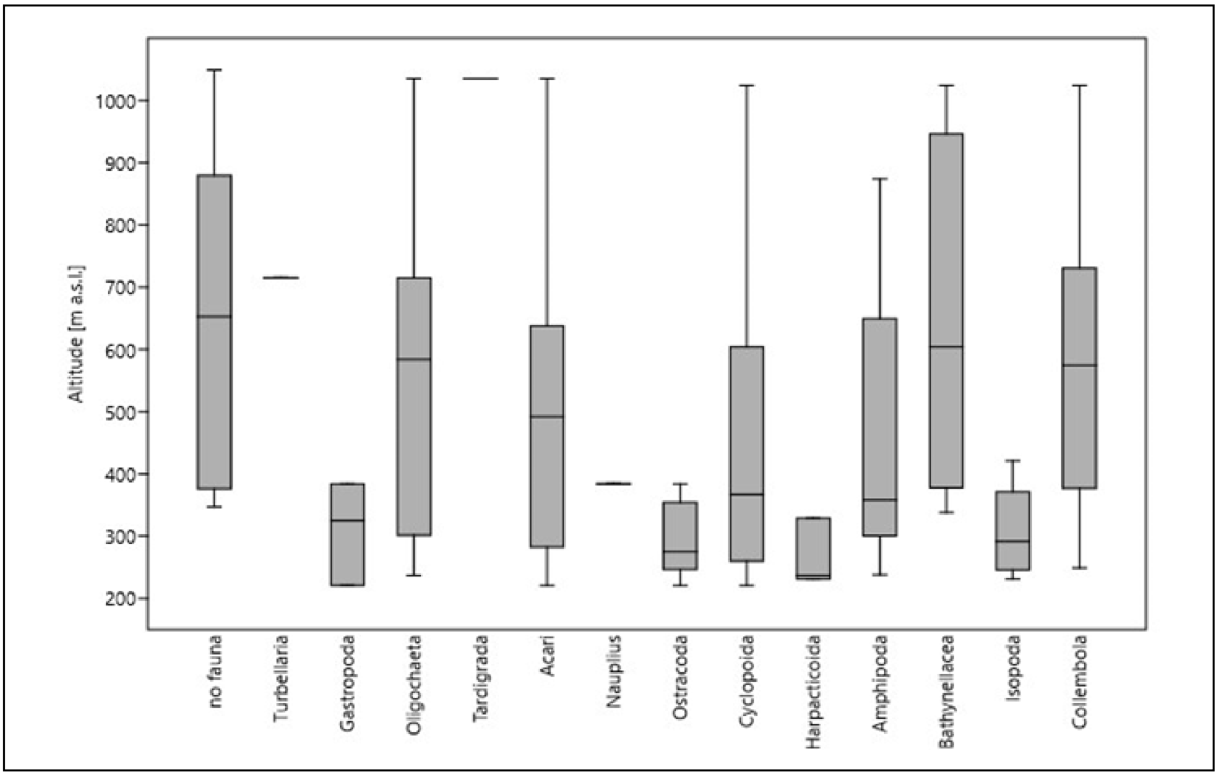

Figure 22: Occurrence of Turbellaria, Gastropoda, Oligochaeta, Tardigrada, Acari, Nauplius-Larvae, Ostracoda, Cyclopoida, Harpacticoida, Amphipoda, Bathynellacea, Isopoda and Collembola as well as samples without fauna in regard to altitude categories (m a.s.l.).

The incidence data of the particular faunal groups showed, that Acari as well as Cyclopoida were more commonly found between 400 and 600 m a.s.l. and less frequently observed in wells above 800 m a.s.l. Amphipoda were most likely to appear in the category 200 – 400 and least likely to occur in the class 800 – 1000(+), while this pattern was reversed regarding Bathynellacea. Isopoda were only found in lower areas (200 – 400 and less present at 400 – 600). Oligochaeta appeared mostly at 600 – 800 m a.s.l. and showed little occurrence at 400 – 600 m a.s.l. Collembola were most commonly observed between 400 and 600 m a.s.l. and less frequent in the category 200 – 400 (Figure 22).

The diversity did not differ significantly in regard to the elevation categories. Wells that were located between 200 and 400 m a.s.l. showed more differences in community structures, than the samples of other elevation categories (Figure 23).

Regarding the hydro-chemical and -physical parameters in relation to altitude, it was shown, that the groundwater temperature was significantly lower and the wells were closer to surface waters in higher elevations than in lower areas. Furthermore, nitrate concentrations were lower in wells that were located in higher altitudes, closer to surface water and delivered colder groundwater (Figure 24). Other correlations between altitude and environmental or chemical factors were not observed.

The groundwater from the wells above 800 m a.s.l. was not or unlikely influenced by municipal waste water. In the altitude category 600 – 800 m a.s.l., only 20 % of the wells were likely to be influenced by waste water. For 40 % of the wells of lower elevation between 200 and 600 m a.s.l., a MWWI of either 2 or 3 was evaluated, while 60 % were categorized to have a MWWI of 0 or 1 (Figure 25).

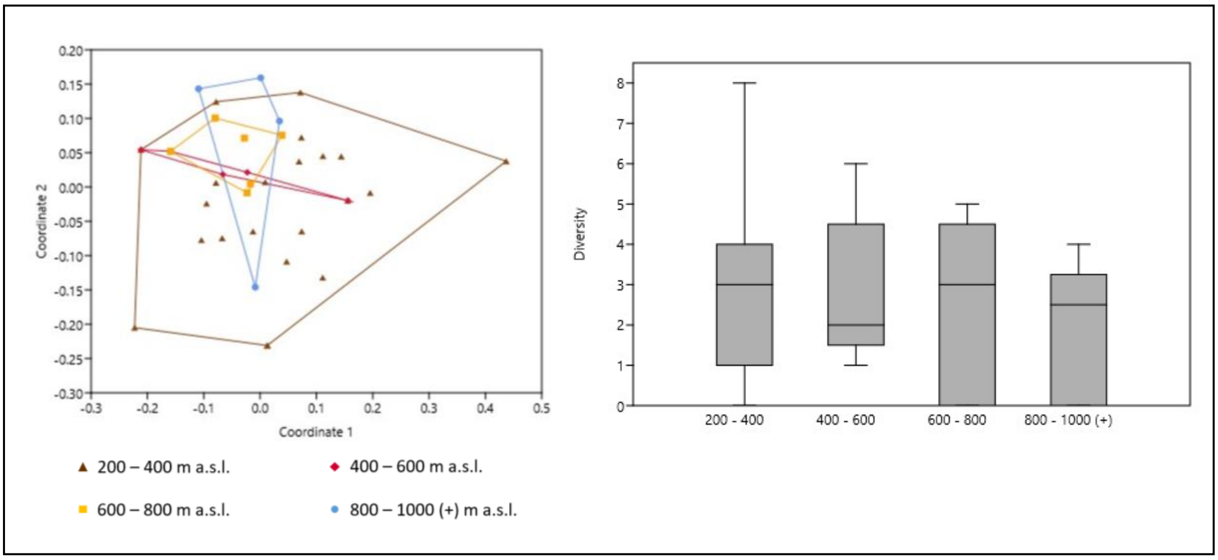

Figure 23: NMDS of the different fauna community structures of each sampling site in regard to altitude categories (Colour-coded: Brown = 200 – 400 m a.s.l., Red = 400 – 600 m a.s.l., Yellow = 600 – 800 m a.s.l. and Blue = 800 – 1000 (+) m a.s.l.) [left] and Boxplot of the comparison of diversity (fauna group incidence) between the elevation categories (in m a.s.l.) [right].

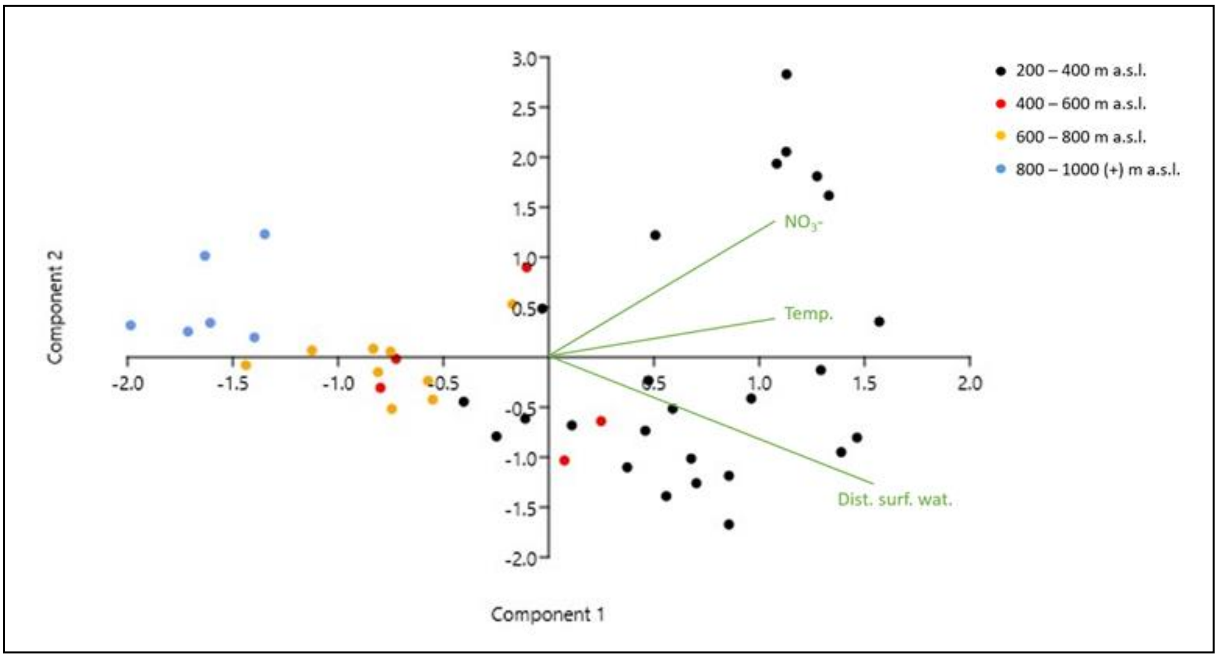

Figure 24: Principal Component Analysis of the 45 samples in relation to concentrations of Nitrate (NO_3^-), Temperature (Temp. in °C) and Distance to surface water (Dist. surf. wat. in m). Colour indicates the altitude categories. (PCA results: PC1 = Eigenvalue 1.91, Variance (%) 63.78. PC2 = Eigenvalue 0.70, Variance (%) 23.49. PC3: Eigenvalue 0.38, Variance (%) 12.73. Correlation-Matrix, Iterative imputation, 9999 Bootstrap).

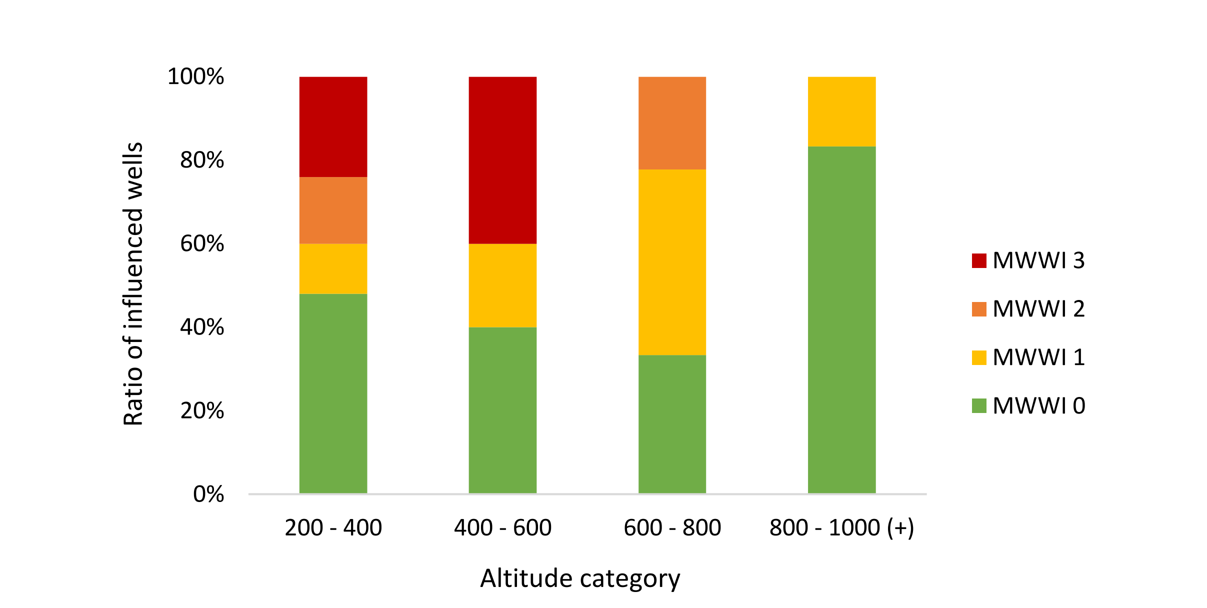

Figure 25: Percentages of samples influenced by municipal waste water (MWWI 0 – 3) categorized by altitude (200 – 400 m a.s.l., 400 – 600 m a.s.l., 600 – 800 m a.s.l. and 800 – 1050 m a.s.l.).

3.5 Faunal distribution across the eight sub-regions and connection with the MWWI

The observed wells were categorized into the eight sub-regions Lungau (4 sample sites), Upper Mur Valley (3 sample sites), Aichfeld (10 sample sites), Middle Mur Valley (1 sample site), Murdurchbruchstal (4 sample sites), Grazer Feld (11 sample sites), Leibnitzer Feld (3 sample sites) and Lower Mur Valley (9 sample sites), that were differentiated by dissimilar hydro-/geological groundwater body types. Wells that were not populated by any macro- or meiofauna were located at the sub-regions Lungau, Upper Mur Valley, Aichfeld and Grazer Feld (Figure 28).

Faunal groups that were present at all sub-regions included Acari, Cyclopoida, Oligochaeta (and Collembola). Amphipoda were found at all sub-regions besides the Lungau and the Leibnitzer Feld. Bathynellacea could only be discovered in areas upstream of the Grazer Feld, while Isopoda were only found at the Mudurchdruchstal and the sub-regions downstream from there. Gastropoda and Ostracoda were present at the Murdurchbruchstal, the Grazer Feld and the Lower Mur Valley, while Harpacticoida were observed only in the Grazer Feld and the Lower Mur Valley (Figure 26).

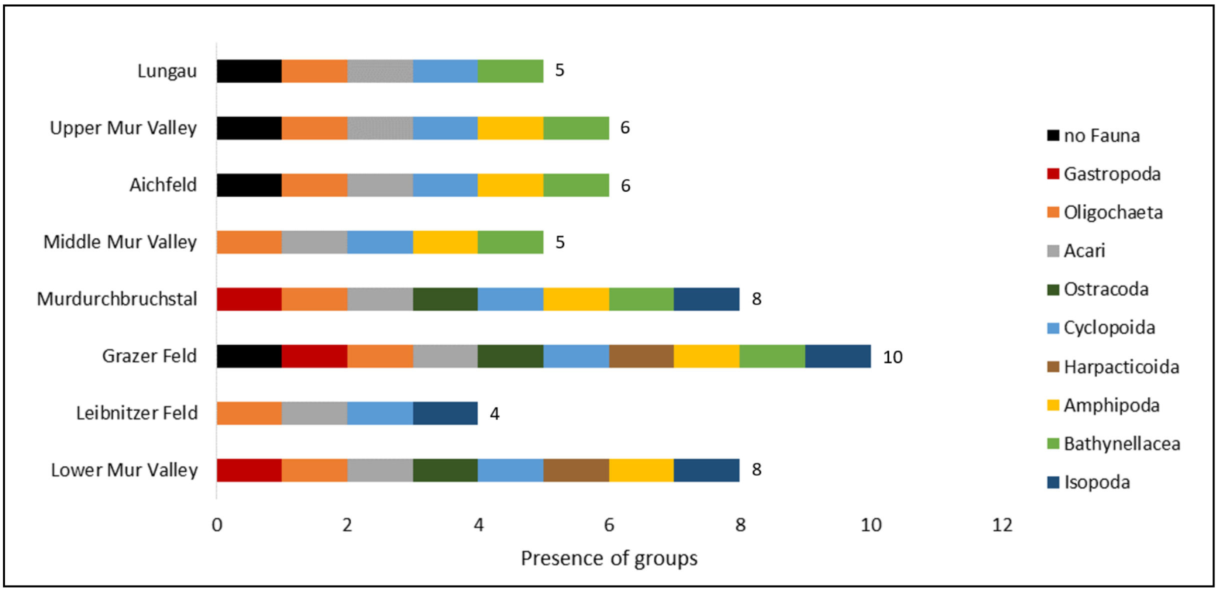

Figure 26: Absence/presence of faunal groups (Gastropoda, Oligochaeta, Acari, Ostracoda, Cyclopoida, Harpacticoida, Amphipoda, Bathynellacea, and Isopoda) in each sub-region of the Mur Valley (Lungau, Upper Mur Valley, Aichfeld, Middle Mur Valley, Murdurchbruchstal, Grazer Feld, Leibnitzer Feld and Lower Mur Valley).

The sample with a single Microturbellaria was situated at the Aichfeld, the well where Nauplii were found was located at the Murdurchbruchstal and the only Tardigrada individual was observed at the Lungau. The sub-regions where the most different faunal groups were present (10 groups) included the Grazer Feld and the Murdurchbruchstal, followed by the Lower Mur Valley (9 groups). At the Aichfeld, 7 groups were found and at the Lungau, the Upper Mur Valley and the Middle Mur Valley, 6 faunal groups were observed. The sub-region with the least faunal units was the Leibnitzer Feld (5 groups). The faunal richness in regard to the particular observed wells did not show any patterns compared to the structure of the sub-regions (Figure 27).

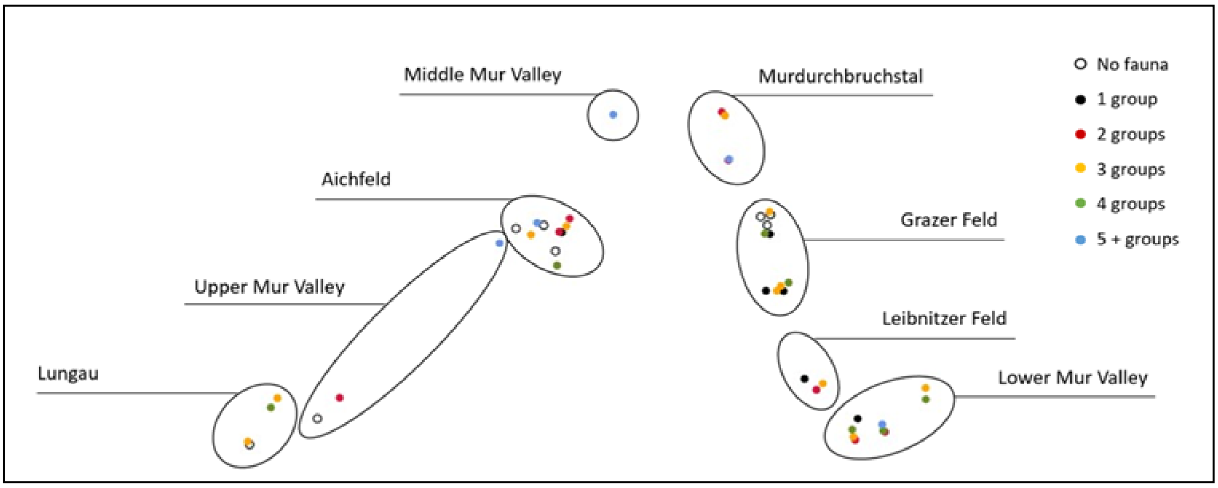

Figure 27: NMDS of the special distribution of the wells in the Mur Valley, differentiated into the eight sub-regions (Lungau, Upper Mur Valley, Aichfeld, Middle Mur Valley, Murdurchbruchstal, Grazer Feld, Leibnitzer Feld and Lower Mur Valley) and categorized by colour-coded richness-classes (from wells with no fauna found to wells with more than 5 observed faunal groups).

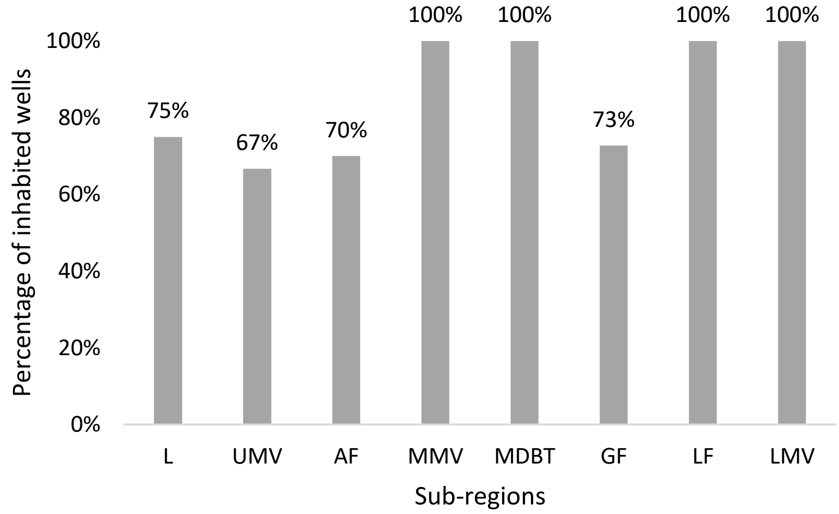

Figure 28: Percentages of groundwater wells that were populated by groundwater fauna in each of the eight sub-regions (L = Lungau, UMV = Upper Mur Valley, AF = Aichfeld, MMV = Middle Mur Valley, MDBT = Murdurchbruchstal, GF = Grazer Feld, LF = Leibnitzer Feld, LMV = Lower Mur Valley).

All fauna (besides single occurring animals Turbellaria, Nauplii and Tardigrada) was found in groundwater that was influenced by municipal waste water as well as water that was not influenced. The wells in which no fauna was present more likely classified as MWWI 2 or 3, although also samples with a MWWI of 0 or 1 were not populated (Figure 29). The majority of Cyclopoida, Oligochaeta and Ostracoda were observed in samples of a MWWI of 0 or 1. Amphipoda, Bathynellacea and Isopoda were slightly more frequently found in wells that were categorized to have a MWWI of 2 or 3. Regarding diversity of faunal groups and community structures, there were no patterns found in relationship to the MWWI.

There were no links shown between the MWWI and the hydro-/physical parameters, apart from a difference in groundwater temperature. Samples that were influenced by municipal waste water were observed to have higher water temperatures than groundwater from wells that was not influenced. The geographical position (sub-regions) of the wells in relationship to the municipal waste water categorization showed a pattern that was mostly consistent with the location of land use types (city of Graz and proximity between Urban areas and likely waste water-influenced wells at Aichfeld). At the most upstream sub-regions Lungau and Upper Mur Valley, as well as the most downstream areas Lower Mur Valley and Leibnitzer Feld, exclusively wells with a MWWI of 0 or 1 were observed. The sub-regions with the most waste water-influenced sites were the Grazer Feld, the Murdurchbruchstal and the Middle Mur Valley (Figure 31).

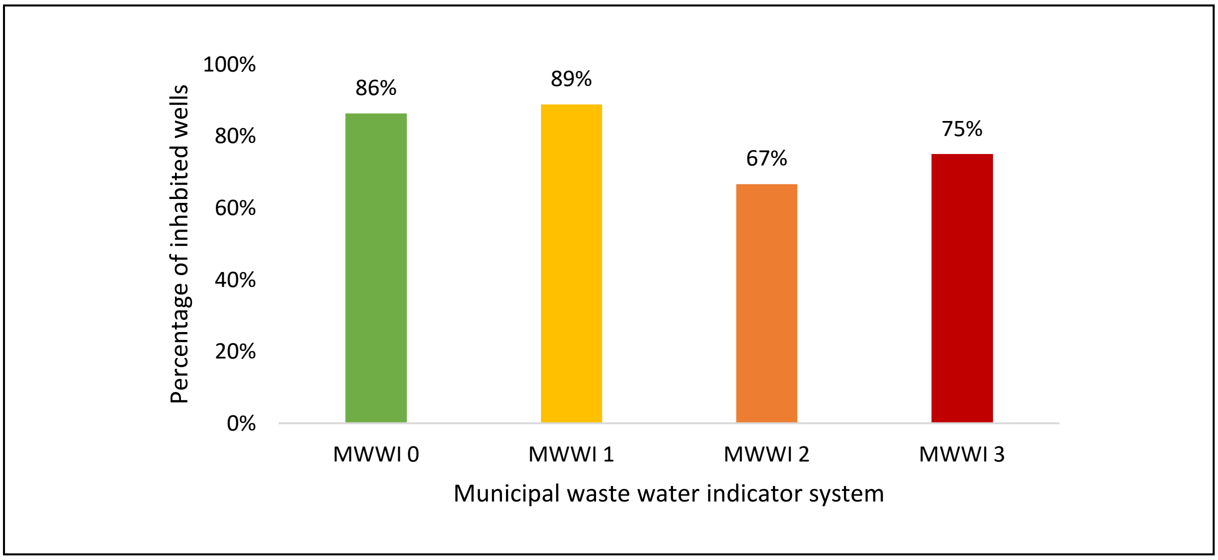

Figure 29: Portion of groundwater wells inhabited by stygofauna with respect to the municipal waste water indicator system (MWWI-test categories 0 – 3).

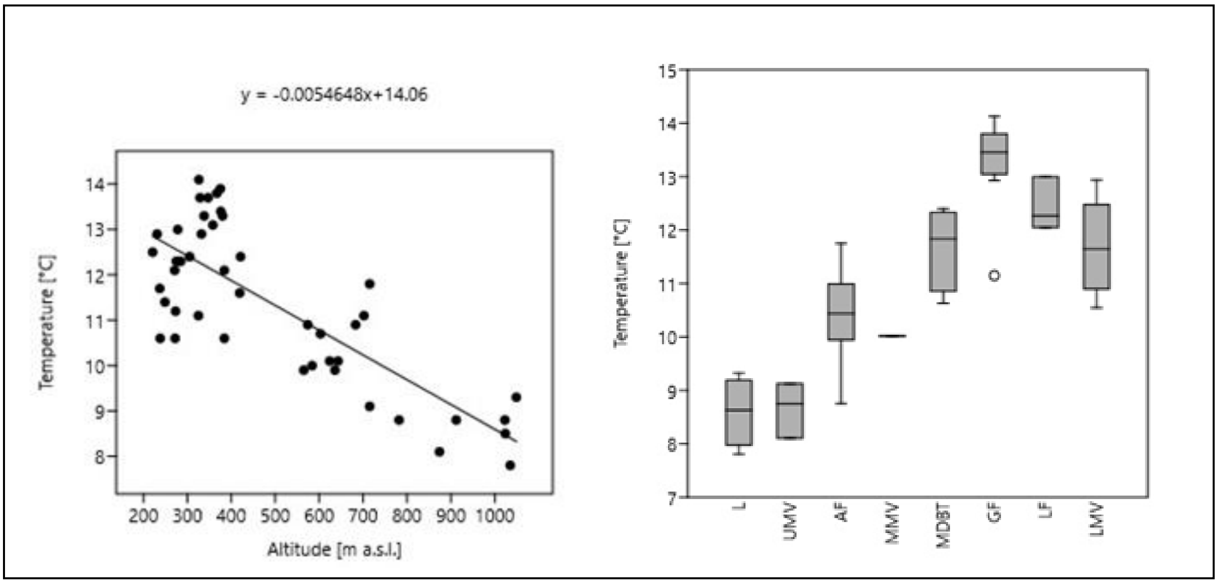

Figure 30: Groundwater temperature differences (°C) in respect to altitude (m a.s.l.) as well as in regard to different sub-regions (LMV = Lower Mur Valley, LF = Leibnitzer Feld, GF = Grazer Feld, MDBT = Murdurchbruchstal, AF = Aichfeld, MMV = Middle Mur Valley, UMV = Upper Mur Valley and L = Lungau).

Analysing the groundwater temperatures between the eight sub-regions, it was shown that the wells of lower altitude (Lower Mur Valley, Leibnitzer Feld, Grazer Feld and Murdurchbruchstal) were observed to have higher temperatures, than the groundwater of higher elevated regions (Lungau and Upper Mur Valley). The difference in water temperature between the coldest region Lungau and the warmest area Grazer Feld accounted for about 4 °C (Figure 30). With respect to the groundwater temperature differences, more wells of the downstream regions (from the Lower Mur Valley to the Murdurchbruchstal) appeared to be located more distant from surface water than wells in higher sub-regions (Middle Mur Valley, Aichfeld, Upper Mur Valley and Lungau). Furthermore, the observed wells were deeper and had a larger water column at the Grazer Feld, the Aichfeld, the Upper Mur Valley and the Middle Mur Valley and have shown to be shallower and have a smaller water column at the Lower Mur Valley, the Leibnitzer Feld, the Murdurchbruchstal and the Lungau (Figure 32). The concentration of dissolved organic carbon appeared to be higher at the Lungau, especially compared to the Aichfeld and the Grazer Feld ($p = 0.0130$, Tukey's Pairwise). Further correlations or patterns between the sub-regions and hydro-/physical parameters could not been found.

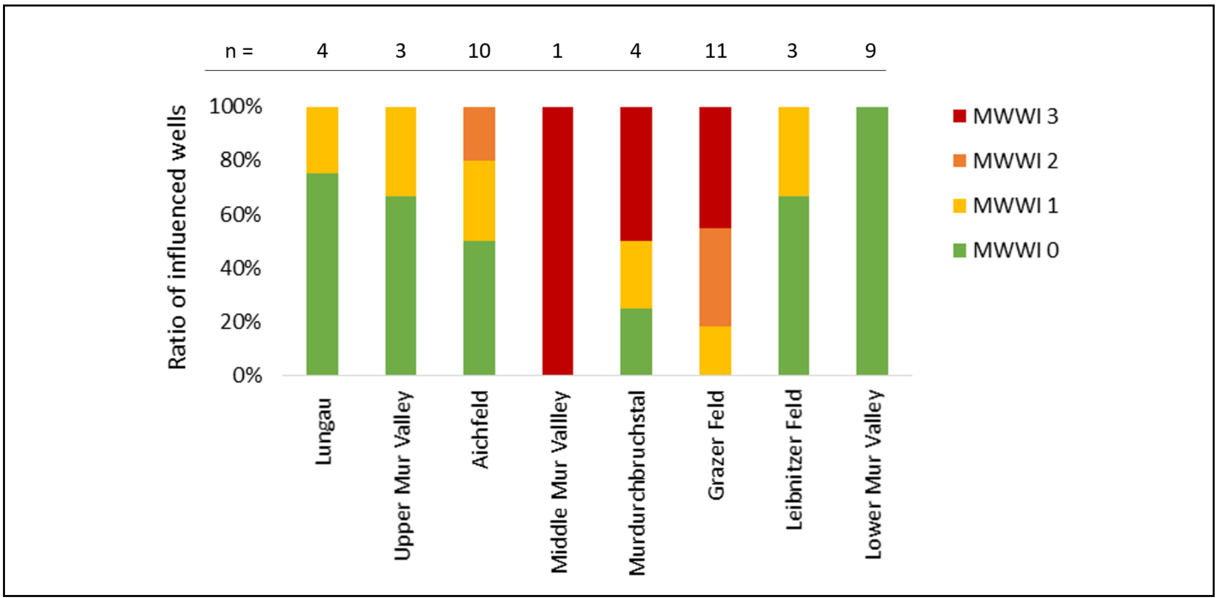

Figure 31: Percentage of groundwater wells influenced by municipal waste water (MWWI 0 – 3) in the sub-regions of the River Mur Valley (Lungau, Upper Mur Valley, Aichfeld, Middle Mur Valley, Murdurchbruchstal, Grazer Feld, Leibnitzer Feld and Lower Mur Valley). n = Total number of groundwater wells investigated per sub-region.

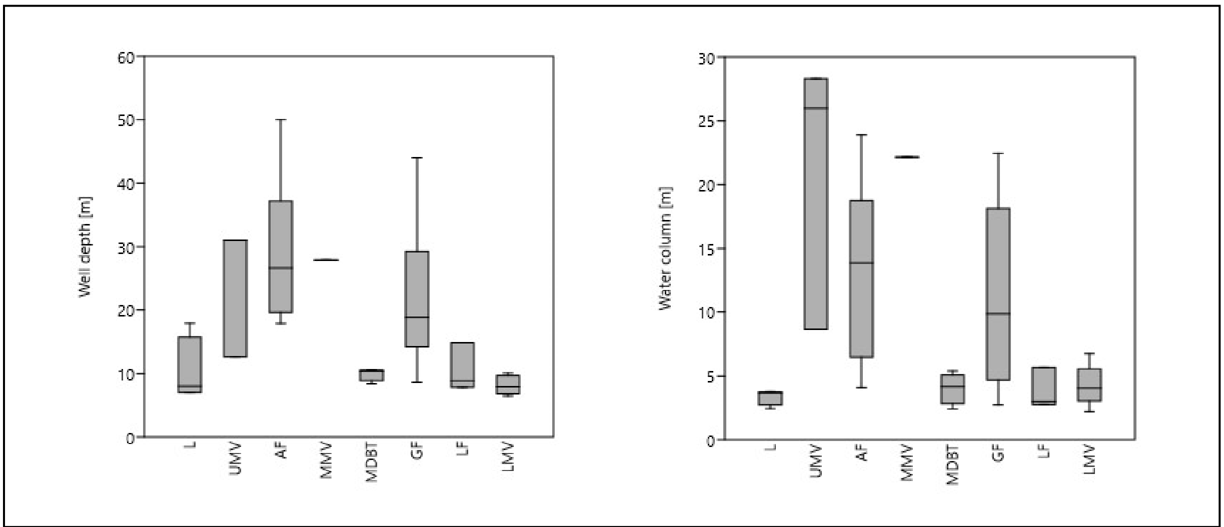

Figure 32: Differences in well depths (m) and water column depths (m) between the eight sub-regions of the Mur Valley (LMV = Lower Mur Valley, LF = Leibnitzer Feld, GF = Grazer Feld, MDBT = Murdurchbruchstal, AF = Aichfeld, MMV = Middle Mur Valley, UMV = Upper Mur Valley and L = Lungau).

4. Discussion

In contrast to the different surface waters of the river Mur Valley in Styria/Salzburg, Austria, the fauna of the groundwater ecosystems in this region was to date poorly investigated. This survey provides a first baseline of macro- and meiofaunal taxonomic groups that can be found in the shallow groundwater habitats of the Mur Valley, from the spring to the Austrian/Slovenian border. The findings include groups that are typically found in groundwater habitats, such as Turbellaria, Gastopoda, Oligochaeta, Acari, Ostracoda, Cyclopoida, Harpacticoida, Amphipoda, Isopoda and Bathynellacea which are most likely to be stygobiont or stygophilous as well as Collembola and Tardigrada which are stygoxen and edaphic/semi-aquatic, in most cases depending on the species (Danielopol, 1989). The vast majority of the observed fauna belongs to the phylum Arthropoda, more precisely Crustacea (Table 2). Although groundwater is highly influenced by nearby surface waters, the composition of faunal communities is quite different. While Hexapoda are the dominant group in limnic systems at the surface, Crustacea are with about 760 stygobiotic species and 122 genera the most common group in the groundwater of Europe (Deharveng et al., 2009).

Although the rates of specialisation and endemism are higher in groundwater ecosystems, biodiversity/richness are lower than in surface waters, especially on the scale of site-diversity. Anticipated values of 2 -3 species per site have been met by the findings in the Mur Valley, where most sites contained 2 to 4 faunal groups (Thulin & Hahn, 2008). Overall, in Europe the taxonomic richness of limnic fauna below ground (2000 stygobiotic species) and above ground (3000 surface water species) is quite comparable (Gibert et al., 2005; Illies, 1978). One of the generally most common groups in groundwater are Copepoda, which also applies to this study, since Cyclopoida was the group that was present in most samples as well as in every sub-region, in every altitude category and in groundwater below all land use types. The second most frequently found group were Acari, from which can be assumed, that the majority of the observed wells offer qualitatively good water conditions (Gerecke & Schwoerbel, 1991). Furthermore, another common group in the groundwater wells of the Mur Valley was Oligochaeta. This mostly stygophilous group is presumed to play a major role in material processing of near-surface groundwater systems (Mösslacher & Hahn, 2003). The last very common group that was present in every sub-region, altitude category and land use type was the group Collembola, which is not recognized as groundwater fauna. However, since these semi-aquatic animals are important members of edaphic communities and play a major role in the control of microorganisms, the degradation of organic material and can be used as indicators for pollution, their impact on groundwater ecosystems should not be neglected (Ponge, 1991; Chang et al., 2013; Bretschko & Christian, 1989). Furthermore, previous research suggests that there exist Collembolan species that are groundwater specialists, indicating that some groups may be recognized as stygophile fauna (Shaw et al., 2011). Therefore, I decided to include Collembola in this survey, where their presence in one third of all observed sites indicates nutrient-/detritus- and oxygen rich conditions due to regular import from surface water and the unsaturated zone (Thulin & Hahn, 2008).

In the groundwater wells of the Mur Valley, a significant correlation was found between the presence of Cyclopoida as well as Oligochaeta and the faunal richness of the sites. Therefore, the assumption that Copepoda and Oligochaeta can act as predictors for richness in groundwater ecosystems, is supported by our findings (Stoch et al., 2009). Considering this, and that the distribution of richness of groundwater fauna is generally very heterogeneous with a patchy distribution of hotspots, the similarity of more diverse communities in comparison to less diverse community structures as well as the very uneven distribution (over sub-regions) of more and less diverse groundwater wells in the Mur Valley are reasonable (Malard et al., 2009; Deharveng et al., 2009).

According to Thulin & Hahn (2008), there are three key factors that shape the distribution patterns of groundwater fauna communities on a local scale. These are the availability of organic matter (food), the concentration of dissolved oxygen and the size of fissures and pores in the sediment matrix (space). With the geological conditions in the Mur Valley consisting mainly of sand/gravel and alluvial fan, the state of the sediments of the observed wells were suitable for macro-/meiofauna. The other factors, availability of organic matter and oxygen content, are highly dependent on hydrological exchange with surface water. The intensity of hydrological exchange is a result of different criteria playing together, which are the composition of soil, the land use type, geomorphology, sediment structure, distance to surface water, flow rate, hydraulic conductivity and the depths of the groundwater table. In most of the sites, hydrological exchange can be assumed to be relatively strong, because the wells are situated proximate to the river Mur and its tributaries, the sediment deposits are well permeable, the majority of groundwater tables were shallower than 10 m below land surface and the content of dissolved oxygen was high in most of the wells (Table 3).

Our data revealed a relationship between the depth of the groundwater table as well as the well depth and the richness of the faunal communities. It was shown, that the communities in less deep wells and with shallower groundwater tables were more diverse in composition. In general, there is a sharp decline of groundwater fauna in terms of abundance and diversity with depth and most of the animals are usually found close to the groundwater table, because the availability of food and oxygen normally is higher there than in deeper zones (Schmidt & Hahn, 2012; Hahn, 2005). Differences in oxygen concentrations did not show any impact on the presence, richness or distribution of the fauna in groundwater, which can be explained by the relatively high oxygen content in most samples (Table 3). Since the critical concentration of dissolved oxygen for the presence of groundwater fauna is 0.5 mg/L to 1 mg/L, the wells of the Mur Valley had too high O_2 concentrations (lowest value was 113 mg/L) as to affect the fauna (Hahn, 2006). However, it remains to be seen if there will be a difference in faunal distribution and composition of communities in the samples that were collected in autumn, because at this time of year, oxygen levels can be drastically lower, below 0.5 mg/L and the animals are then highly influenced by such seasonal changes.

As opposed to hydrological exchange, hydrochemistry is presumed not to have a big impact on the distribution patterns and community structures of groundwater fauna (Paran et al., 2005; Dumas et al., 2001). Therefore, it is not surprising that there were no correlations observed between the distribution of faunal communities and basic hydrochemical parameters. However, the group of Harpacticoida was the only one that could be differentiated from the others in regard of the preferred pH and DIC values. In contrast to the other groups, Harpacticoida were found only in slightly acidic conditions (pH between 6 and 7) and in wells with relatively low concentrations of dissolved inorganic carbon (below 30 mg/L). Since the tolerances for these parameters vary depending on the species, it will be of value to identify the Harpacticoida individuals to species level and see if there can be a connection found, or if these findings are just by chance, since Harpacticoida were generally scarcely found (single individuals in three wells) in the Mur Valley (Fryer, 1993; Gottstein et al., 2007).

Furthermore, Gastropoda as well as Ostracoda, Harpacticoida and Isopoda were only found in sub-regions downstream of the Murdurchbruchstal. These sub-regions (Murdurchbruchstal, Grazer Feld, Leibnitzer Feld and Lower Mur Valley) differ in several factors from the upstream sub-regions (Middle Mur Valley, Aichfeld, Upper Mur Valley and Lungau). Firstly, below the quaternary Valley fillings of the Mur Valley, in the upper regions fractured, crystalline aquifers exist, while the subjacent aquifer of the lower regions is categorized as porous. In the middle part (Murdurchbruchstal and Grazer Feld) there is limestone aquifer (including the Grazer Highlands) below the quaternary, porous cover (Figure 4). With the spring of the river Mur being located at approximately 1900 m a.s.l., the sampling sites of the upper four sub-regions are higher in elevation (500 – 1050 m a.s.l.) than the wells downstream of the Murdurchbruchstal, which are located at sites between 200 and 500 m a.s.l.. This altitude difference is strongly linked to a gradient in groundwater temperature, showing that wells of respectively lower altitude had higher water temperatures. Moreover, the municipal waste water indicator test detected higher values in regions of respectively lower altitude. Although this relation is presumably a by-product of the correlation between the MWWI and the land use. Naturally, wells in urban areas were most influenced by municipal waste water and considering the location of the wells categorized as urban areas, the correlation between altitude/sub-regions and MWWI can be explained. Additionally, it should be mentioned, that the MWWI values of the river Mur were elevated in the Lower Mur Valley, while the values of the groundwater were not.

Lastly, the concentration of NO_3^- in groundwater were higher in the sub-regions downstream of the Murdurchbruchstal, than in sub-regions of higher altitudes. It can be speculated, that these differences may be the result of more intense agriculture and fertilisation. As the width of the Mur Valley becomes broader as the course of the river progresses, the distances between the wells and the surface waters become greater in downstream areas (below Murdurchbruchstal). Therefore, lower nitrate concentrations in higher located wells could also be explained by stronger water exchange and shorter water residence time in the aquifer. Natural background concentrations of nitrate in groundwater are typically below 2 mg/L, with concentrations over 3 mg/L being considered to indicate anthropogenic influences (Burkart & Stoner, 2008). Since the majority of the wells in the River Mur Valley were influenced by agriculture, it is unsurprising that the mean concentrations of nitrate in groundwater were with 18 mg/L above that referential value and could be categorized as environments that are according to the freshwater nitrate toxicity guidelines chronic-highly disturbed systems (Hickey, 2013) (Table 4).

On the other hand, the differences in nitrate concentration did not seem to have an influence on the distribution of fauna. Reasons for that could be, that the impact of agricultural activities in the Mur Valley is uniformly spread, that the differences in nitrate concentrations are too minor to affect faunal communities, or that other factors have a much greater influence on the distribution patterns of fauna. In fact, the general impact of nitrate on groundwater fauna is still unclear and widely discussed. Some research suggests that there are limits to the tolerance of different groundwater species (e.g. nitrate concentrations around the guidelines for drinking water of about 50 to 100 mg/L and higher have been shown to have a negative impact on groundwater species), or that a shift in community composition from stygobiont and stygophile to stygoxenous is likely in nitrate influenced wells, while others imply that the influence of nitrate on groundwater fauna may be less severe than currently expected (Gerhardt, 2020; Stein et al., 2010; Di Lorenzo et al., 2021). It cannot be excluded, that a relationship between faunal distribution and nitrate influence will be revealed in the River Mur Valley when the fauna has been identified to species level.

Some of the most significant mentioned factors in relation to the particular distribution of Gastropoda, Ostracoda, Harpacticoida and Isopoda are supposedly the spatial differences regarding altitude and aquifer structure and its likely connection to historical events like glacial periods, as well as differences in groundwater temperature, which are factors that are also related to each other (Mazor, 2003). The ice ages of the Pleistocene era had a great impact on the richness and distribution of limnic fauna. Many species became extinct or missing from certain areas and some animals sought refuge in groundwater or migrated to warmer regions. This led to the adaption and specialisation of some species into real stygobiota and the occurrence of endemic relict species in some formerly glaciated regions on the one hand and the depletion of species richness in some of those areas on the other hand (Gibert & Deharveng, 2002). The Riss-Würm glaciations, which were the last glacial periods in the Alpine region, had a direct effect on the upper third of the Mur Valley which was for an extended period of time ice covered. An indirect effect to the lower parts of the Mur Valley were movements of sediment (Hewitt, 1999).

It can be speculated, that the Bathynellacea populations or communities (individuals not yet determined to species level), which were found in the upper sub-regions (all besides the Leibnitzer Feld and the Lower Mur Valley) may consist of relict species that outlasted the glaciations in the groundwater. Harpacticoida, Gastropoda, Isopoda and Ostracoda, which were only found in sub-regions located between 200 and 500 m a.s.l. (below the Murdurchbruchstal), are assumed to have re-colonized these groundwater bodies. The re-colonization may have occurred from local refugia, or over mid- to long distance dispersal from unglaciated southern regions (Martin et al., 2009). A scenario like this would for example be very likely for Isopoda, since their groundwater communities commonly consist of stygobiont, endemic species with their main distribution area in regions that were not affected by glaciation, namely in Mediterranean areas and the Balkans (Mösslacher, 2003). However, to verify this hypothesis, more detailed analysis on species level are required.

Between the faunal groups themselves, a correlation in the presence of Gastropoda and Ostracoda was found, but since no other study mentions this relationship, it can be assumed that this result is either coincidence (especially because Gastropoda were only found in three wells), or there appear to be similar requirements on species level. This aspect asks for further research. Furthermore, the opposed distribution patterns of the groups Bathynellacea and Harpacticoida are especially interesting, since Hasenhüttl (1972) observed Bathynellacea in separate water basins than Harpacticoida in the Odelsteinhöhle in Styria. Hasenhüttl suggested possible exclusion effects between those two groups but also considered other reasons for his findings, since the basins in which Bathynellacea were found in his observations exhibited colder temperatures, than the waters in which Harpacticoida were found. In our findings, Bathynellacea were also found in comparably colder groundwater of higher altitudes. Therefore, it may be assumed that Bathynellacea prefer colder conditions. Further research is suggested on the community pattern differences between Bathynellacea and Harpacticoida.

Reasons for the limit of distribution of the mentioned groups at the Murdurchbruchstal could be the altitude, a geological or chemical barrier within the aquifer in this area, and/or the groundwater temperature differences. Since the area of this faunal distribution front (around Murdurchbruchstal) is the same and only area where the deeper aquifer is karstic, and the subjacent aquifer types differ between upstream (fractured) and downstream regions (porous), it cannot be neglected that this may at least partially act as a physical distribution barrier for some groundwater fauna groups. Furthermore, most of the groundwater wells in the mid-section of the Mur Valley (Middle Mur Valley, Murdurchbruchstal and Grazer Feld) were influenced by municipal waste water, indicating a possible chemical distribution barrier. In terms of altitude, there have been observations showing, that the biodiversity of groundwater fauna is highest between 200 and 500 m a.s.l. and scarcer in higher elevations (Dole-Olivier et al., 2009), but other reports state that there are no large-scale influences of altitude in fauna composition (Mösslacher, 2003). Our results indicate, that there is no significant difference in richness depending on elevation alone, although community structures showed a bigger variety in lower regions, than in higher areas. Therefore, we may assume that this faunal boundary is the product of altitude and temperature in combination with spatial distribution factors (historically/geologically).

Groundwater temperature is usually relatively stable and does not exhibit significant diurnal or annual variations, although the occurrence of temperature fluctuations is highly depending on discharge rates, geology and hydrological exchange with the surface (Silliman & Booth, 1993). In shallow aquifers, represented by most of the groundwater wells in the Mur Valley, groundwater temperature is typically one to two degrees Celsius above the annual surface temperature (Parsons, 1970). According to the Klimaatlas Steiermark, the annual surface temperature in the lower parts of the Mur Valley (downstream Murdurchbruchstal) were between 8 and 10 °C, while the temperature in the sub-regions Middle Mur Valley and Aichfeld showed temperatures between 6 and 7 °C and the upper most regions of the Mur Valley (Upper Mur Valley and Lungau) had temperatures of 5 °C and below. The temperature map of the province Styria reveals that the annual surface temperatures generally are higher in the flat, south-eastern regions than in the more alpine north-western areas, with a temperature shift around the Murdurchbruchstal (Wakonigg, 2010; [Digital Atlas Styria], 2021). Considering the anthropogenic influences in the River Mur Valley, the slightly elevated groundwater temperatures that we observed may be explained. These slight temperature differences between the upper and the lower parts of the Mur Valley support that temperature limitations could also be a reason for the distribution pattern of the macro- and meiofauna in the Mur Valley.

However, the main factor regarding temperature in relation to its impact on fauna distribution is the fluctuation rate, because it is an indicator for the strength of hydrological exchange in a groundwater ecosystem and as mentioned, hydrological exchange together with oxygen and food availability are the main three factors that govern faunal distribution patterns. Hahn (2006) established a GW-Fauna-Index that considers temperature deviation, oxygen concentrations and detritus amount and thereby aims to calculate a value for hydrological exchange. In relation to those connections, it will be informative to compare the results of this survey with the data of the samples taken in autumn, with special focus on dynamics in faunal community patterns, oxygen concentrations and temperature values.

Another factor that possibly influences the distribution and richness of groundwater fauna is the anthropogenic alteration of the landscape. While the hydrological exchange and hydrochemical as well as physical integrity of the groundwater ecosystem in natural areas is mainly influenced by annual fluctuations and natural phenomena like floods, in agriculturally used and urban areas other factors impact groundwater systems additionally (Hahn, 2002). Around 30 % of all groundwater wells sampled in Central Europe were found not to be inhabited by macro- or meiofauna (Fuchs et al., 2006). In the Mur Valley, only 20 % of the sampled groundwater wells were obviously free of fauna. The reasons for that may be, that most of the observed wells had high oxygen concentrations, were relatively proximate to surface waters, were assumed to have suitable geological conditions for fauna and the chemical contamination was below levels that would disturb faunal communities.

However, the wells that did not contain fauna, were mostly located in urban areas or at high altitudes. In high altitudes (fauna-less wells in the Upper Mur Valley and the Lungau), fauna presumably got extinct from some areas during the last glacial period. The reasons for the absence of fauna in urban areas (Aichfeld and Grazer Feld) could be soil sealing, relatively high discharge of groundwater in combination with low recharge, low hydrological exchange, pollution (e.g. sewage, industrial effluents, heavy metals), higher temperatures and/or nutrient loads (Khatri & Tyagi, 2015). In the Mur Valley, wells in urban areas had significantly higher temperatures, the sample sites were more distant to surface water than in natural or agricultural areas and the majority of urban wells were influenced by municipal waste water (especially wells near the city of Knittelfeld, in the sub-region Aichfeld and wells near the city of Graz, in the sub-region Grazer Feld). Interestingly, although 42 % of wells in urban areas were not populated, Amphipoda which are assumed to be indicators for good water quality and high biodiversity in European groundwater ecosystems, were the group that was most commonly found in this land use type (Stoch et al., 2009). Further taxonomic classification to species level will help to clarify if those individuals are stygophile or stygoxenous species that dominated the faunal communities due to influencing anthropogenic factors like temperature or pollutants.

In urban areas as well as in agriculturally influenced areas (especially in the Aichfeld, Grazer Feld and Upper Mur Valley), wells were deeper than in natural areas. It can be speculated, that this may be due to higher anthropogenic use of groundwater in those areas. Higher discharge of groundwater often results in the vanishing of microhabitats by depletion of fine sediments and the reducing of available surface for bacterial biofilms which function as food sources for fauna and have an important role in self-purification processes of aquifers. These factors have an impact on species abundances and richness, which explains that the richness trend for groundwater fauna in the Mur Valley is higher in natural areas (Lungau and Lower Mur Valley), than in agricultural or urban areas (Di Lorenzo & Galassi, 2013; Murray et al., 2003). Furthermore, the groups that were most frequently found in wells of natural areas (which were all populated) were Cyclopoida, followed by Oligochaeta and as mentioned above, these groups can be used as indicators for richness in Central European groundwater ecosystems (Stoch et al., 2009).

Some wells in agriculturally used areas were also influenced by municipal waste water, probably because of being located near urban areas too. However, the more common chemical influence on groundwater systems in agricultural areas are inorganic fertilizers, the application of manure and/or pesticides. Components like ammonium or nitrate can have a lethal or depleting effect on groundwater fauna, if their concentrations are above specific limits. In the Mur Valley, the measurements of those chemical compounds were far below critical concentrations (e.g. all wells had ammonium concentrations below 0.03 mg/L, which after the freshwater ammoniacal-N toxicity guidelines is categorized as NOF attribute state A - a pristine environment with high biodiversity and conservation values), indicating comparatively high water quality even though the majority of groundwater wells were located in agricultural areas (Hickey, 2014) (Table 4). However, since there still is relatively little research on the impact of contaminants on groundwater fauna, especially regarding the long-term chronic impact of elevated non-critical concentrations, conclusions must be drawn carefully (Di Lorenzo & Galassi, 2013; Di Lorenzo et al., 2015).

In groundwater systems that have a very high hydrological exchange (can be maintained by discharge and irrigation in agricultural areas) and a high availability of nutrients/food, stygobiota can be replaced by stygophile and stygoxenous fauna (Hahn, 2006; Korbel et al., 2013). In wells that were located in agriculturally used regions, the groups that were most common were Acari, Cyclopoida, Oligochaeta and Collembola. The frequent presence of Acari, being a group that can to some extent be used as an indicator for environmental changes and anthropogenic influences in a groundwater ecosystem, and the preference of Collembola for wells in agricultural areas, being an edaphic, stygoxenous group, suggest that the GW-Fauna-Index in the agricultural areas of the Mur Valley is expected to be high (Di Sabatino et al., 2003).

In conclusion, it can be said that the groundwater fauna of the River Mur Valley is relatively rich with regard to the presence of common groundwater groups and the assumption, that Copepoda and Oligochaeta can act as indicators for richness/diversity is supported by our data. There appear to be spatial distribution patterns for Gastopoda, Ostracoda, Harpacticoida, Isopoda and probably Bathynellacea that seem to be influenced by the combination of geology, the last glacial episode, temperature and altitude. Furthermore, key factors like hydrological exchange seem to have an impact on the distribution patterns of fauna in relation to the land use type and factors like well depth and the depth of the groundwater table appeared to have more effect on community structures, than differences in hydrochemistry (to some extent). It is expected to gain more detailed knowledge on the faunal patterns, as soon as species are identified and there are reference values for important parameters that are expected to fluctuate annually (e.g. oxygen concentrations). Ultimately, it is suggested to follow up on hydrochemical changes and pollution of groundwater on a long-term basis to detect possible influences to the crucial stability of groundwater fauna communities.

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