

Crustacean stygofauna in the urban shallow aquifers of Vienna, Austria – biodiversity, community dynamics and environmental drivers

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This study investigates the diversity and ecological patterns of stygobiont and stygophile crustaceans in shallow groundwater of the city of Vienna, Austria, with a particular focus on amphipods. In total, 47 crustacean taxa were identified, revealing an unexpectedly high diversity in this urban setting. Among these, seven amphipod species were confirmed, largely matching known biogeographic distribution patterns. Molecular analyses based on COI gene sequencing refined amphipod species identification based on morphology and revealed the presence of a potentially undescribed taxon (*Niphargus* aff. *aquilex*) as well as the most eastern record of *Niphargus tonywhitteni*. Availability of dissolved oxygen and associated redox conditions, as well as microbiological-ecological indicators were key in explaining the spatial distribution of taxa. Groundwater temperature and hydrogeology (aquifer type) further influenced occurrence patterns, particularly with respect to amphipods, which preferred lower temperatures and larger sediment pore space. In addition, community-level analyses identified the influence of surface waters as a significant determinant of community composition. Seasonal effects were almost absent. The high overall community homogeneity as well as the limited species-specific environmental preferences among amphipods provide evidence that basic conservation measures are likely to benefit multiple taxa concurrently. However, rare species warrant further targeted investigations. Overall, this study highlights the ecological value of urban aquifers.

Nilles C, Englisch C, Zittra C, Scharhauser F, Fuchs A, Gaviria S, Kaminsky E, Stumpp C, Steiner C, Buga-Nyéki E, Karwautz C, Griebler C (2026) Crustaceen Stygofauna in den urbanen, oberflächennahen Grundwasserleitern von Wien, Österreich – Biodiversität, Gemeinschaftsdynamiken und umweltbedingte Einflussfaktoren

Diese Studie untersucht die Diversität und die ökologischen Muster stygobionter und stygophiler Krebstiere im oberflächennahen Grundwasser der Stadt Wien, Österreich, mit besonderem Fokus auf Amphipoden. Insgesamt wurden 47 Krebstiertaxa identifiziert, was eine unerwartet hohe Diversität für diesen urbanen Lebensraum offenbart. Darunter konnten sieben Amphipodenarten nachgewiesen werden, deren Vorkommen weitgehend den bekannten biogeographischen Verbreitungsmustern entspricht. Molekulargenetische Analysen auf Grundlage des COI-Gens präzisierten die auf morphologischen Merkmalen basierende Artbestimmung der Amphipoden und unterstreichen das Vorkommen eines potenziell unbeschriebenen Taxons (*Niphargus* aff. *aquilex*) sowie den bislang östlichsten Nachweis von *Niphargus tonywhitteni*.

Die Verfügbarkeit von gelöstem Sauerstoff und damit verbundene Redoxbedingungen sowie mikrobiologisch-ökologische Indikatoren erwiesen sich als zentrale Faktoren zur Erklärung der räumlichen Verteilung der Fauna. Auch die Grundwassertemperatur und die Hydrogeologie (Aquifertyp) beeinflussten die Verteilungsmuster, insbesondere bei Amphipoden, die niedrigere Temperaturen und größere Porenräume im Sediment bevorzugten. Gemeinschaftsanalysen identifizierten darüber hinaus den Einfluss von Oberflächengewässern als einen signifikanten Treiber für die Zusammensetzung der Gemeinschaften. Saisonale Effekte waren weitestgehend nicht nachweisbar.

Die insgesamt hohe Homogenität der Lebensgemeinschaften sowie die begrenzten artspezifischen Umweltpreferenzen der Amphipoden legen nahe, dass grundlegende Schutzmaßnahmen wahrscheinlich vielen Taxa gleichzeitig zugutekommen würden. Seltene

Arten erfordern jedoch weiterhin gezielte weiterführende Untersuchungen. Insgesamt unterstreicht diese Studie den ökologischen Wert urbaner Grundwasser-Systeme.

Keywords: stygofauna, groundwater biodiversity, urban aquifers, COI barcoding, environmental drivers, crustacea, groundwater ecology, Amphipoda.

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Introduction

Groundwater is a globally essential resource, supplying drinking water, irrigation, and industrial uses, and represents the most extracted raw material worldwide (Wada et al. 2010; Lall et al. 2020). Beyond its societal importance, groundwater sustains aquatic and terrestrial ecosystems and constitutes the largest continental aquatic biome (Kløve et al. 2011; Griebler et al. 2023a). These ecosystems harbor largely unexplored biodiversity, including microorganisms and groundwater fauna, which perform key ecological functions such as water purification, contaminant attenuation, pathogen control, and the maintenance of subsurface connectivity through bioturbation (Griebler & Avramov 2015; Feichtmayer et al. 2017; Stumpp & Hose 2017; Hose & Stumpp 2019; Mammola et al. 2026). In this sense, ecosystem integrity is maintained by the activity of groundwater fauna, which creates and reserves open pore space essential for water flow, transport of materials and replenishment of dissolved oxygen and organic matter, thereby governing geochemical processes.

Due to restricted energy input, long water residence times, limited dispersal, and high ecological specialization, groundwater ecosystems are energy-poor and particularly vulnerable to disturbance (Hose et al. 2022). While they often host comparatively high numbers of rare, relict, and endemic species (Gibert et al. 2009; Humphreys 2009; Mammola et al. 2019, 2022; Wynne et al. 2021; Di Lorenzo et al. 2024; Englisch et al. 2024), their biodiversity remains poorly documented and is largely underrepresented in conservation and management frameworks (Sánchez-Fernández et al. 2021; Mammola et al. 2022, 2024). The limited accessibility of subsurface habitats and the scarcity of systematic surveys across

different land use types constrain our understanding of species distributions and ecological requirements.

In urban areas, like the city of Vienna, Austria, groundwater ecosystems are influenced by a multitude of anthropogenic stressors. In addition to the environmental drivers that are present in natural systems (e.g. oxygen and food availability, hydrogeological conditions like pore space, geohistorical background, hydrological exchange), groundwater fauna in urban aquifers is impacted by pressures like pollution, reduced groundwater recharge due to surface sealing, subsurface infrastructure, groundwater warming and subsurface urban heat islands, all potentially resulting in habitat loss and biodiversity decline, and in the worst case leading to a disturbance and loss of ecosystem functioning (Griebler et al. 2019; Castaño-Sánchez et al. 2020; Becher et al. 2022; Englisch et al. 2024). To date, groundwater fauna communities in urban areas have received particularly little attention, as only a few studies have been conducted in cities in Central Europe (Koch et al. 2021; Dumnicka et al. 2025a, 2025b). In Vienna specifically, only single species records of groundwater fauna existed from single wells with exception of the Lobau National Park area (Vornatscher 1938, 1972; Danielopol 1984; Pospisil 1994; Griebler et al. 2023b). Therefore, one aim of this study was to compile a comprehensive species inventory of the groundwater crustacean fauna in Vienna. The focus was on crustaceans (including Cladocera, Ostracoda, Harpacticoida, Cyclopoida, Amphipoda, Isopoda, Bathynellacea), because they generally constitute the majority of stygofauna and determination to species level can be achieved for most groups.

Invertebrate fauna in groundwater includes organisms that exclusively inhabit aquifers (stygobionts), preferentially or temporarily inhabit subsurface aquatic environments (stygophiles), or lack adaptations to the subterranean habitats and are either accidentally transported there or act as invasive colonizers from the surface (stygoxenes). Groundwater habitats are characterized by permanent darkness, which creates unique ecological niches and nutrient cycling processes that differ substantially from surface ecosystems (Thulin & Hahn 2008). Although subterranean habitats are environmentally stable, they depend largely on surface-derived organic matter, often resulting in low oxygen concentrations due to limited replenishment (Hahn 2002). Adaptations such as eye reduction, depigmentation, body elongation, low metabolic rates, extended lifespans, and reduced reproductive output reflect specialization to energy-poor and spatially constrained environments (Hahn 2002; Boulton et al. 2008; Hose et al. 2022). These adaptations are reflected in phylogenetic diversity and cryptic species. In this study, particular emphasis in combining morphological and molecular identification was placed on stygobiont amphipods to delineate species and link them to specific niches. Amphipods were chosen to first expand the knowledge on their distribution, and second, making use of recently established protocols for molecular analysis (Fišer et al. 2019; Alther et al. 2021; Borko et al. 2021).

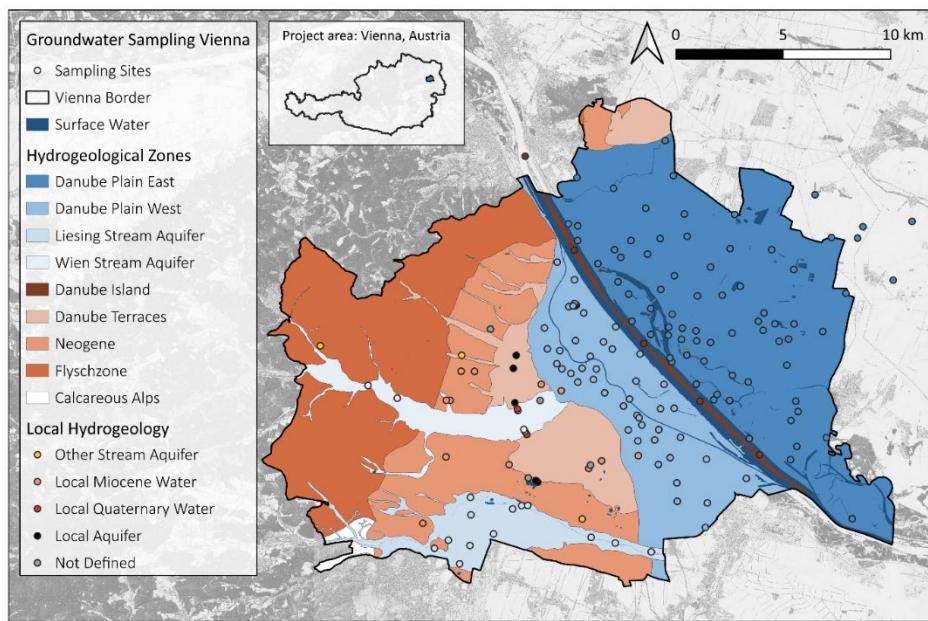
Beyond providing new insights into the crustacean biodiversity of Vienna's urban shallow groundwater, this study aims to identify environmental factors key to the distribution and community assembly of groundwater crustaceans in general and amphipods in particular. By this, we seek to expand our understanding of urban groundwater ecosystems and their associated crustacean fauna, thereby providing support for the development of sustainable groundwater management strategies and effective conservation planning.

Materials and Methods

Study area

Vienna, capital of Austria and fifth-largest city in the European Union with more than two million inhabitants, is located in the northeastern part of Austria at the Danube River. The city spans 414.87 km², with elevations from 151 m a.s.l. in the Lobau floodplain to 543 m at the Hermannskogel (MA 23 der Stadt Wien, 2024). Green areas and water bodies cover 53.1% of the city area. A total of 40,5% of the city is designated as protected, notably the Lobau wetland within the Danube Floodplain National Park and the Vienna Forest (Wienerwald) (MA 23 der Stadt Wien 2024). Land use comprises approximately 10.9% agriculture, 19.6% traffic, and 16.4% built-up areas (MA 23 der Stadt Wien 2024). Vienna's hydrogeology is highly heterogeneous (Fig 1), described in detail in Kaminsky et al. (2025a).

Vienna has a temperate climate, with 2021 averaging 11.1 °C and 583 mm of precipitation, and 2022 being warmer and drier at 12.2 °C and 461 mm (MA 23 der Stadt Wien 2024). Long-term precipitation averages around 650 mm per year (Stadt Wien 2025), but there



Data sources: Basemap Austria (<https://www.basemap.at/wmts/1.0.0/WMTSCapabilities.xml>), Open Government Data (data.wien.gv.at), Municipal Department 45- Water Management Vienna, GeoSphere Austria, BOKU University, University of Vienna. Map Author: Constanze Englisch.

Fig. 1: The city of Vienna, with its boundaries outlined in black. Sampling sites are indicated by circles. Hydrogeological zones are shown using colour-coded background areas representing the main continuous aquifers. The fill colour of the circles indicates the local aquifer type at each sampling site (local hydrogeology or main hydrogeological zone). | **Abb. 1:** Die Stadt Wien und ihre Stadtgrenzen (schwarze Linie). Die Probennahmepunkte sind als Kreise dargestellt. Die unterschiedlichen Grundwasserleiter sind farblich unterschieden und so auch die Zugehörigkeit der einzelnen Probennahmepunkte zu den hydrogeologischen Einheiten.

is pronounced spatial variability, ranging from up to 800 mm annually in the Wienerwald to less than 600 mm in parts of the southeast. These climatic gradients, together with the city's mosaic of urbanized areas, extensive protected landscapes, and heterogeneous hydrogeology, create diverse environmental conditions that make Vienna well suited for studying groundwater ecosystems, the distribution of groundwater fauna, and its ecological responses to anthropogenic pressures.

Groundwater sampling, on-site measurements and fauna collection

Two comprehensive groundwater sampling campaigns were conducted in autumn 2021 (September – December) and spring 2022 (February – April) collecting fauna from 163 groundwater observation wells distributed citywide. Sites were selected based on geology/aquifer types and availability (Fig. 1). Fauna was sampled with a miniaturized Cvetkov net (100 μm mesh; weighted angling mechanism with 50 mL tube; Jäger et al. 2022). Samples were preserved in ethanol ($\geq 70\%$ final conc.). Targeted resampling of selected wells in June 2023 provided fresh material for sequencing, this time immediately preserved in molecular grade EtOH (96%) and stored at -80°C .

For physical-chemical and microbiological analysis, each well was purged 2–3 times with a submersible pump (Grundfos MP1; Eijkelkamp Soil & Water, Giesbeek, Netherlands) to remove stagnant well water before collecting fresh groundwater samples. On-site we measured groundwater temperature, dissolved oxygen, electrical conductivity, and pH with field sensors (Multi 3620; WTW, Weilheim, Germany), and recorded the depth to the groundwater table (distance between ground surface and water level), well depth, and water column height. Water samples dedicated to DOC/DIC analysis were filtered on-site through 0.45 μm syringe filters into muffled 40 mL glass vials, with DOC samples further acidified to pH 2 using HCl. All samples were stored at 8°C in darkness until processing.

Laboratory analysis

Abiotic and microbial analyses

Collected water samples were analyzed for its stable water isotopes signature (D/H, $^{18}\text{O}/^{16}\text{O}$) and electrical conductivity to evaluate the surface water influence (Kaminsky et al. 2025a). Based on its hydrochemical composition, groundwater samples were categorized into four redox categories. These categories ranged from 1, indicating oxic conditions, to 4, indicating highly reduced conditions. A detailed description is found in Kaminsky et al. (2025a).

Concentrations of major ions and nutrients (NO_3^- , NO_2^- , NH_4^+ , PO_4^{3-} , SO_4^{2-} , Cl^- , Na^+ , K^+ , Ca^{2+} , Mg^{2+}), as well as ferrous iron (Fe^{2+}) and reduced manganese (Mn^{2+}) were determined after filtration through 0.45 μm membrane filters using ion chromatography (DIONEX ICS-1100; Thermo Fisher Scientific, Waltham, USA) and standardized photometric assays, respectively (Kaminsky et al. 2025a). Dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were measured using catalytic oxidation with non-dispersive infrared detection (Shimadzu TOC-L Analyzer, Shimadzu, Kyoto, Japan) (Kaminsky et al. 2025a). For chemical species with values below the detection limit, i.e. nitrate and ammonium, the detection limits of 0.04 mg/L for NO_3^- and 0.01 mg/L for NH_4^+ were used for statistical analysis.

Microbial activity in groundwater was assessed by means of intracellular ATP concentration in fresh groundwater samples using a luciferin-oxidation method with a luminometer plate reader (Promega, Madison, WI, USA) in triplicate. Total prokaryotic cell counts (TCC) were determined from glutaraldehyde fixed samples by means of flow cytometry in combination with SYBR Green staining (Amnis CellStream, Luminex, Austin, TX, USA) (Retter et al. 2021).

We measured the distance of all sampling points to the nearest surface water using the state map of Vienna (Open Data Austria 2024). Surface sealing was quantified as mean percent sealed area in the upstream zone of each well using triangular catchments extending 300 m upstream with a 120° opening, oriented by groundwater flow from isopiestic lines (GeoSphere Austria; Englisch et al. 2025, in revision). Mean imperviousness was extracted from the Copernicus Imperviousness Density HRL 2018 (10 × 10 m) and assigned to sites. For wells outside a continuous aquifer with no isopiestic lines, we used a circular area of radius 222.6 m (area 155,772 m²), following Böttcher & Zosseder (2022). Hydraulic conductivity (K_f) was extracted from a 10 × 10 m raster (GeoSphere Austria); gaps (isolated western wells) were filled using sediment distribution data (Grupe et al. 2021; Pfeiderer et al. 2019).

Taxonomic analysis of groundwater fauna

Prior to sorting, fauna samples fixed in EtOH were stained with eosin to enhance the visibility of small and unpigmented meiofauna. All specimens were then sorted into major taxonomic groups (phylum to order) under a stereomicroscope and morphologically identified to the lowest possible taxonomic level. Morphological identification of amphipods was genetically evaluated. Where the morphological and genetic identifications differed, specimens were re-examined morphologically for definite identification.

Barcoding of mitochondrial cytochrome c oxidase subunit I (COI) gene fragments from each morphotype (if possible, $n > 1$ from multiple sampling sites) confirmed or revised the morphological identification. Whole genomic DNA was extracted from one to several Speedvac-dried legs of each individual (Eppendorf, Hamburg, Germany) using a modified QIAamp UCP DNA Micro Kit (50) (QIAGEN, Hilden, Germany) Quick-Start Protocol (step 1: 100 µL AUT buffer; step 4: 56 °C for 10–14 h; step 2: reuse of 20 µL initial eluate). Partial mtCOI amplification was done using the primers LCO1490/HCO2198 (Folmer et al. 1994). 50 µL standard reactions with 0.5–2 µL template (Q5 High-Fidelity DNA Polymerase, New England Biolabs, USA) were run following Geller et al. (2013) (5' 95 °C; 35 x (30" 95 °C, 30" 48 °C, 45" 72 °C); 5' 72 °C) and visualized using SYBR (SYBR Safe/Gold; Thermo Fisher Scientific) after electrophoretic separation in agarose gels. Products were Sanger-sequenced with Microsynth Austria GmbH. *Phred* (Ewing & Green 1998; Ewing et al. 1998) and *Geneious* (Kearse et al. 2012) were used to process raw chromatograms. We BLASTed sequences against NCBI for confirmation of identifications (Altschul et al. 1990). The final alignment (E-INS-I MAFFT; Katoh & Toh 2008; Katoh & Standley 2013) contained the new COI sequences and reference data (Weber et al. 2022: *Niphargus* aff. *aquilex* NCBI OK380849; Griebler et al. 2023b) and was used to infer phylogenetic relationships via IQTREE (Nguyen et al. 2015) estimating branch support through UFBoot (Minh et al. 2013), aBayes (Anisimova et al. 2011), and SH-aLRT (Guindon et al. 2010). The resulting tree was visualized with iTOL (Letunic & Bork 2021), with isopods serving as the outgroup. All sequences can be accessed at GenBank PZ503198 to PZ503226.

Statistical Analyses

Statistical analyses and visualizations were done in R (R Core Team 2021) via RStudio 2023.9.1.494 (Posit Team 2023) using the packages *vegan* (Oksanen et al. 2022), *tidyr* (Wickham & Girlich 2022), *DescTools* (Signorell et al. 2022), *ggplot2* (Wickham 2016), *reshape2* (Wickham 2007), *FSA* (Ogle et al. 2023), *TOSTER* (Lakens 2017; Caldwell 2022). An Akaike Information Criterion (AIC)-based permutational multivariate analysis of variance (PERMANOVA) approach was done using the code from kdyson (2017). GIS analyses and visualizations were done in QGIS 3.22.11 (QGIS Development Team 2024). The significance threshold was set at $p < 0.05$; p-values adjustment used a False Discovery Rate (FDR; Benjamini & Hochberg 1995) approach.

Community composition and associated environmental drivers

We included only taxa occurring in >5 samples and pooled subspecies (i.e. of *Nitocrella hirta*) at the species level. After preselecting environmental variables to reduce noise/multicollinearity, the community matrix was log- and then Hellinger-transformed and Bray–Curtis dissimilarities were computed. Continuous predictors were screened with Mantel tests and categorical ones with ANOSIM; significant variables were checked for multicollinearity via Spearman, retaining proxies (e.g., electrical conductivity for major ions; dissolved oxygen for redox). The final PERMANOVA analysis set comprised electrical conductivity, geology/aquifer type, groundwater temperature, surface-water influence, and dissolved oxygen; the best model was chosen using an adapted AIC, and variable order was set by R^2 , single-variable AIC, and ecological relevance (geology/aquifer > dissolved oxygen > surface-water influence). Community patterns were visualized with NMDS in two runs (all samples; outliers removed), with outliers defined as distances from the NMDS centroid above the 95th percentile.

Environmental drivers of crustacean occurrence and distribution in the shallow aquifers

We classified samples into presence versus absence groups (amphipods; extended to Bat-hynellacea, Cyclopoida, Harpacticoida, Isopoda, Ostracoda). Continuous variables were visualized with boxplots (log-scaled y where appropriate) and tested using the two-sample Brunner–Munzel permutation test (10,000 permutations; robust to unequal variances/sample sizes). Categorical variables were evaluated by comparing observed versus expected counts under the null hypothesis of no effect, with significance from G-tests. Cladocera were excluded due to the occurrence of a single specimen ($n=1$).

Species-specific environmental associations in amphipods

Environmental affinities among amphipod species were assessed using continuous variables based on sample presence, including a “no Amphi” category. Differences were tested with Kruskal–Wallis rank sum tests followed by Dunn’s post hoc tests. Boxplots (log-scaled y where appropriate) display selected ecologically relevant variables with significant interspecific differences. Categorical variables were analyzed using pairwise Fisher’s exact tests. A map illustrating the spatial distribution of amphipod species across Vienna was generated.

Results

Crustacean diversity in Vienna's groundwater

A broad diversity of stygobiont and stygophile crustaceans was detected across Vienna. Of the 306 groundwater fauna samples, amphipods occurred in 100 samples, whereas crustaceans in general were detected in 220. Bathynellacea occurred in 13 samples, Cyclopoida in 123, Harpacticoida in 18, Isopoda in 95, Ostracoda in 128, and Cladocera in only a single sample. In 27 samples, no fauna was detected at all, considering all fauna groups, not only crustaceans (Fig. 2 & 3).

When resolved taxonomically, these records comprised a total of 47 taxa representing 23 genera and 15 families, including three undescribed *Nitrocrella hirta* subspecies (Santiago Gaviria-Melo pers. comm.; detailed morphological differences are listed in the supplementary material) (Tab. 1).

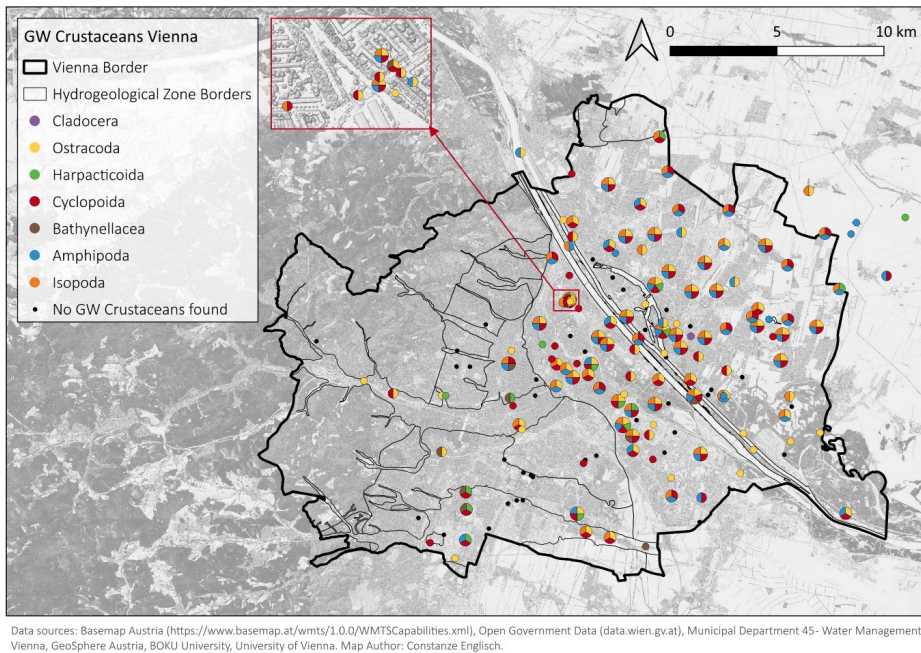


Fig. 2: Spatial distribution of crustacean groups (including stygophile and stygobiont taxa) across the study area in Vienna. Each point represents a sampled site. At sites where crustaceans were found, pie charts indicate the occurrence of the different groups, with colours corresponding to the respective group. Sites without crustaceans are shown as simple black points. Fine black lines indicate the borders of the major hydrogeological zones in Vienna which are depicted in detail in Figure 1. | **Abb. 2:** Räumliche Verteilung der einzelnen Crustaceengruppen unter Wien, stygophile und stygobionte Taxa. Jedes Symbol repräsentiert einen Probenahmepunkt. Das Auftreten einzelner Großgruppen ist in Form von Tortendiagrammen dargestellt. Standorte die keine Crustaceennachweise lieferten sind als einfach schwarze Punkte dargestellt. Dünne schwarze Linien sind Grenzen der hydrogeologischen Zonen. Siehe auch Abb. 1.

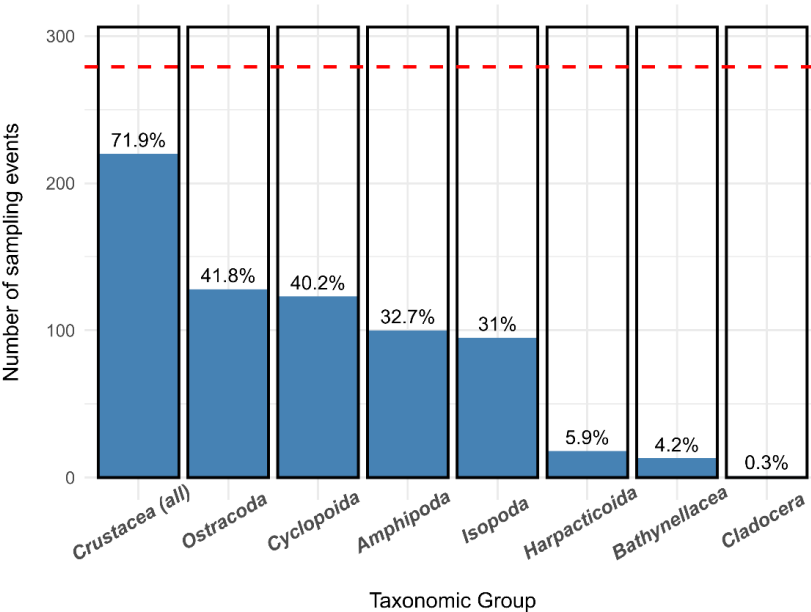


Fig. 3: Occurrence of taxonomic groups across 306 samples. The bar chart shows the number of samples in which each taxonomic group was detected, with percentage values above the bars relative to the maximum of 306 samples. Groups are sorted by frequency of occurrence. The red dashed line marks the 27 samples with a complete absence of fauna, including non-crustacean groups. | **Abb. 3:** Auftreten der taxonomischen Großgruppen. Das Säulendiagramm zeigt in wievielen Proben (aus 306) Vertreter einzelner Gruppen gefunden wurden. Die taxonomischen Gruppen sind absteigend nach Häufigkeit des Auftretens geordnet. Die rote gestrichelte Linie markiert die 27 Proben die keine Fauna enthielten.

Tab. 1: List of stygobiont and stygophile taxa identified in Vienna, based on data from the sampling campaigns conducted in 2021 and 2022. | **Tab. 1:** Liste aller in den Jahren 2021 und 2022 in Wiens Grundwasser gefundenen Crustaceen.

CLASS	ORDER	FAMILY	TAXA
Branchiopoda	Cladocera	Chydoridae	<i>Peracantha truncata</i> (O. F. Müller, 1785)
Ostracoda	Podocopida	Candonidae	<i>Cryptocandona kieferi danubialis</i> Namiotko et al. 2005
			<i>Marmocandona zschokkei</i> (Wolf, 1920)
			<i>Mixtacandona laisi</i> (Klie, 1938)
			<i>Mixtacandona spandli</i> Rogulj & Danielopol, 1993
			<i>Mixtacandona transleithanica</i> (Löffler, 1960)
			<i>Pseudocandona lobipes</i> (Hartwig, 1900)
			<i>Schellencandona belgica</i> (Klie, 1937)
			<i>Typhlocypris eremita</i> (Vejdovsky, 1882)
		Cyprididae	<i>Heterocypris incongruens</i> (Ramdohr, 1808)
		Limnocytheridae	<i>Kovalevskiella elisabethae</i> Gaviria, 2022

CLASS	ORDER	FAMILY	TAXA
Copepoda	Harpacticoida	Ameiridae	<i>Nitocra</i> n. sp.
			<i>Nitocrella hirta hirta</i> (Chappuis, 1924)
			<i>Nitocrella hirta</i> ssp. 1
			<i>Nitocrella hirta</i> ssp. 2
			<i>Nitocrella hirta</i> ssp. 3
		Chappuisiidae	<i>Chappuisius inopinus</i> Kiefer, 1938
		Parastenocarididae	<i>Fontinalicaris fontinalis borea</i> (Kiefer, 1960)
			<i>Fontinalicaris fontinalis fontinalis</i> (Schnitter & Chappuis, 1915)
			<i>Parastenocaris gertrudae</i> Kiefer, 1968
			<i>Parastenocaris husmanni</i> Chappuis, 1953
		Phyllognathopodidae	<i>Phyllognathopus viguieri</i> (Maupas, 1892)
	Cyclopoida	Cyclopidae	<i>Acanthocyclops gmeineri</i> Pospisil, 1989
			<i>Acanthocyclops hispanicus</i> Kiefer, 1937
			<i>Acanthocyclops kieferi</i> (Chappuis, 1925)
			<i>Acanthocyclops rhenanus</i> Kiefer, 1936
			<i>Acanthocyclops sensitivus</i> (Graeter & Chappuis, 1914)
			<i>Acanthocyclops venustus</i> (Norman & Scott, 1906)
			<i>Diacyclops charon</i> (Kiefer, 1931)
			<i>Diacyclops cohabitatus</i> Monchenko, 1980
			<i>Diacyclops danielopoli</i> Pospisil & Stoch, 1999
			<i>Diacyclops felix</i> Pospisil & Stoch, 1999
			<i>Diacyclops languidoides goticus</i> Kiefer, 1931
			<i>Diacyclops languidoides languidoides</i> (Lilljeborg, 1901)
			<i>Eucyclops graeteri</i> (Chappuis, 1927)
			<i>Paracyclops fimbriatus</i> (Fischer, 1853)
			<i>Paracyclops imminutus</i> Kiefer, 1929
Malacostraca	Bathynellacea	Bathynellidae	<i>Bathynellidae</i> gen. sp.
		Parabathynellidae	<i>Parabathynellidae</i> gen. sp.
	Amphipoda	Bogidiellidae	<i>Bogidiella albertimagni</i> Hertzog, 1933
		Crangonyctidae	<i>Crangonyx subterraneus</i> (Bate, 1859)
		Niphargidae	<i>Niphargus</i> aff. <i>aquilex</i> Schiødte, 1855
			<i>Niphargus bajuvaricus</i> Schellenberg, 1932
			<i>Niphargus caspary</i> (Pratz, 1866)
			<i>Niphargus inopinatus</i> Schellenberg, 1932
			<i>Niphargus tonywhitteni</i> Fišer et al. 2018
	Isopoda	Asellidae	<i>Proasellus slavus</i> (Remy, 1948)



Fig. 4: Phylogenetic tree based on COI sequences from amphipod species sequenced in this study (coloured by species), alongside isopod sequences (outgroup), amphipod sequences from a previous study (Griebler et al. 2023b), and one additional *Niphargus* aff. *aquilex* sequence from Luxembourg (Weber et al. 2022). Species from this study (coloured) are described by species name followed by the project title (HBT C = 'Heat below the City'), and a project-specific consecutive well number. Several published sequences were collapsed into clusters for clarity, with the species included in each cluster indicated alongside the number of sequences per species in parentheses. Well-supported branches are marked by orange circles (UFBoot $\geq 95\%$, aBayes ≥ 0.9 , SH-aLRT $\geq 85\%$), with circle size proportional to support strength. | **Abb. 4:** Phylogenetischer Baum auf Basis von COI Sequenzen für ausgewählte Amphipoden aus der Projektstudie. Zusätzlich wurden Sequenzen aus vorangehenden Untersuchungen im Raum Wien (Griebler et al. 2023) berücksichtigt und eine Sequenz von *Niphargus* aff. *aquilex* aus Luxemburg (Weber et al. 2022). Die Arten aus dem Projekt sind farblich markiert und der Code setzt sich aus Artnamen gefolgt vom Projektkürzel (HBT C = Heat below the City) und fortlaufender Standortnummer zusammen. Weitere publizierte Amphipodenarten sind im Baum als Cluster (mehrere Arten zusammengefasst) integriert. Äste mit gut dokumentierten Arten sind durch einen orangen Kreis markiert (UFBoot $\geq 95\%$, aBayes ≥ 0.9 , SH-aLRT $\geq 85\%$).

Taxonomic richness and composition varied among groups: Cyclopoida were richest (15 taxa in a single family); Harpacticoida had eleven taxa across four families (highest family diversity); Ostracoda exhibited ten taxa from three families, dominated by Candonidae (8/10); Amphipoda comprised seven taxa of three families, with Niphargidae dominating (5/7); Bathynellacea included at least two taxa from two families, i.e. Bathynellidae and Parabathynellidae. Both Isopoda and Cladocera were each represented by a single taxon.

Stygobiont amphipods in the shallow aquifers of Vienna

Most amphipod species collected showed consistent morphological, BLAST, and phylogenetic results (*Bogidiella albertimagni*, *Crangonyx subterraneus*, *Niphargus bajuvaricus*, *Niphargus caspary*, and most specimens of *Niphargus inopinatus*). In this respect, molecular analyses largely confirmed expert-based morphological identifications, with only a few exceptions. Some individuals were reassigned based on molecular evidence: specimens identified as *Niphargus* cf. *fontanus* were confirmed as *Niphargus tonywhitteni*, and several specimens initially suspected to belong to *Niphargus laisi* were genetically identified as *N. inopinatus*. One taxon, morphologically identified as *Niphargus aquilex*, could not be confidently assigned to any described species and is therefore referred to as *N. aff. aquilex*. BLAST searches revealed no close genetic matches except for a single, likewise unresolved *N. aff. aquilex* sequence from Luxembourg (Weber et al. 2022, Accession Number: OK380849; NCBI). Phylogenetic analyses placed this lineage clearly apart from *N. aquilex* and other published *N. aff. aquilex* lineages, indicating an unresolved taxonomic status (Fig. 4). Overall, the phylogenetic tree shows that amphipod sequences generated in this study cluster with sequences of the same species from previous studies, reflecting clear species-level groupings. Throughout this paper, the spelling *Niphargus caspary* is used, although both *N. caspary* and *N. casparyi* occur in the literature and the correct orthography remains unresolved.

Environmental structuring of crustacean community composition

The final PERMANOVA model revealed geology/aquifer type, dissolved oxygen concentration and surface water influence as the most important explanatory variables for differences in groundwater crustacean community compositions (Tab. 2).

Tab. 2: Results of the Permutational Multivariate Analysis of Variance (PERMANOVA) for the ecological variables geology/aquifer type, dissolved oxygen concentration, and surface water influence. The analysis is based on 999 permutations. | **Tab. 2:** Ergebnisse der PERMANOVA für die ökologischen Variablen Geologie/Aquifertyp, gelöster Sauerstoff und Oberflächenwassereinfluss. Die Analyse basiert auf 999 Permutationen.

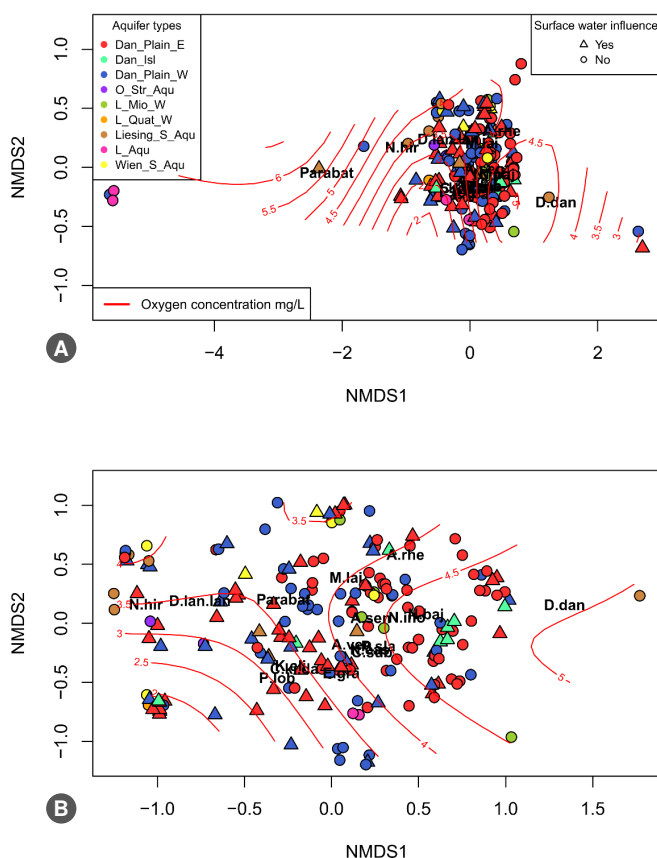
Ecological parameter	df	SumOfSqs	MeanSqs	R ²	F	p-value
Geology/Aquifer type	8	7.377	0.9221	0.10558	3.0090	0.001
Dissolved oxygen (mg/L)	1	1.743	1.7430	0.02494	5.6863	0.001
Surface water influence	1	1.608	1.6080	0.02301	5.2465	0.001
Residual	193	59.143	0.3064	0.84647		
Total	203	69.870		1.00000		

Fig. 5: Non-metric Multidimensional Scaling (NMDS) ordination of crustacean community composition. (A) Ordination including all samples. (B) Ordination excluding identified outliers. Points represent individual samples and are coloured by aquifer type. Surface water influence is indicated by point shape. Dissolved oxygen concentration is shown as smooth red isolines fitted across the ordination. Weighted average species scores are overlaid in black. Stress values: 0.1346 (A) and 0.1741 (B).

Taxa abbreviations: N.cas = *Niphargus caspary*, N.baj = *Niphargus bajuvaricus*, N.ino = *Niphargus inopinatus*, C.sub = *Crangonyx subterraneus*, A.rhe = *Acanthocyclops rhenanus*, A.sen = *Acanthocyclops sensitivus*, A.ven = *Acanthocyclops venustus*, D.dan = *Diacyclops danielopolis*, D.lan.lan = *Diacyclops languidoides languidoides*, E.gra = *Eucyclops graeteri*, C.ki.da = *Cryptocandona kieferi danubialis*, M.lai = *Mixtacandona laisi*, P.lob = *Pseudocandona lobipes*, K.eli = *Kovalevskiella elisabethae*, Parabat = *Parabathynellidae* gen. sp., N.hir = *Nitocrella hirta*, P.sla = *Proasellus slavus*.

Aquifer type abbreviations: Dan_Plain_E = Danube Plain East, Dan_Plain_W = Danube Plain West, Dan_Isl = Danube Island, O_Str_Aqu = Other Stream Aquifer, L_Mio_W = Local Miocene Water, L_Quat_W = Local Quaternary Water, Wien_S_Aqu = Wien Stream Aquifer, Liesing_S_Aqu = Liesing Stream Aquifer, L_Aqu = Local Aquifer (see Fig. 1).

Abb. 5: NMDS Ordination der Crustaceengemeinschaften. (A) Ordination inklusive aller Proben. (B) Ordination exklusive einzelner Ausreißer. Die Symbole repräsentieren individuelle Proben und sind entsprechend ihrer Zugehörigkeit zu den Aquifertypen gefärbt. Der Oberflächenwassereinfluss ist durch die Form der Symbole repräsentiert. Die Konzentrationen an gelöstem Sauerstoff sind als rote Isolinen in das Ordinationsdiagramm eingefügt. Gewichtete durchschnittliche Werte für einzelne Arten sind in schwarzer Schrift eingefügt. Stresswerte: 0.1346 (A) und 0.1741 (B). **Abkürzungen für die Taxa:** N.cas = *Niphargus caspary*, N.baj = *Niphargus bajuvaricus*, N.ino = *Niphargus inopinatus*, C.sub = *Crangonyx subterraneus*, A.rhe = *Acanthocyclops rhenanus*, A.sen = *Acanthocyclops sensitivus*, A.ven = *Acanthocyclops venustus*, D.dan = *Diacyclops danielopolis*, D.lan.lan = *Diacyclops languidoides languidoides*, E.gra = *Eucyclops graeteri*, C.ki.da = *Cryptocandona kieferi danubialis*, P.lob = *Pseudocandona lobipes*, K.eli = *Kovalevskiella elisabethae*, Parabat = *Parabathynellidae* gen. sp., N.hir = *Nitocrella hirta*, P.sla = *Proasellus slavus*. **Abkürzungen für Aquifertypen:** Dan_Plain_E = Danube Plain East, Dan_Plain_W = Danube Plain West, Dan_Isl = Danube Island, O_Str_Aqu = Other Stream Aquifer, L_Mio_W = Local Miocene Water, L_Quat_W = Local Quaternary Water, Wien_S_Aqu = Wien Stream Aquifer, Liesing_S_Aqu = Liesing Stream Aquifer, L_Aqu = Local Aquifer (siehe Abb. 1).



Geology/aquifer type explained the largest proportion of variation ($R^2 = 0.106$, $F = 3.01$, $p = 0.001$), making it the strongest predictor in the best-fitting model. Dissolved oxygen concentration ($R^2 = 0.025$, $F = 5.69$, $p = 0.001$) and surface water influence ($R^2 = 0.023$, $F = 5.25$, $p = 0.001$) also showed significant but smaller effects, with dissolved oxygen emerging as the slightly stronger of the two. Despite these significant relationships, most variation in community composition remained unexplained (residual $R^2 = 0.846$), suggesting that additional disregarded environmental factors and/or stochastic processes likely contribute to the observed community differences. Notably, sampling season (autumn vs. spring) did not have a significant effect.

Community-level patterns and environmental gradients were further explored using NMDS ordination, incorporating the three variables identified in the PERMANOVA (Fig. 5). Stress values were 0.1346 (including outliers) and 0.1741 (excluding outliers), both indicating an acceptable representation of the underlying dissimilarity structure. The NMDS ordinations revealed a dense central cluster encompassing the majority of samples, accompanied by a small number of distinct outliers. Samples associated with *Diacyclops danielopolis* were positioned toward the positive end of the NMDS1 axis, whereas those linked to *Parabathynellidae* gen. sp. occurred toward negative NMDS1 values. Following removal of outliers, *Parabathynellidae* gen. sp. shifted slightly toward the central cluster, while the overall spatial configuration of the different samples remained largely unchanged. Across aquifer types, no distinct groupings or clearly separated community clusters were evident in the NMDS ordinations. Samples from different aquifer types overlapped strongly when outliers were included. Outlier events originated from multiple aquifer types. Only *Parabathynellidae* gen. sp., *Diacyclops danielopolis*, and *Nitocrella hirta* appeared spatially associated with individual samples from the Liesing Stream Aquifer. After excluding outliers, the general lack of well-defined clustering persisted, however, more subtle spatial patterns became discernible. Samples from the Danube Plain aquifer (east and west) remained broadly scattered throughout the ordination space, while samples from the Danube Island were mainly aligned along the positive NMDS1 axis and occurred in proximity to the species scores of *Niphargus bajuvaricus* and *Niphargus inopinatus*. Samples from the Liesing Stream Aquifer were situated mainly in the negative NMDS1 region, with NMDS2 values approximately around zero. Samples from the Wien Stream Aquifer were concentrated primarily in the positive NMDS2 and negative NMDS1 region.

Regarding dissolved oxygen concentration, the NMDS ordination, including outliers, revealed a gradient with a range of approximately 2.5–5.5 mg/L, with *Parabathynellidae* gen. sp. associated with the highest oxygen levels. Due to the dense central clustering, finer patterns were easier to resolve after excluding outliers, but the overall range of the gradient remained almost unchanged. In this ordination, *D. danielopolis* was associated with the highest oxygen concentrations (≈ 5 mg/L). Several species including *M. laisi*, *A. rhenanus*, *A. sensitivus*, *A. venustus*, *E. graeteri*, *C. subterraneus*, *N. bajuvaricus*, *N. caspary*, *N. inopinatus*, and *P. slavus* were associated with intermediate oxygen levels (≈ 4.0 – 4.5 mg/L), whereas lower oxygen concentrations (≈ 3.0 – 3.5 mg/L) were linked to *C. kieferi danubialis*, *P. lobipes*, *K. elisabethae*, *D. languidoides languidoides* and *N. hirta*. *Parabathynellidae* gen. sp., previously among the high oxygen samples, shifted toward the central cluster after outlier removal, corresponding to moderate oxygen levels (≈ 3.5 mg/L).

Surface water influence did not produce a distinct structure within the main cluster when outliers were included, while outlier samples were mostly not affected by surface water. After excluding outliers, a moderate separation became apparent, with surface water influenced samples occurring predominantly in the negative quadrant of the NMDS plot, where *P. lobipes*, *C. kieferi danubialis*, and *K. elisabethae* were positioned.

Overall, compositional tendencies were detectable for all three variables, which may reflect the PERMANOVA results. However, the NMDS ordinations indicate a largely homogeneous community structure, and clearly separated community clusters based on these variables were not observed.

Environmental drivers shaping the occurrence and distribution of amphipods and other crustaceans

Presence-absence patterns of total Crustacea and individual crustacean groups were analysed in relation to environmental variables. After False Discovery Rate (FDR) correction, seven continuous parameters emerged as significant for Amphipoda (Fig. 6) and eight for total Crustacea, with largely overlapping sets of predictors. Both Amphipoda and total Crustacea preferably occurred in samples with higher dissolved oxygen concentrations (Amphipoda: $p < 0.001$; total Crustacea: $p < 0.001$) and lower values of microbial activity (ATP; Amphipoda: $p < 0.001$; total Crustacea: $p < 0.001$), bacterial abundance (TCC; Amphipoda: $p < 0.001$; total Crustacea: $p < 0.001$), dissolved organic carbon (DOC; Amphipoda: $p = 0.002$; total Crustacea: $p = 0.006$), ammonium (Amphipoda: $p < 0.001$; total Crustacea: $p = 0.001$), and ferrous iron (Amphipoda: $p < 0.001$; total Crustacea: $p < 0.001$), compared to samples where they were absent. Amphipoda additionally occurred more frequently at significantly lower temperatures ($p = 0.006$), whereas this pattern was not observed for total Crustacea. However, no crustaceans were found at temperatures above 21.94°C. In contrast, total Crustacea showed significant associations with lower dissolved inorganic carbon (DIC; $p = 0.043$) and higher nitrate concentrations ($p = 0.019$), relationships that were not significant for Amphipoda.

For the remaining crustacean groups, many of the directional patterns observed for Amphipoda and total Crustacea were likewise evident. Dissolved oxygen concentrations were consistently higher in samples containing Bathynellacea ($p = 0.001$), Cyclopoida ($p < 0.001$), Harpacticoida ($p < 0.001$), and Isopoda ($p < 0.001$). Microbial activity (ATP), bacterial abundance (TCC), and DOC were lower at sites where Cyclopoida (ATP: $p = 0.001$; TCC: $p = 0.029$; DOC: $p < 0.001$) and Isopoda (ATP: $p < 0.001$; TCC: $p < 0.001$; DOC: $p = 0.038$) occurred. Nitrate showed the same positive association as in the total Crustacea analysis with higher concentrations in samples containing Isopoda ($p = 0.002$), Harpacticoida ($p = 0.035$), and Cyclopoida ($p < 0.001$). Ammonium concentrations were lower at sites where Bathynellacea ($p < 0.001$), Cyclopoida ($p < 0.001$), and Isopoda ($p < 0.001$) were present, and ferrous iron likewise showed a negative association with the occurrence of Cyclopoida ($p < 0.001$) and Isopoda ($p < 0.001$).

Beyond these shared patterns, several significant relationships were restricted to individual crustacean groups. Water column height was negatively associated with Bathynellacea ($p = 0.019$), Harpacticoida ($p = 0.013$), and Cyclopoida ($p < 0.001$). Hydraulic conductivity

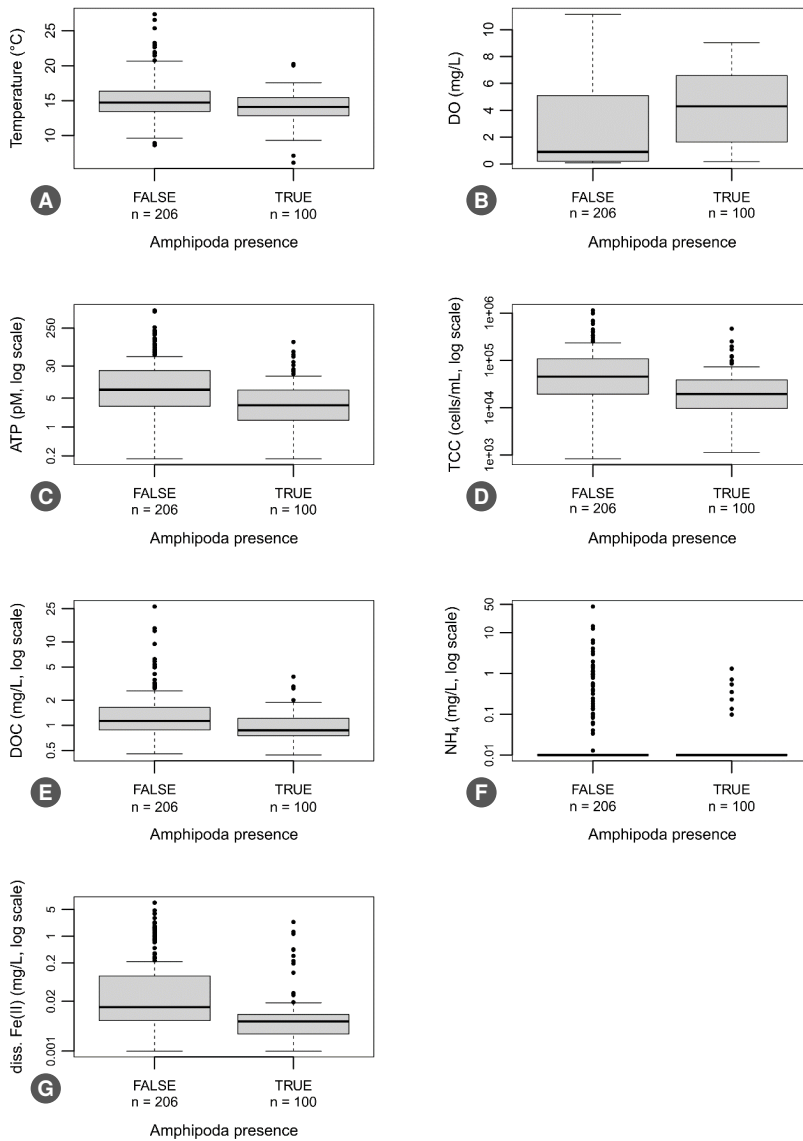


Fig. 6: Boxplots of continuous environmental variables that showed significant associations with Amphipoda presence across all samples (“FALSE” = absent, “TRUE” = present). Plots display median, interquartile range (IQR), whiskers (1.5 × IQR), and outliers. Variables: A) groundwater temperature (Temperature), B) dissolved oxygen (DO). Variables with log-scaled y-axes: C) microbial activity (ATP), D) bacterial abundance (TCC), E) dissolved organic carbon (DOC), F) ammonium (NH₄⁺), and G) ferrous iron (diss. Fe²⁺). | **Abb. 6:** Boxplots von Umweltvariablen die einen signifikanten Zusammenhang mit der Anwesenheit von Amphipoden zeigen (“FALSE” = abwesend, “TRUE” = anwesend). Die Abbildungen zeigen Medianwerte, den interquartilen Bereich (IQR), Whiskers (1.5 × IQR), und Ausreißer. Variablen: A) Grundwassertemperatur (Temperature), B) gelöster Sauerstoff (DO), C) mikrobielle Aktivität (ATP), D) Gesamtbakterienzahl (TCC), E) gelöster organischer Kohlenstoff (DOC), F) Ammonium (NH₄⁺), und G) reduziertes Eisen (diss. Fe²⁺).

was negatively associated with Bathynellacea ($p = 0.013$) and Harpacticoida ($p = 0.035$), while surface sealing was positively associated with Harpacticoida ($p = 0.036$). Sulfate concentrations were positively associated with Harpacticoida ($p = 0.020$) and Cyclopoida ($p = 0.037$) and negatively with Ostracoda ($p = 0.014$). Chloride was positively associated with Harpacticoida ($p = 0.013$), and calcium positively with Harpacticoida ($p = 0.035$) but negatively with Ostracoda ($p = 0.036$). Magnesium ($p = 0.014$) and electrical conductivity ($p = 0.048$) were negatively associated with Ostracoda. Orthophosphate, sodium, potassium, and distance to surface water were positively associated with Cyclopoida ($p = 0.037$; $p = 0.037$; $p = 0.037$; $p < 0.001$).

In addition to continuous variables, three categorical environmental variables – surface water influence, geology/aquifer type, and redox conditions – were analysed. Surface water influence was only significant for Ostracoda, which exhibited higher-than-expected occurrences at sites classified as influenced by surface water ($p = 0.036$).

Geology/aquifer type showed no significant associations for total Crustacea, whereas Amphipoda exhibited significant differences. Amphipod occurrences were higher than expected in the Danube Plain East ($p = 0.046$) and Danube Island ($p = 0.046$) aquifers, and lower than expected in the Liesing Stream Aquifer ($p = 0.046$) and the Wien Stream Aquifer ($p = 0.046$). Bathynellacea showed lower-than-expected occurrences in the Danube Plain East aquifer ($p = 0.002$) but higher-than-expected occurrences in the Liesing Stream Aquifer ($p = 0.002$) and Local Aquifers ($p = 0.006$), while Isopoda exhibited lower-than-expected occurrences in the Local Miocene Water aquifer type ($p = 0.025$).

Redox conditions showed significant effects on most groups. For reference, redox categories were defined as: 1 = $O_2 > 2 \text{ mg/L}$; 2 = $O_2 < 2 \text{ mg/L}$; 3 = $Mn^{2+} > 0.1 \text{ mg/L}$ or $Fe^{2+} > 0.1 \text{ mg/L}$; 4 = $HS^- > 0.01 \text{ mg/L}$. Redox category 1 was associated with higher-than-expected occurrences (Amphipoda: $p = 0.005$; total Crustacea: $p < 0.001$; Cyclopoida: $p = 0.001$; Isopoda: $p = 0.034$). From category 2 onwards, only significant negative associations were observed: category 2 showed lower-than-expected occurrences for total Crustacea ($p = 0.024$), category 3 for Amphipoda ($p = 0.004$), total Crustacea ($p < 0.001$), Cyclopoida ($p < 0.001$), Harpacticoida ($p = 0.032$), and Isopoda ($p < 0.001$), and category 4 for Amphipoda ($p = 0.039$), Cyclopoida ($p = 0.017$), and Isopoda ($p = 0.046$).

It should be noted that sample sizes across categories were highly uneven, as was also the case for the occurrence of the individual crustacean groups (Fig. 4). For geology/aquifer type, sample numbers ranged from $n = 2$ (Local Quaternary Water & Not Defined) to $n = 141$ (Danube Plain East), with intermediate values for Danube Plain West ($n = 95$), Danube Island ($n = 10$), Liesing Stream Aquifer ($n = 15$), Wien Stream Aquifer ($n = 7$), Local Aquifers ($n = 7$), Local Miocene Water ($n = 21$), and Other Stream Aquifers ($n = 6$). Redox categories were similarly unbalanced (category 1: $n = 163$, category 2: $n = 79$, category 3: $n = 58$, category 4: $n = 6$), whereas surface water influence classes were moderately balanced (no: $n = 184$; yes: $n = 122$). Results should therefore be interpreted with appropriate caution.

Amphipod species-specific associations with environmental conditions

The most frequently recorded species, based on presence per sample, was *Niphargus inopinatus* ($n = 76$), followed by *N. caspary* (26), *N. bajuvaricus* (15), *Crangonyx subterraneus* (6), *Bogidiella albertimagni* (4), *N. tonywhitteni* (3), and *N. aff. aquilex* (2), while samples without amphipods were included as “no Amphi” ($n = 206$). These differences in occurrence, together with the low sample size for some species, should be considered when interpreting the results.

Dissolved oxygen (DO) (Fig. 7A) and ferrous iron (Fe^{2+}) both differed significantly between groups (KW: DO $p < 0.001$, Fe^{2+} $p < 0.001$). Dunn’s test for multiple comparisons, however, identified significant differences only between sites without amphipods and those with *N. bajuvaricus* (DO: $p = 0.009$, Fe^{2+} : $p = 0.034$), *N. caspary* (Fe^{2+} : $p = 0.011$), and *N. inopinatus* (DO: $p < 0.001$, Fe^{2+} : $p < 0.001$). Fe^{2+} showed an inverse pattern to DO, with species associated with lower DO concentrations tending to also be associated with higher Fe^{2+} values. No significant differences were observed between individual species for these variables, although some moderate differences are visible in the plot.

Microbial activity (ATP) (Fig. 7B) and bacterial abundance (TCC) also varied significantly between species/groups (KW: ATP $p < 0.001$, TCC $p < 0.001$), with both variables exhibiting comparable patterns. Sites without amphipods had higher ATP and TCC values than those where *N. inopinatus* (ATP: $p < 0.001$, TCC: $p < 0.001$), *N. caspary* (ATP: $p = 0.007$, TCC: $p = 0.001$), *N. bajuvaricus* (ATP: $p = 0.015$, TCC: $p = 0.001$), and *Bogidiella albertimagni* (ATP: $p = 0.024$) were found. Additionally, a significant difference in microbial activity (ATP) was observed specifically between two species, namely *B. albertimagni*, which had the lowest median, and *N. aff. aquilex*, which had the highest, among all species ($p = 0.037$).

Similarly, water column height (Wco) (Fig. 7C) and well depth (Wede) differed significantly between groups (KW: Wco $p = 0.007$, Wede $p = 0.032$), showing similar patterns. Here, most significant pairwise differences occurred between species. *B. albertimagni* exhibited the highest median values and differed from *N. aff. aquilex* (Wco: $p = 0.023$, Wede: $p = 0.030$), *N. tonywhitteni* (Wco: $p = 0.013$, Wede: $p = 0.041$), and “no Amphi” (Wco: $p = 0.049$, Wede: $p = 0.048$). Significant pairwise differences specific to water column height were also observed for *N. caspary*, which was associated with higher values than *N. tonywhitteni* ($p = 0.037$) and *N. aff. aquilex* ($p = 0.045$).

Hydraulic conductivity (K_f) (Fig. 7D) was significant overall (KW: $p = 0.034$), but no pairwise comparisons remained significant after FDR adjustment. Nevertheless, a gradient across species was apparent, with all species exhibiting higher K_f median values than “no Amphi,” except *C. subterraneus* and, notably, *N. aff. aquilex*, which was associated with particularly low values. Dissolved organic carbon (DOC) showed a significant KW result ($p = 0.001$). Subsequent Dunn’s test revealed only one significant difference: *N. inopinatus* was associated with lower DOC values than “no Amphi” ($p = 0.003$), while differences between other species were relatively minor. Electrical conductivity (EC) was not significant; however, it is worth mentioning that *N. aff. aquilex* exhibited a pronounced deviation with substantially higher values compared to other species. Similar patterns were observed for sulfate, chloride, sodium, and magnesium.

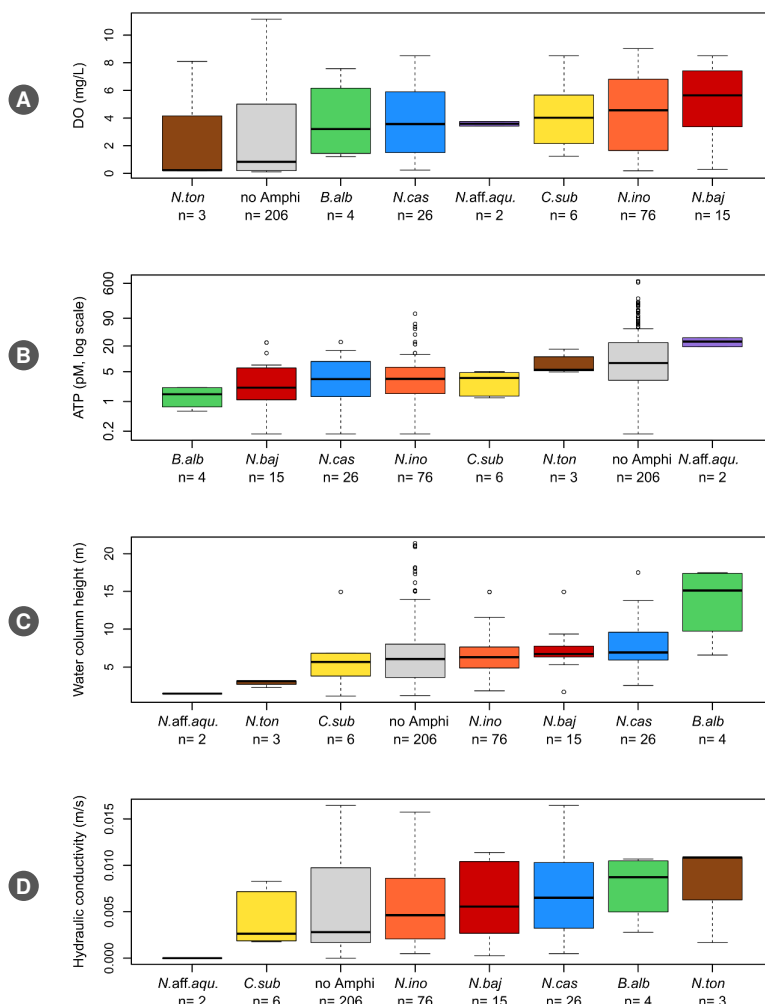


Fig. 7: Distribution of selected continuous environmental variables across all samples for different amphipod species and samples without amphipods (no Amphi). Panels show: (A) dissolved oxygen (DO), (B) microbial activity measured as cellular ATP, (C) water column height, and (D) hydraulic conductivity (K_f). The x-axis indicates amphipod species, labelled with the number of samples in which each species occurred, and the y-axis represents the respective parameter. Boxplots are arranged in order of increasing median. **Species abbreviations:** B.alb = *Bogidiella albertimaghi*, C.sub = *Crangonyx subterraneus*, N.aff.aqu. = *Niphargus aff. aquilex*, N.baj = *Niphargus bajuvaricus*, N.cas = *Niphargus caspary*, N.ino = *Niphargus inopinatus*, N.ton = *Niphargus tonywhitteni*. “No Amphi” represents samples in which no amphipods were recorded. | **Abb. 7:** Variabilität ausgewählter Umweltfaktoren bei einzelnen Amphipodenarten im Vergleich zu den Standorten die keine Amphipoden (no Amphi) lieferten. Gezeigt sind: (A) gelöster Sauerstoff (DO), (B) mikrobielle Aktivität gemessen als intrazelluläres ATP, (C) Höhe der Wassersäule, und (D) hydraulische Leitfähigkeit (K_f). Die Amphipodenarten sind auf der x-Achse aufgetragen und entsprechend eines steigenden Medianwertes geordnet. **Abkürzung der Arten:** B.alb = *Bogidiella albertimaghi*, C.sub = *Crangonyx subterraneus*, N.aff.aqu. = *Niphargus aff. aquilex*, N.baj = *Niphargus bajuvaricus*, N.cas = *Niphargus caspary*, N.ino = *Niphargus inopinatus*, N.ton = *Niphargus tonywhitteni*. “No Amphi” steht für Proben die keine Amphipoden enthielten.

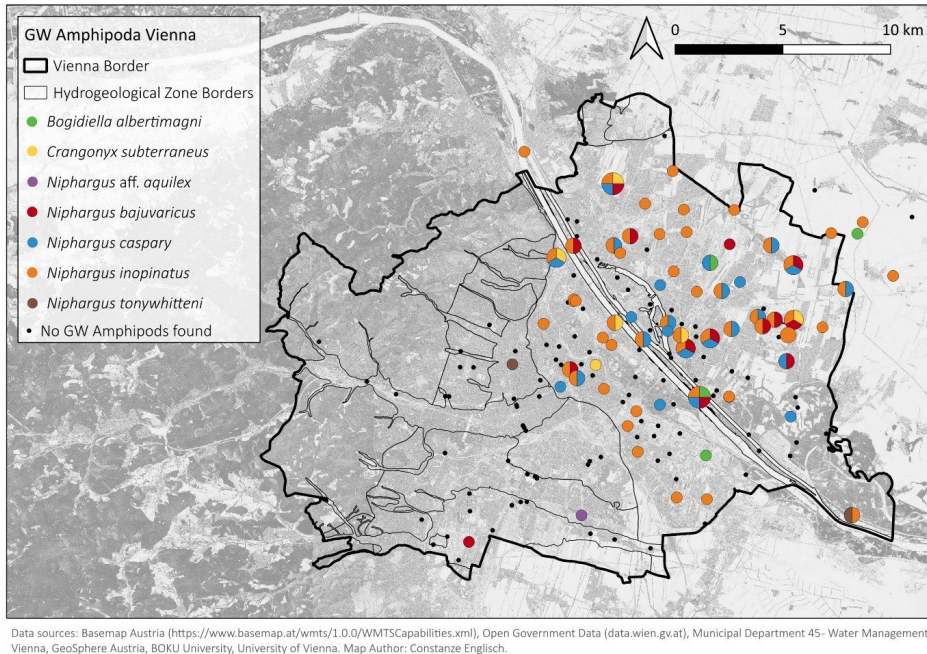


Fig. 8: Spatial distribution of amphipod species across the study area in Vienna. Each point represents a sampled site. At sites where amphipods were detected, pie charts indicate the occurrence of the different species, with colours corresponding to the respective species. Sites without amphipods are shown as black points. Fine black lines indicate the borders of the major hydrogeological zones in Vienna which are depicted in detail in Figure 1. | **Abb. 8:** Räumliche Verbreitung der Amphipodenarten im Untersuchungsgebiet. Standorte ohne Amphipodennachweis während der Studie sind als schwarze Punkte dargestellt. Dünne schwarze Linien sind Grenzen der hydrogeologischen Zonen. Siehe auch Abb. 1.

Additionally, some differences were observed with respect to the categorical variables. For geology/aquifer type, significant pairwise differences in species occurrence were identified, indicating that the respective species pairings differ in their distribution across aquifer types. Specifically, *N. aff. aquilex* differed from *N. caspary* ($p = 0.025$), *N. inopinatus* ($p = 0.009$), and “no Amphi” ($p = 0.042$). Additionally, *N. inopinatus* differed from “no Amphi” ($p = 0.002$). In this context, it is noteworthy that *N. aff. aquilex* was recorded at only a single site, with one occurrence in spring and one in autumn, and represented the only amphipod species detected within the aquifer type Other Stream Aquifer. The spatial distribution of amphipod species across the study area is illustrated in Figure 8.

With respect to the redox conditions, a single significant difference was detected, with *N. inopinatus* differing from “no Amphi” ($p = 0.002$). Across the redox gradient, the likelihood of detecting *N. inopinatus* increased from redox category 4 (highly reduced conditions) to category 1 (oxic conditions). A very similar, however non-significant trend was observed for most of the other amphipod species, whereas the probability of finding no amphipods increased from redox category 1 to 4. For surface water influence, no significant differences in amphipod species distributions were observed.

Discussion

Groundwater crustacean diversity in Vienna

Considering the multitude of stressors present in an urban area (Becher et al. 2022), the present study recorded an unexpectedly high diversity of groundwater crustaceans within the city of Vienna, with a total of 47 stygobiont/stygophile taxa identified. Figure 9 depicts a selection of species that represent the major taxonomic groups of crustaceans. Direct comparisons with other cities are limited, as only a few studies in Central European cities have previously investigated stygofauna (Buder 2019; Koch et al. 2021; Dumnicka et al. 2025a, 2025b). Most of these other studies have not analysed the urban stygofauna to species level, but rather on a coarser taxonomic level as well as with a lower spatial resolution and sampling density. However, comparison suggests that the groundwater fauna biodiversity in Vienna is exceptionally high compared to other cities, as well as to rural and more natural groundwater ecosystems of a similar size, including well accepted hotspots of subterranean biodiversity (Ferreira et al. 2007; Culver et al. 2021; Griebler et al. 2023b).

The exceptional richness observed in Vienna is likely rooted in its geological and ecological history. The city developed on a formerly dynamic floodplain shaped by the unregulated Danube River. Over long periods of time, this generated a diverse array of groundwater habitats and facilitated the diversification of groundwater fauna. Today, pronounced environmental heterogeneity, including geological variability, spatially heterogeneous hydrological conditions, and differing degrees of surface water influence, helps to explain how such high levels of diversity have been maintained. Moreover, the linkage between the city of Vienna and the Lobau wetland as part of the Danube Floodplain National Park (DFNP) is expected to facilitate faunal exchange and recolonization, supporting regional genetic exchange and adaptation. In addition, groundwater across large parts of Vienna exhibited oligotrophy, i.e. a low productivity mirrored by low amounts of organic matter and microbial biomass, and did at the same time contain sufficient amounts of dissolved oxygen, providing favourable conditions for groundwater crustaceans. Overall, the combination of historical habitat formation and present-day environmental heterogeneity appears to have enabled the persistence of an exceptionally diverse groundwater crustacean community within a densely populated urban area, with a taxon richness comparable to other groundwater biodiversity hotspots (Ferreira et al. 2007; Culver et al. 2021; Griebler et al. 2023b).

Comparing the groundwater crustacean species list compiled in this study with the species that were previously known for Vienna and adjacent regions, some species were rediscovered, some could not be found again, some have never been found in this area before, and some are even new for science. Species that have been rediscovered include *Cryptocandona kieferi danubialis*, *Mixtacandona laisi*, *M. spandli*, *Pseudocandona lobipes*, *Typhlocypris eremita* and *Kovalevskiella elisabethae* (Ostracoda - previous records compiled by Griebler et al. 2023b), *Nitocrella hirta hirta*, *Fontinalicaris fontinalis fontinalis* and *Parastenocaris* sp. (Harpacticoida - previous records by Vornatscher, 1972; Danielopol & Pospisil, 2001), *Acanthocyclops gmeineri*, *A. kieferi*, *A. rhenanus*, *A. sensitivus*, *A. venustus*, *Diacyclops cohabitatus*, *D. danielopoli*, *D. felix*, *D. languidoides goticus*, *D. languidoides languidoides*, *Eucyclops graeteri* and *Paracyclops fimbriatus* (Cyclopoida - previous records compiled by Griebler et al. 2023b),

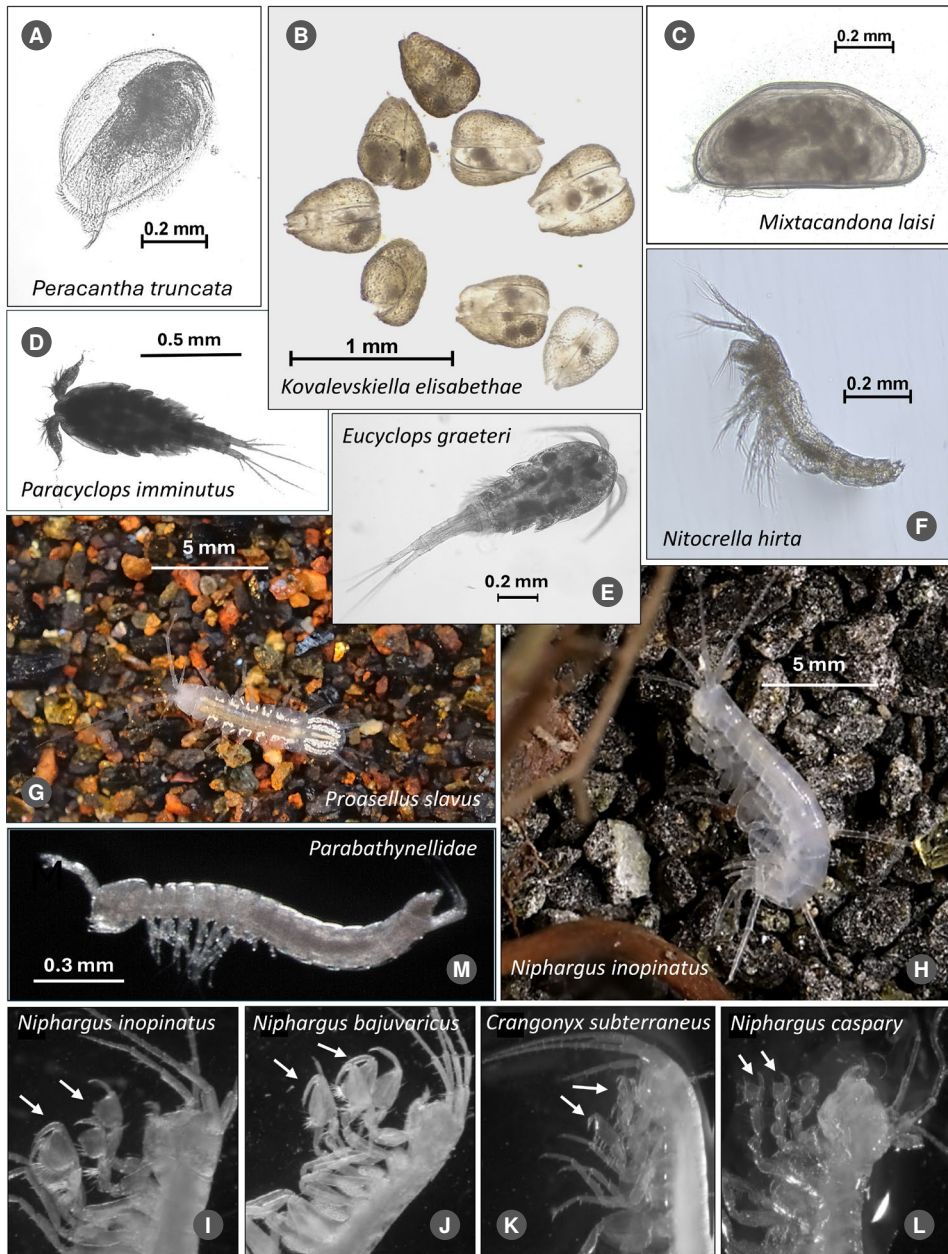


Fig. 9: The composition of pictures shows stygobiont and stygophile crustaceans from Viennese shallow groundwater habitats, representing the taxonomic groups (A) Cladocera, (B+C) Ostracoda, (D+E) Cyclopoida, (F) Harpacticoida, (G) Isopoda, (H–L) Amphipoda, and (M) Bathynellacea. The pictures I–L depict the gnathopods (white arrows) of four different stygobiont amphipod species occurring below Vienna. The gnathopods are modified first two pairs of walking legs mainly used for handling food & prey. Their shape and size help in identification of different taxa and provide evidence for feeding

behavior and food sources. © (A–F) Santiago Gaviria, (G) Günther Teichmann, (H) Kurt Kracher, (I–L) Ayac Iuan Jiménez Salvador, (M) Constanze Englisch. | **Abb. 9:** Die Bildertafel zeigt stygobionte und stygophile Crustaceen aus oberflächennahen Grundwasserlebensräumen unter Wien. Folgende taxonomische Gruppen sind vertreten: (A) Cladocera (Wasserflöhe), (B+C) Ostracoda (Muschelkrebse), (D+E) Cyclopoida (Hüpfertinge), (F) Harpacticoida (Rauenhüpfertinge), (G) Isopoda (Asseln), (H–L) Amphipoda (Flohkrebse), and (M) Bathynellacea (Brunnenkrebse). Die Bilder I–L zeigen den Kopf und die Gnathopoden (weiße Pfeile) von vier in Wien vorkommenden Grundwasserflohkrebsarten. Die Gnathopoden sind die vordersten beiden Schreitbeinpaare die zu einer Art Händen umfunktioniert sind. Die Form und Größe sind ein wichtiges Bestimmungsmerkmal und geben Auskunft über die Ernährungsweise der Tiere.

Bathynellidae gen. sp. and Parabathynellidae gen. sp. (Bathynellacea - previous records by Strouhal, 1964; Vornatscher, 1972; Danielopol, 1976; Serban, 1989;), *Bogidiella alberti-magni*, *Crangonyx subterraneus*, *Niphargus bajuvaricus*, *Niphargus caspary* and *Niphargus inopinatus* (Amphipoda - previous records compiled by Griebler et al. 2023b), and *Proasellus slavus* (Isopoda - previous records compiled by Griebler et al. 2023b). Therefore, the majority of the crustacean taxa found in this study (60 %) were known from Vienna and surrounding regions, however, with some that were last found several decades ago. A large portion (40 %) of taxa was newly discovered for the Vienna area. This underlines the necessity and relevance of high-resolution sampling efforts.

Besides the rediscovered and newly discovered taxa, some species were previously found in the Vienna area but could not be confirmed in this study. Reasons can be manifold, including local rarity or extinction. One reason can be taxonomic uncertainty, as the groundwater ecosystem in Vienna hosts several cryptic species and not all historic records may withstand a verification by means of modern methods. For example, the case of *Niphargus* aff. *aquilex* highlights taxonomic uncertainty, as historical records of *N. aquilex* in the Vienna area exist, but there is evidence that the formerly assigned and newly analysed specimens do not belong to the *N. aquilex* species complex. The phylogenetic analyses revealed that the sequences obtained in this study do not cluster with any confirmed *N. aquilex* sequences, but only with a single unresolved *N. aff. aquilex* sequence from Luxembourg (Weber et al. 2022). Given the lack of a monophyletic *N. aquilex* clade and the ongoing revision of this species complex (Weber et al. 2023, 2025), the Vienna population may represent an undescribed species or subspecies.

In the cases of several other taxa, historical records only describe a genus or species, while our current findings report corresponding species or subspecies, and *vice versa*. Historical records describe several *Proasellus* species and subspecies of *P. slavus*. Current molecular analyses, including specimen from this study (exclusively *P. slavus*) as well as data collected independently of this study, indicate a wide-spread occurrence of *P. slavus* throughout the city with less abundant occurrences of *P. strouhali* (C. Novotny pers. comm.). With respect to the main focus on amphipod assemblages in this study, the combination of morphological and molecular analyses was successfully implemented. This is reflected in the largely coherent identification (morphologically and molecularly) of seven amphipod species in Vienna, as well as new insights in the *N. aff. aquilex* case, the first detection of *Niphargus tonywhitteni* in Vienna, and the first molecular verification of *Bogidiella alberti-magni* in Vienna. Overall, the groundwater amphipod fauna of Vienna largely conforms established biogeographic

distribution patterns within Central Europe and the Danube catchment. An exception is *N. tonywhitteni*, as this species was described only quite recently from Upper and Lower Austria, Tyrol (Fišer et al. 2018), Switzerland (Alther & Altermatt 2021), and southern Bavaria (Weber 2024). The present record further extends its known distribution to the east. Molecular analyses indicate that specimens previously identified morphologically as *N. fontanus* cluster with *N. tonywhitteni*, supporting recent evidence that historical records of *N. fontanus* may represent misidentifications (Weber 2024). Consequently, the historical record of *N. fontanus* from 1972 (Vornatscher 1972) in Vienna may in fact correspond to *N. tonywhitteni*, implying that this species really has been confirmed rather than newly detected for Vienna.

Community compositions and environmental influences

Understanding the environmental factors that shape groundwater communities is essential for explaining patterns of subterranean biodiversity. We show a key influence of geology/aquifer type, dissolved oxygen (DO) concentration, and surface water influence (Tab. 2). Nevertheless, our model explained only a small fraction of the observed variation ($\approx 15\%$). The geology/aquifer type emerged as the most influential variable with respect to crustacean community composition. Different aquifer types provide distinct environmental conditions and habitat qualities that may be favourable for certain taxa, while unfavourable for others. Smaller groundwater crustaceans with particularly elongated bodies (Bathynellacea and Harpacticoida) were found more frequent in habitats with lower K_f values, despite groundwater fauna generally preferring larger pore space and higher permeability, which provides a better replenishment of oxygen and food (Hose et al. 2017; Korbel et al. 2013, 2019; Korbel & Hose 2015). In fact, crustaceans with larger body sizes (Amphipoda and Isopoda) were mostly found in gravel aquifers with high K_f values. Finer sediments may serve as refugia for stygofauna with smaller body sizes, limiting access for larger predators and competition. However, sample sizes for bathynellids and harpacticoids were relatively small, and further data is needed to confirm these patterns. The role of geological heterogeneity and sediment structure in shaping subsurface invertebrate communities is generally well established (Schmidt & Hahn 2012; Korbel and Hose 2015). However, in our study the limited explanatory power is consistent with the subsequent occurrence-based analyses, which showed no significant associations between aquifer type and total crustacean occurrences. Only a few patterns emerged but were largely restricted to specific taxonomic groups or species.

Dissolved oxygen emerged as the most consistent predictor across the statistical analyses. Higher DO levels tended to be associated with communities dominated by Amphipoda, Cyclopoida, Bathynellacea, and Isopoda, while lower DO coincided more with Ostracoda-dominated assemblages. Most crustacean groups occurred primarily under oxic conditions and were negatively associated with low oxygen and reduced conditions indicated by elevated ammonium and ferrous iron concentrations. This is also mirrored in the relationship with the redox categorical variable, as most crustacean groups, were confined to oxic environments and absent under highly reduced conditions, while moderately reducing conditions seemed to limit, but not entirely prevent, their occurrence. This suggests that DO levels ≤ 2 mg/L already begin to negatively affect crustacean occurrence, with progressively reducing conditions further aggravating this effect. Nevertheless, crustaceans were

occasionally also found at very low DO levels, pointing at short-term tolerance of hypoxic conditions, consistent with previous reports (Danielopol 1989; Malard & Hervant 1999; Mösslacher 2000). While DO concentrations below 1 or 0.5 mg/L are defined as critical (Malard & Hervant 1999; Strayer 1994; Hahn 2006), levels below 0.1 mg/L are believed to strictly limit longer survival (Strayer et al. 1997). Stygobionts can endure fluctuating DO availability through rapid recovery from anaerobic stress, allowing them to exploit a range of suboxic (< 0.3 mg/L), dysoxic (0.3–3.0 mg/L), and oxic (> 3 mg/L) habitats (Danielopol et al. 1994; Malard & Hervant 1999). This likely explains their presence across a broad range of DO availability, although they may clearly prefer well-oxygenated conditions, which is confirmed by our study and reported in other studies as well (Pospisil 1994; Pospisil et al. 1994; Malard et al. 1996b, 1997).

In accordance with the findings regarding DO and redox-sensitive variables is the positive association of total crustacean occurrences, and specifically isopods, harpacticoids and cyclopoids, with nitrate. Nitrate is microbially only turned over at hypoxic to anoxic conditions. As such presence of nitrate generally assumes the presence of dissolved oxygen. Despite being one of the most prominent groundwater pollutants (Becher et al. 2022), nitrate appears to influence groundwater crustaceans less negatively than previously assumed (Dumas & Lescher-Moutoué 2001; Galassi et al. 2009; Di Lorenzo & Galassi 2013; Di Lorenzo et al. 2021; Gerhardt 2020; Jemec-Kokalj et al. 2026). On the contrary, groundwater crustaceans regularly exhibit a strong sensitivity to ammonium (Becher et al. 2022), which is in accordance with the negative correlation between ammonium and the presence of total crustaceans, and specifically amphipods, bathynellids, cyclopoids, and isopods observed in this study. Ammonium is quite stable under reduced conditions but microbially oxidized to nitrite and nitrate upon availability of dissolved oxygen. Additionally, microbiological variables and proxies, including microbial activity (intracellular ATP), bacterial abundance (TCC), and dissolved organic carbon (DOC), showed a significant negative relationship with the presence of total crustaceans, and specifically amphipods, cyclopoids, and isopods. These variables have been proposed bioindicators of groundwater ecosystem status sensitively hinting at disturbances such as surface water influence and organic contamination (Fillinger et al. 2019), with elevated values frequently linked to oxygen depletion and reducing conditions (Kunkel et al. 2002). Moreover, higher levels of energy enhancing food availability, have been shown to promote the invasion of stygoxen and epigean species (Malard et al. 1994, 1996a; Sket 1999).

Amphipoda showed a significant preference for lower temperatures, whereas no significant effects were detected for the other groups of crustaceans. Elevated groundwater temperatures are widely recognized as a major stressor in groundwater ecosystems (Briellmann et al. 2009; Griebler et al. 2016). Urban aquifers regularly experience groundwater warming due to extensive surface sealing, geothermal installations, district heating systems, heat emission from sewage pipes and underground infrastructure (Taniguichi et al. 2005; Menberg et al. 2013; Kaminsky et al. 2025b). Exposure to temperatures outside the natural thermal range (usually 10 - 12°C in low altitude regions of Central Europe) induces physiological stress (e.g. suppresses reproduction) in groundwater invertebrates (Bannick et al. 2008; Thulin & Hahn 2008; Griebler et al. 2016). Above 14°C, groundwater crustacean biodiversity was found to significantly change in the Upper Rhine Valley (Spengler 2017). Briellmann et al. (2011) reported strong negative effects of prolonged thermal stress with *Niphargus*

inopinatus exhibiting an LT_{50} (temperature causing 50 % mortality with the test organisms) of 20.2 °C after 20 days of exposure, which is very close to the upper temperature threshold observed for amphipods in this study. Worth mentioning, the LT_{50} values were much lower with *Proasellus cavaticus* (Briellmann et al. 2011). Besides direct effects, increasing water temperatures are linked to reduced oxygen solubility while simultaneously increasing metabolic demand/rates of microbes and metazoans reducing oxygen levels further. The overall absence of crustaceans at temperatures above 22 °C is consistent with the findings by Foulquier et al. (2011), who reported an almost complete absence of groundwater fauna above this threshold.

Regarding interactions with surface water, distance to surface water affected groundwater Crustacea distribution, but effects were limited. Only Cyclopoida showed a significant preference for habitats farther from surface waters. In areas close to surface water, stronger hydrological exchange also increases the likelihood of pollutant entry from infiltrating surface water, which may reduce habitat suitability and outweigh potential benefits, such as higher food availability. With respect to the variable “surface water influenced”, only Ostracoda exhibited a significant positive association. Ostracoda also appeared to tolerate varying DO levels and microbial loads and may thus benefit from additional organic material imported. Surface water influence played only a comparatively minor role in structuring groundwater communities. Previous studies suggest that strong surface water influence can promote entry of stygophile and hyporheic species, whereas non-influenced samples tend to be dominated by stygobionts (Schmidt & Hahn, 2012); notably, in this study, the stygophile species *P. lobipes* was predominantly found in wells influenced by surface water, supporting these observations.

Several hydrochemical parameters were significantly associated with individual crustacean groups, however, it was found that these patterns likely simply reflect the geological background rather than direct physiological effects from water components. Due to the observed values being well below thresholds that were shown to negatively impact groundwater species, hydrochemical variables appeared to shape taxa distribution rather indirectly through geological and hydrogeological context, than through toxicity. The modest explanatory power of the aquifer type for several taxa further suggests that small-scale heterogeneity and groundwater mixing processes may override coarse geological classifications.

Certain parameters showed statistically significant associations with crustacean occurrence, but their ecological interpretation remains challenging. Water column height was negatively associated with occurrences of Bathynellacea, Cyclopoida, and Harpacticoida, with lower water levels corresponding to higher occurrences. Although a positive effect could be explained by larger filter sections facilitating fauna entry from different depths, the observed negative relationship remains difficult to interpret. The percentage of surface sealing had a significant but counterintuitive effect with Harpacticoida. This group unexpectedly showed higher occurrence in more strongly sealed areas, despite sealed surfaces generally reducing groundwater recharge, and contributing to groundwater warming (Tissen et al. 2019). Given the low overall occurrence of Harpacticoida in this study, this finding should be interpreted with caution and requires confirmation.

Seasonality had no significant effect on groundwater crustacean community composition, which is consistent with the findings of Korbelt & Hose (2015). While groundwater habitats are environmentally more stable than surface waters, dependent to depth they are subject to seasonal fluctuations, often with a certain delay (Steiner et al. 2024). Stygofauna species appear to be well adapted to moderate fluctuations, particularly those living in shallow aquifers. Nevertheless, the rapid pace of climate change may challenge this fragile adjustment, potentially leading to a cumulative negative effect on groundwater fauna biodiversity in the future (Englisch et al. 2024; Kaminsky et al. 2025b) – an aspect that requires further research.

Species-specific patterns among amphipods

Assessing species-specific environmental associations is challenging due to strongly uneven sample sizes between species, with several species being represented by only a few records. The most significant differences emerged from comparisons between samples with and without amphipods rather than between single amphipod species. Consistent patterns could be observed for the variables DO, ferrous iron, redox category, TCC, ATP and DOC, with amphipods generally occurring more frequently under oxic conditions with lower microbial activity/abundance as well as lower ferrous iron and DOC concentrations. Although mainly the most frequently recorded amphipod species (*Niphargus inopinatus*, *N. caspary* and *N. bajuvaricus*) reached statistical significance for most variables, similar trends were exhibited by nearly all amphipod species, suggesting broadly comparable environmental associations rather than strong niche segregation.

Interspecific differences were most notably observed in the cases of *Bogidiella albertimagni* and *Niphargus* aff. *aquilex*. *B. albertimagni* occurred at greater well depths and water column heights as well as lower microbial activity, which could indicate a higher affinity to somewhat deeper, more stable groundwater habitats. *N.* aff. *aquilex* was detected exclusively at a single site with very distinct hydrogeochemical characteristics (e.g. a unique aquifer type, elevated ATP, TCC, and DOC concentrations, moderate to high DO levels, a very short water column, shallow well depth, high surface sealing, very low hydraulic conductivity, and exceptionally high electrical conductivity). The differences in environmental associations of *N.* aff. *aquilex* could indicate a highly specialized habitat preference or distinctive tolerances of this species, but more likely reflect site-specific conditions rather than ecological specialization. Given the low number of records these patterns should be interpreted cautiously.

With respect to aquifer type, the results indicated an overall association of amphipods (especially of the most frequently found *N. inopinatus*) with high interstitial connectivity (Danube Plains), in contrast to samples without amphipods, which were more frequent in settings with low hydraulic conductivity. This underlines the importance of hydraulic connectivity and pore space for groundwater amphipods. Interestingly, *N. tonywhitteni* was the only species recorded in a Local Aquifer, whereas the Liesing Stream Aquifer was exclusively inhabited by *N. bajuvaricus*. In contrast to *N.* aff. *aquilex*, however, these species were not restricted to single sites or aquifer types. Overall, this suggests that at least the more frequently encountered amphipod species occupy similar aquifer types and do not exhibit strong species-specific segregation at this scale. Ultimately, the results suggest that common amphipod species show largely similar environmental preferences, while apparent differences involving

rarer taxa are likely influenced by the limited sample sizes. Larger datasets or meta-analysis are proposed to determine whether these patterns reflect genuine ecological differentiation or statistical artefacts. Worth mentioning in this context is the difference in morphological traits related to feeding found with commonly occurring amphipod species (Fig. 9).

Conclusion and Outlook

This study advances our understanding of groundwater crustacean fauna, particularly in urban environments such as the city of Vienna. The discovery of 47 stygobiont and stygophile crustacean taxa, which is an exceptionally high richness for an urban setting, highlights substantial knowledge gaps in the distribution and diversity of these organisms and underscores the need for continued research. Despite their ecological importance, subterranean ecosystems/species remain poorly represented in conservation frameworks. Limited knowledge on stygofauna distribution and ecological requirements, combined with the restricted accessibility of groundwater ecosystems, continues to hinder effective conservation planning. Recent initiatives aim to address these gaps. At the European level, the Biodiversa+ project “DarCo – The vertical dimension of conservation” seeks to integrate subterranean ecosystems into the EU Biodiversity Strategy for 2030, while national projects such as “Stygofauna Austriaca” and “Heat below the City” focussed on groundwater biodiversity, resilience and protection, the latter emphasizing urban aquifers in Vienna. Despite increasing attention, specifically urban groundwater ecosystems remain understudied. Ecologically, the identification of key environmental drivers is crucial for developing effective conservation strategies. Our study showed that maintaining oxygen-rich, oligotrophic conditions and avoiding excessive warming represent impactful and feasible conservation measures. Similar preferences for environmental conditions suggest that basic conservation strategies could effectively protect groundwater crustacean fauna without multiple species- or community-specific measures. However, rare taxa, including *B. albertimagni*, *N. tonywhitteni*, *C. subterraneus* and particularly *N. aff. aquilex*, require specific attention in future research to uncover their environmental demands and ecological roles. In the future, the combined application of morphological and molecular methods, as demonstrated here, offers a promising approach to resolve taxonomic uncertainties. Moreover, interdisciplinary approaches, integrating ecological, geochemical and hydrological perspectives are crucial in advancing our understanding of groundwater ecosystems and its biodiversity.

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Author contributions

CN contributed to conceptualization, data curation, analysis and visualization, interpretation and discussion of results, took lead in writing of the original draft, and assisted in reviewing and editing. CE contributed to data curation, analysis and visualization, interpretation and discussion of results, assisted in writing of the original draft, as well as in reviewing and editing, and acquired additional funding. CZ contributed to conceptualization, data analysis and visualization, interpretation and discussion of results, and assisted in reviewing and editing. FS supported in molecular analysis and assisted in reviewing and editing. AF contributed with morphological species identification and assisted in reviewing and editing. SG contributed with morphological species identification and assisted in reviewing and editing. EK contributed to data curation and analysis and assisted in reviewing and editing. CStumpp contributed to acquisition of funding, project coordination, data curation, and assisted in reviewing and editing. CSteiner contributed to data analysis and assisted in reviewing and editing. EBN contributed to data analysis and assisted in reviewing and editing. CK contributed to data analysis and assisted in reviewing and editing. CG was project leader, contributed to conceptualization, data curation, analysis and visualization, interpretation and discussion of results, guided the reviewing and editing process, and acquired the main funding.

Electronic Supplement

S1: Project “Heat below the City2. Groundwater environmental data of the city of Vienna from 2021 and 2022. | **S1:** Projekt „Hitze unter der Stadt“. Daten zur Grundwasserfauna in Wien aus 2021 und 2022.

https://www.zobodat.at/pdf/supp/Nilles_et_al_Supplement_1.xlsx

S2: Project “Heat below the City”. Groundwater fauna data of the city of Vienna from 2021 and 2022. | **S2:** Projekt „Hitze unter der Stadt“. Daten zur Grundwasserfauna in Wien aus 2021 und 2022.

https://www.zobodat.at/pdf/supp/Nilles_et_al_Supplement_2.xlsx

S3: Uncollapsed phylogenetic tree based on COI sequences from amphipod species sequenced in the HBTC project. | **S3:** Detaillierter Phylogenetischer Baum auf Basis von COI Sequenzen für ausgewählte Amphipoden aus der Projektstudie HBTC.

https://www.zobodat.at/pdf/supp/Nilles_et_al_Supplement_3.pdf

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